The complex evolution of the metazoan HSP70 gene family

Er-meng Yu[1,2], Tatsuki Yoshinaga[3], Frank L. Jalufka[1], Hashimul Ehsan[1], David B. Mark Welch[4*], Gen Kaneko[1*]

[1]School of Arts & Sciences, University of Houston-Victoria, Victoria, TX.

[2]Key Laboratory of Tropical & Subtropical Fishery Resource Application & Cultivation, Key Laboratory of Aquatic Animal Immune Technology of Guangdong Province, Pearl River Fisheries Research Institute of CAFS, Guangzhou, China.

[3]School of Marine Biosciences, Kitasato University, Kanagawa, Japan.

[4]Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, Marine Biological Laboratory, MA.

***Corresponding author:**

Gen Kaneko, kanekog@uhv.edu

David Mark Welch, dmarkwelch@mbl.edu

Abstract

The metazoan 70-kDa heat shock protein (HSP70) family contains several members localized in different subcellular compartments. The cytosolic members have been classified into inducible HSP70s and constitutive heat shock cognates (HSC70s), but their distinction and evolutionary relationship remain unclear because of occasional reports of "constitutive HSP70s" and the lack of cross-phylum comparisons. Here we provide novel insights into the evolution of these important molecular chaperones. Phylogenetic analyses of ~100 full-length HSP70s revealed an ancient duplication that gave rise to two lineages from which all metazoan cytosolic HSP70s descend. One lineage (A) contains a relatively small number of Lophotrochozoan and Ecdysozoan genes, none of which have been shown to be constitutively expressed (i.e., either inducible or unknown). The other lineage (B) included both inducible and constitutive genes from diverse phyla. Species-specific duplications are present in both lineages, and Lineage B contains well-supported phylum-specific clades for Rotifera, Nematoda, and Chordata. Some genes in Lineage B have likely independently acquired inducibility, which may explain the sporadic distribution of "HSP70" or "HSC70" in previous analyses. Consistent with the diversification history within each group, inducible members show lower purifying selection pressure compared to constitutive members. These results illustrate the evolutionary history of the HSP70 family, encouraging us to propose a new nomenclature: "HSP70 + subcellular localization + linage + copy number in the organism + inducible or constitutive, if known." e.g., HSP70cA1i for cytosolic Lineage A, copy 1, inducible.

Key words: heat shock cognates, molecular evolution, rotifer, stress inducibility, synonymous and nonsynonymous substitution.

Introduction

The 70-kDa heat shock protein (HSP70) family members play important roles in various cellular processes including heat shock response, folding of newly synthesized proteins, protein transport, and protein degradation. These apparently diverse functions are attributed to their chaperone activity (Daugaard et al. 2007; Hartl et al. 2011), by which they prevent the aggregation and misfolding of target proteins. Typically, HSP70 family members bind to denaturing or newly synthesized proteins by recognizing up to ten hydrophobic amino acid residues exposed to the protein surface because of the misfolding. The release of the HSP70 members, which is triggered by ATP hydrolysis, facilitates the proper folding of the target proteins (Zuiderweg et al. 2017). The HSP70 family contains organelle-specific members localized in cytosol, endoplasmic reticulum (ER), mitochondria, and chloroplasts (Miernyk 1997), and these organelle-specific types not only perform chaperoning functions in the organelles but are also known to contribute to protein transport across organelle membranes.

HSP70 family members are often upregulated upon various stresses that disrupt protein folding, such as heat treatment, exposure to toxic materials, ultraviolet irradiation, and pathogen attack (Sørensen et al. 2003; Baird et al. 2006). The upregulation is primarily regulated at the transcription level by transcription factors called heat shock factors (HSFs), particularly HSF1. Under unstressed conditions, HSF1 remains in a monomeric state. Various stresses lead to the formation of HSF1 trimers, which in turn bind to heat shock elements (HSEs) in the promoter region of HSP70 family member genes and promote their transcription. The HSF1-dependent transactivation system is generally conserved among eukaryotes, although species-specific differences in HSF1 function have been reported in many organisms such as the fruit fly *Drosophila melanogaster* (Jedlicka et al. 1997; Marchler and Wu 2001) and yeast *Saccharomyces cerevisiae* (Sorger and Pelham 1988). The expression of HSP70 family member is also regulated by many other mechanisms such as the unfolded protein response, chromatin modification, and other transcription factors depending on the type of stress and subcellular localization (De Nadal et al.

67 2011; Garbuz 2017). HSP70 family member genes have therefore been used as biomarkers of
68 environmental stresses both in laboratory and field experiments (Sanders 1993; Ceyhun et al. 2010;
69 Judge et al. 2011).

70 On the other hand, several cytosolic HSP70 family members show constitutive expression
71 patterns. These proteins are involved in the folding of newly synthesized proteins and are
72 traditionally called 70-kDa heat shock cognates (HSC70). However, the distinction between
73 HSP70s and HSC70s remains unclear because of the occasional reports of "constitutive HSP70s,"
74 especially from invertebrates (Jayasena et al. 1999; Piano et al. 2002; Liu et al. 2017). Stress-
75 induced upregulation of HSC70 has also been reported in several animals including shrimps (Luan
76 et al. 2010), snails (Zheng et al. 2012), and fish (Yabu et al. 2011). Furthermore, little is known for
77 the evolutionary relationship between metazoan HSP70s and HSC70s because of the lack of cross-
78 phylum comparisons. A previous phylogenetic analysis indicated that metazoan HSP70 family
79 members can be classified into invertebrate HSP70s, vertebrate HSP70s, and HSC70s from both
80 vertebrates and invertebrates (Kourtidis et al. 2006), but this study comprises only a few phyla. In-
81 depth phylogenetic analysis on HSP70 family members with a broad sampling would offer further
82 insight into the classification of this important group of molecular chaperones.

83 In this study, we sought to trace the evolutionary history of metazoan HSP70 family members
84 by cross-phylum phylogenetic analyses with a particular attention to their stress inducibility. The
85 specific hypothesis was that the cross-phylum analysis would provide the essential information to
86 solve the evolutionary relationship between metazoan HSP70s and HSC70s. In order to add insights
87 from an emerging model in evolutionary biology to the analyses, we first cloned two stress-
88 inducible HSP70 family member genes from a monogonont rotifer, *Brachionus plicatilis*. The
89 phylum Rotifera, composed of mostly microscopic aquatic animals with about 1,000 cells and a
90 ciliated head structure, is part of the Gnathifera, a group of basal-branching phyla related to
91 Lophotrochozoa, which contains Mollusca and Annelida. Together these groups form a sister-clade
92 to Ecdysozoa, which contains the established invertebrate models *Caenorhabditis elegans* and *D.*

93 *melanogaster* (Struck et al. 2014; Fröbius and Funch 2017). Genetic information from Gnathifera
94 remains limited, making rotifers useful models in evolutionary studies. Our results added novel
95 groups of stress-inducible HSP70, providing important insight into the evolutionary history of the
96 metazoan HSP70 family.
97
98 **Results**
99 Molecular Characterization of Rotifer HSP70 Genes
100 A combination of 3' and 5' RACE identified two *B. plicatilis* HSP70 genes consisting of 2,121
101 and 2,248 bp, designated as HSP70cB1i and HSP70cB2i, respectively (see Discussion for the
102 nomenclature). The nucleotide sequence of the open reading frames, as well as the absence of
103 introns, were ascertained by single PCR experiments using cDNA or genomic DNA, respectively,
104 followed by DNA sequencing. The deduced amino acid sequences of HSP70-1 and HSP70-2 genes
105 were more than 99% identical to each other (supplementary fig. S1). Both contain the HSP70
106 protein family signatures, IDLGTTYS, IFDLGGGTFDVSIL, and IVLVGGSTRIPKVQK (Prosite
107 motifs PS00297, PS00329, and PS01036, respectively), as well as the non-organelle stress protein
108 motif RARFEEL found in several cytosolic HSP70s (Lo et al. 2004; Cottin et al. 2008; Simoncelli
109 et al. 2010; Zheng et al. 2012). The major difference between *B. plicatilis* HSP70 genes was the
110 number of GGMP repeats, which are involved in binding to the HSP70-HSP90-organizing protein
111 (Hop), a cofactor of HSP70 (Demand et al. 1998). The nucleotide sequences of *B. plicatilis*
112 HSP70cB1i and HSP70cB2i genes were registered into the DDBJ/EMBL/GenBank databases with
113 accession numbers AB775784 and AB775785, respectively. The two HSP70 genes were also found
114 in the *B. plicatilis* genomic sequence.
115 We next examined whether the mRNA levels of *B. plicatilis* HSP70s are increased by heat
116 stress. Due to the high sequence identity between *B. plicatilis* HSP70 genes and difficulty in
117 designing a TaqMan probe, we employed semi-quantitative RT-PCR to measure their respective
118 mRNA levels. Primers were designed to amplify DNA fragments of 112 and 124 bp from

119 HSP70cB1i and HSP70cB2i cDNAs, respectively (fig. 1A), and the PCR products were separated
120 using a polyacrylamide gel. The mRNA levels of HSP70cB1i and HSP70cB2i in heat-treated
121 rotifers were 2.9 and 7.5 times higher, respectively, than those in control rotifers (fig. 1B and 1C).
122 These results indicate that both genes encode heat-inducible HSP70.
123 Since the two *B. plicatilis* HSP70 genes showed a similar expression pattern in response to
124 heat stress, we used quantitative real-time PCR primers that amplify both HSP70cB1i and
125 HSP70cB2i genes in the subsequent time-course expression analyses (fig. 1D). The relative mRNA
126 levels were increased by heat stress at 40˚C for 10 min and reached at the maximum level 2 h after
127 the heat stress. The difference in mRNA levels between the control and 2 h groups was statistically
128 significant. The mRNA levels of *B. plicatilis* HSP70s remained twofold 8 h after the heat stress. We
129 then performed *in situ* hybridization using DIG-labeled RNA probes (fig. 1E). The probe region
130 contained only five base pair differences between the two HSP70 genes, and thus the RNA probes
131 were likely to hybridize with both HSP70cB1i and HSP70cB2i mRNAs. The hybridization signals
132 increased in all tissues of heat-treated rotifers. Altogether, these results indicate that the *B. plicatilis*
133 HSP70cB1i and HSP70cB2i genes are heat-inducible and have similar expression profiles.
134
135 Phylogenetic Analysis of HSP70 Family Members
136 To assess the relationship of the *B. plicatilis* HSP70 genes with those of other rotifers and
137 other metazoans, we constructed gene trees of more than 100 HSP70 family members, including
138 members associated with the mitochondria and ER and many sequences known only from
139 automated genome annotation as "HSP70." To assess the robustness of the results we used
140 alignments made by three different methods [Clustal-Omega (Sievers et al. 2011), M-Coffee
141 (Wallace et al. 2006), and Expresso (Armougom et al. 2006)] to construct consensus trees with
142 maximum-likelihood (RAxML) and Bayesian (MrBayes) approaches. The RAxML
143 PROTGAMMAAUTO function found that the LG model of protein evolution (Le and Gascuel
144 2008) was optimal for all three alignments and this model was used to construct ML and Bayesian

consensus trees. The six trees were in general agreement for major nodes; fig. 2 shows the Bayesian consensus tree of the Expresso alignment and support for major nodes from each approach is shown in table 1. In all analyses HSP70 family members known to be mitochondria- and ER-specific formed two monophyletic groups (nodes 11 and 12 in fig. 2) in agreement with previous reports (Boorstein et al. 1994; Miernyk 1997; Nikolaidis and Nei 2004). All family members known to be cytosolic formed a third group (node 1; clades 1–6 in fig. 2), with yeast cytosolic HSP70s (clade 6) basal to all metazoan cytosolic HSP70s. The tree topology generally supported the species phylogeny. Within the metazoan cytosolic HSP70s (node 2; clades 1–5) there were two lineages: Lineage A was well supported and contained of genes from arthropods, molluscs, and rotifers (node 3; clade 1); Lineage B was more poorly resolved and represented by many diverse vertebrate and invertebrate phyla (node 4; clades 2–5). There are two groups of vertebrate cytosolic HSP70s (nodes 8 and 9), which form a monophyletic group (node 7; clade 5) in Bayesian and RAxML analyses of Expresso and Clustal alignments. Unexpectedly, we found two additional clusters of cytosolic HSP70 family members in all analyses: one composed of HSP70 genes from the phylum Rotifera (node 5; clade 2) and the other from the phylum Nematoda (node 6; clade 3). This suggests the evolution of two unique subgroups of HSP70, distinct from conventional vertebrate and invertebrate HSP70s. We also constructed phylogenetic trees mainly using HSP70 family members of known expression patterns (supplementary figs. S2 and S3). Both of the two HSP70 lineages as well as most of the nodes described above were identified, supporting the robustness of the tree shown in fig. 2, although several clades were poorly resolved in supplementary figs. 2 and 3 that have limited number of samples.

The phylogenetic breadth of our analyses provided the opportunity to search for sequence motifs characteristic of different groups within the HSP70 family. We identified a single region near the N terminus (but after signal peptide motifs) that discriminates between metazoan mitochondrial, ER, and cytosolic HSP70s (fig. 3), which enables better discrimination for organelle-specific HSP70s together with the well-known C-terminal motifs for cytosolic (EEVD) and ER

171 (HDEL/KDEL) HSP70s (supplementary fig. S4). We also found the RARFEEL motif, previously
172 described as possibly cytosolic-specific in conjunction with the EEVD motif (Cottin et al. 2008;
173 Simoncelli et al. 2010; Zheng et al. 2012), in several invertebrate genes that are found with high
174 confidence in the ER-associated node 12 (fig. 2; *B. ibericus* HSC70, *C. gigas* GRP, and *B.*
175 *manjavacas* HSC71). This result suggests that the RARFEEL is not a reliable predictor of cytosol
176 association. Indeed, this motif was identified by a comparison of few sequences (Lo et al. 2004) and
177 appears to have no strong scientific support.
178 Stress-inducible invertebrate HSP70s are reported to have an extra serine residue in their
179 ATPase domain (Kourtidis et al. 2006; Garbuz et al. 2011). In our analysis this insertion occurs only
180 in Lineage A irrespective of the stress-inducibility, where it is present in all sequences except *B.*
181 *ibericus* HSP70 and one of four *Lottia* HSP70s; in *Priapulus* HSP70-1 the serine has been replaced
182 by alanine (fig. 4). These results suggest that the extra serine residue is a characteristic of this
183 lineage and may be associated with the stress-inducibility of the ancestral HSP70, which will be
184 tested by broader sampling focusing on this residue. The alignment result supports the idea that
185 HSP70s from Nematoda and Rotifera evolved separately from the those in Annelida, Mollusca and
186 Arthropoda, which have been generally recognized as "invertebrate HSP70s."
187
188 Synonymous and Nonsynonymous Substitution Rates
189 Lastly, we calculated the synonymous and nonsynonymous substitution rates of 34
190 representative cytosolic HSP70 member genes. We primarily selected genes with known expression
191 patterns (stress-inducible or constitutive) from each clade, hypothesizing that constitutive HSP70
192 family members have been under stronger purifying selection compared to stress-inducible
193 members. Only well-conserved regions were used for the calculation (supplementary fig. S5). We
194 noted that *B. ibericus* HSP70 (GU574486) has an insertion at nucleotide position 403 and a
195 nucleotide deletion at position 563, causing a frame shift. Deduced amino acid sequences encoded
196 by nucleotides 403–563 were markedly different from those of other HSP70 family members

197 because of the frame shift, which resulted in the long genetic distance in phylogenetic trees (fig. 2).
198 Because the frame shift greatly affects the synonymous and nonsynonymous substitution rates, this
199 sequence was excluded from our analysis.

200 For the selected 34 sequences, we calculated synonymous and nonsynonymous substitution
201 rates (Ks and Ka) for all combinations of representative cytosolic members of the HSP70 family
202 from metazoans (fig. 5A). Synonymous substitution rates (Ks) were saturated in many combinations
203 and only a limited number of combinations produced meaningful results (229 out of 465
204 combinations, supplementary material 1). The Ka/Ks values calculated for the 229 combinations
205 were averaged for each clade (fig. 5B). Overall, Ka/Ks values were markedly lower than 1,
206 indicating that cytosolic HSP70 family member genes have been under purifying selection. The
207 purifying selection pressure appears to have been less in Lineage A (clade 1) than in Lineage B
208 (clades 2-5). The effect size between these lineages ranged from 0.19 to 1.64 (fig. 6C). Since the
209 effect size larger than 0.8 indicate large difference in multiple comparisons, these results support
210 our hypothesis that constitutive HSP70 family members [clade 4 and clade 5 (node 8)] have been
211 under stronger purifying selection.

212 Within Lineage B, the selection pressure was strongest in Nematode HSP70s (clade 3),
213 invertebrate HSC70/HSP70s (clade 4), and vertebrate HSC70s (clade 5, node 8). In particular,
214 vertebrate HSC70s (clade 5, node 8) and HSP70s (clade 5, node 9) showed significant difference in
215 the Ka/Ks with a large effect size of 0.86 despite their close phylogenetic relationship. These results
216 also support the above hypothesis. To confirm how much this finding depends on the sampling, we
217 conducted the same analysis with a larger sample size, including most genes used in the
218 phylogenetic analysis, and obtained consistent results (supplementary fig. S6).

219

220 **Discussion**

221 A previous phylogenetic analysis by Kourtidis et al. (2006) classified metazoan cytosolic
222 HSP70s into three groups, invertebrate HSP70, vertebrate HSP70, and HSC70 including both

223 vertebrate and invertebrate orthologues using data from a few taxa (Kourtidis et al. 2006). The
224 monophyletic origin of HSC70s was also proposed by Nikolaidis and Nei (2004) based on their
225 analysis using *Drosophila* and nematode sequences (Nikolaidis and Nei 2004). Surprisingly, to our
226 knowledge, there has been no significant update on the evolution of metazoan HSP70 family after
227 these small-scale studies despite the continuing expansion of available genomic data. Here we show
228 that there are at least two types of ancestral cytosolic HSP70 family member genes in all analyzed
229 phyla of metazoans, one (node 3; clade 1) giving birth to Lineage A of invertebrate HSP70s, and a
230 second (node 4; clade 5) to Lineage B of both vertebrate and invertebrate HSP70s and all HSC70
231 genes. This second lineage has further diversified within diverse phyla: Rotifera (node 5; clade 2),
232 Nematoda (node 6; clade 3), and Chordata (node 7; clade 5) (fig. 2), which has not been identified
233 in the previous reports. The tree topology is generally consistent with the species phylogeny,
234 indicating that the effect of sequence contamination is very limited.

235 Inter-clade comparison of the synonymous and nonsynonymous substitution rates shows that
236 while all HSP70 family members are under strong purifying selection, the pressure to conserve
237 amino acid sequence varies across clades. The presence of purifying selection has been reported for
238 mammalian (Hess et al. 2018), nematode (Nikolaidis and Nei 2004), and molluscan (Kourtidis et al.
239 2006) HSP70 genes, and our cross-phylum analysis was consistent with these reports. Most
240 dramatically, the canonical HSC70 genes of vertebrates (node 8; clade 5) showed stronger purifying
241 selection pressure than the HSP70 family members in node 9 (clade 5). This concept can be further
242 generalized by thoroughly investigating the saturation rate between each HSP70 gene (Pollock and
243 Larkin 2004). It is thus speculated that the role of HSC70 protein, the folding of newly synthesized
244 proteins, is more essential for cell survival compared to the role of the HSP70 cognates (node 9;
245 clade 5). In line with this speculation, cells deficient in the HSC70 gene are non-viable (Florin et al.
246 2004), whereas HSP70 knockout mice are mostly viable and fertile, although they are known to be
247 sensitive to stress (Daugaard et al. 2007). Meanwhile, genetic distance between each clade is not
248 uniform, and the gene conversion may also affect the accuracy of this calculation. In this regard our

249 data should be interpreted in a semiquantitative manner.

250 It is known that nematode, mammalian, and molluscan HSP70 families have experienced gene
251 conversion events (Nikolaidis and Nei 2004; Kourtidis et al. 2006; Hess et al. 2018). In addition to
252 the purifying selection, gene conversion also likely contributes to the highly conserved nature of the
253 HSP70 family members found in this study. The tree topology might have been affected by the gene
254 conversion in closely related paralogues and orthologues such as human HSPA1A and HSPA1B
255 (Hess et al. 2018). However, gene conversion generally functions to conserve the sequence in a
256 species, and thus the overall topology of the metazoan tree is unlikely to be significantly affected.
257 Gene conversion rate should be about 10 times higher than the mutation rate to significantly affect
258 the topological distance according to a genomic simulation study (Touchon et al. 2009), but the
259 nucleotide identity between sequences is often lower than 70% in our phylogenetic analysis.

260 Given the presence of inducible and constitutively expressed genes in many clades of
261 metazoan cytosolic HSP70, it is difficult to predict the ancestral state of this phenotype, and
262 whether a gene is inducible or constitutive expression does not predict its relationship to other
263 family members. Phylogenetic analyses, the novel signature sequences near the N terminus (fig. 4),
264 and the extra serine residue (fig. 5) would provide better clues for the classification of HSP70
265 family members than their expression patterns under stress.

266 Based on the largest-ever cross-phylum phylogenetic analysis on HSP70 family, we propose a
267 new nomenclature that reflects the phylogenetic relationship of the HSP70 family members (fig. 6).
268 The proposed name of an HSP70 family member protein consists of "HSP70 + subcellular
269 localization + linage + copy number found in the organism + inducible or constitutive, if known."
270 For example, HSP70cA1i represents a cytosolic HSP70 in Lineage A, copy 1, inducible; and
271 HSP70mA1 for mitochondrial linage of unknown inducibility. Although the current study found
272 only one lineage in mitochondrial and ER members, the use of "A" in these organelle-specific
273 isoforms would be beneficial. Systematic lineage names such as HSP70cD and HSP70mB can be
274 assigned for novel lineages that may be discovered in future. Gene names are represented by

275 lowercase italics of protein names (e.g., *hsp70cb2i* and *hsp70era1*).

276 In conclusion, the present study identified two novel HSP70 family members from a
277 monogonont rotifer, an emerging model in evolutionary biology. The subsequent phylogenetic
278 analyses illustrated the evolutionary history of the metazoan HSP70 family, in which stress
279 inducibility does not reflect evolutionary history. The proposed nomenclature based on molecular
280 evolution will help us understand the true nature of the HSP70 family.

281

282 **Materials and Methods**

283 Culture

284 We used the *B. plicatilis* Ishikawa strain (also called *Brachionus* sp. ISKW), originally
285 isolated from a Japanese eel culture pond (Yoshinaga et al. 2004), for cDNA cloning, semi-
286 quantitative RT-PCR, quantitative real-time PCR, and *in situ* hybridization. The cytochrome oxidase
287 subunit I gene sequence has been registered for detailed identification of this strain (GenBank
288 accession number LC422762). Rotifers were cultured at 25 ˚C by a standard protocol (Kaneko et al.
289 2016) using half-diluted Brujewicz artificial seawater and fed the algae *Chlorella regularis* (Nikkai
290 Center, Tokyo, Japan) at a final concentration of approximately 7×10^6 cells/mL. See
291 supplementary material 2 for details of molecular characterization of rotifer HSP70 genes.

292

293 Phylogenetic Analysis

294 Metazoan HSP70 family genes were selected based on the following criteria: 1) genes from
295 diverse metazoan phyla are included; 2) HSP70 genes with known expression patterns are
296 prioritized; 3) both Lineage A and Lineage B genes from the same organism are included when
297 possible; and 4) the number of genes from a single species is limited to ~5 because within-phylum
298 gene duplications do not change the tree topology. Annotated and unannotated genome and
299 transcriptome databases were used. A fasta file containing sequences used for the tree is provided as
300 supplementary material 3.

301 A multiple sequence alignment of HSP70 and HSC70 amino acid sequences was produced
302 using Clustal Omega, Expresso, and M-Coffee with default parameters. Bayesian phylogenetic
303 analysis was performed using MrBayes (v3.2.6) on the CIPRES scientific gateway v3.3 (Miller et
304 al. 2010) with four chains each for 10^7 generations. Every 1000th trees were sampled, and the first
305 25% of samples were discarded as a burn-in. Bayesian consensus trees were visualized with the
306 TreeView software v1.4.3.
307
308 Synonymous and Nonsynonymous Substitution Rates
309 R version 4.0.4 was used for the calculation of the synonymous and nonsynonymous
310 substitution rates (the kaks function), Kruskal-Wallis test (kruskal.test function), and post-hoc test
311 (pairwise.wilcox.test function) on the Macintosh platform. Heat maps were created in Microsoft
312 Excel 2016 (Redmond, MA).
313
314 **Author Contributions**
315 Conceived and designed the experiments: TY, GK. Performed experiments and data analyses: EMY,
316 FLJ, DMW, GK. Contributed reagents/materials/analysis tools: EH, DMW, GK. Wrote the paper:
317 DMW, GK. Read and approved the final manuscript: EMY, TY, FLJ, EH, DMW, GK.
318
319 **Acknowledgements**
320 We express our sincere thanks to Dr. Kristin Gribble, Marine Biological Laboratory, for helpful
321 discussions and comments on the manuscript. The authors are grateful to Cynthia Rodriguez and
322 Jamie Wilson, University of Houston-Victoria, for their help in gene expression analyses. GK was
323 supported by funding from M.G. and Lillie A. Johnson Foundation, Victoria, Texas.
324
325 **Figure Legends**
326 **Fig. 1.** Molecular characterization of *Brachionus plicatilis* HSP70 genes. (A-C) Semi-quantitative

327 reverse transcription-PCR. A representative result is shown out of three independent experiments.
328 Location of primers rHSP70_gapF and rHSP70_gapR that amplify cDNA fragment of 112 and 124
329 bp from HSP70cB1i and HSP70cB2i cDNAs, respectively (A). Polyacrylamide gel electrophoresis
330 patterns of the RT-PCR product. Cycle numbers within a linear range of PCR amplification were
331 determined to be 24 to 28 cycles for both cDNAs by preliminary experiments on the basis of signal
332 intensities of amplified products by RT-PCR (B). Signal intensities of HSP70cB1i and HSP70cB2i
333 genes standardized to those of β-actin (C). (D) Heat stress-induced expression of HSP70 quantified
334 by real-time PCR. Beta-actin gene was used as the internal control. Bars represent standard errors
335 from three replications. One-way ANOVA detected significant effects of heat treatment ($F = 3.281$,
336 $df = 5$, $P = 0.0426$). Dunnett's multiple comparison was used to detect significant differences
337 between the control and other groups. Difference between control and two hours group was
338 statistically significant ($t = 3.246$, $P = 0.028$). (E) *In situ* hybridization for *B. plicatilis* HSP70
339 genes. Rotifers were fixed 4 h after the heat treatment.
340
341 **Fig. 2.** Bayesian consensus tree of HSP70 family members. Nodes discussed in the text are
342 indicated. Numbers on the branches indicate the posterior probability support for each node. Stress-
343 inducible and constitutive genes are shown in red and blue, respectively. No data on the stress
344 inducibility is available for genes shown in black. Approved HGNC names of human HSP70 family
345 members are shown in yellow boxes for reference. The DDBJ/EMBL/GenBank accession numbers
346 and other information are summarized in supplementary table S2. Clades 1 to 6 include only
347 cytosolic members of the HSP70 family.
348
349 **Fig. 3.** Logo visualization of mitochondrial-, endoplasmic reticulum-, and cytosolic-specific
350 sequence near the N terminus of the HSP70 family members. Compared to the cytosolic form, ER-
351 specific forms have several conserved amino acid substitutions: Q8K, H9N, K11R, and T23I.
352 Similarly, mitochondrial forms have Y1N, G5A, F7M, Q8E, H9G, G10K, K11T/Q, V12P/A, E13K,

353 I14V, I15L, A16E, D17A, and Q18E.

354

355 **Fig. 5.** Comparison of the amino acid sequences of the HSP70 family members from invertebrates.
356 Nodes in the phylogenetic tree (fig. 3) are indicated on the right margin, and serine residues specific
357 to node 3 are highlighted. Stress-inducible and non-inducible genes are shown in red and blue,
358 respectively. No data on the stress inducibility is available for genes shown in black. The accession
359 numbers and other information are summarized in supplementary table S2.

360

361 **Fig. 6.** Selection pressure on cytosolic HSP70 family member genes. (A) Nonsynonymous
362 substitution rates (Ka values) between 34 HSP70 family member genes. Gene number 1 is the fruit
363 fly HSP70 Bb (AF295957) gene. The substitution rate of fruit fly HSP70 Aa and Bb gene was
364 00395526, which is shown as 0.00 in the figure. Accession numbers for other genes are indicated in
365 the figure. Clades and nodes correspond to those in fig. 2. (B) Bee swarm boxplots of Ka/Ks values
366 for each clade. Only Ka/Ks values calculated from inter-cluster pairs were averaged. Statistical
367 differences were calculated by Kruskal-Wallis test (chi-squared = 88.704, df = 5, $p < 2.2e-16$)
368 followed by the non-parametric post-hoc tests (pairwise Wilcox test with P value adjustment by the
369 Holm method). Clades sharing same letters are not significantly different at the 5% level of
370 significance. One outlier in clade 3 (0.53) is not included in the plot (see supplementary material 1)
371 although this value was used for all statistical analyses. (C) Effect sizes (Cohen's d) for all
372 comparisons. Combinations where significant differences were found in the pairwise Wilcox test are
373 shown in bold.

374

375 **References**

376 Armougom F, Moretti S, Poirot O, Audic S, Dumas P, Schaeli B, Keduas V, Notredame C. 2006.
377 Expresso: automatic incorporation of structural information in multiple sequence alignments using
378 3D-Coffee. *Nucleic Acids Res* 34:W604-W608.
379 Baird NA, Turnbull DW, Johnson EA. 2006. Induction of the heat shock pathway during hypoxia

380 requires regulation of heat shock factor by hypoxia-inducible factor-1. *J Biol Chem* 281:38675-
381 38681.
382 Boorstein WR, Ziegelhoffer T, Craig EA. 1994. Molecular evolution of the HSP70 multigene
383 family. *J Mol Evol* 38:1-17.
384 Ceyhun SB, Sentürk M, Ekinci D, Erdoğan O, Ciltaş A, Kocaman EM. 2010. Deltamethrin
385 attenuates antioxidant defense system and induces the expression of heat shock protein 70 in
386 rainbow trout. *Comp Biochem Physiol, C Toxicol Pharmacol* 152:215-223.
387 Cottin D, Ravaux J, Leger N, Halary S, Toullec JY, Sarradin PM, Gaill F, Shillito B. 2008. Thermal
388 biology of the deep-sea vent annelied *Paralvinella grasslei*: in vivo studies. *J Exp Biol* 211:2196-
389 2204.
390 Daugaard M, Rohde M, Jäättelä M. 2007. The heat shock protein 70 family: Highly homologous
391 proteins with overlapping and distinct functions. *FEBS Lett* 581:3702-3710.
392 De Nadal E, Ammerer G, Posas F. 2011. Controlling gene expression in response to stress. *Nat Rev*
393 *Genet* 12:833-845.
394 Demand J, Lüders J, Höhfeld J. 1998. The carboxy-terminal domain of Hsc70 provides binding
395 sites for a distinct set of chaperone cofactors. *Mol Cell Biol* 18:2023-2028.
396 Florin L, Becker KA, Sapp C, Lambert C, Sirma H, Müller M, Streeck RE, Sapp M. 2004. Nuclear
397 translocation of papillomavirus minor capsid protein L2 requires Hsc70. *J Virol* 78:5546-5553.
398 Fröbius AC, Funch P. 2017. Rotiferan Hox genes give new insights into the evolution of metazoan
399 bodyplans. *Nat Commun* 8:9.
400 Garbuz D. 2017. Regulation of heat shock gene expression in response to stress. *Mol Biol* 51:352-
401 367.
402 Garbuz DG, Yushenova IA, Zatsepina OG, Przhiboro AA, Bettencourt BR, Evgen'ev MB.
403 2011. Organization and evolution of hsp70 clusters strikingly differ in two species of Stratiomyidae
404 (Diptera) inhabiting thermally contrasting environments. *BMC Evol Biol* 11:74.
405 Hartl FU, Bracher A, Hayer-Hartl M. 2011. Molecular chaperones in protein folding and
406 proteostasis. *Nature* 475:324-332.
407 Hess K, Oliverio R, Nguyen P, Le D, Ellis J, Kdeiss B, Ord S, Chalkia D, Nikolaidis N. 2018.
408 Concurrent action of purifying selection and gene conversion results in extreme conservation of the
409 major stress-inducible Hsp70 genes in mammals. *Sci Rep* 8:1-16.

410 Jayasena S, Chandrasekharan N, Karunanayake EH. 1999. Molecular characterisation of a hsp70
411 gene from the filarial parasite *Setaria digitata*. *Int J Parasitol* 29:581-591.
412 Jedlicka P, Mortin MA, Wu C. 1997. Multiple functions of *Drosophila* heat shock transcription
413 factor *in vivo*. *EMBO J* 16:2452-2462.
414 Judge ML, Botton ML, Hamilton MG. 2011. Physiological consequences of the supralittoral fringe:
415 microhabitat temperature profiles and stress protein levels in the tropical periwinkle *Cenchritis*
416 *muricatus* (Linneaus, 1758). *Hydrobiologia* 675:143-156.
417 Kaneko G, Yoshinaga T, Gribble KE, Mark Welch D, Ushio H. 2016. Measurement of survival time
418 in *Brachionus* rotifers: synchronization of maternal conditions. *J Vis Exp* 113:e54126.
419 Kourtidis A, Drosopoulou E, Nikolaidis N, Hatzi VI, Chintiroglou CC, Scouras ZG. 2006.
420 Identification of several cytoplasmic HSP70 genes from the Mediterranean mussel (*Mytilus*
421 *galloprovincialis*) and their long-term evolution in Mollusca and Metazoa. *J Mol Evol* 62:446-459.
422 Le SQ, Gascuel O. 2008. An improved general amino acid replacement matrix. *Mol Biol Evol*
423 25:1307-1320.
424 Liu L, Cheng T-y, Yang Y. 2017. Cloning and expression pattern of a heat shock cognate protein 70
425 gene in ticks (*Haemaphysalis flava*). *Parasitol Res* 116:1695-1703.
426 Lo W-Y, Liu K-F, Liao I-C, Song Y-L. 2004. Cloning and molecular characterization of heat shock
427 cognate 70 from tiger shrimp (*Penaeus monodon*). *Cell Stress Chaperones* 9:332.
428 Luan W, Li F, Zhang J, Wen R, Li Y, Xiang J. 2010. Identification of a novel inducible cytosolic
429 Hsp70 gene in Chinese shrimp *Fenneropenaeus chinensis* and comparison of its expression with the
430 cognate Hsc70 under different stresses. *Cell Stress Chaperones* 15:83-93.
431 Marchler G, Wu C. 2001. Modulation of *Drosophila* heat shock transcription factor activity by the
432 molecular chaperone DROJ1. *EMBO J* 20:499-509.
433 Miernyk JA. 1997. The 70 kDa stress-related proteins as molecular chaperones. *Trends Plant Sci*
434 2:180-187.
435 Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of
436 large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE);
437 New Orleans, LA. p. 1-8.
438 Nikolaidis N, Nei M. 2004. Concerted and nonconcerted evolution of the Hsp70 gene superfamily
439 in two sibling species of nematodes. *Mol Biol Evol* 21:498-505.

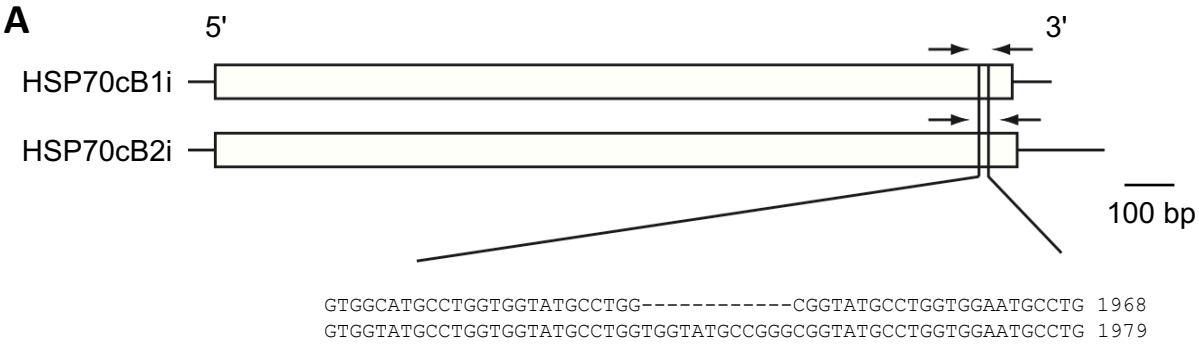
440 Piano A, Asirelli C, Caselli F, Fabbri E. 2002. Hsp70 expression in thermally stressed *Ostrea edulis*,
441 a commercially important oyster in Europe. *Cell Stress Chaperones* 7:250.
442 Pollock DD, Larkin JC. 2004. Estimating the degree of saturation in mutant screens. *Genetics*
443 168:489-502.
444 Sanders BM. 1993. Stress proteins in aquatic organisms - an environmental perspective. *Crit Rev*
445 *Toxicol* 23:49-75.
446 Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M,
447 Söding J. 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using
448 Clustal Omega. *Mol Syst Biol* 7:539.
449 Simoncelli F, Morosi L, Di Rosa I, Pascolini R, Fagotti A. 2010. Molecular characterization and
450 expression of a heat-shock cognate 70 (Hsc70) and a heat-shock protein 70 (Hsp70) cDNAs in Rana
451 (Pelophylax) lessonae embryos. *Comp Biochem Physiol, A Mol Integr Physiol* 156:552-560.
452 Sørensen JG, Kristensen TN, Loeschcke V. 2003. The evolutionary and ecological role of heat
453 shock proteins. *Ecol Lett* 6:1025-1037.
454 Sorger PK, Pelham HR. 1988. Yeast heat shock factor is an essential DNA-binding protein that
455 exhibits temperature-dependent phosphorylation. *Cell* 54:855-864.
456 Struck TH, Wey-Fabrizius AR, Golombek A, Hering L, Weigert A, Bleidorn C, Klebow S,
457 Iakovenko N, Hausdorf B, Petersen M. 2014. Platyzoan paraphyly based on phylogenomic data
458 supports a noncoelomate ancestry of Spiralia. *Mol Biol Evol* 31:1833-1849.
459 Touchon M, Hoede C, Tenaillon O, Barbe V, Baeriswyl S, Bidet P, Bingen E, Bonacorsi S, Bouchier
460 C, Bouvet O. 2009. Organised genome dynamics in the *Escherichia coli* species results in highly
461 diverse adaptive paths. *PLoS genet* 5:e1000344.
462 Wallace IM, O'sullivan O, Higgins DG, Notredame C. 2006. M-Coffee: combining multiple
463 sequence alignment methods with T-Coffee. *Nucleic Acids Res* 34:1692-1699.
464 Yabu T, Imamura S, Mohammed MS, Touhata K, Minami T, Terayama M, Yamashita M. 2011.
465 Differential gene expression of HSC70/HSP70 in yellowtail cells in response to chaperone-
466 mediated autophagy. *FEBS J* 278:673-685.
467 Yoshinaga T, Minegishi Y, Rumengan IFM, Kaneko G, Furukawa S, Yanagawa Y, Tsukamoto K,
468 Watabe S. 2004. Molecular phylogeny of the rotifers with two Indonesian *Brachionus* lineages.
469 *Coast Mar Sci* 29:45-56.

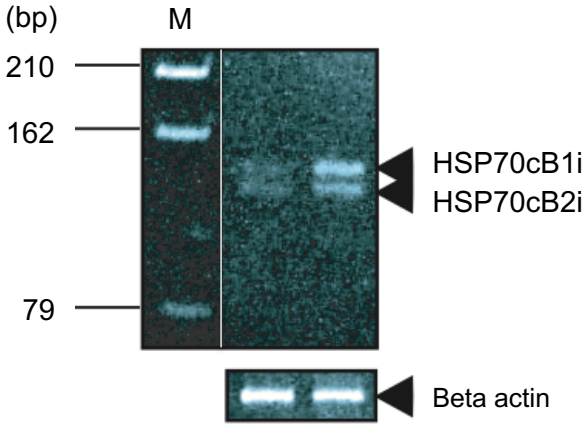
470 Zheng G, Dong S, Hou Y, Yang K, Yu X. 2012. Molecular characteristics of HSC70 gene and its
471 expression in the golden apple snails, *Pomacea canaliculata* (Mollusca: Gastropoda). *Aquaculture*
472 358-359:41-49.
473 Zuiderweg ER, Hightower LE, Gestwicki JE. 2017. The remarkable multivalency of the Hsp70
474 chaperones. *Cell Stress Chaperones* 22:173-189.
475

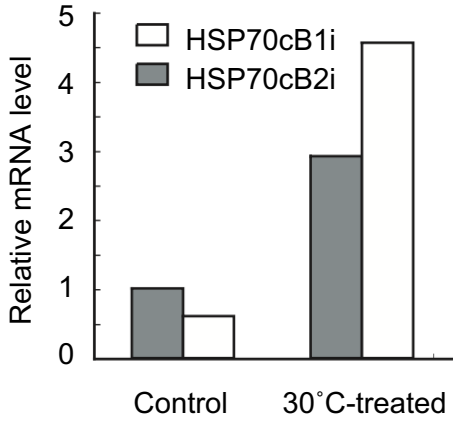
Table 1. Support for major nodes from each approach

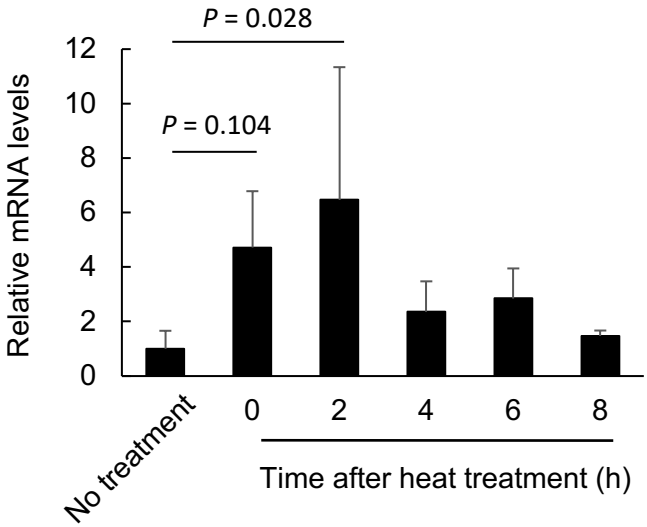
		ClustalOmega		Expresso		M-Coffee	
		MrBayes	RAxML	MrBayes	RAxML	MrBayes	RAxML
1	Cytosolic	100	99	100	99	100	100
2	Metazoan	100	95	100	99	100	94
3	Lineage 1	(a)	99	100	99	100	100
4	Lineage 2	(b)	41	85	67	(b)	(c)
5	Rotifer	100	48	74	48	100	65
6	Nematode	94	93 (d,e)	100 (e)	96 (e)	100 (e)	86 (e)
7	Chordate		42	85	35		
8	HSC	100	91	100	86	100	31
9	HSP	100	88	100	83	100	87
10	Outgroup	100	99	100	99	100	100
11	Mitochondrial	100	100	100	97	100	100
12	ER	100	99	100	100	100	100

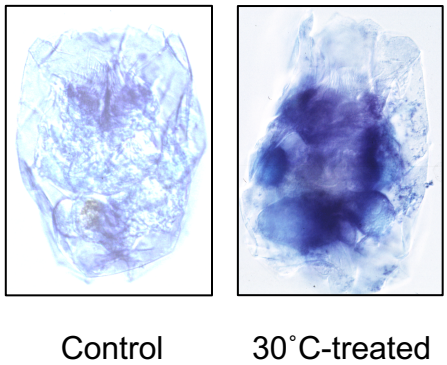
(a) Rotifer genes (node 5) is included in node 3 with 83% support
(b) Rotifer genes (node 5) sister group to node 3 with 80% support, other paraphyly
(c) Nematode genes (node 6) basal; rotifer genes sister group to node 3 with 46% support, other paraphyly
(d) 36% with Trichuris (immediate outgroup to node 6)
(e) Trichuris not in node 6

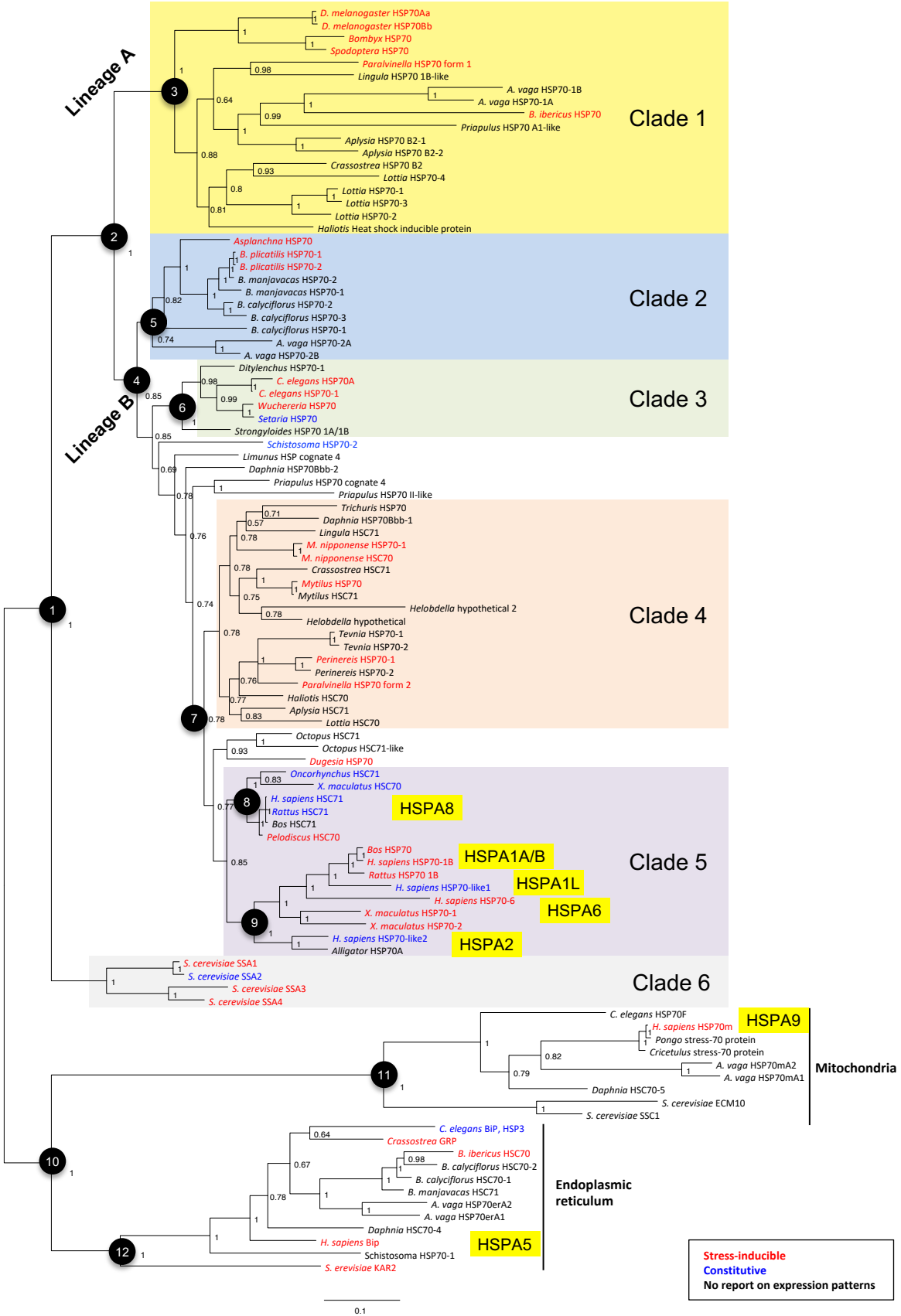

A
5'
3'
HSP70cB1i
HSP70cB2i
100 bp
GTGGCATGCCTGGTGGTATGCCTGG----------CGGTATGCCTGGTGGAATGCCTG 1968
GTGGTATGCCTGGTGGTATGCCTGGTGGTATGCCGGGCGGTATGCCTGGTGGAATGCCTG 1979


B
(bp)
M
210
162
79
HSP70cB1i
HSP70cB2i
Beta actin

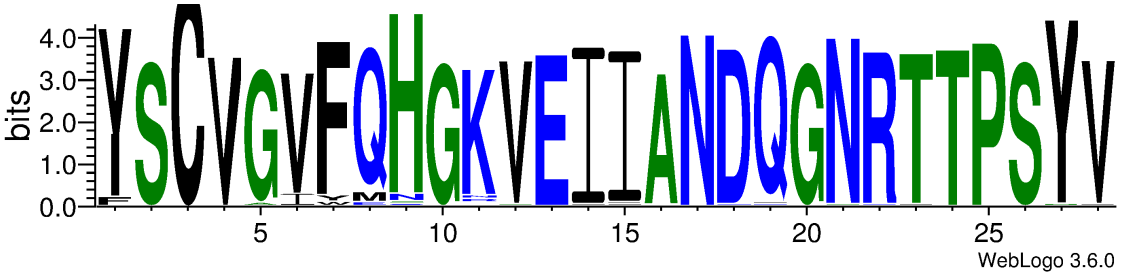

C
Relative mRNA level
0
1
2
3
4
5
HSP70cB1i
HSP70cB2i
Control
30˚C-treated


D
P = 0.028
P = 0.104
Relative mRNA levels
0
2
4
6
8
10
12
No treatment
0
2
4
6
8
Time after heat treatment (h)

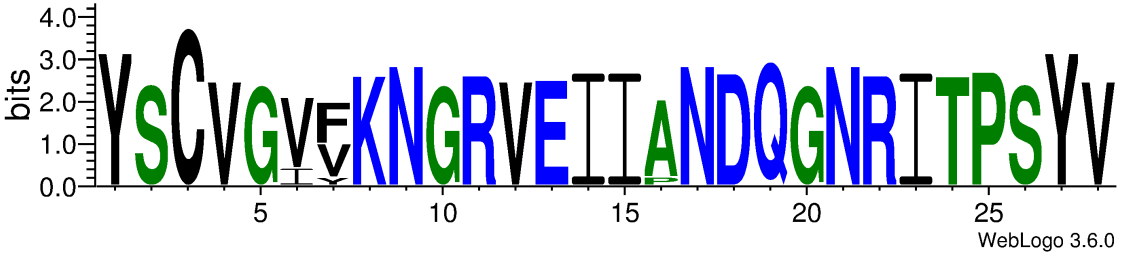

E
Control
30˚C-treated


Lineage A
Lineage B
Clade 1
Clade 2
Clade 3
Clade 4
Clade 5
Clade 6
D. melanogaster HSP70Aa
D. melanogaster HSP70Bb
Bombyx HSP70
Spodoptera HSP70
Paralvinella HSP70 form 1
Lingula HSP70 1B-like
A. vaga HSP70-1B
A. vaga HSP70-1A
B. ibericus HSP70
Priapulus HSP70 A1-like
Aplysia HSP70 B2-1
Aplysia HSP70 B2-2
Crassostrea HSP70 B2
Lottia HSP70-4
Lottia HSP70-1
Lottia HSP70-3
Lottia HSP70-2
Haliotis Heat shock inducible protein
Asplanchna HSP70
B. plicatilis HSP70-1
B. plicatilis HSP70-2
B. manjavacas HSP70-2
B. manjavacas HSP70-1
B. calyciflorus HSP70-2
B. calyciflorus HSP70-3
B. calyciflorus HSP70-1
A. vaga HSP70-2A
A. vaga HSP70-2B
Ditylenchus HSP70-1
C. elegans HSP70A
C. elegans HSP70-1
Wuchereria HSP70
Setaria HSP70
Strongyloides HSP70 1A/1B
Schistosoma HSP70-2
Limunus HSP cognate 4
Daphnia HSP70Bbb-2
Priapulus HSP70 cognate 4
Priapulus HSP70 II-like
Trichuris HSP70
Daphnia HSP70Bbb-1
Lingula HSC71
M. nipponense HSP70-1
M. nipponense HSC70
Crassostrea HSC71
Mytilus HSP70
Mytilus HSC71
Helobdella hypothetical 2
Helobdella hypothetical
Tevnia HSP70-1
Tevnia HSP70-2
Perinereis HSP70-1
Perinereis HSP70-2
Paralvinella HSP70 form 2
Haliotis HSC70
Aplysia HSC71
Lottia HSC70
Octopus HSC71
Octopus HSC71-like
Dugesia HSP70
Oncorhynchus HSC71
X. maculatus HSC70
H. sapiens HSC71
Rattus HSC71
Bos HSC71
Pelodiscus HSC70
HSPA8
Bos HSP70
H. sapiens HSP70-1B
HSPA1A/B
Rattus HSP70 1B
H. sapiens HSP70-like1
HSPA1L
H. sapiens HSP70-6
HSPA6
X. maculatus HSP70-1
X. maculatus HSP70-2
H. sapiens HSP70-like2
Alligator HSP70A
HSPA2
S. cerevisiae SSA1
S. cerevisiae SSA2
S. cerevisiae SSA3
S. cerevisiae SSA4
C. elegans HSP70F
H. sapiens HSP70m
HSPA9
Pongo stress-70 protein
Cricetulus stress-70 protein
A. vaga HSP70mA2
A. vaga HSP70mA1
Daphnia HSC70-5
S. cerevisiae ECM10
S. cerevisiae SSC1
Mitochondria
C. elegans BiP, HSP3
Crassostrea GRP
B. ibericus HSC70
B. calyciflorus HSC70-2
B. calyciflorus HSC70-1
B. manjavacas HSC71
A. vaga HSP70erA2
A. vaga HSP70erA1
Daphnia HSC70-4
H. sapiens Bip
Schistosoma HSP70-1
S. erevisiae KAR2
HSPA5
Endoplasmic reticulum
Stress-inducible
Constitutive
No report on expression patterns
0.1

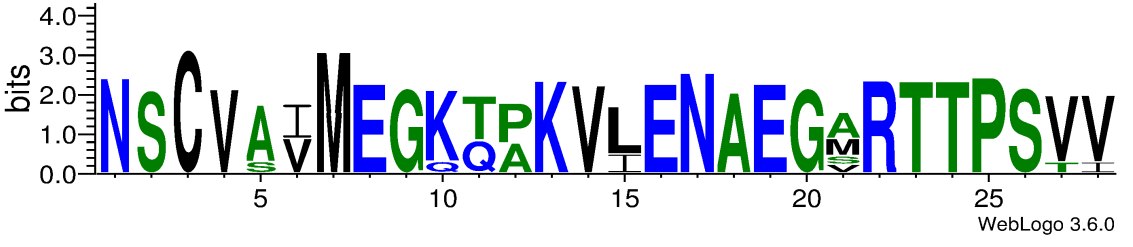
Cytosol



Endoplasmic reticulum



Mitochondrial



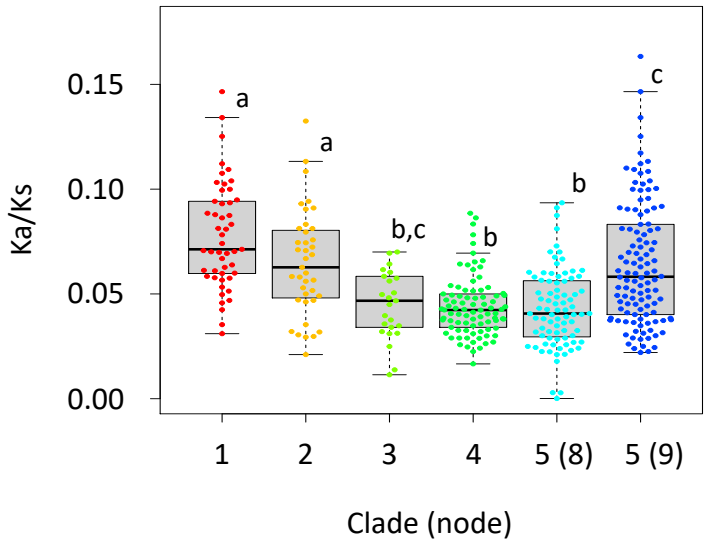
			Node
D. melanogaster HSP70A	KGERNVLIFDLGGGTFDVSILTIDE----G**S**LFEVRSTAGDT	224	
D. melanogaster HSP70B	KGERNVLILDLGGGTFDVSILTIDE----G**S**LFEVRSTAGDT	224	
Bombyx HSP70	KGERNVLIFDLGGGTFDVSILTIDE----G**S**LFEVKSTAGDT	224	
Spodoptera HSP70	KGERNVLIFDLGGGTFDVSILTIDE----G**S**LFEVRATAGDT	224	
Paralvinella HSP70-1	SGEKNVLIFDLGGGTFDVSILSIAD----G**S**LFEVKSTAGDT	229	
Lingula HSP71	QGEKNVLIFDLGGGTFDVSILSIAD----G**S**LFEVRSTAGDT	228	
A. vaga HSP70-1B	HRDQNILIYDLGGGTFDVSVLTISGGDVSG**S**VFEVKATAGDT	238	
A. vaga HSP70-1A	QRDQNILIYDLGGGTFDVSILTISGGDAGG**S**VFEVRSTAGDT	238	
B. ibericus HSP70	KRRKNVLIYDLGGGTFDVSVLSIND----G-LFEVLSTNGNT	224	
Priapulus HSP70-1	AGERNVLVYDLGGGTFDVSVLTIDE----GAMFEVRSTAGDT	226	**3**
Aplysia HSP70-1	KGEKHVLIFDLGGGTFDVSVLAIDE----G**S**IFEVKATAGDT	227	
Aplysia HSP70-2	SGEKNVLIFDLGGGTFDVSVLTIDE----G**S**MFEVKATAGDT	230	
Crassostrea HSP70	SGEKNVLIFDLGGGTFDVSILTIDE----G**S**IFEVRSTAGDT	228	
Lottia HSP70-4	DTEQHILIFDLGGGTFDVSILAIED----G-VFEVLATAGDT	227	
Lottia HSP70-1	SGEKNVLIFDLGGGTFDVSILTIDE----G**S**LFEVKSTAGDT	225	
Lottia HSP70-3	SGEKNVLIFDLGGGTFDVSILTIDE----G**S**LFEVKSTAGDT	226	
Lottia HSP70-2	SGEKNVLIFDLGGGTFDVSILAIDE----G**S**LFEVKSTAGDT	226	
Haliotis HSP70	KGEKNVLIFDLGGGTFDVPVLTIDE----G**S**MFEVRSTAGDT	231	
B. plicatilis HSP70-1	GGEKNILIFDLGGGTFDVSILTIEE----G-IFEVKSTAGDT	226	
B. manjavacas HSP70-1	AGEKNILIFDLGGGTFDVSILTIED----G-IFEVKATAGDT	226	**5**
A. vaga HSP70-2A	SGERNILIFDLGGGTFDVSVLKIEE----G-IFEVKSTAGDT	184	
C. elegans HSP70A	HGERNVLIFDLGGGTFDVSILTIED----G-IFEVKSTAGDT	227	
Wuchereria HSP70	HGERNVLIFDLGGGTFDVSILTIED----G-IFEVKSTAGDT	226	**6**
Setaria HSP70	HGERNVLIFDLGGGTFDVSILTIED----G-IFEVKSTAGDT	226	
Strongyloides HSP70	ATERNVLIFDLGGGTFDVSVLTIED----G-IFEVKSTAGDT	227	
Schistosoma HSP70-2	GGERNVLIFDLGGGTFDVSILTIED----G-IFEVKSTAGDT	224	
M. nipponense HSP70-1	GGERNVLIFDLGGGTFDVSILTIED----D-IFEVKSTAGDT	226	
Mytilus HSP70	GGERNVLIFDLGGGTFDVSILTIKD----G-IFEVKSTSGDT	227	
Tevnia1 HSP70-1	GGEQNVLIFDLGGGTFDVSILTIED----G-IFEVKSTAGDT	226	
Perinereis1 HSP70-1	GGERNVLIFDLGGGTFDVSILTIED----G-IFEVKSTAGDT	225	
Lottia HSP70c	GGERNVLIFDLGGGTFDVSILTIED----G-IFEVKSTAGDT	226	
Dugesia HSP70	GIERNVLIFDLGGGTFDVSILTIED----G-IFEVKSTAGDT	226	
Oncorhynchus HSC71	GAERNVLIFDLGGGTFDVSILTIED----G-IFEVKSTAGDT	226	
H. sapiens HSC71	GAERNVLIFDLGGGTFDVSILTIED----G-IFEVKSTAGDT	226	**8**
Pelodiscus HSC70	GAERNVLIFDLGGGTFDVSILTIED----G-IFEVKSTAGDT	226	
H. sapiens HSP70-1B	KGERNVLIFDLGGGTFDVSILTIDD----G-IFEVKATAGDT	226	
H. sapiens HSP70-like1	QGERHVLIFDLGGGTFDVSILTIDD----G-IFEVKATAGDT	228	**9**
X. maculatus HSP70-1	RGERNVLIFDLGGGTFDVSILTIED----G-IFEVKSTAGDT	228	
Alligator HSP70A	AGEKNVLIFDLGGGTLDVSILTIED----G-IFEVKSTAGDT	229	

A

Ka			Clade 1					Clade 2			Clade 3				Clade 4						Clade 5 (node 8)					Clade 5 (node 9)						
	Gene No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Clade 1	2	*D. melanogaster* HSP70Aa (AF295933)	0.00																													
	3	*Spodoptera* HSP70 (FJ754276)	0.09	0.09																												
	4	*Bombyx* HSP70 (AB035326)	0.10	0.10	0.03																											
	5	*Paralvinella* HSP70-1 (EF580992)	0.16	0.16	0.14	0.14																										
Clade 2	6	*B. plicatilis* _HSP70-1 (AB775784)	0.15	0.15	0.14	0.14	0.15																									
	7	*B. plicatilis* _HSP70-2 (AB775785)	0.15	0.15	0.14	0.14	0.15	0.00																								
	8	*A. brightwelli* _HSP70 (KX119429)	0.16	0.15	0.15	0.16	0.15	0.06	0.06																							
Clade 3	9	*Wuchereria* (AF167352)	0.16	0.15	0.15	0.16	0.16	0.08	0.09	0.09																						
	10	*Setaria* (AF079360)	0.15	0.15	0.15	0.15	0.16	0.08	0.08	0.09	0.01																					
	11	*C. elegans* HSP70A (M18540)	0.16	0.16	0.16	0.16	0.18	0.09	0.09	0.11	0.05	0.05																				
	12	*C. elegans* HSP-1 (NM_070667)	0.15	0.15	0.15	0.15	0.17	0.08	0.08	0.09	0.04	0.03	0.01																			
Clade 4	13	*M. nipponense* HSP70-1 (KC460343)	0.16	0.15	0.15	0.15	0.15	0.07	0.07	0.08	0.08	0.08	0.09	0.08																		
	14	*M. nipponense* HSP70-2 (DQ660140)	0.15	0.15	0.15	0.15	0.15	0.07	0.07	0.07	0.08	0.08	0.09	0.08	0.01																	
	15	*Mytilus* HSP70 (AY861684)	0.16	0.16	0.16	0.16	0.15	0.09	0.09	0.07	0.09	0.09	0.11	0.10	0.07	0.07																
	16	*Perinereis* HSP70-1 (KU255783)	0.15	0.15	0.17	0.16	0.15	0.09	0.09	0.08	0.10	0.10	0.11	0.10	0.07	0.07	0.06															
	17	*Paralvinella* HSP70-2 (EF580993)	0.17	0.17	0.17	0.17	0.16	0.10	0.10	0.09	0.11	0.11	0.12	0.11	0.08	0.08	0.07	0.06														
	18	*Haliotis* HSP70c (FJ812176)	0.15	0.15	0.16	0.16	0.16	0.10	0.10	0.09	0.10	0.10	0.12	0.11	0.08	0.08	0.06	0.06	0.07													
Clade 5 (node 8)	19	*Oncorhynchus* HSC71 (AAB21658)	0.16	0.16	0.16	0.16	0.16	0.08	0.08	0.08	0.09	0.09	0.11	0.09	0.06	0.06	0.07	0.06	0.07	0.08												
	20	*X. maculatus* HSC70 (AB062115)	0.18	0.17	0.16	0.16	0.15	0.09	0.09	0.08	0.11	0.11	0.12	0.11	0.07	0.07	0.07	0.08	0.09	0.08	0.04											
	21	Human_HSPA8 (NP_006588)	0.15	0.15	0.14	0.15	0.15	0.07	0.07	0.07	0.09	0.09	0.10	0.09	0.07	0.06	0.06	0.07	0.07	0.07	0.03	0.05										
	22	*Rattus* HSC71 (NM_024351)	0.15	0.15	0.14	0.15	0.15	0.07	0.07	0.07	0.09	0.09	0.10	0.09	0.06	0.06	0.06	0.07	0.07	0.07	0.03	0.05	0.00									
	23	*Pelodiscus* HSC70 (HQ219723)	0.15	0.15	0.14	0.15	0.14	0.07	0.07	0.07	0.09	0.08	0.10	0.09	0.06	0.06	0.06	0.07	0.07	0.06	0.03	0.04	0.00	0.00								
Clade 5 (node 9)	24	*Bos* HSP70 (U09861)	0.15	0.15	0.15	0.15	0.16	0.10	0.10	0.09	0.10	0.10	0.12	0.11	0.09	0.09	0.09	0.10	0.11	0.10	0.08	0.09	0.07	0.07	0.07							
	25	Human_HSPA1A (NM_005345)	0.15	0.15	0.15	0.15	0.15	0.10	0.09	0.09	0.10	0.09	0.11	0.10	0.08	0.08	0.09	0.09	0.11	0.10	0.07	0.09	0.06	0.06	0.06	0.00						
	26	*Rattus* HSP70_1A (NM_031971)	0.15	0.15	0.15	0.15	0.16	0.10	0.10	0.09	0.10	0.10	0.12	0.11	0.09	0.09	0.09	0.09	0.11	0.10	0.08	0.09	0.07	0.07	0.07	0.01	0.01					
	27	*X. maculatus* HSP70-1 (AB062113)	0.17	0.17	0.17	0.16	0.16	0.10	0.10	0.09	0.11	0.10	0.12	0.11	0.09	0.09	0.09	0.10	0.09	0.10	0.07	0.08	0.06	0.06	0.06	0.07	0.07	0.07				
	28	*X. maculatus* HSP70-2 (AB062114)	0.16	0.16	0.16	0.17	0.16	0.10	0.10	0.09	0.11	0.10	0.12	0.11	0.09	0.09	0.09	0.10	0.11	0.10	0.08	0.09	0.07	0.07	0.07	0.07	0.07	0.07	0.06			
	29	Human_HSPA1L (NM_005527)	0.15	0.15	0.15	0.16	0.16	0.10	0.10	0.10	0.10	0.10	0.12	0.11	0.09	0.09	0.10	0.10	0.11	0.11	0.09	0.10	0.08	0.08	0.08	0.04	0.04	0.04	0.07	0.07		
	30	Human_HSPA6 (NP_002146)	0.16	0.16	0.16	0.17	0.17	0.12	0.12	0.11	0.12	0.12	0.14	0.13	0.12	0.11	0.11	0.11	0.13	0.12	0.12	0.12	0.10	0.10	0.10	0.07	0.07	0.07	0.12	0.11	0.09	
	31	Alligator_HSP70 (AB306279)	0.16	0.16	0.16	0.16	0.17	0.09	0.09	0.08	0.10	0.10	0.12	0.10	0.09	0.09	0.09	0.09	0.10	0.10	0.08	0.09	0.07	0.07	0.07	0.09	0.09	0.09	0.08	0.09	0.10	0.11

0 — 0.18
Ka

B



C

Clade (node)

Clade (node)	2	3	4	5 (8)	5 (9)
1	0.51	0.19	**1.64**	**1.55**	**0.49**
2		**0.01**	**1.01**	**0.98**	**0.02**
3			0.28	0.29	0.01
4				0.05	**0.87**
5 (8)					**0.86**

1: Invertebrate HSP70s (Lineage A)
2: Rotifera HSP70s (Lineage B)
3: Nematode HSP70s (Lineage B)
4: Invertebrate HSP/HSC70s (Lineage B)
5 (8): Vertebrate HSC70s (Lineage B)
5 (9): Vertebrate HSP70s (Lineage B)

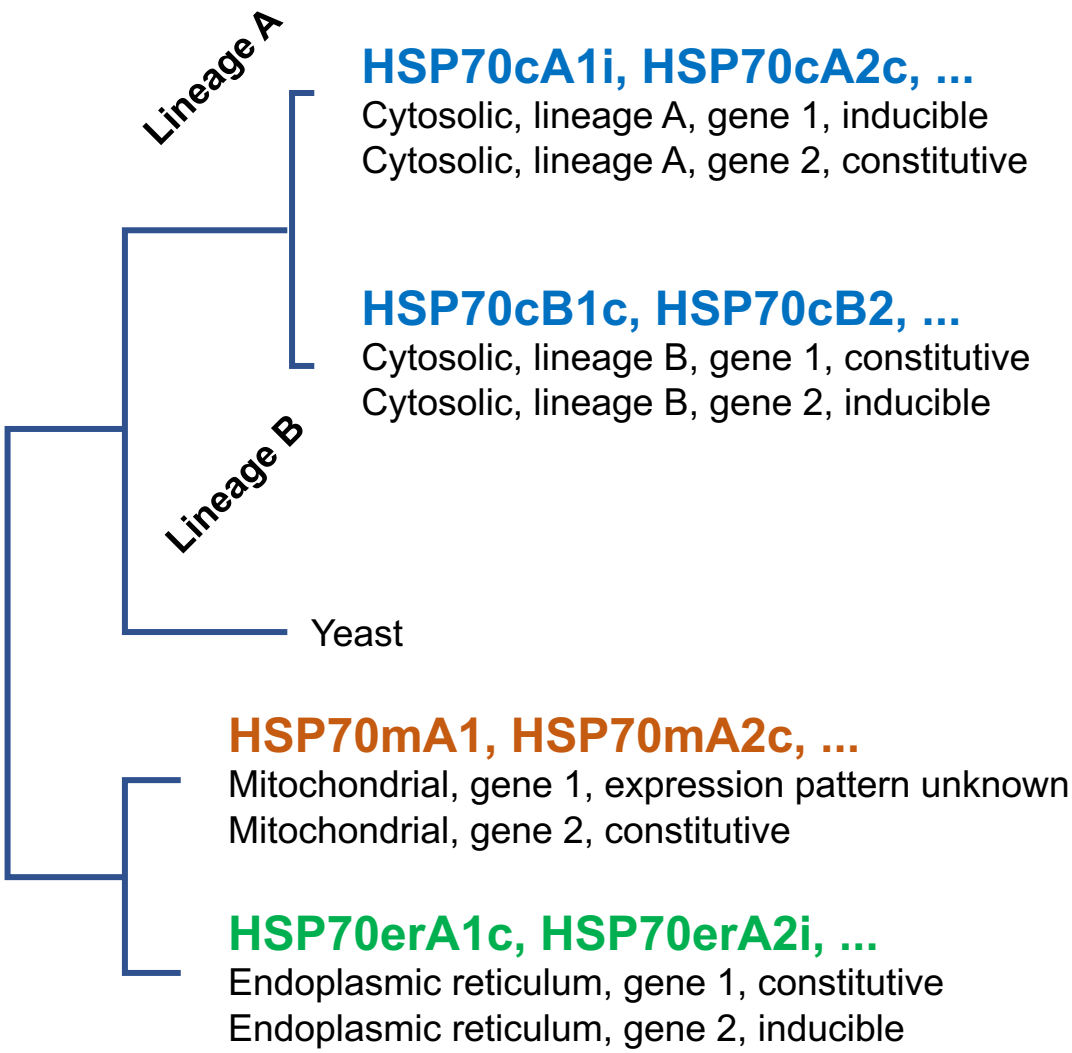

Lineage A
HSP70cA1i, HSP70cA2c, ...
Cytosolic, lineage A, gene 1, inducible
Cytosolic, lineage A, gene 2, constitutive
HSP70cB1c, HSP70cB2, ...
Cytosolic, lineage B, gene 1, constitutive
Cytosolic, lineage B, gene 2, inducible
Lineage B
Yeast
HSP70mA1, HSP70mA2c, ...
Mitochondrial, gene 1, expression pattern unknown
Mitochondrial, gene 2, constitutive
HSP70erA1c, HSP70erA2i, ...
Endoplasmic reticulum, gene 1, constitutive
Endoplasmic reticulum, gene 2, inducible

Table 1. Nucleotide sequences of primers used in this study

Primer name	Sequence (5'-3')	Nucleotide position	Gene
cDNA cloning			
rHSP70_5RACE1	AAAAGTCCTGGAGCAGCTT	1107-1125*	HSP70-1, 2
rHSP70_5RACE2	GAACTCCTCGACAAAATGGTT	780-800*	HSP70-1, 2
rHSP70_5RACE3	CAGGTCAAAGATCAGGATGTT	645-665*	HSP70-1, 2
rHSP70_3RACE1	AGGCCAACATCGAAATAGA	901-919*	HSP70-1, 2
rHSP70_3RACE2	GACAAGTCAAGTGTGAACGAG	1044-1064*	HSP70-1, 2
rHSP70_full_f1	ACATACACCCAGACACACAA	1-20*	HSP70-1, 2
rHSP70_full_r1	GTCGACTTCATCAATGGTGG	2002-2021*	HSP70-1, 2
GeneRacer 5' RACE	CGACTGGAGCACGAGGACACTGA	-	-
GeneRacer 5' RACE Nested	GGACACTGACATGGACTGAAGGAGTA	-	-
Oligo-dT adapter	GGCCACGCGTCGACTAGTAC $(T)_{17}$	-	-
AUAP	GGCCACGCGTCGACTAGTAC	-	-
Semi-quantitative RT-PCR			
rHSP70_gapF	CGATTATTACTCAGTTGTATCAG	1879-1901*	HSP70-1, 2
rHSP70_gapR	ATCAATGGTAGGTCCACTGC	1993-2012*	HSP70-1, 2
rActinF	CCTGCCATGTACGTAGCC	2-19	Actin
rActinR	ATCACTTGACCATCGGGC	340-357	Actin
Quantitative real-time PCR			
RT-hsp70-140F	AGTGGAAATAATCGCCAACGA	140-160*	HSP70
RT-hsp70-208R	TCGGTGTCGGTGAACGCTA	190-208*	HSP70
RT-AC-306F	CGTCCAGTTCCTCGTTGGAA	306-325	Actin
RT-AC-373R	CTCGTTGCCAATGGTGATCA	354-373	Actin
In situ hybridization			
rHSP70_insituF	CCATTTACAAACACAAATATT	2026-2046*	HSP70-1, 2
rHSP70_insituR	GTCAAACAAATCTTTTATC	2085-2103*	HSP70-1, 2

*Nuceltide positions in HSP70-1 gene

) family members used for the construction of phylogenetic trees

Organism and protein name	Proposed name	Nucleotide accession number	Protein ID	Tissue	Treatment	Quantification method	Expression	Reference for expression patterns
D. melanogaster HSP70Aa	HSP70cA1i	AF295933.1:486-2411	AAG26887	Whole body of larvae	Heat	-	Inducible	UniProtKB, ID P82910
D. melanogaster HSP70Bb	HSP70cA2i	AF295957.1:215-2140	AAG26911	Whole body of larvae	Heat	-	Inducible	UniProtKB, ID Q9BIS2
Bombyx HSP70	HSP70cA1i	AB035326.1:102-2024	BAF69068	Diapause egg	Heat	Real-time PCR	Inducible	Moribe et al., 2010
Spodoptera HSP70	HSP70cA1i	FJ754276.1:151-2154	ACN78407	Fat body	Heat and cold shock	Northern blot	Inducible	Xu et al., 2011
Paralvinella HSP70 form 1	HSP70cA1i	EF580992.1:1-1947	ABU63808	Posterior part of the body	Heat	Western blot	Inducible, but the data may contain other members	Cottin et al., 2008
Lingula HSP70 1B-like	HSP70cA1	XM_013527000.2:123-2033	XP_013382454	-	-	-	-	-
A. vaga HSP70-1B	HSP70cA2	-	GSADVT00016830001	-	-	-	-	-
A. vaga HSP70-1A	HSP70cA1	-	GSADVT00001811001	-	-	-	-	-
B. ibericus HSP70	HSP70cA1i	GU574486.1:1-1860	ADR79281	Whole body	Ultraviolet	Real-time PCR	Inducible	Kim et al., 2011
Priapulus HSP70 A1-like	HSP70cA1	XM_014826376.1:219-2171	XP_014681862	-	-	-	-	-
Aplysia HSP70 B2-1	HSP70cA1	XM_005103777.2:352-2253	XP_005103834	-	-	-	-	-
Aplysia HSP70 B2-2	HSP70cA2	XM_005100297.2:191-2110	XP_005100354	-	-	-	-	-
Crassostrea HSP70 B2	HSP70cA1	JH818426.1:39359-41263	EKC30019	-	-	-	-	-
Lottia HSP70-4 (hypothetical protein LOTGIDRAFT_190284)	HSP70cA4	XM_009058212.1:120-2033	XP_009056460	-	-	-	-	-
Lottia HSP70-1 (hypothetical protein LOTGIDRAFT_181897)	HSP70cA1	XM_009053468.1:343-2253	XP_009051716	-	-	-	-	-
Lottia HSP70-3 (hypothetical protein LOTGIDRAFT_209056)	HSP70cA3	XM_009053469.1:216-2129	XP_009051717	-	-	-	-	-
Lottia HSP70-2 (hypothetical protein LOTGIDRAFT_198956)	HSP70cA2	XM_009047345.1:307-2226	XP_009045593	-	-	-	-	-
Haliotis heat shock inducible protein 70	HSP70cA1	FJ812177.1:169-2079	ACO36048	-	-	-	-	-
Asplanchna HSP70	HSP70cB1i	KX119429.1:81-2009	ARU12813	Whole body of larvae	Oxidation stressors (Paraquat and juglone)	Real-time PCR	Inducible	Xu et al., 2017
B. plicatilis HSP70cB1i	HSP70cB1i	AB775784.1:66-2024	BAO23806	Whole body of larvae	Heat	Real-time PCR and in situ hybridization	Inducible	This study
B. plicatilis HSP70cB2i	HSP70cB2i	AB775785.1:65-2035	BAO23807	Whole body of larvae	Heat	Real-time PCR and in situ hybridization	Inducible	This study
B. manjavacas HSP70-1	HSP70cB1	-	BmTi01_isotig01884_1998_175_2172_f_0 BmTi01_isotig01885_1998_27_2024_f_2	-	-	-	-	-
B. manjavacas HSP70-2	HSP70cB2	-	BmTi01_isotig03034_2025_165_2189_r_2	-	-	-	-	-
B. calyciflorus HSP70-2	HSP70cB2	-	NTPY01000792	-	-	-	-	-
B. calyciflorus HSP70-3	HSP70cB3	-	NTPY01000899	-	-	-	-	-
B. calyciflorus HSP70-1	HSP70cB1	-	NTPY01000620	-	-	-	-	-
A. vaga HSP70-2A	HSP70cB1	-	GSADVT00032221001	-	-	-	-	-
A. vaga HSP70-2B	HSP70cB2	-	GSADVT00050272001	-	-	-	-	-
Ditylenchus HSP70-1	HSP70cB1	HQ386232.1:49-1986	AEP19214	-	-	-	-	-
C. elegans HSP70A	HSP70cB1i	M18540.1:423-627,677-1464,1659-2339,2395-2643	AAA28078	Whole body	Heat	Northern blot	Inducible	Snutch et al., 1988
C. elegans HSP70-1	HSP70cB2i	NM_070667.4:1-1923	NP_503068	Whole body	-	-	Inducible	WormBase ID: WBGene00002005
Wuchereria HSP70	HSP70cB1i	AF167352.1:973-1069,1451-1555,1731-2092,2179-2302,2418-2571,2690-2948,3076-3297,3400-3747,3830-4016,4116-4195	AAF32254	Whole body	Heat	SDS-PAGE and blotting, ELISA	Inducible, but the data may contain other members	Ravi et al., 2004

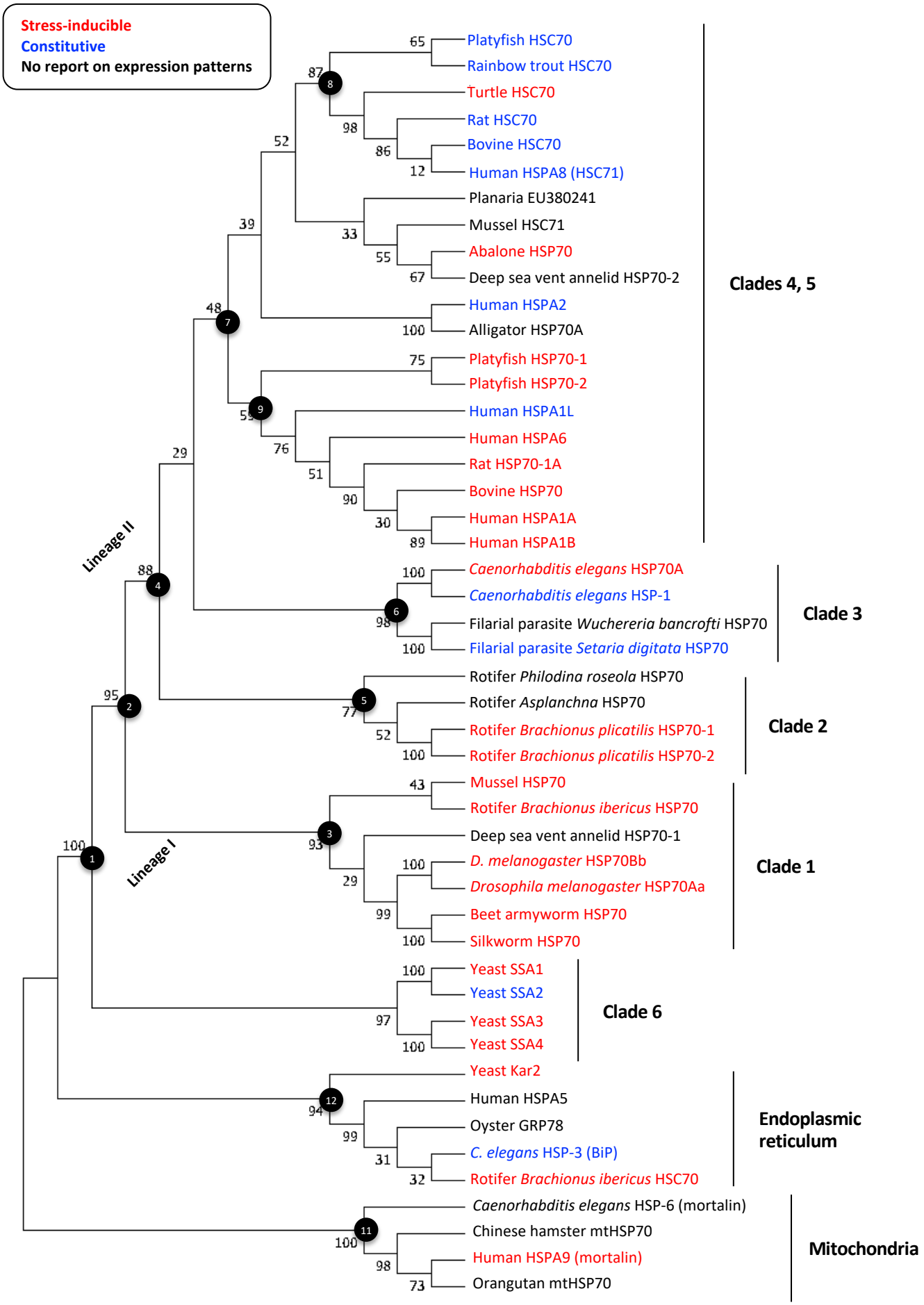
Setaria HSP70	HSP70cB1c	AF079360.1:711-807,1003-1107,1212-1573,1654-1777,1864-2017,2103-2361,2450-2671,2772-3119,3198-3384,3499-3578	AAD13154	Whole body	Heat	Northern blot	Non-inducible	Jayasena et al., 1999
Strongyloides HSP70 1A/1B	HSP70cB1	XM_024648782.1:1-1935	XP_024502726	-	-	-	-	-
Schistosoma HSP70-2	HSP70cB1	L02415.1:533-2446	AAA29898	Whole body	Heat	Northern blot	Non-inducible	Neumann et al., 1993
Limunus HSP cognate 4	HSP70cB1	XM_013923214.2:160-2091	XP_013778668	-	-	-	-	-
Daphnia HSP70Bbb-2	HSP70cB2	LRGB01001774.1:337-903,963-1285,1342-1597,1655-1858,1928-2025,2086-2194,2252-2641	gi|1022765301|gb|KZS10545.1|	-	-	-	-	-
Priapulus HSPc	HSP70cB1	XM_014812613.1:143-2113	XP_014668099	-	-	-	-	-
Priapulus HSP70 II-like	HSP70cB2	XM_014817626.1:190-2052	XP_014673112	-	-	-	-	-
Trichuris HSP70	HSP70cB1	KL363195.1:c58762-57407,c57345-57028,c56967-56683	KFD56098	-	-	-	-	-
Daphnia HSP70Bbb-1	HSP70cB1	LRGB01002121.1:c532073-531866,c531808-531464,c531400-530010	gi|1022763482|gb|KZS09073.1|	-	-	-	-	-
Lingula HSP71c	HSP70cB1	XM_013525464.2:202-2139	XP_013380918	-	-	-	-	-
M. nipponense HSP70-1	HSP70cB1i	KC460343.1:253-2202	AGM50430	Gill	Heat	Real-time PCR	Inducible	Xiu et al., 2014
M. nipponense HSC70	HSP70cB2i	DQ660140.1:99-2048	ABG45886	Gill	Heat	Real-time PCR	Inducible	Xiu et al., 2014
Crassostrea HSP71c	HSP70cB1	AB122064.1:33-2012	BAD15287	-	-	-	-	-
Mytilus HSP70	HSP70cB1i	AY861684.1:71-2035	AAW52766	Whole body	Heat, heat-killed bacteria	Real-time PCR	Inducible	Cellura et al., 2006
Mytilus HSP71c	HSP70cB2	AJ783714.1:475-682,1087-1292,1752-1904,2462-3017,5566-5967,6815-7254	CAH04109	-	-	-	-	-
Helobdella hypothetical protein 2 HELRODRAFT	HSP70cB1	XM_009023904.1:1-1878	XP_009022152	-	-	-	-	-
Helobdella hypothetical protein HELRODRAFT	HSP70cB2	XM_009031197.1:188-2125	XP_009029445	-	-	-	-	-
Tevnia HSP70-1	HSP70cB1	FN860147.1:1-411,757-2100,2515-2709	CBM42052	-	-	-	-	-
Tevnia HSP70-2	HSP70cB2	FN860148.1:1-411,865-2208,2623-2817	CBM42053	-	-	-	-	-
Perinereis HSP70-1	HSP70cB1i	KU255783.1:49-2019	AND99892	Whole body	Cu^{2+}	Real-time PCR	Inducible	Zhang et al., 2016
Perinereis HSP70-2	HSP70cB2	HQ449186.1:89-2050	ADR66514	-	-	-	-	-
Paralvinella HSP70 form 2	HSP70cB1i	EF580993.1:1-1962	ABU63809	Posterior part of the body	Heat	Western blot	Inducible, but the data may contain other members	Cottin et al., 2008
Haliotis HSC70	HSP70cB1	FJ812176.1:77-2032	ACO36047	-	-	-	-	-
Aplysia HSC71	HSP70cB1	XM_005097955.2:103-2067	XP_005098012	-	-	-	-	-
Lottia HSC70 (hypothetical protein LOTGIDRAFT_177837)	HSP70cB1	XM_009048116.1:1-1962	XP_009046364	-	-	-	-	-
Octopus HSC71	HSP70cB1	XM_014927588.1:112-2067	XP_014783074	-	-	-	-	-
Octopus HSP71-like	HSP70cB2	XM_014929288.1:100-2058	XP_014784774	-	-	-	-	-
Dugesia HSP70	HSP70cB1	EU380241.1:53-1999	ABY83101	Whole body	Heat, amputation and starvation	Semi-quantitative RT-PCR	Inducible	Ma et al., 2009
Oncorhynchus HSC71	HSP70cB1c	S85730.1:2774-2978,3222-3427,3528-3680,3904-4459,4686-4888,5108-5306,5536-5768,5965-6165	AAB21658	Gonad cell line	Heat	Northern blot	Non-inducible	Zafarullah et al., 1992
X. maculatus HSC70	HSP70cB1c	AB062115.1:135-2048	BAB72169	Muscle and liver	Heat	Northern blot	Non-inducible	Yamashita et al., 2004
H. sapiens HSC71 (HSPA8)	HSP70cB6c	NM_006597.6:79-2019	NP_006588	-	-	-	-	-
Rattus HSC71	HSP70cB1c	NM_024351.2:81-2021	NP_077327	Brain	Axonal synapsin	Two-color superresolution microscopy	Non-inducible	Ganguly et al., 2017
Bos HSC71	HSP70cB1	NM_174345.4:76-2028	P19120	-	-	-	-	-
Pelodiscus HSC70	HSP70cB1i	HQ219723.1:81-2021	ADO17794	Liver, lung, heart, and skeletal muscle	Heat	Western blot, Real-time PCR	Inducible	Li et al., 2012
Bos HSP70	HSP70cB2i	U09861.1:157-2082	AAA73914	Skeletal muscle	Bacteria	Two-dimensional gel electrophoresis	Inducible	Gutierrez and Guerriero, 1995

H. sapiens HSP70-1B (HSPA1B)	HSP70cB2i	NM_005346.5:214-2139	NP_005337	-	-	-	Inducible	Uniprot P0DMV8
Rattus HSP70	HSP70cB2i	NM_031971.2:1248-3173	NP_114177	Pancreas	Heat	Western blot	Inducible	Lee et al., 2018
H. sapiens HSP70-like1 (HSPA-1L)	HSP70cB3c	NM_005527.4:409-2334	NP_005518	HeLa cells	Heat	Northern blot	Non-inducible	Uniprot P34931, Milner and Campbell, 1990
H. sapiens HSP70-6 (HSPA6)	HSP70cB5i	NM_002155.5:120-2051	NP_002146	Neuronal cells	Aarimoclomol	Immunostaining, Western blot	Inducible	Deane and Brown, 2018, UniProt P17066
X. maculatus HSP70-1	HSP70cB1i	AB062113.1:83-1999	BAB72167	Muscle and liver	Heat	Northern blot	Inducible	Yamashita et al., 2004
X. maculatus HSP70-2	HSP70cB2i	AB062114.1:131-2050	BAB72168	Muscle and liver	Heat	Northern blot	Inducible	Yamashita et al., 2004
H. sapiens HSP70-like2 (HSPA2)	HSP70cB4c	NM_021979.3:383-2302	NP_068814	Lung carcinoma cell	Heat	Immunostaining, Western blot	Non-inducible	Ścieglińska et al., 2008
Alligator HSP70A	HSP70cB1	AB306279.1:43-1962	BAF94142	-	-	-	-	
S. cerevisiae SSA1	-	NC_001133.9:c141431-139503	YAL005C	-	Heat	-	Inducible	Boorstein et al., 1994
S. cerevisiae SSA2	-	NC_001144.5:c97485-95566	YLL024C	-	Heat	-	Non-inducible	Boorstein et al., 1994
S. cerevisiae SSA3	-	NC_001134.8:c86448-84499	YBL075C	-	Heat	-	Inducible	Boorstein et al., 1994
S. cerevisiae SSA4	-	NC_001137.3:364589-366517	YER103W	-	Heat	-	Inducible	Boorstein et al., 1994
C. elegans HSP70F	HSP70mA1	NM_071890.4:1-1974	NP_504291	-	-	-	-	-
H. sapiens HSP70m (HSPA9)	HSP70mA1i	NM_004134.7:84-2123	NP_004125	Cancer cells	Ovarian carcinoma	Immunostaining, Western blot	Inducible	Xu et al., 2019
Pongo stress-70 protein	HSP70mA1	NM_001133388.1:101-2140	NP_001126860	-	-	-	-	-
Cricetulus stress-70 protein		NM_001246829.1:1-2040	NP_001233758	-	-	-	-	-
A. vaga HSP70mA2	HSP70mA2	-	GSADVT00049157001	-	-	-	-	-
A. vaga HSP70mA1	HSP70mA1	-	GSADVT00003702001	-	-	-	-	-
Daphnia HSC70-5	HSP70mA1	LRGB01000716.1:c3467-3389,c3326-3203,c3078-2880,c2819-2228,c2144-1297	gi|1022772237|gb|KZS16423.1|	-	-	-	-	-
Scerevisiae ECM10	-	NC_001137.3:94644-96578	YEL030W	-	-	-	-	-
Scerevisiae SSC1	-	NC_001142.9:c521602-519638	YJR045C	-	-	-	-	-
C. elegans BiP, heat shock protein 3	HSP70erA1c	M26604.1:442-628,675-1148,1387-2132,2237-2815	AAA28074	Whole body of larvae	Heat	Not mentioned	Non-inducible	Heschl and Baillie, 1989
Crassostrea GRP	HSP70erA1i	AB122065.1:108-2093	BAD15288	Gill and adductor muscle	Heat	Northern blot	Inducible	Yokoyama et al., 2006
B. ibericus HSC70	HSP70erA1i	GU574487.1:1-1962	ADR79282	Whole body	Ultraviolet	Real-time PCR	Inducible	Kim et al., 2011
B. calyciflorus HSC70-2	HSP70erA2	NTPY01000000	NTPY01001024	-	-	-	-	-
B. calyciflorus HSC70-1	HSP70erA1	NTPY01000000	NTPY01001024	-	-	-	-	-
B. manjavacas HSC71	HSP70erA1	-	1977_126_2102_r_0_1 BmTi01_isotig03139	-	-	-	-	-
A. vaga HSP70erA2	HSP70erA2	-	GSADVT00030804001	-	-	-	-	-
A. vaga HSP70erA1	HSP70erA1	-	GSADVT00008959001	-	-	-	-	-
Daphnia HSC70-4	HSP70erA1	LRGB01000024.1:1105780-1105910,1105969-1106200,1106260-1106476,1106539-1106876,1106942-1107467,1107528-1108063	gi|1022777923|gb|KZS21417.1|	-	-	-	-	-
H. sapiens Bip	HSP70erA1i	NM_005347.5:218-2182	NP_005338	Umbilical vein endothelial cells	Thapsigargin (endoplasmic stress)	Western blot	Inducible	Uniprot P11021, Milner and Campbell, 1990
Schistosoma HSP70-1	HSP70erA1	XM_018794726.1:55-2001	XP_018649109	-	-	-	-	-
S. cerevisiae KAR2	-	NC_001142.9:381327-383375	YJL034W	-	Heat	-	Inducible	Boorstein et al., 1994

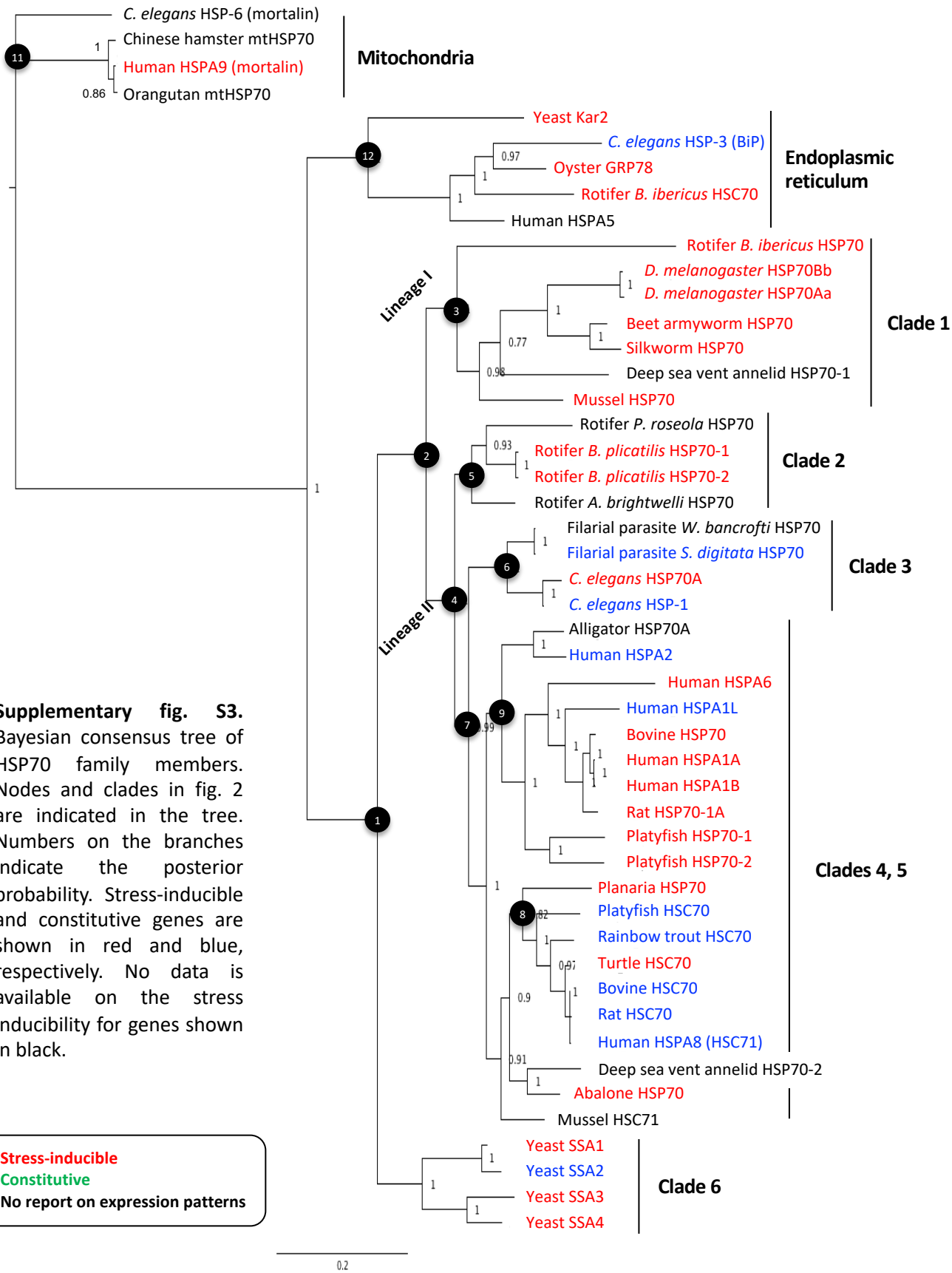
```
Bp_HSP70-1   --------------------------MSKGPAVGIDLGTTYSCVGVFQHGKVEIIANDQG 34
Bp_HSP70-2   --------------------------MSKGPAVGIDLGTTYSCVGVFQHGKVEIIANDQG 34
Bi_HSC70     MNKLGLFLLLVLSISSYLNAQKDEKKESYGTVIGIDLGTTYSCVGVVKNGRVEIIANDQG 60
Bi_HSP70     ----------------------------MSPAIGIDLGTTFSCVAVFENGKVEIIANDQG 32
Ab_HSP70     ---------------------------MAKTAIGIDLGTTYSCVGVFQHGKVEIIANDQG 33
Pr_HSP70     ----------------------------MARAIGIDLGTTYSCVGIFQHGKVEIIANDQG 32
                                          .:*******:***.:.::*:*********

Bp_HSP70-1   NRTTPSYVAFT-DTERLIGDAAKNQVAMNPNNTVFDAKRLIGRKFDDLTVQADMKHWPFT 93
Bp_HSP70-2   NRTTPSYVAFT-DTERLIGDAAKNQVAMNPNNTVFDAKRLIGRKFDDPTVQADMKHWPFT 93
Bi_HSC70     NRITPSYVAFTSDGERLIGDAAKNQLTSNPENTIFDAKRLIGREFKDSSVQGDMKYWPFK 120
Bi_HSP70     NRTTPSYVAFT-DTERIIGDAAKAQVATNPTNTVFDAKRLIGRRFDEHTVQSDMKHWPFK 91
Ab_HSP70     NRTTPSYVAFT-DTERLIGDAAKNQVAMNPTNTVFDAKRLIGRRFDDSTVQSDMKHWPFT 92
Pr_HSP70     NRTTPSYVAFT-DSERLIGDAAKNQVAMNPNNTIFDAKRLIGRKFDDATVQADMKHWPFK 91
             ** ******** * **:****** *:: ** **:*********.*.: :**.***:***.

Bp_HSP70-1   VISDSGRPKIQVEFKGETKSFYPEEVSSMVLTKMKETAEAYLGKKVTDAVVTVPAYFNDS 153
Bp_HSP70-2   VISDSGRPKIQVEFKGETKSFYPEEVSSMVLTKMKETAEAYLGKKVTDAVVTVPAYFNDS 153
Bi_HSC70     VVDKNNKPHIKVKSADEEKLFAAEEVSAMVLGKMKEIAEAYLGKPVTHAVVTVPAYFNDA 180
Bi_HSP70     LVNVNGKPKIQVEFKNSSKTFSPEEISSMVLTKMKETAESYLRKKSKKCSHHSTGLFQRF 151
Ab_HSP70     VVNEGGKPKIQVEFKGEKKTFFPEEISSMVLIKMKEIAEAYLGKKVNDAVITVPAYFNDS 152
Pr_HSP70     VISEGGKPKIQVEYKNETKSFTPEEVSSMVLTKMKEIAEAYLGKKISEAVITVPAYFNDS 151
             ::. ..:*:*:*: .. * * **:*:*** **** **:** * ... . *:

Bp_HSP70-1   QRQATKDAGAIAGLNVLRIINEPTAAAIAYGLDKKGGGEKNILIFDLGGGTFDVSILTIE 213
Bp_HSP70-2   QRQATKDAGAIAGLNVLRIINEPTAAAIAYGLDKKGGGEKNILIFDLGGGTFDVSILTIE 213
Bi_HSC70     QRQATKDAGTIAGMTVMRIINEPTAAVYCLRLGQK-EGEKNILVFDLGGGTFDVSLLTID 239
Bi_HSP70     ATTSYQRCWINSRFKCATYYQRAYCCCSCLWLRKKFKRRKNVLIYDLGGGTFDVSVLSIN 211
Ab_HSP70     QRQATKDAGAISGLNVLRIINEPTAAAIAYGLDKKVGGEKNVLIFDLGGGTFDVSILTIE 212
Pr_HSP70     QRQATKDAGVIAGLNVLRIINEPTAAAIAYGLDKKVTGERNILIFDLGGGTFDVSVLKIE 211
                : : .   : :.     :.  ..  .  * :*   .:*:*::**********:*.*:

Bp_HSP70-1   EGIFEVKSTAGDTHLGGEDFDNRLVNHFVEEFKRKNKKDITSNKRALRRLRTACERAKRT 273
Bp_HSP70-2   EGIFEVKSTAGDTHLGGEDFDNRLVNHFVEEFKRKNKKDITSNKRALRRLRTACERAKRT 273
Bi_HSC70     NGVFEVVATNGDTHLGGEDFDNRVMEHFIKLFKKKTGKDIRKDNRAVQKLRREVEKAKRT 299
Bi_HSP70     DGLFEVLSTNGNTHLGGEDFDNNLVNFFASEFRRKHGKDLSRNQKALRRLRTACERAKRT 271
Ab_HSP70     EGIFEVKSTAGDTHLGGEDFDNRLVNHFVEEFKRKHKKDITGNKRAIRRLRTACERAKRT 272
Pr_HSP70     EGIFEVKSTAGDTHLGGEDFDNRMVSHFVQEFKRKNNKDLSQNKRGLRRLRTACERAKRT 271
             :*:*** :* *:**********.::..* . *::*  **:  :::.:::**   *:****

Bp_HSP70-1   LSSSAQANIEIDSLHEGVDFYTSITRARFEELCADLFRGTLDPVEKALRDAKMDKSSVNE 333
Bp_HSP70-2   LSSSAQANIEIDSLHEGVDFYTSITRARFEELCADLFRGTLDPVEKALRDAKMDKSSVNE 333
Bi_HSC70     LSTQFDTRIEIESFFDGEDFSETFTRARFEELNMDLFRNTLKPVQKVLEDAGLKKTEIDE 359
Bi_HSP70     LSSSAEATIEVDSLYEGTDFNSKISRARFEELNMDLFRSTLHLVEQALKDARITKTKIDE 331
Ab_HSP70     LSSSAQANIEIDSLHEGVDFYTNITRARFEELNADLFRGCLDPVEKSLRDAKLDKAQIHE 332
Pr_HSP70     LSSSSQASIEIDSLHEGIDFYSTITRARFEELCADLFRSTLEPVEKALRDAKMDKASIHE 331
             **:. :: **::*:.:* **  .::*******  ****. *. *:: *.** : *:.:.*

Bp_HSP70-1   IVLVGGSTRIPKVQKLLQDFFNGKELNKSINPDEAVAYGAAVQAAILTGDKSEAVQDLLL 393
Bp_HSP70-2   IVLVGGSTRIPKVQKLLQDFFNGKELNKSINPDEAVAYGAAVQAAILTGDKSEAVQDLLL 393
Bi_HSC70     IVLVGGSTRIPKIQQLVKEYFDGKEPSRGINPDEAVAYGAAVQGGVLSGEDVG--GEVVL 417
Bi_HSP70     IVLVGGSTRIPRIQKAFRGFFNGKQLNKSINPDEAVAYGAAVQAAILSGDKSREIKDVLL 391
Ab_HSP70     IVLVGGSTRIPKVQKLLQDFFNGKELNKSINPDEAVAYGAAVQAAILTGDKSEAVQDLLL 392
Pr_HSP70     IVLVGGSTRIPKVQKLLQDFFNGKELNKSINPDEAVAYGAAVQAAILTGDKSEEVKDVLL 391
             ************::*: .: :*:**: .:.**************..:*:*:.     :::*

Bp_HSP70-1   LDVAPLSLGIETAGGVMTALIKRNTTIPTKQTQTFTTYADNQPGVLIQVYEGERAMTKDN 453
Bp_HSP70-2   LDVAPLSLGIETAGGVMTALIKRNTTIPTKQTQTFTTYADNQPGVLIQVYEGERAMTKDN 453
Bi_HSC70     LDVNPLTMGIETVGGVMTKLIPRNTVIPTKNP-KFSTASDNQPTVTIAVYEGERPMTKDN 476
Bi_HSP70     VDVAPLSLGIETAGGVMTNLINRNSRIPVKTSQIFTTYSDNQTAVSIRVFEGERALVQDN 451
Ab_HSP70     LDVAPLSLGIETAGGVMTSLIKRNTTIPTKQTQVFTTYSDNQPGVLIQVYEGERAMTKDN 452
Pr_HSP70     LDVAPLSLGIETAGGVMTPLIKRNTTIPTKQTQTFTTYSDNQPGVDIKVFEGERSMTRDN 451
             :** **::****.***** ** **: **.*    *:* :***  * * *:**** :.:**
```

```
Bp_HSP70-1    HLLGKFELSGIPPAPRGVPQIEVTFDIDANGILNVSAADKSTGKTNKITITNDKGRLSKE 513
Bp_HSP70-2    HLLGKFELSGIPPAPRGVPQIEVTFDIDANGILNVSAADKSTGKTNKITITNDKGRLSKE 513
Bi_HSC70      HLLGKFDLTGIPPAPRGVPQIEVTFEIDANGILKVSAEDKGTGNKNNIVINNNQNRLSPE 536
Bi_HSP70      HLLGNFDLVGIPPAPRGIPQIDVTFDIDANGILSVSAKENSSGKSNNITIKNDKGRLSKE 511
Ab_HSP70      NLLGKFELTGIPPAPRGVPQIEVTFDIDANGILNVSACDKSTGKQNKITITNDKGRLSKE 512
Pr_HSP70      HLLGNFELSGIPPAPRGVPQIEVTFDIDANGILNVTAVDKSTGRENKITITNDKGRLSKD 511
              :***:*:* ********:***:***:*******.*:* ::.:*. *:*.*.*::.*** :

Bp_HSP70-1    EIDRMVNEAEKYKKDDEEQRDKVAAKNSLESYCFNMKQTVED-EKLAAKISADDKKKILD 572
Bp_HSP70-2    DIDRMVNEAEKYKKDDEEQRDKVAAKNSLESYCFNMKQTVED-EKLAAKISADDKKKILD 572
Bi_HSC70      EIERMIKDAEKFADEDKKVKEKVEAKNELESYVYSLKNQLNDKEKLGNKLSEDEKETINS 596
Bi_HSP70      EIERMINEAERFRAEDDRQRDRIASKNKLETYIFAVKQALDD-AK---NLSSSDKKVCQE 567
Ab_HSP70      DIERMVNDAEKYKKDDEQQRERITAKNSLESYCFNMKQTIED-EKLAAKVSADEKKKILD 571
Pr_HSP70      QIEQMVAEAEKYKKEDEAQRDRISAKNSLESYCFNMKTSIND-DKIGAKISADDKAKITE 570
              :*::*: :**::  :*.  :::: :**.**:* : :*  ::*  *   ::* .:*    .

Bp_HSP70-1    ACEEALKWLDSNQTAEKDEFEHKMKEVEKICSPIITQLYQGAGGAPGGMPGGM----PGG 628
Bp_HSP70-2    ACEEALKWLDSNQTAEKDEFEHKMKEVEKICSPIITQLYQGAGGAPGGMPGGMPGGMPGG 632
Bi_HSC70      AVDEKIKWLESNSDAEVDDFKEQKKALEEIVNPIMTKLYQQNSENAGSSDSQTNDEL--- 653
Bi_HSP70      ACRKELKWLEANQMADKDEFAFHYKELSRKCMPLMKKIHSGERNGPTVEEVE-------- 619
Ab_HSP70      ACENALKWLDANQTAEKDEFEHKLKEIEKTCSPIITKLYQG--GAPGGMPGGMPGGAGA- 628
Pr_HSP70      TIESALKWMETNQLAEKDEFEHKLKEVEKICSPIMTKLYGGEAGGHAG-PGGHAGGA--- 626
              :  . :**:::*. *: *:*  : * :..   *::.:::

Bp_HSP70-1    MPGGMPGAGPESAGRSGPTIDEVD 652
Bp_HSP70-2    MPGGMPGAGPESAGRSGPTIDEVD 656
Bi_HSC70      ------------------------ 653
Bi_HSP70      ------------------------ 619
Ab_HSP70      --GGAS--------GAGPKIEEVD 642
Pr_HSP70      -PGGS------KSGGKGPTIEEVD 643
```

Supplementary fig. S1. Comparison of the deduced amino acid sequences of cDNAs encoding HSP70-1 and HSP70-2 of *Brachionus plicatilis* with those of the HSP70 family members from other rotifers. Dashes denote gaps introduced to maximize homology. The HSP70 protein family signatures are meshed, and the EEVD motifs are boxed. The non-organelle consensus motifs and bipartite nuclear localization motifs are shown in blue and red letters, respectively. The numbers in the right margin of the sequences represent residues from the N-terminus. Bi, *B. ibericus* (ADR79281, ADR79282); Ab, *Asplanchna brightwelli* (ARU12813).


Stress-inducible
Constitutive
No report on expression patterns
Platyfish HSC70
Rainbow trout HSC70
Turtle HSC70
Rat HSC70
Bovine HSC70
Human HSPA8 (HSC71)
Planaria EU380241
Mussel HSC71
Abalone HSP70
Deep sea vent annelid HSP70-2
Human HSPA2
Alligator HSP70A
Platyfish HSP70-1
Platyfish HSP70-2
Human HSPA1L
Human HSPA6
Rat HSP70-1A
Bovine HSP70
Human HSPA1A
Human HSPA1B
Clades 4, 5
Caenorhabditis elegans HSP70A
Caenorhabditis elegans HSP-1
Filarial parasite Wuchereria bancrofti HSP70
Filarial parasite Setaria digitata HSP70
Clade 3
Rotifer Philodina roseola HSP70
Rotifer Asplanchna HSP70
Rotifer Brachionus plicatilis HSP70-1
Rotifer Brachionus plicatilis HSP70-2
Clade 2
Mussel HSP70
Rotifer Brachionus ibericus HSP70
Deep sea vent annelid HSP70-1
D. melanogaster HSP70Bb
Drosophila melanogaster HSP70Aa
Beet armyworm HSP70
Silkworm HSP70
Clade 1
Yeast SSA1
Yeast SSA2
Yeast SSA3
Yeast SSA4
Clade 6
Yeast Kar2
Human HSPA5
Oyster GRP78
C. elegans HSP-3 (BiP)
Rotifer Brachionus ibericus HSC70
Endoplasmic reticulum
Caenorhabditis elegans HSP-6 (mortalin)
Chinese hamster mtHSP70
Human HSPA9 (mortalin)
Orangutan mtHSP70
Mitochondria
Lineage II
Lineage I

Supplementary fig. S2. Maximum likelihood tree of HSP70 family members. A bootstrap consensus tree was constructed by the maximum likelihood method using the LG + G model. The bootstrap values from a 1000-replicate analysis are given at the nodes in percentage. Nodes and clades in fig. 2 are indicated in the tree. Stress-inducible and constitutive genes are shown in red and blue, respectively. No data on the stress inducibility is available for genes shown in black.



Supplementary fig. S3. Bayesian consensus tree of HSP70 family members. Nodes and clades in fig. 2 are indicated in the tree. Numbers on the branches indicate the posterior probability. Stress-inducible and constitutive genes are shown in red and blue, respectively. No data is available on the stress inducibility for genes shown in black.

Stress-inducible
Constitutive
No report on expression patterns

Supplementary fig. S3. Bayesian consensus tree of HSP70 family members. Nodes and clades in fig. 2 are indicated in the tree. Numbers on the branches indicate the posterior probability. Stress-inducible and constitutive genes are shown in red and blue, respectively. No data is available on the stress inducibility for genes shown in black.

```
Aplysia_HSP70-2          ----------------------------------------------MTKGMKTPAIGIDL 14
Haliotis_HSP70           ----------------------------------------------MAPKGKSPAIGIDL 14
Lottia_HSP70-4           ---------------------------------------------MAPKGPAIGIDL 12
Bplicatilis_HSP70-1      --------------------------------------------MSKGPAVGIDL 11
Avaga_HSP70erA           -----------------------MKIFTALSLIALTTIFTFSSASEDAKKDIGTVIGIDL 37
Bibericus_HSC70          -----------------------MNKLGLFLLLVLSISSYLNAQKDEKKESYGTVIGIDL 37
BcHSC70-2                -------------------------------------------------GTVIGIDL 8
Celegans_HSP70F          MLSARS--------------------FLSSARTIA---RSSLMSARSLSDKPKGHVIGIDL 38
Daphnia_HSP70c5-1        ------------------------------------------------------------ 0
Pongo_HSP70m             MISASRAVAARLVGAAASRGPTAARYQDGWNGLSHEAFRIVSRRDYASEAIKGAVVGIDL 60
Hsapiens_HSP70m          MISASRAAAARLVGAAASRGPTAARHQDSWNGLSHEAFRLVSRRDYASEAIKGAVVGIDL 60


Aplysia_HSP70-2          GTTYSCVGIFQHGKVEIIANDQGNRTTPSYVAFT-DTERLVGDAAKNQAALNPSNTIFDA 73
Haliotis_HSP70           GTTYSCVGVFQNGQVEIIANDQGNRTTPSYVAFT-DTERLIGDAAKNQVALNPQNTVFDA 73
Lottia_HSP70-4           GTTFSCVGVFQHGAVEIIANDQGNRTTPSYVAFT-DSERLIGDSAKNQVALNPSNTIFDA 71
Bplicatilis_HSP70-1      GTTYSCVGVFQHGKVEIIANDQGNRTTPSYVAFT-DTERLIGDAAKNQVAMNPNNTVFDA 70
Avaga_HSP70erA           GTTYSCVGIFKNGRVEIIANDQGNRITPSYVAFTAEGERLIGDAAKNQLTSNPENTVFDA 97
Bibericus_HSC70          GTTYSCVGVVKNGRVEIIANDQGNRITPSYVAFTSDGERLIGDAAKNQLTSNPENTIFDA 97
BcHSC70-2                GTTYSCVGVVKNGRVEIIANDQGNRITPSYVAFTSEGERLIGDAAKNQLTSNPENTIFDA 68
Celegans_HSP70F          GTTNSCVSIMEGKTPKVIENAEGVRTTPSTVAFTADGERLVGAPAKRQAVTNSANTLFAT 98
Daphnia_HSP70c5-1        ---------MEGQTPKVIENAEGSRTTPSVIAFTKDGERLAGMPAKRQAVTNAQNTLYAT 51
Pongo_HSP70m             GTTNSCVAVMEGKQAKVLENAEGARTTPSVVAFTADGERLVGMPAKRQAVTNPNNTFYAT 120
Hsapiens_HSP70m          GTTNSCVAVMEGKQAKVLENAEGARTTPSVVAFTADGERLVGMPAKRQAVTNPNNTFYAT 120
                                  .:    ::: * :* * *** :*** : *** *  **.* . *  **.: :

Aplysia_HSP70-2          KRLIGRKFTDKSVQSDMKHWPFQVAE-VDSRPKIEAEYKGERKLFAPEEVSSMVLSKMKE 132
Haliotis_HSP70           KRLIGRQFDDSAVQKDMKHWPFRVVNSSGSKPKLQVEYKGEKKTFAPEEISSMVLNKMKE 133
Lottia_HSP70-4           KRLIGRKFDDATIQSDMKHWPFKVVK-DGDKPKLQAEFKNELKTFSPEEVSAMVLTKMKE 130
Bplicatilis_HSP70-1      KRLIGRKFDDLTVQADMKHWPFTVIS-DSGRPKIQVEFKGETKSFYPEEVSSMVLTKMKE 129
Avaga_HSP70erA           KRLIGREFSDSSVQQDMKHFPFKVVE-KNSKPAIQISTGKEQKLFTPEEISAMVLGKMRE 156
Bibericus_HSC70          KRLIGREFKDSSVQGDMKYWPFKVVD-KNNKPHIKVKSADEEKLFAAEEVSAMVLGKMKE 156
BcHSC70-2                KRLIGREFSDPSVQGDMKYWPFKVVE-KNKKPYIKVKSGDQDKLFAPEEISAMVLGKMKE 127
Celegans_HSP70F          KRLIGRRYEDPEVQKDLKVVPYKIVKASNGDAWVE----AQGKVYSPSQVGAFVLMKMKE 154
Daphnia_HSP70c5-1        KRLIGRKFEDPEVKKDMKTVSYKIVRASNGDAWVE----AQGKLYSPSQIGAFVLTKMKE 107
Pongo_HSP70m             KRLIGRRYDDPEVQKDIKNVPFKIVRASNGDAWVE----AHGKLYSPSQIGAFVLMKMKE 176
Hsapiens_HSP70m          KRLIGRRYDDPEVQKDIKNVPFKIVRASNGDAWVE----AHGKLYSPSQIGAFVLMKMKE 176
                         ******.: *  :: *:*   : :    .    ::     . * :  .::.::** **:*

Aplysia_HSP70-2          TAEAYLGQKVTEAVITVPAYFNDSQRQATKDAGAIAGLNVLRMINEPTAAALAYGLDKGQ 192
Haliotis_HSP70           TAEAYLGQQVTDAVITVPAYFNDSQRQATKDAGAIAGLNVLRIINEPTAAALAYGLDKNL 193
Lottia_HSP70-4           TAEAYLGENVTNAVITVPAYFTDSQRSATKDAGVIAGLNVLRIINEPTAAALAYGLDKKT 190
Bplicatilis_HSP70-1      TAEAYLGKKVTDAVVTVPAYFNDSQRQATKDAGAIAGLNVLRIINEPTAAAIAYGLDKKG 189
Avaga_HSP70erA           IAEAYLGKKVTHAVVTVPAYFNDAQRQATKDAGTISGLNVLRIINEPTAAAIAYGLDKK- 215
Bibericus_HSC70          IAEAYLGKPVTHAVVTVPAYFNDAQRQATKDAGTIAGMTVMRIINEPTAAVYCLRLGQK- 215
BcHSC70-2                IAEAYLGKNVTHAVVTVPAYFNDAQRQATKDAGTISGMTVMRIINEPTAAAIAYGLDKK- 186
Celegans_HSP70F          TAESYLGTTVNNAVVTVPAYFNDSQRQATKDAGQISGLNVLRVINEPTAAALAYGLDKD- 213
Daphnia_HSP70c5-1        TAEAYLGTPVKNAVVTVPAYFNDSQRQATKDAGQISGLNVLRVINEPTAAALAYGMDKS- 166
Pongo_HSP70m             TAENYLGHTAKNAVITVPAYFNDSQRQATKDAGQISGLNVLRVINEPTAAALAYGLDKS- 235
Hsapiens_HSP70m          TAENYLGHTAKNAVITVPAYFNDSQRQATKDAGQISGLNVLRVINEPTAAALAYGLDKS- 235
                          ** ***  ...**:******.*:**.****** *:*:.*:*:*******. .  :.:

Aplysia_HSP70-2          SGEKNVLIFDLGGGTFDVSVLTIDEGSMFEVKATAGDTHLGGEDFDNRLVSHFLQEFKRK 252
Haliotis_HSP70           KGEKNVLIFDLGGGTFDVPVLTIDEGSMFEVRSTAGDTHLGGEDFDNRLVEHFLQEFQRK 253
Lottia_HSP70-4           DTEQHILIFDLGGGTFDVSILAIEDG-VFEVLATAGDTHLGGEDFDNRMVNYFTQEFKRK 249
Bplicatilis_HSP70-1      GGEKNILIFDLGGGTFDVSILTIEEG-IFEVKSTAGDTHLGGEDFDNRLVNHFVEEFKRK 248
Avaga_HSP70erA           EGEKNILVFDLGGGTFDVSLLTIDNG-VFEVVATNGDTHLGGEDFDQRVMEHFIKLFKKK 274
Bibericus_HSC70          EGEKNILVFDLGGGTFDVSLLTIDNG-VFEVVATNGDTHLGGEDFDNRVMEHFIKLFKKK 274
BcHSC70-2                EGEKNILVFDLGGGTFDVSLLTIDNG-VFEVVATNGDTHLGGEDFDNRVMEHFIKLFKKK 245
Celegans_HSP70F          AGDKIIAVYDLGGGTFDVSILEIQKG-VFEVKSTNGDTFLGGEDFDHALVHHLVGEFKKE 272
Daphnia_HSP70c5-1        -DDKIIAVYDLGGGTFDISILEIQKG-VFEVKSTNGDTFLGGEDFDNALVNFLVNEFKRD 224
Pongo_HSP70m             -EDKVIAVYDLGGGTFDISILEIQKG-VFEVKSTNGDTFLGGEDFDQALLRHIVKEFKRE 293
Hsapiens_HSP70m          -EDKVIAVYDLGGGTFDISILEIQKG-VFEVKSTNGDTFLGGEDFDQALLRHIVKEFKRE 293
                           :: : ::********: :* *:.* :*** :* ***.*******: :: .:   *::.

Aplysia_HSP70-2          HNKDISKNARATRRLRTACERAKRTLSSSSEASIEIDSLFE----GMDYYTKISRARFEE 308
Haliotis_HSP70           TRKDISNNTRAMRRLHTACERAKRTLSSSTEASIEIDSLYE----GVDFYSKISRARFEE 309
Lottia_HSP70-4           FGKDLSKSSRALRRLRTACERAKRTLSSSAEANIEIDALFE----GFDFYSKITRARFED 305
Bplicatilis_HSP70-1      NKKDITSNKRALRRLRTACERAKRTLSSSAQANIEIDSLHE----GVDFYTSITRARFEE 304
Avaga_HSP70erA           TGKDVRKDHRAVQKLRREVEKAKRTLSSQHQTKIEIESFFD----NEDFSETLTRAKFEE 330
Bibericus_HSC70          TGKDIRKDNRAVQKLRREVEKAKRTLSTQFDTRIEIESFFD----GEDFSETFTRARFEE 330
```

```
BcHSC70-2                TGKDIRKDNRAVQKLRREVEKAKRTLSTQFDTRIEIESFFD----SEDFSETFTRASFEK 301
Celegans_HSP70F          QGVDLTKDPQAMQRLREAAEKAKCELSSTTQTDINLPYITMDQSGPKHLNLKLTRAKFEQ 332
Daphnia_HSP70c5-1        QGLDITKDPMAMQRVKEAAEKAKIELSSSMQTDINLPYLTMDSSGPKHMNLKMSRSKLES 284
Pongo_HSP70m             TGVDLTKDNMALQRVREAAEKAKCELSSSVQTDINLPYLTMDSSGPKHLNMKLSRAQFEG 353
Hsapiens_HSP70m          TGVDLTKDNMALQRVREAAEKAKCELSSSVQTDINLPYLTMDSSGPKHLNMKLTRAQFEG 353
                            *: ..  * ::::   *:**  **:  :: *::  :        .   .::*: :*

Aplysia_HSP70-2          LCADLFRSTLEPVESALRDAKLDKGKIDEVVLVGGSTRIPKIQKLLSDFFNGKELNKSIN 368
Haliotis_HSP70           LCSDLFRSTLEPVEKALRDAKLDKSCIHDVVLVGGSTRIPKIQKLLQNFMNGKDLNKSIN 369
Lottia_HSP70-4           LCADLFRNTMVPVEKALKDAKLDKSKIDEVVLVGGSTRIPKIQKLLKDFMNGKELNKSIN 365
Bplicatilis_HSP70-1      LCADLFRGTLDPVEKALRDAKMDKSSVNEIVLVGGSTRIPKVQKLLQDFFNGKELNKSIN 364
Avaga_HSP70erA           LNMDLFRSTMKPVQKVLEDADMKKSDIAEVVLVGGSTRIPKVQQLVKDFFDGKEPSRGIN 390
Bibericus_HSC70          LNMDLFRNTLKPVQKVLEDAGLKKTEIDEIVLVGGSTRIPKIQQLVKEYFDGKEPSRGIN 390
BcHSC70-2                LNIELFRNTLKPVQKVLEDAGLKKTDIDEIVLVGGSTRIPKVQELVKEFFNGKEPSRGIN 361
Celegans_HSP70F          IVGDLIKRTIEPCRKALHDAEVKSSQIADVLLVGGMSRMPKVQATVQEIF-GKVPSKAVN 391
Daphnia_HSP70c5-1        LVGDLIKRTVGPCQKALKDAEVSKNDIGDVLLVGGMTRMPKVQETVKDIF-GRVPSKAVN 343
Pongo_HSP70m             IVTDLIRRTIAPCQKAMQDAEVSKSDIGEVILVGGMTRMPKVQQTVQDLF-GRAPSKAVN 412
Hsapiens_HSP70m          IVTDLIRRTIAPCQKAMQDAEVSKSDIGEVILVGGMTRMPKVQQTVQDLF-GRAPSKAVN 412
                         :  :*:: *: * ...:.** :..  : :::**** :*:**:*  :.: : *:  .:.:*

Aplysia_HSP70-2          PDEAVAYGAAVQAAVLTGDKSEAIRDVLLVDVAPLSLGIETAGGVMTSLIKRGSTIPAKT 428
Haliotis_HSP70           PDEAVAYGAAVQAAVLSGDSSDAIKDVLLVDVAPLSLGIETAGGVMTKLIDRNTRIPTKA 429
Lottia_HSP70-4           PDEAVAYGAAVQAAVLIGDKSEKIKDVLLVDVAPLSLGIETAGGVMTRIIERNTRIPTKH 425
Bplicatilis_HSP70-1      PDEAVAYGAAVQAAILTGDKSEAVQDLLLLDVAPLSLGIETAGGVMTALIKRNTTIPTKQ 424
Avaga_HSP70erA           PDEAVAYGAAVQAGVLSGEEN--TGDIVLLDVNPLTMGIETVGGVMTKIIPRNTVIPTKK 448
Bibericus_HSC70          PDEAVAYGAAVQGGVLSGEDV--GGEVVLLDVNPLTMGIETVGGVMTKLIPRNTVIPTKN 448
BcHSC70-2                PDEAVAYGAAVQGGVLSGEDV--GGDVVLLDVNPLTMGIETVGGMMTKLISRNTVIPTKK 419
Celegans_HSP70F          PDEAVAMGAAIQGAVLAGD----VTDVLLLDVTPLSLGIETLGGIMTKLITRNTTIPTKK 447
Daphnia_HSP70c5-1        PDEAVAVGAAIQGGVLSGG----VTDILLLDVTPLSLGIETLGGVFTKLIQRNTTIPTKK 399
Pongo_HSP70m             PDEAVAIGAAIQGGVLAGD----VTDVLLLDVTPLSLGIETLGGVFTKLINRNTTIPTKK 468
Hsapiens_HSP70m          PDEAVAIGAAIQGGVLAGD----VTDVLLLDVTPLSLGIETLGGVFTKLINRNTTIPTKK 468
                         ****** ***:*..:* *       :::*:** **::**** **::* :* *.: **:*

Aplysia_HSP70-2          SQVFTTYSDNQPGVDIQVFEGERAMTKDNNLLGKFQLSGIPPAPRGVPQIEVTFDVDANG 488
Haliotis_HSP70           SQIFTTYSDNQPGVSIQVFEGERALTKDNNILGKFELTGIPPAPRGVPQIEVTFNIDANG 489
Lottia_HSP70-4           SQIFTTYSDNQPGVSIQVYEGERTMTKDNHPMGNFELSGIPPAPRGVPQIEVSFDIDANG 485
Bplicatilis_HSP70-1      TQTFTTYADNQPGVLIQVYEGERAMTKDNHLLGKFELSGIPPAPRGVPQIEVTFDIDANG 484
Avaga_HSP70erA           SQVFSTAAENQPSVTIQVFEGERPMTKDNHVLGKFDLTGIPPAPRGVPQIEVTFEIDVNG 508
Bibericus_HSC70          -PKFSTASDNQPTVTIAVYEGERPMTKDNHLLGKFDLTGIPPAPRGVPQIEVTFEIDANG 507
BcHSC70-2                SQVFSTAADNQPSVTIAVYEGERPMTKDNHLLGKFDLTGIPPAPRGVPQIEVTFEIDVNG 479
Celegans_HSP70F          SQVFSTAADGQTQVQIKVFQGEREMATSNKLLGQFSLVGIPPAPRGVPQVEVTFDIDANG 507
Daphnia_HSP70c5-1        SQVFSTAADGQTQVEIKVYQGEREMAADNKILGQFSLIGIPPAPRGVPQIEVTFDIDANG 459
Pongo_HSP70m             SQVFSTAADGQTQVEIKVCQGEREMAGDNKLLGQFTLIGIPPAPRGVPQIEVTFDIDANG 528
Hsapiens_HSP70m          SQVFSTAADGQTQVEIKVCQGEREMAGDNKLLGQFTLIGIPPAPRGVPQIEVTFDIDANG 528
                            *:* ::.*  * * * :*** :: .*: :*:* * ***********:**:*::*.**

Aplysia_HSP70-2          ILNVTAQDKSTGKSSHITIKNERGRLSQAEIDRMLADADKYKDEDEKQRERVSARNQLET 548
Haliotis_HSP70           ILNVSAVDKSTGKSNNVTISDDQSRLSKADIDRMVSEAERYKEEDEKHKERIAARNHLEN 549
Lottia_HSP70-4           ILNVLAEDKSTGKSNKITITNDKGRLSSEEIEKLVQEAEKYKDEDEKQKRKISARNQLES 545
Bplicatilis_HSP70-1      ILNVSAADKSTGKTNKITITNDKGRLSKEEIDRMVNEAEKYKKDDEEQRDKVAAKNSLES 544
Avaga_HSP70erA           ILKVTAEDKGTGNKNNIVINSNTNRLSPDEIDRMIKDSEKFADEDKKVKERVDAKNELES 568
Bibericus_HSC70          ILKVSAEDKGTGNKNNIVINNNQNRLSPEEIERMIKDAEKFADEDKKVKEKVEAKNELES 567
BcHSC70-2                ILKVTAEDKGTGNKNNIVINNNQNRLSPEEIEKMIKDAEKFADEDKKVKEKVEAKNELES 539
Celegans_HSP70F          IVNVSARDRGTGKEQQIVIQS-SGGLSKDQIENMIKEAEKNAAEDAKRKELVEVINQAEG 566
Daphnia_HSP70c5-1        IVHVSARDKGTGKEQQIVIQS-SGGLSKDEIENMVRNAEQFAKEDQVKRDRVEAVNHAEG 518
Pongo_HSP70m             IVHVSAKDKGTGREQQIVIQS-SGGLSKDDIENMVKNAEKYAEEDRRKKERVEAVNMAEG 587
Hsapiens_HSP70m          IVHVSAKDKGTGREQQIVIQS-SGGLSKDDIENMVKNAEKYAEEDRRKKERVEAVNMAEG 587
                         *::* * *:.**. .::.* .  . **  :*:.:: ::::   :*   :  : . *  *

Aplysia_HSP70-2          YIFHVKQAV-DA--AGDKLPSPDKDAVLKACNDSLSWLDNNSLADKEEVEDRLKELQKTT 605
Haliotis_HSP70           YVFSVRASV-DE--MGSKQEDVDKQTVSKACEETLRWLDNNSLAEKEEFEQQYKQLEKLC 606
Lottia_HSP70-4           YVFNVKQVL-DNG-GADKICDSDKETVKQCCDETIQWLDNNALAEKDEYEHRLDEVQKVC 603
Bplicatilis_HSP70-1      YCFNMKQTV-EDEKLAAKISADDKKKILDACEEALKWLDSNQTAEKDEFEHKMKEVEKIC 603
Avaga_HSP70erA           YAYSLKTQLNDKEKLGGKLSSDDKETIEKAVEEQIKWLESNQGAEVDELKEHKKQLEEIV 628
Bibericus_HSC70          YVYSLKNQLNDKEKLGNKLSEDEKETINSAVDEKIKWLESNSDAEVDDFKEQKKALEEIV 627
BcHSC70-2                YVYSLKNQLSDKEKLGGKLSEEDKEKIQSSVEEKIKWLESNSNAEVEEFKDQKKELEEIV 599
Celegans_HSP70F          IIHDTEAKMT---EFADQLPKDECEALRTKIADTKKILDNKDNETPEAIKEACNTLQQQS 623
Daphnia_HSP70c5-1        IVHDTESKME---EFKEQLPAEECSKLREQISAVRDMLVNKDSKTPEEIKKTTNDLQQAS 575
Pongo_HSP70m             IIHDTETKME---EFKDQLPADECNKLKEEISKMRELLARKDSETGENIRQAASSLQQAS 644
Hsapiens_HSP70m          IIHDTETKME---EFKDQLPADECNKLKEEISKMRELLARKDSETGENIRQAASSLQQAS 644
                           .  .  :        :    : . :          *  :     :  ..  . :::
```

```
Aplysia_HSP70-2          SSVMAKLHTQGQSQGQGSS---------------QGSSGPHGPSVEEMD  639
Haliotis_HSP70           SPVMSKLHNKGCGQQ-------------------QQGGSSKGPTVEEMD  636
Lottia_HSP70-4           SPIMTKLHGEANGQSTD-PTG--------------RSGGNQGPTVEEID  637
Bplicatilis_HSP70-1      SPIITQLYQGAGGAPGGMPGGMPGGMPGGMPGAGPESAGRSGPTIDEVD  652
Avaga_HSP70erA           TPIMTKLYGQGGAGAGAGPEDVPPPPPHGHDDDSL--------------  663
Bibericus_HSC70          NPIMTKLYQQNSENAGSSDSQTNDEL-----------------------  653
BcHSC70-2                NPIMSKLYQQSG--------------------------------------  611
Celegans_HSP70F          LKLFEAAYKNMAAKNSGGDAQEAKT---AEE-----------PKKEQN-  657
Daphnia_HSP70c5-1        LKLFEMAYKKMAADRESSGGSTNSGDSTGST-----------DKKEDKQ  613
Pongo_HSP70m             LKLFEMAYKKMASEREGSGSSGTGEQ---KE-----------DQKEEKQ  679
Hsapiens_HSP70m          LKLFEMAYKKMASEREGSGSSGTGEQ---KE-----------DQKEEKQ  679
                           ::   :
```

Supplementary fig. S4. Alignment of several HSP70 family members with variations in the cytosolic (EEVD), endoplasmic reticulum (ER; HDEL/KDEL), and mitochondrial (PEAEYEEAKK) motifs. The three motifs are shown in bold. Distinctive amino acid residues in the newly identified motifs are shown in red (cytosolic), blue (ER), and green (mitochondrial). The discrimination of organelle-specific HSP70 family members becomes more evident by using multiple motifs.

```
Drosophila_Bb_AF295957          ---------------ATGCCTGCTATTGGAATCGATCTGGGCACCACCTACTCCTGCGTG 45
Drosophila_Aa_AF295933          ---------------ATGCCTGCTATTGGAATCGATCTGGGCACCACCTACTCCTGCGTG 45
Spodoptera_FJ754276             ---------------ATGCCAGCCATTGGAATAGATCTGGGTACCACATACTCGTGCGTC 45
Bombyx_AB035326                 ---------------ATGCCAGCTATCGGAATCGATTTGGGAACGACATACTCATGTGTT 45
Paralvinella_EF580992           ATGGCACAAGGAAAAACACCGGCAATTGGCATTGATCTTGGCACCACCTATTCCTGTGTT 60
B_plicatilis_HSP70-1_AB775784   ------ATGAGCAAAGGACCCGCTGTAGGCATCGACTTGGGCACCACGTACTCTTGCGTG 54
B_plicatilis_HSP70-2_AB775785   ------ATGAGCAAAGGACCCGCTGTAGGCATCGACTTGGGCACCACGTACTCTTGCGTG 54
A_brightwelli_HSP70_KX119429    ---------ATGGCCAAAACAGCGATCGGTATTGATTTGGGCACGACATATTCGTGTGTG 51
Wuchereria_AF167352             ---------ATGTCAAAGAATGCCATTGGTATCGATCTTGGAACCACGTATTCCTGCGTG 51
Setaria_AF079360                ---------ATGTCAAAGAACGCAATCGGCATTGATCTTGGGACTACTTACTCGTGCGTG 51
C_elegans_HSP70A_M18540         ------ATGAGTAAGCATAACGCTGTTGGAATCGATTTGGGAACTACCTACTCCTGCGTG 54
C_elegans_HSP-1_NM_070667       ------ATGAGTAAGCATAACGCTGTTGGAATCGATTTGGGAACTACCTACTCCTGCGTG 54
M_nipponense_KC460343           ------ATGGCTAAAAGTGCAGCTGTCGGGATTGACCTGGGTACCACCTACTCATGTGTA 54
M_nipponense_DQ660140           ------ATGGCTAAAAGTGCAGCTGTCGGGATTGACCTGGGTACCACCTACTCATGTGTA 54
Mytilus_HSP70_AY861684          ---ATGGCAAAAACAGGACCAGCAATTGGAATAGATCTTGGAACTACATACTCCTGTGTT 57
Perinereis_KU255783             ---------ATGGCAGCACCTGCAGTAGGTATTGATCTCGGCACCACATACTCCTGCGTT 51
Paralvinella_HSP70-2_EF580993   ------ATGGCGAAGGCAAGTGCTGTGGGTATCGACCTTGGAACCACGTACTCTTGCGTT 54
Haliotis_FJ812176               ------ATGGCCAAGGCACCAGCTATTGGTATAGATCTGGGGACCACCTACTCGTGTGTA 54
Oncorhynchus_HSC71_AAB21658     ------ATGTCTAAGGGACCAGCAGTCGGCATCGATCTCGGGACCACCTACTCCTGCGTG 54
X_maculatus_HSC70_AB062115      ------ATGTCCAAGGGACCAGCTGTAGGCATTGACCTGGGCACTACCTACTCTTGTGTG 54
Human_HSPA8_HSC71_NP_006588     ------ATGTCCAAGGGACCTGCAGTTGGTATTGATCTTGGCACCACCTACTCTTGTGTG 54
Rattus_HSC71_NM_024351          ------ATGTCTAAGGGACCTGCAGTTGGCATTGATCTTGGCACCACCTACTCCTGTGTG 54
Pelodiscus_HSC70_HQ219723       ------ATGTCTAAGGGACCAGCAGTTGGAATTGATCTTGGCACCACATACTCCTGTGTT 54
Bos_HSP70_U09861                ------ATGGCGAAAAACATGGCTATCGGCATCGACCTGGGCACCACCTACTCCTGCGTA 54
Human_HSPA1A_NM_005345          ------ATGGCCAAAGCCGCGGCGATCGGCATCGACCTGGGCACCACCTACTCCTGCGTG 54
Rattus_HSP70_1A_NM_031971       ------ATGGCCAAGAAAACAGCGATCGGCATCGACCTGGGCACCACCTACTCGTGCGTG 54
X_maculatus_HSP70-1_AB062113    ATGTCATCAGCTAAGGGAATATCCATTGGCATCGATCTGGGCACCACCTACTCTTGTGTC 60
X_maculatus_HSP70-2_AB062114    ATGTGTGCAGCTAAAAACGTGGCCATCGGCATCGACCTGGGCACCACCTACTCCTGCGTG 60
Human_HSPA1L_NM_005527          ATGGCTACTGCCAAGGGAATCGCCATAGGCATCGACCTGGGCACCACCTACTCCTGTGTG 60
Human_HSPA6_NP_002146           ATGCAGGCCCCACGGGAGCTCGCGGTGGGCATCGACCTGGGCACCACCTACTCGTGCGTG 60
Alligator_HSP70_AB306279        ---ATGTCGGGCAAAGGGCCTGCGATCGGCATCGACTTGGGCACCACGTACTCCTGCGTG 57
                                                          *  * ** ** **  * ** ** ** ** ** ** **

Drosophila_Bb_AF295957          GGTGTCTACCAGCATGGCAAGGTTGAGATTATCGCCAATGACCAGGGCAACCGCACCACG 105
Drosophila_Aa_AF295933          GGTGTCTACCAGCATGGCAAGGTGGAGATTATCGCCAACGACCAGGGCAACCGCACCACG 105
Spodoptera_FJ754276             GGCGTGTGGCAGCACGGCAACGTGGAGATCATCGCCAACGACCAGGGCAACCGCACCACA 105
Bombyx_AB035326                 GGAGTATGGCAGCACGGGAACGTGGAGATCATCGCGAACGACCAGGGCAACCGTACCACA 105
Paralvinella_EF580992           GGTGTCTTCCAACATGGAAAAGTGGAGATTATTGCCAACGACCAAGGAAATAGGACGACA 120
B_plicatilis_HSP70-1_AB775784   GGCGTATTCCAGCACGGCAAAGTGGAAATAATCGCCAACGACCAAGGCAACCGCACAACC 114
B_plicatilis_HSP70-2_AB775785   GGCGTATTCCAGCACGGCAAAGTGGAAATAATCGCCAACGACCAAGGCAACCGCACAACC 114
A_brightwelli_HSP70_KX119429    GGCGTGTTCCAACATGGTAAAGTTGAGATCATTGCCAACGACCAAGGTAATAGAACAACG 111
Wuchereria_AF167352             GGTGTGTTCATGCACGGCAAAGTGGAAATCATTGCTAATGATCAAGGTAATCGTACAACA 111
Setaria_AF079360                GGTGTTTTCATGCACGGTAAAGTGGAAATCATTGCTAACGATCAAGGTAATCGAACAACA 111
C_elegans_HSP70A_M18540         GGAGTTTTCATGCACGGAAAGGTAGAAATCATTGCCAACGATCAAGGAAACCGTACAACT 114
C_elegans_HSP-1_NM_070667       GGAGTTTTCATGCACGGAAAGGTAGAAATCATTGCCAACGATCAAGGAAACCGTACAACT 114
M_nipponense_KC460343           GGTGTGTTCCAGCATGGAAAGGTAGAAATCATTGCCAATGATCAGGGCAACCGAACCACT 114
M_nipponense_DQ660140           GGTGTGTTCCAGCATGGAAAGGTAGAAATCATTGCCAATGATCAGGGCAACCGAACCACT 114
Mytilus_HSP70_AY861684          GGAGTATTCCAGCATGGCAAAGTTGAAATCATTGCCAATGATCAAGGAAACAGAACAACC 117
Perinereis_KU255783             GGGGTTTTCCAGCACGGGAAAGTGGAAATCATTGCAAACGACCAGGGTAACAGGACCACG 111
Paralvinella_HSP70-2_EF580993   GGTGTATTCCAGCATGGCAAGGTGGAAATCGTAGCTAATGATCAGGGAAACCGAACGACG 114
Haliotis_FJ812176               GGGGTATTTCAACACGGAAAGGTAGAAATCATTGCCAATGACCAAGGAAACAGAACAACA 114
Oncorhynchus_HSC71_AAB21658     GGTGTGTTCCAGCATGGCAAGGTTGAAATCATTGCCAACGACCAAGGCAACAGGACCACT 114
X_maculatus_HSC70_AB062115      GGAGTGTTTCAGCACGGGAAAGTTGAGATCATTGCTAATGACCAGGGGAACAGGACCACG 114
Human_HSPA8_HSC71_NP_006588     GGTGTTTTCCAGCACGGAAAAGTCGAGATAATTGCCAATGATCAGGGAAACCGAACCACT 114
Rattus_HSC71_NM_024351          GGTGTCTTCCAGCATGGAAAGGTGGAAATAATTGCCAATGACCAGGGTAACCGCACCACG 114
Pelodiscus_HSC70_HQ219723       GGGGTCTTCCAACATGGCAAGGTGGAGATCATTGCCAATGATCAGGGCAACAGGACCACG 114
Bos_HSP70_U09861                GGGGTGTTCCAGCACGGCAAGGTGGAGATCATCGCCAACGACCAGGGCAACCGCACCACC 114
Human_HSPA1A_NM_005345          GGGGTGTTCCAACACGGCAAGGTGGAGATCATCGCCAACGACCAGGGCAACCGCACCACC 114
Rattus_HSP70_1A_NM_031971       GGCGTGTTCCAGCACGGCAAGGTGGAGATCATCGCCAACGACCAGGGCAACCGCACGACC 114
X_maculatus_HSP70-1_AB062113    GGGGTCTTCCAGCATGGAAAAGTGGAAATTATCGCCAATGATCAAGGCAACAGGACAACT 120
X_maculatus_HSP70-2_AB062114    GGGGTTTTCCAGCACGGAAAAGTGGAAATCATCGCCAACGACCAGGGCAACAGGACCACC 120
Human_HSPA1L_NM_005527          GGGGTGTTCCAGCACGGCAAGGTGGAGATCATCGCCAACGACCAGGGCAACCGCACCACC 120
Human_HSPA6_NP_002146           GGCGTGTTTCAGCAGGGCCGCGTGGAGATCCTGGCCAACGACCAGGGCAACCGCACCACG 120
Alligator_HSP70_AB306279        GGCGTCTTCCAGCATGGCAAGGTGGAGATCATTGCCAACGACCAGGGCAACCGCACCACG 117
                                ** ** *     ** **    ** ** **  * ** ** ** ** ** **  * ** **

Drosophila_Bb_AF295957          CCGTCCTACGTGGCTTTCACAGACTCGGAACGCCTCATTGGTGATCCGGCCAAGAACCAG 165
Drosophila_Aa_AF295933          CCGTCCTACGTGGCTTTCACAGATTCGGAACGCCTCATCGGCGATCCGGCTAAGAACCAG 165
Spodoptera_FJ754276             CCATCCTATGTGGCGTTCACGGACACGGAGCGCCTCATCGGAGACGCAGCCAAGAACCAG 165
Bombyx_AB035326                 CCATCGTACGTCGCGTTCACGGACACGGAGCGTCTCATCGGCGACGCAGCCAAGAACCAG 165
Paralvinella_EF580992           CCAAGTTATGTGGCATTTACAGATACCGAGAGACTGATAGGTGATGCGGCAAAGAACCAA 180
B_plicatilis_HSP70-1_AB775784   CCCTCCTACGTAGCGTTCACCGACACCGAGCGCTTGATCGGCGACGCGGCCAAAAACCAA 174
B_plicatilis_HSP70-2_AB775785   CCCTCCTACGTAGCGTTCACCGACACCGAGCGCTTGATCGGCGACGCGGCCAAAAACCAA 174
A_brightwelli_HSP70_KX119429    CCGAGTTACGTGGCGTTCACTGATACTGAACGTTTGATCGGAGACGCGGCCAAGAACCAA 171
Wuchereria_AF167352             CCATCGTATGTGGCATTTACCGATACTGAACGTCTTATTGGCGATGCAGCCAAAAATCAG 171
Setaria_AF079360                CCATCATATGTGGCATTTACCGATACTGAACGTCTTATTGGTGATGCAGCCAAAAATCAG 171
C_elegans_HSP70A_M18540         CCATCATATGTGGCTTTCACCGACACCGAGCGTCTCATCGGAGATGCTGCCAAGAATCAA 174
C_elegans_HSP-1_NM_070667       CCATCATATGTGGCTTTCACCGACACCGAGCGTCTCATCGGAGATGCTGCCAAGAATCAA 174
M_nipponense_KC460343           CCATCATATGTTGCTTTCACCGATACAGAGCGTCTTATTGGTGATGCTGCCAAGAACCAA 174
```

```
M_nipponense_DQ660140              CCATCATATGTTGCTTTCACCGACACAGAGCGTCTTATTGGTGATGCTGCCAAGAACCAA 174
Mytilus_HSP70_AY861684             CCAAGCTATGTCGCCTTCACAGACACCGAAAGATTAATAGGTGATGCTGCCAAGAACCAA 177
Perinereis_KU255783                CCCAGTTATGTGGCATTCACAGACTCCGAGCGTCTCATTGGAGATGCCGCAAAGAACCAA 171
Paralvinella_HSP70-2_EF580993      CCAAGTTATGTGGCGTTTACGGATTCCGAACGTATTATTGGAGATGGAGCCAAGAACCAA 174
Haliotis_FJ812176                  CCCAGCTATGTTGCCTTCACCGACACAGAGCGTCTCATTGGTGACGCTGCAAAGAACCAG 174
Oncorhynchus_HSC71_AAB21658        CCAAGCTACGTTGCCTTCACTGACTCTGAGAGGCTCATCGGTGATGCTGCCAAGAATCAG 174
X_maculatus_HSC70_AB062115         CCCAGTTATGTGGCCTTTACTGACACAGAGAGGCTGATTGGAGATGCAGCCAAGAACCAG 174
Human_HSPA8_HSC71_NP_006588        CCAAGCTATGTCGCCTTTACGGACACTGAACGGTTGATCGGTGATGCCGCAAAGAATCAA 174
Rattus_HSC71_NM_024351             CCGAGCTATGTTGCTTTCACCGACACAGAACGATTAATTGGGGATGCGGCCAAGAATCAG 174
Pelodiscus_HSC70_HQ219723          CCAAGTTACGTTGCCTTCACAGATACAGAGAGGTTGATCGGTGATGCTGCAAAGAACCAA 174
Bos_HSP70_U09861                   CCCAGCTACGTGGCCTTCACCGATACCGAGCGGCTCATCGGCGATGCGGCCAAGAACCAG 174
Human_HSPA1A_NM_005345             CCCAGCTACGTGGCCTTCACGGACACCGAGCGGCTCATCGGGGATGCGGCCAAGAACCAG 174
Rattus_HSP70_1A_NM_031971          CCCAGCTACGTGGCCTTCACCGACACCGAGCGGCTCATCGGGGACGCCGCCAAGAACCAG 174
X_maculatus_HSP70-1_AB062113       CCCAGCTACGTGGCCTTTACCGACACAGAGAGGCTCATCGGAGATGCTGCCAAGAACCAA 180
X_maculatus_HSP70-2_AB062114       CCCAGCTACGTGGCCTTCACCGACACCGAGAGGCTGATCGGGGACGCGGCCAAGAACCAG 180
Human_HSPA1L_NM_005527             CCCAGCTACGTGGCCTTCACAGACACCGAGCGGCTCATTGGGGATGCGGCCAAGAACCAG 180
Human_HSPA6_NP_002146              CCCAGCTACGTGGCCTTCACCGACACCGAGCGGCTGGTCGGGGACGCGGCCAAGAGCCAG 180
Alligator_HSP70_AB306279           CCCAGCTACGTGGCCTTCACGGACACGGAGCGCCTCATCGGCGACGCGGCCAAGAACCAG 177
                                   **    ** ** ** ** ** **  * **  *  *  * ** **    ** ** *  **

Drosophila_Bb_AF295957             GTGGCCATGAACCCCAGAAACACAGTGTTTGACGCCAAGCGACTCATCGGCCGAAAATAC 225
Drosophila_Aa_AF295933             GTGGCCATGAACCCCAGAAACACAGTGTTTGACGCCAAGCGACTCATCGGCCGAAAATAC 225
Spodoptera_FJ754276                GTCGCCCTCAACCCCAACAACACTGTGTTCGACGCCAAGCGACTGATCGGAAGGAAATTC 225
Bombyx_AB035326                    GTCGCCTTGAACCCTAACAACACCGTGTTCGACGCGAAGGGGCTGATCGGGAGGAAATTC 225
Paralvinella_EF580992              GTGGCGCTGAATCCGAGCAACACGGTATTTGATGCGAAGAGGCTGATTGGTCGAAGATTT 240
B_plicatilis_HSP70-1_AB775784      GTGGCCATGAACCCCAATAACACGGTGTTCGACGCCAAGCGTCTCATCGGCCGCAAGTTC 234
B_plicatilis_HSP70-2_AB775785      GTGGCCATGAACCCCAATAACACGGTGTTCGACGCCAAGCGTCTCATCGGCCGCAAGTTC 234
A_brightwelli_HSP70_KX119429       GTGGCCATGAATCCGACGAACACAGTGTTCGACGCGAAACGTCTCATCGGACGCAGATTC 231
Wuchereria_AF167352                GTTGCAATGAATCCGCACAACACTGTCTTCGACGCTAAACGACTGATTGGTCGTAAGTTT 231
Setaria_AF079360                   GTTGCGATGAATCCGCACAATACTGTTTTCGATGCGAAACGATTGATCGGCCGTAAATTT 231
C_elegans_HSP70A_M18540            GTTGCCATGAACCCACATAACACTGTTTTCGATGCCAAACGTCTTATTGGACGCAAGTTC 234
C_elegans_HSP-1_NM_070667          GTTGCCATGAACCCACATAACACTGTTTTCGATGCCAAACGTCTTATTGGACGCAAGTTC 234
M_nipponense_KC460343              GTTGCTATGAATCCCAACAACACTGTATTTGATGCCAAGAGGCTCATTGGTCGTAAGTTT 234
M_nipponense_DQ660140              GTTGCTATGAATCCCAACAACACTGTATTTGATGCCAAGAGGCTCATTGGTCGTAAGTTT 234
Mytilus_HSP70_AY861684             GTGGCAATGAACCCAGTCAACACGGTTTTTGATGCCAAGAGATTAATTGGTAGAAAGTTT 237
Perinereis_KU255783                GTGGCCATGAACCCCGAGAACACAGTCTTCGACGCCAAACGTTTGATCGGACGCAAGTTT 231
Paralvinella_HSP70-2_EF580993      GTGGCCATGAACCCCAGTAACACGGTGTTCGATGCGAAACGTCTCATTGGTCGTAGGCTC 234
Haliotis_FJ812176                  GTTGCCATGAACCCAGAGAACACTATCTTCGATGCCAAACGTCTGATTGGTAGAAGATTT 234
Oncorhynchus_HSC71_AAB21658        GTTGCCATGAACCCCTGCAACACAGTATTCGATGCTAAGAGACTGATTGGCCGCAGGTTT 234
X_maculatus_HSC70_AB062115         GTGGCCCTCAACCCCAACAACACCGTTTTTGATGCAAAGCGGCTTATTGGGCGTCGTTTT 234
Human_HSPA8_HSC71_NP_006588        GTTGCAATGAACCCCACCAACACAGTTTTTGATGCCAAACGTCTGATTGGACGCAGATTT 234
Rattus_HSC71_NM_024351             GTTGCAATGAACCCCACCAACACAGTTTTTGATGCCAAACGTCTGATCGGACGTAGGTTC 234
Pelodiscus_HSC70_HQ219723          GTTGCAATGAATCCTACCAACACGGTTTTTGATGCAAAGCGGCTGATCGGTCGTAGATTT 234
Bos_HSP70_U09861                   GTGGCGCTGAACCCGCAGAACACGGTGTTCGACGCGAAGCGGCTGATCGGCCGCAAGTTC 234
Human_HSPA1A_NM_005345             GTGGCGCTGAACCCGCAGAACACCGTGTTTGACGCGAAGCGGCTGATTGGCCGCAAGTTC 234
Rattus_HSP70_1A_NM_031971          GTGGCGCTGAACCCGCAGAACACCGTGTTCGACGCGAAGCGGCTGATCGGCCGCAAGTTC 234
X_maculatus_HSP70-1_AB062113       GTTGCCATGAACCCCACCAACACGATCTTTGATGCCAAACGGCTCATTGGAAGAAGGTTT 240
X_maculatus_HSP70-2_AB062114       GTGGCCCTGAACCCCAGCAACACGGTGTTTGACGCCAAGAGGCTGATCGGCAGAAAGTTT 240
Human_HSPA1L_NM_005527             GTAGCAATGAATCCCCAGAACACTGTTTTTGATGCTAAACGTCTGATCGGCAGGAAATTT 240
Human_HSPA6_NP_002146              GCGGCCCTGAACCCCCACAACACCGTGTTCGATGCCAAGCGGCTGATCGGGCGCAAGTTC 240
Alligator_HSP70_AB306279           GTGGCCATGAACCCCACCAACACCATCTTCGACGCCAAGCGCCTCATCGGCCGCAAGTAC 237
                                   *  **  * ** **    ** **  * ** ** ** **  *  * ** **  *

Drosophila_Bb_AF295957             GACGATCCCAAAATCGCAGAGGACATGAAGCACTGGCCTTTCAAAGTTGTAAGCGACG-- 283
Drosophila_Aa_AF295933             GACGACCCCAAGATCGCAGAGGACATGAAGCACTGGCCTTTCAAGGTGGTAAGCGACG-- 283
Spodoptera_FJ754276                GATGACCCCAAGATCCAGGCAGACATGAAGCACTGGCCCTTCAGGGTGGTCAGCGACT-- 283
Bombyx_AB035326                    GACGACCCCAAGATTCAGCAGGACATGAAGCACTGGCCCTTCAAAGTAATCAACGACT-- 283
Paralvinella_EF580992              GATGACGACAATGTCCAGAAGGACATCAAACATTGGCCTTTTAAAGTACTTAACGACG-- 298
B_plicatilis_HSP70-1_AB775784      GACGACCTGACCGTCCAGGCCGACATGAAGCACTGGCCCTTCACGGTGATCAGCGACA-- 292
B_plicatilis_HSP70-2_AB775785      GACGACCCGACCGTCCAGGCCGACATGAAGCACTGGCCCTTCACGGTGATCAGCGACA-- 292
A_brightwelli_HSP70_KX119429       GACGACTCCACTGTCCAGTCCGACATGAAACACTGGCCGTTCACTGTCGTCAATGAAG-- 289
Wuchereria_AF167352                GATGATGGATCAGTGCAATCGGATATGAAACATTGGCCATTTAAAGTAGTGAATGCTGGT 291
Setaria_AF079360                   GATGATGGATCAGTACAGTCAGACATGAAACATTGGCCGTTTAAAGTTATGAATGCTGGT 291
C_elegans_HSP70A_M18540            GACGATCCAGCAGTTCAGTCTGACATGAAGCATTGGCCATTCAAGGTCATCTCTGCCGAA 294
C_elegans_HSP-1_NM_070667          GACGATCCAGCAGTTCAGTCTGACATGAAGCATTGGCCATTCAAGGTCATCTCTGCCGAA 294
M_nipponense_KC460343              GAAGATCATGTAGTGCAGTCCGACATGAAACATTGGCCCTTTACAGTCATTAATGACA-- 292
M_nipponense_DQ660140              GAAGATCATGTAGTGCAGTCCGACATGAAACATTGGCCCTTTACAGTCATTAATGACA-- 292
Mytilus_HSP70_AY861684             GATGACGCTACAGTACAATCAGACATGAAGCATTGGCCTTTCACTGTTGTCAATGATG-- 295
Perinereis_KU255783                GACGACTCAGCCGTTCAGTCAGACAAAAAGCATTGGCCATTCGATGTGGTCAGTGAGG-- 289
Paralvinella_HSP70-2_EF580993      GATGATTCGTCCGTCCAATCAGATATGAAGTTCTGGCCCTTCAAGGTCATCAGTGAGA-- 292
Haliotis_FJ812176                  GATGAGACAAATGTTCAATCAGACATGAAGCACTGGCCATTCAATGTGTTGAGTGATG-- 292
Oncorhynchus_HSC71_AAB21658        GATGATGGAGTTGTTCAATCGGACATGAAGCATTGGCCCTTTGAAGTTATCAATGATT-- 292
X_maculatus_HSC70_AB062115         GACGATAGTATTGTCCAATCAGACATGAAACATTGGCCCTTCACCATCATCAATGATA-- 292
Human_HSPA8_HSC71_NP_006588        GATGATGCTGTTGTCCAGTCTGATATGAAACATTGGCCCTTTATGGTGGTGAATGATG-- 292
Rattus_HSC71_NM_024351             GATGATGCTGTTGTTCAGTCTGACATGAAGCACTGGCCCTTCATGGTGGTGAACGATG-- 292
Pelodiscus_HSC70_HQ219723          GATGATGCTGTTGTCCAGTCGGACATGAAACATTGGCCATTCACTGTGGTGAATGATG-- 292
Bos_HSP70_U09861                   GGAGACCCGGTGGTGCAGTCGGACATGAAGGAGTGGCCTTTCCGCGTCATCAACGACG-- 292
Human_HSPA1A_NM_005345             GGCGACCCGGTGGTGCAGTCGGACATGAAGCACTGGCCTTTCCAGGTGATCAACGACG-- 292
Rattus_HSP70_1A_NM_031971          GGCGACCCGGTGGTGCAGTCGGACATGAAGCACTGGCCCTTCCAGGTGGTGAACGACG-- 292
```

```
X_maculatus_HSP70-1_AB062113        GATGACCTGGTGGTCCAGTCTGACATGAAACTCTGGCCGTTCAAGGTGATCAACGACA-- 298
X_maculatus_HSP70-2_AB062114        GAAGAGCCAGTGGTGCAGGCCGACATGAAGCACTGGCCCTTCGAGGTGCTTTCAGACG-- 298
Human_HSPA1L_NM_005527              AATGATCCTGTTGTACAAGCAGATATGAAACTTTGGCCTTTTCAAGTGATTAATGAAG-- 298
Human_HSPA6_NP_002146               GCGGACACCACGGTGCAGTCGGACATGAAGCACTGGCCCTTCCGGGTGGTGAGCGAGG-- 298
Alligator_HSP70_AB306279            GACGACCCCACGGTGCAGGCGGACATGAAGCACTGGCCCTTCCACGTAGTGTCCGAGG-- 295
                                       **        *       ** *  **    ***** **     *  *    *

Drosophila_Bb_AF295957              -GCGGAAAGCCCAAGATCGGGGTGGAGTATAAGGGTGAGTCCAAGAGATTTGCTCCCGAG 342
Drosophila_Aa_AF295933              -GCGGAAAGCCCAAGATCGGGGTGGAGTATAAGGGTGAGTCCAAGAGATTTGCCCCCGAG 342
Spodoptera_FJ754276                 -GTGGCAAACCGAAGATCCAAGTGGAGTTCAAGGGTGAAACGAAACGGTTCGCGCCCGAG 342
Bombyx_AB035326                     -GCGGCAAACCGAAAATACAGATCGAGTTCAAAGGTGAGACGAAACGATTTGCGCCAGAA 342
Paralvinella_EF580992               -GTGGAAAGCCAAAGATTGACGTAGAATACAAAAGTGAGAGGAAACGTTTTACTCCAGAA 357
B_plicatilis_HSP70-1_AB775784       -GCGGCCGGCCCAAAATCCAGGTCGAGTTCAAAGGCGAGACCAAGAGCTTCTACCCGGAA 351
B_plicatilis_HSP70-2_AB775785       -GCGGCCGGCCCAAAATCCAGGTCGAGTTCAAAGGCGAGACCAAGAGCTTCTACCCGGAA 351
A_brightwelli_HSP70_KX119429        -GCGGAAAACCAAAAATCCAAGTCGAGTTCAAAGGCGAGAAAAAGACCTTCTTCCCTGAA 348
Wuchereria_AF167352                 GGTGGTAAACCTAAAGTGCAGGTTGAATACAAGGGTGAGACAAAGACCTTTACTCCAGAA 351
Setaria_AF079360                    GGAGGCAAGCCAAAAGTTCAAGTGGAGTATAAGGGGGAGACGAAAACTTTCACGCCCGGA 351
C_elegans_HSP70A_M18540             GGAGCTAAGCCAAAGGTCCAAGTTGAGTACAAAGGAGAGAACAAGATCTTCACTCCAGAA 354
C_elegans_HSP-1_NM_070667           GGAGCTAAGCCAAAGGTCCAAGTTGAGTACAAAGGAGAGAACAAGATCTTCACTCCAGAA 354
M_nipponense_KC460343               -GCACGAAGCCCAAAATTCAAGTTGATTACAAAGGAGAGACCAAAACCTTTTTCCCAGAA 351
M_nipponense_DQ660140               -GCACGAAGCCCAAAATTCAAGTTGATTACAAAGGAGAGACCAAAACCTTTTTCCCAGAA 351
Mytilus_HSP70_AY861684              -CCTCCAAACCTAAAGTTACGGTAGACTACAAAGGAGAAACAAAAACATTTTTCCCTGAG 354
Perinereis_KU255783                 -GTGGCAAACCAAAGATCTCTGTTGATTACAAGGGGGAGAAGAAATCATTTTACCCAGAA 348
Paralvinella_HSP70-2_EF580993       -ATGGCAAGCCAAAAATCCAAGTGGAGTACAAGGGGGAGTTGAAGACCTTCTATCCCGAA 351
Haliotis_FJ812176                   -GAGGCAAACCCAAGATCCAAGTAAATTACAAAGATGAACCAAAAACTTTCTACCCTGAA 351
Oncorhynchus_HSC71_AAB21658         -CTACTCGGCCTAAGCTCCAAGTTGAATACAAAGGAGAGACTAAGTCCTTCTACCCAGAA 351
X_maculatus_HSC70_AB062115          -GCTCGAGGCCCAAAGTGAAAGTGGAGTACAAAGGGGAGACGAAGACCTTCTACCCAGAG 351
Human_HSPA8_HSC71_NP_006588         -CTGGCAGGCCCAAGGTCCAAGTAGAATACAAGGGAGAGACCAAAAGCTTCTATCCAGAG 351
Rattus_HSC71_NM_024351              -CAGGCAGGCCCAAGGTCCAAGTCGAATACAAAGGGGAGACAAAAAGTTTCTATCCTGAG 351
Pelodiscus_HSC70_HQ219723           -CTGGCAGGCCGAAAGTCCAGGTTGAGTACAAAGGGGAGACCAAGAGCTTCTATCCAGAG 351
Bos_HSP70_U09861                    -GAGACAAGCCTAAGGTGCAGGTGAGCTACAAAGGGGAGACCAAGGCGTTCTACCCGGAG 351
Human_HSPA1A_NM_005345              -GAGACAAGCCCAAGGTGCAGGTGAGCTACAAGGGGGAGACCAAGGCATTCTACCCCGAG 351
Rattus_HSP70_1A_NM_031971           -GCGACAAGCCCAAGGTGCAGGTGAACTACAAGGGCGAGAACCGGTCGTTCTACCCGGAG 351
X_maculatus_HSP70-1_AB062113        -ACGGAAAGCCCAAAGTCCAGGTGGAATATAAAGGAGAAATTAAGACATTCTGTCCTGAA 357
X_maculatus_HSP70-2_AB062114        -GAGGCAGGCCCAAAATTCAGGTGGAGTACAAAGGGGAGAACAAAGCCTTCTTCCCTGAG 357
Human_HSPA1L_NM_005527              -GAGGCAAGCCCAAAGTCCTTGTGTCCTACAAAGGGGAGAATAAAGCTTTCTACCCTGAG 357
Human_HSPA6_NP_002146               -GCGGCAAGCCCAAGGTGCGCGTATGCTACCGCGGGGAGGACAAGACGTTCTACCCCGAG 357
Alligator_HSP70_AB306279            -GCGGCAAGCCCAAGGTGCAGGTGGAGTATAAGGGCGAGGCCAAGACCTTCTTCCCCGAA 354
                                             ** **  *     *    *        **          **    ** *

Drosophila_Bb_AF295957              GAGATCAGTTCGATGGTGCTGACCAAGATGAAGGAGACGGCGGAGGCGTATCTGGGCGAG 402
Drosophila_Aa_AF295933              GAGATCAGCTCGATGGTGCTGACCAAGATGAAGGAGACGGCGGAGGCATATCTGGGCGAG 402
Spodoptera_FJ754276                 GAGATCAGCAGCATGGTGCTGACGAAGATGAAGGAGACGGCGGAAGCTTACCTCGGAACG 402
Bombyx_AB035326                     GAAATTAGCAGCATGGTGCTGACAAAAATGAAGGAGACGGCGGAAGCCTATCTGGGAAGT 402
Paralvinella_EF580992               GAAATCAGCTCGATGATACTTACCAAGATGAAAGAGACGGCCGAGGCCTATCTCGGTAAT 417
B_plicatilis_HSP70-1_AB775784       GAAGTCTCATCCATGGTACTGACCAAAATGAAGGAGACGGCCGAGGCCTACCTGGGCAAA 411
B_plicatilis_HSP70-2_AB775785       GAAGTCTCATCCATGGTACTGACCAAAATGAAGGAGACGGCCGAGGCCTACCTGGGCAAA 411
A_brightwelli_HSP70_KX119429        GAAATCTCATCAATGGTCTTAATTAAAATGAAAGAGATCGCCGAGGCTTATTTGGGAAAG 408
Wuchereria_AF167352                 GAGATTTCGTCGATGGTTTTGGTTAAAATGAAGGAGACGGCTGAAGCTTTTCTGGGTCAT 411
Setaria_AF079360                    GAAATTTCTTCAATGGTTTTAGTTAAAATGAAAGAAACAGCTGAGGCATTTCTTGGTCAC 411
C_elegans_HSP70A_M18540             GAGATCTCCTCAATGGTTCTGCTGAAGATGAAGAAGACTGCCGAGGCTTTCCTTGAACCG 414
C_elegans_HSP-1_NM_070667           GAGATCTCCTCAATGGTTTTGCTGAAGATGAAGGAGACTGCCGAGGCTTTCCTTGGAACC 414
M_nipponense_KC460343               GAGATCTCCTCAATGGTGCTTATTAAGATGAAAGAAACTGCAGAAGCCTTTTTGGGTGGT 411
M_nipponense_DQ660140               GAGATCTCCTCAATGGTGCTTATTAAGATGAAAGAAACTGCAGAAGCCTTTTTGGGTGGT 411
Mytilus_HSP70_AY861684              GAAATTTCATCAATGGTGCTGGTAAAAATGAAAGAAACTGCAGAAGCATATCTAGGAAAG 414
Perinereis_KU255783                 GAGATCTCCTCCATGGTATTGGTTAAGATGAAGGAAACAGCCGAGGCTTACATCGGCAAG 408
Paralvinella_HSP70-2_EF580993       GAAATTTCCTCCATGGTACTTTTGAAAATGAAAGAAACTGCTGAAGCCTATCTTGGGAAG 411
Haliotis_FJ812176                   GAGATTTCCTCCATGGTGCTAACAAAGATGAAGGAAACTGCAGAACAGTATTTGGGAAAG 411
Oncorhynchus_HSC71_AAB21658         GAAATTTCATCTATGGTTCTGGTCAAGATGAAGGAGATTGCTGAGGCCTACCTTGGGAAA 411
X_maculatus_HSC70_AB062115          GAGATCTCCTCCATGGTGCTGCTGAAGATGAAGGAGATTGCTGAGGCATACCTTGGCAAG 411
Human_HSPA8_HSC71_NP_006588         GAGGTGTCTTCTATGGTTCTGACAAAGATGAAGGAAATTGCAGAAGCCTACCTTGGGAAG 411
Rattus_HSC71_NM_024351              GAAGTGTCTTCAATGGTTCTGACAAAAATGAAGGAAATTGCAGAAGCTTACCTTGGAAAG 411
Pelodiscus_HSC70_HQ219723           GAAATATCTTCCATGGTTTTGACAAAGATGAAAGAGATAGCAGAAGCATATCTTGGGAAG 411
Bos_HSP70_U09861                    GAGATCTCGTCGATGGTGCTGACCAAGATGAAGGAGATCGCCGAGGCGTACCTGGGCCAC 411
Human_HSPA1A_NM_005345              GAGATCTCGTCCATGGTGCTGACCAAGATGAAGGAGATCGCCGAGGCGTACCTGGGCTAC 411
Rattus_HSP70_1A_NM_031971           GAGATCTCGTCCATGGTGCTGACCAAGATGAAGGAGATCGCCGAGGCGTACCTGGGCCAC 411
X_maculatus_HSP70-1_AB062113        GAGATTTCCTCGATGGTCCTGGTTAAAATGAGAGAAATAGCTGAGGCCTTTCTGGGACAG 417
X_maculatus_HSP70-2_AB062114        GAGATCTCCTCCATGGTCCTGGTGAAGATGAAGGAAATCGCCGAGGCCTACCTGGGCCAC 417
Human_HSPA1L_NM_005527              GAAATCTCTTCGATGGTATTGACTAAGTTGAAGGAGACTGCTGAGGCCTTTTTGGGCCAC 417
Human_HSPA6_NP_002146               GAGATCTCGTCCATGGTGCTGAGCAAGATGAAGGAGACGGCCGAGGCGTACCTGGGCCAG 417
Alligator_HSP70_AB306279            GAGATCAGCTCCATGGTGCTGACTAAGATGAAGGAGATCGCCGAAGCCTACCTGGGCCGC 414
                                    **  *       *** *  *    **  ***   * *  ** **    *   * *

Drosophila_Bb_AF295957              AGCATCACGGATGCAGTCATCACAGTTCCAGCTTACTTCAACGACTCTCAGCGCCAGGCT 462
Drosophila_Aa_AF295933              AGCATCACAGACGCAGTCATCACAGTTCCAGCCTACTTCAACGACTCCCAGCGCCAGGCT 462
Spodoptera_FJ754276                 ACAGTACGCGACGCAGTGATCACAGTGCCGGCGTACTTCAACGACTCTCAGCGCCAGGCC 462
Bombyx_AB035326                     ACAGTGCGGGATGCGGTAGTCACAGTTCCGGCATACTTCAACGACTCCCAGCGTCAGGCC 462
Paralvinella_EF580992               AAAGTGTTAGATGCCGTCATAACTGTCCCCGCTTACTTCAACGACTCTCAGAGACAAGCT 477
B_plicatilis_HSP70-1_AB775784       AAGGTCACGGACGCAGTGGTGACCGTGCCCGCCTACTTCAACGACTCGCAGCGCCAAGCG 471
```

```
B_plicatilis_HSP70-2_AB775785      AAGGTCACGGACGCAGTGGTGACCGTGCCCGCCTACTTCAACGACTCGCAGCGCCAAGCG 471
A_brightwelli_HSP70_KX119429       AAAGTAAACGACGCCGTGATCACAGTCCCGGCCTATTTCAACGACTCTCAAAGACAGGCC 468
Wuchereria_AF167352                GCGGTCAAGGATGCTGTTATTACAGTCCCCGCATATTTTAATGACTCACAGCGACAAGCC 471
Setaria_AF079360                   GCAGTCAAGGATGCTGTGATCACAGTTCCAGCTTATTTCAATGACTCACAGCGACAGGCA 471
C_elegans_HSP70A_M18540            ACCGTCAAGGATGCCGTTGTCACTGTCCCGACTTACTTCAACGACTCGCAGCGTCAAGCC 474
C_elegans_HSP-1_NM_070667          ACCGTCAAGGATGCCGTTGTCACTGTCCCAGCTTACTTCAACGACTCGCAGCGTCAAGCC 474
M_nipponense_KC460343              ACTGTAAAGGATGCTGTCGTCACAGTCCCTGCCTATTTCAATGACTCCCAGCGTCAGGCC 471
M_nipponense_DQ660140              ACTGTAAAGGATGCTGTCGTCACAGTCCCTGCCTATTTCAATGACTCCCAGCGTCAGGCC 471
Mytilus_HSP70_AY861684             TTAGTCAACAACAGTGTCATTACAGTCCCAGCTTATTTTAATGATTCACAAGGACAAGCA 474
Perinereis_KU255783                ACTGTTCTAAATGCCGTCGTAACAGTGCCTGCCTACTTCAACGATTCTCAGCGCCAAGCA 468
Paralvinella_HSP70-2_EF580993      AACGTACAGAGCGCTGTGATCACAGTACCAGCCTATTTCAATGACAGCCAGCGTCAGGCC 471
Haliotis_FJ812176                  ACTATAACAGACGCTGTTGTAACAGTCCCAGCTTACTTCAACGACTCTCAGCGACAGGCC 471
Oncorhynchus_HSC71_AAB21658        ACTGTCAACAATGCTGTTGTTACCGTACCTGCCTACTTCAATGACTCCCAGCGCCAGGCA 471
X_maculatus_HSC70_AB062115         ACTATAACAAATGCCGTGGTGACTGTACCTGCCTACTTCAATGACTCTCAGCGTACGGCC 471
Human_HSPA8_HSC71_NP_006588        ACTGTTACCAATGCTGTGGTCACAGTGCCAGCTTACTTTAATGACTCTCAGCGTCAGGCT 471
Rattus_HSC71_NM_024351             ACTGTTACCAATGCCGTGGTCACCGTGCCAGCTTACTTCAATGACTCTCAGCGACAGGCA 471
Pelodiscus_HSC70_HQ219723          ACTGTTACTAATGCAGTGGTCACGGTACCAGCCTACTTCAATGACTCCCAGCGCCAGGCC 471
Bos_HSP70_U09861                   CCGGTGACCAACGCGGTGATCACCGTGCCGGCCTACTTCAACGACTCGCAGCGGCAGGCC 471
Human_HSPA1A_NM_005345             CCGGTGACCAACGCGGTGATCACCGTGCCGGCCTACTTCAACGACTCGCAGCGCCAGGCC 471
Rattus_HSP70_1A_NM_031971          CCGGTGACCAACGCGGTGATCACCGTGCCCGCCTACTTCAACGACTCGCAGCGGCAGGCC 471
X_maculatus_HSP70-1_AB062113       AGGGTGTCGAATGCGGTCATCACAGTGCCAGCTTACTTTAATGATTCCCAAAGGCAAGCC 477
X_maculatus_HSP70-2_AB062114       AAGGTGTCCAACGCCGTGATCACGGTCCCCGCCTATTTCAACGACTCCCAGCGACAGGCC 477
Human_HSPA1L_NM_005527             CCTGTCACCAATGCAGTGATTACCGTGCCAGCCTATTTCAATGACTCTCAACGTCAGGCT 477
Human_HSPA6_NP_002146              CCCGTGAAGCACGCAGTGATCACCGTGCCCGCCTATTTCAATGACTCGCAGCGCCAGGCC 477
Alligator_HSP70_AB306279           AAGGTGCAGAACGCCGTCATCGCGGTGCCCGCCTACTTCAACGACTCGCAGCGCCAGGCC 474
                                       *          **  *  * ** **  * ** ** ** **    **  *    **

Drosophila_Bb_AF295957             ACCAAAGACGCCGGTCACATCGCCGGCCTGAATGTGCTCCGCATCATCAATGAGCCCACG 522
Drosophila_Aa_AF295933             ACCAAAGACGCCGGTCACATCGCCGGCCTGAATGTGCTCCGCATCATCAATGAGCCCACG 522
Spodoptera_FJ754276                ACCAAGGACGCGGGAGCCATCGCCGGGCTGAACGTGCTCAGGATCATCAACGAGCCCACA 522
Bombyx_AB035326                    ACCAAGGACGCCGGAGCCATCGCCGGCCTGAACGTGCTTCGCATCATCAACGAGCCCACA 522
Paralvinella_EF580992              ACAAAAGATGCTGGTCTCATCTCCGGATTGAACGTTCTCCGAGTGATTAACGAGCCAACA 537
B_plicatilis_HSP70-1_AB775784      ACCAAGGACGCGGGCGCCATCGCCGGCCTCAACGTGCTGCGCATCATCAACGAGCCCACT 531
B_plicatilis_HSP70-2_AB775785      ACCAAGGACGCGGGCGCCATCGCCGGCCTCAACGTGCTGCGCATCATCAACGAGCCCACT 531
A_brightwelli_HSP70_KX119429       ACAAAAGACGCCGGCGCCATTTCAGGTCTGAACGTGTTACGTATCATAAACGAGCCAACA 528
Wuchereria_AF167352                ACTAAAGATTCCGGTGCAATTGCTGGTCTAAATGTCTTGCGTATTATCAACGAACCAACA 531
Setaria_AF079360                   ACGAAAGATTCGGGTGCCATTGCGGGTTTGAACGTTCTGCGTATTATCAACGAACCAACC 531
C_elegans_HSP70A_M18540            ACCAAGGATGCCGGAGCCATCGCTGGACTCAACGTTCTCCGTATCATCAACGAGCCAACC 534
C_elegans_HSP-1_NM_070667          ACCAAGGATGCCGGAGCCATCGCTGGACTCAACGTTCTCCGTATCATCAACGAGCCAACC 534
M_nipponense_KC460343              ACCAAAGATGCTGGTACAATCTCTGGTCTCAATGTGCTCCGTATCATCAATGAGCCCACT 531
M_nipponense_DQ660140              ACCAAAGATGCTGGTACAATCTCTGGTCTCAATGTGCTCCGTATCATCAATGAGCCCACT 531
Mytilus_HSP70_AY861684             ACAAAGGATGCTGGTACCATCTCTGGAATGAATGTTCTACGTATTATCAATGAGCCAACT 534
Perinereis_KU255783                ACCAAAGATGCCGGAACTATCTCTGGCTTGAATGTATTACGTATCATCAATGAACCAACG 528
Paralvinella_HSP70-2_EF580993      ACCAAAGATGCTGGTACCATCTCTGGTATGAATGTGCTGCGTATCATCAATGAGCCCACA 531
Haliotis_FJ812176                  ACTAAAGATGCAGGGACAATCTCTGGTCTCAATGTTTTACGTATCATCAATGAGCCTACT 531
Oncorhynchus_HSC71_AAB21658        ACCAAAGATGCTGGTACCATCTCGGGGCTGAATGTGCTGCGTATCATCAATGAGCCAACT 531
X_maculatus_HSC70_AB062115         ACCAAAGACGCTGGGACCATTTCTGGGCTCAATGTTCTGCGTATCATTAACGAGCCAACG 531
Human_HSPA8_HSC71_NP_006588        ACCAAAGATGCTGGAACTATTGCTGGTCTCAATGTACTTAGAATTATTAATGAGCCAACT 531
Rattus_HSC71_NM_024351             ACAAAAGATGCTGGAACTATTGCTGGCCTCAACGTACTTCGAATTATCAATGAGCCAACT 531
Pelodiscus_HSC70_HQ219723          ACAAAAGATGCTGGAACCATTGCAGGTCTTAATGTGCTCAGGATCATCAATGAACCAACT 531
Bos_HSP70_U09861                   ACCAAGGACGCGGGGGTGATCGCGGGGCTGAACGTGCTGAGGATCATCAACGAGCCCACG 531
Human_HSPA1A_NM_005345             ACCAAGGATGCGGGTGTGATCGCGGGGCTCAACGTGCTGCGGATCATCAACGAGCCCACG 531
Rattus_HSP70_1A_NM_031971          ACCAAGGACGCGGGCGTGATCGCGGGTCTGAACGTGCTGCGGATCATCAACGAGCCCACG 531
X_maculatus_HSP70-1_AB062113       ACAAAGGATGCTGGAGTGATCTCCGGACTAAATGTTCTCCGCATCATAAATGAGCCGACA 537
X_maculatus_HSP70-2_AB062114       ACCAAAGACGCAGGCGTCATTGCGGGACTCAACGTCTTGAGGATCATCAACGAGCCCACG 537
Human_HSPA1L_NM_005527             ACTAAGGATGCAGGTGTGATTGCTGGACTTAATGTGCTAAGAATCATCAATGAGCCCACG 537
Human_HSPA6_NP_002146              ACCAAGGACGCGGGGGCCATCGCGGGGCTCAACGTGTTGCGGATCATCAATGAGCCCACG 537
Alligator_HSP70_AB306279           ACCAAGGACGCGGGCACCATCACGGGCCTCAACGTGATGCGCATCATCAACGAGCCCACG 534
                                   ** ** **  * **    **  * **  * ** **  *  *  * ** ** ** ** **

Drosophila_Bb_AF295957             GCGGCAGCATTGGCCTACGGACTGGACAAGAACCTCAA------GGGTGAGCGCAATGTG 576
Drosophila_Aa_AF295933             GCGGCAGCACTGGCCTACGGACTGGACAAGAACCTCAA------GGGTGAGCGCAATGTG 576
Spodoptera_FJ754276                GCCGCTGCGCTCGCCTACGGCCTCGACAAGAACCTCAA------GGGCGAGAGGAACGTC 576
Bombyx_AB035326                    GCCGCCGCGCTCGCGTACGGTCTCGACAAGAACCTCAA------GGGTGAGAGAAATGTT 576
Paralvinella_EF580992              GCAGCCGCTCTGGCTTATGGCTTGGATAAGAACCTATC------TGGAGAGAAGAACGTC 591
B_plicatilis_HSP70-1_AB775784      GCGGCCGCCATCGCCTACGGCCTTGACAAAAAGGGCGG------CGGCGAAAAAAACATC 585
B_plicatilis_HSP70-2_AB775785      GCGGCCGCCATCGCCTACGGCCTTGACAAAAAGGGCGG------CGGCGAAAAAAACATC 585
A_brightwelli_HSP70_KX119429       GCAGCTGCGATCGCTTACGGTCTTGATAAAAAAGTCGG------CGGTGAGAAAAACGTG 582
Wuchereria_AF167352                GCAGCTGCTATTGCTTATGGTTTGGACAAGAAGGGCCA------TGGTGAACGCAACGTT 585
Setaria_AF079360                   GCAGCAGCTATTGCATATGGGTTGGACAAGAAGGGCCA------TGGTGAGCGTAATGTC 585
C_elegans_HSP70A_M18540            GCTGCAGCTATCGCTTACGGACTTGACAAGAAGGGACA------CGGAGAACGCAACGTT 588
C_elegans_HSP-1_NM_070667          GCTGCAGCTATCGCTTACGGACTTGACAAGAAGGGACA------CGGAGAACGCAACGTT 588
M_nipponense_KC460343              GCTGCTGCCATCGCATATGGGCTGGACAAGAAAGTTGG------TGGAGAACGTAATGTT 585
M_nipponense_DQ660140              GCTGCTGCCATCGCATATGGGCTGGACAAGAAAGTTGG------TGGAGAACGTAATGTT 585
Mytilus_HSP70_AY861684             GCAGCTGCTATTGCTTATGGTTTGGATAAGAAAGTAGG------TGGAGAAAGAAATGTA 588
Perinereis_KU255783                GCCGCCGCCATCGCTTACGGTCTTGATAAGAAGGTGGG------AGGAGAGAGAAATGTC 582
Paralvinella_HSP70-2_EF580993      GCTGCCGCCATTGCTTACGGTTTGGACAAGAAGGTTGG------TGGAGAGCGTCATGTG 585
Haliotis_FJ812176                  GCCGCAGCTATTGCATACGGTCTTGACAAGAAGGTTGG------TGGTGAACGCAACGTT 585
Oncorhynchus_HSC71_AAB21658        GCTGCTGCCATTGCCTACGGCCTGGACAAGAAGGTCGG------TGCTGAAAGGAATGTC 585
```

```
X_maculatus_HSC70_AB062115          GCTGCTGCCATTGCTTACGGTTTGGACAAAAAGGTCGG------GTGCGAGAAAAACGTC 585
Human_HSPA8_HSC71_NP_006588         GCTGCTGCTATTGCTTACGGCTTAGACAAAAAGGTTGG------AGCAGAAAGAAACGTG 585
Rattus_HSC71_NM_024351              GCTGCTGCTATTGCCTATGGCTTAGATAAGAAGGTCGG------GGCTGAAAGGAATGTG 585
Pelodiscus_HSC70_HQ219723           GCTGCTGCTATTGCTTATGGCTTGGACAAGAAGGTTGG------AGCGGAAAGGAATGTC 585
Bos_HSP70_U09861                    GCCGCCGCCATCGCCTACGGCCTGGACAGGACGGGCAA------GGGGGAGCGCAACGTG 585
Human_HSPA1A_NM_005345              GCCGCCGCCATCGCCTACGGCCTGGACAGAACGGGCAA------GGGGGAGCGCAACGTG 585
Rattus_HSP70_1A_NM_031971           GCGGCCGCCATCGCCTACGGGCTGGACCGGACCGGCAA------GGGCGAGCGCAACGTG 585
X_maculatus_HSP70-1_AB062113        GCTGCAGCCATTGCCTACGGCCTTGATAAAGGCAAAAG------AGGAGAGCGCAATGTC 591
X_maculatus_HSP70-2_AB062114        GCGGCCGCCATCGCCTACGGTCTGGACAAAGGCAAGTC------GGGAGAGAGAAACGTC 591
Human_HSPA1L_NM_005527              GCTGCTGCCATTGCCTATGGTTTAGATAAAGGAGGTCA------AGGAGAACGACATGTC 591
Human_HSPA6_NP_002146               GCAGCTGCCATCGCCTATGGGCTGGACCGGCGGGGCGC------GGGAGAGCGCAACGTG 591
Alligator_HSP70_AB306279            GCGGCCGCCATCGCCTACGGGCTGGACAAGAAGGGCGCGCGCGCCGGCGAGAAGAACGTG 594
                                    ** ** **  * ** ** **  * **                      **     *  *

Drosophila_Bb_AF295957              CTTATCCTCGACTTGGGCGGCGGCACCTTCGATGTCTCCATCCTGACCATCGACGAGGGA 636
Drosophila_Aa_AF295933              CTTATCTTCGACTTGGGCGGCGGCACCTTCGATGTCTCCATCCTGACCATCGACGAGGGA 636
Spodoptera_FJ754276                 CTCATTTTCGACCTCGGCGGCGGAACGTTCGACGTGTCGATCCTGACCATCGACGAAGGT 636
Bombyx_AB035326                     CTTATCTTCGATCTGGGTGGAGGAACCTTCGACGTGTCCATCCTGACTATCGACGAGGGA 636
Paralvinella_EF580992               TTGATATTTGACCTCGGAGGAGGAACGTTCGATGTGTCGATTCTCTCGATTGCCGATGGT 651
B_plicatilis_HSP70-1_AB775784       CTGATCTTTGACCTGGGCGGCGGCACCTTCGACGTCTCCATCCTGACCATCGAAGAGGG- 644
B_plicatilis_HSP70-2_AB775785       CTGATCTTTGACCTGGGCGGCGGCACCTTCGACGTCTCCATCCTGACCATCGAAGAGGG- 644
A_brightwelli_HSP70_KX119429        TTAATCTTCGACTTGGGCGGCGGTACTTTCGATGTGTCGATCTTGACCATTGAAGAAGG- 641
Wuchereria_AF167352                 CTTATCTTTGATCTTGGTGGTGGTACATTTGATGTGTCTATCCTAACTATCGAGGATGG- 644
Setaria_AF079360                    CTTATATTTGACCTTGGTGGTGGCACATTTGATGTGTCTATCCTGACTATAGAGGATGG- 644
C_elegans_HSP70A_M18540             CTTATCTTCGATCTTGGAGGTGGTACCTTCGATGTCTCCATTCTTACCATTGAGGACGG- 647
C_elegans_HSP-1_NM_070667           CTTATCTTCGATCTTGGAGGTGGTACCTTCGATGTCTCCATTCTTACCATTGAGGACGG- 647
M_nipponense_KC460343               CTGATTTTTGATTTAGGTGGTGGCACTTTTGATGTATCTATTCTAACTATTGAAGATGA- 644
M_nipponense_DQ660140               CTGATTTTTGATTTAGGTGGTGGCACTTTTGATGTATCTATTTTAACTATTGAAGATGG- 644
Mytilus_HSP70_AY861684              CTCATCTTTGACTTGGGTGGTGGAACTTTTGATGTGTCAATCCTTACAATTAAGGATGG- 647
Perinereis_KU255783                 CTGATTTTTGATCTCGGTGGTGGAACCTTCGATGTGTCCATCTTGACCATTGAGGATGG- 641
Paralvinella_HSP70-2_EF580993       CTGATCTTCGATCTGGGTGGAGGCACCTTTGATGTGTCCGTTCTGACCATAGAGGATGG- 644
Haliotis_FJ812176                   CTCATCTTTGATCTTGGCGGTGGTACATTTGATGTGTCTATCTTGACAATCGAAGATGG- 644
Oncorhynchus_HSC71_AAB21658         CTTATCTTTGATCTGGGTGGCGGCACCTTTGACGTGTCCATCTTGACCATCGAGGATGG- 644
X_maculatus_HSC70_AB062115          CTCATCTTCGACCTGGGCGGCGGCACTTTCGACGTCTCCATCCTGACCATCGAGGACGG- 644
Human_HSPA8_HSC71_NP_006588         CTCATCTTTGACCTGGGAGGTGGCACTTTTGATGTGTCAATCCTCACTATTGAGGATGG- 644
Rattus_HSC71_NM_024351              CTCATTTTTGACTTGGGAGGTGGCACTTTTGATGTGTCAATCCTCACTATCGAGGATGG- 644
Pelodiscus_HSC70_HQ219723           CTTATCTTTGACCTTGGGGGTGGCACTTTCGATGTTTCAATCCTCACTATTGAGGATGG- 644
Bos_HSP70_U09861                    CTCATCTTTGATCTGGGAGGGGGCACGTTCGACGTGTCCATCCTGACGATCGACGACGG- 644
Human_HSPA1A_NM_005345              CTCATCTTTGACCTGGGCGGGGGCACCTTCGACGTGTCCATCCTGACGATCGACGACGG- 644
Rattus_HSP70_1A_NM_031971           CTCATCTTCGACCTGGGGGGCGGCACGTTCGACGTGTCCATCCTGACGATCGACGACGG- 644
X_maculatus_HSP70-1_AB062113        CTCATCTTTGATCTTGGTGGAGGCACTTTTGACGTGTCCATCCTGACCATTGAGGATGG- 650
X_maculatus_HSP70-2_AB062114        CTGATCTTTGACCTGGGCGGAGGCACCTTCGACGTGTCCGTCCTGACCATCGAGGACGG- 650
Human_HSPA1L_NM_005527              CTGATTTTTGATCTGGGTGGAGGCACATTTGATGTGTCAATTCTGACCATAGATGATGG- 650
Human_HSPA6_NP_002146               CTCATTTTTGACCTGGGTGGGGGCACCTTCGATGTGTCGGTTCTCTCCATTGACGCTGG- 650
Alligator_HSP70_AB306279            CTGATCTTCGACCTGGGCGGCGGCACCCTCGACGTGTCCATCCTCACCATCGAGGACGG- 653
                                     * **  * **  * ** ** ** **  * ** ** **  *  *  * **    *  *

Drosophila_Bb_AF295957              TCTCTGTTCGAGGTGCGCTCCACAGCCGGAGACACACACTTGGGCGGCGAGGACTTTGAC 696
Drosophila_Aa_AF295933              TCTCTGTTCGAGGTGCGCTCCACCGCCGGAGACACGCACTTGGGCGGCGAGGACTTTGAC 696
Spodoptera_FJ754276                 TCATTGTTCGAAGTGAGGGCGACGGCGGGCGACACGCACCTCGGAGGCGAGGACTTCGAC 696
Bombyx_AB035326                     TCGTTGTTCGAAGTGAAGTCCACAGCCGGCGACACGCACCTGGGAGGCGAAGACTTTGAT 696
Paralvinella_EF580992               TCGCTGTTCGAGGTGAAGTCGACGGCCGGAGACACGCATCTCGGTGGCGAGGACTTCGAC 711
B_plicatilis_HSP70-1_AB775784       --CATCTTCGAGGTCAAGTCCACTGCCGGCGACACTCATTTAGGCGGCGAGGACTTCGAC 702
B_plicatilis_HSP70-2_AB775785       --CATCTTCGAGGTCAAGTCCACTGCCGGCGACACTCATTTAGGCGGCGAGGACTTCGAC 702
A_brightwelli_HSP70_KX119429        --TATCTTCGAGGTAAAATCCACGGCCGGAGACACACATTTGGGCGGCGAGGACTTTGAT 699
Wuchereria_AF167352                 --TATTTTCGAAGTCAAATCTACAGCTGGAGATACTCATCTTGGAGGAGAAGATTTCGAT 702
Setaria_AF079360                    --TATTTTCGAAGTGAAATCTACTGCTGGTGACACTCATCTTGGAGGCGAAGATTTCGAT 702
C_elegans_HSP70A_M18540             --AATCTTCGAAGTCAAGTCTACCGCTGGAGACACTCATCTTGGAGGAGAGGACTTCGAT 705
C_elegans_HSP-1_NM_070667           --AATCTTCGAAGTCAAGTCTACCGCTGGAGACACTCATCTTGGAGGAGAGGACTTCGAT 705
M_nipponense_KC460343               --CATTTTTGAAGTGAAGTCTACTGCTGGTGACACACATTTAGGTGGTGAAGATTTTGAT 702
M_nipponense_DQ660140               --CATTTTTGAAGTGAAGTCTACTGCTGGTGACACACATTTAGGTGGTGAAGATTTTGAT 702
Mytilus_HSP70_AY861684              --TATTTTTGAAGTTAAATCAACCTCTGGTGATACCCACTTGGGTGGTGAAGACTTTGAC 705
Perinereis_KU255783                 --TATCTTTGAAGTGAAGTCAACAGCCGGAGACACCCATTTGGGAGGAGAGGATTTCGAC 699
Paralvinella_HSP70-2_EF580993       --CATCTTTGAAGTGAAATCCACATCCGGTGACACTCACTTGGGTGGTGAGGACTTCGAC 702
Haliotis_FJ812176                   --TATCTTTGAGGTGAAATCTACAGCTGGAGACACCCACTTGGGTGGTGAGGACTTTGAC 702
Oncorhynchus_HSC71_AAB21658         --CATCTTTGAGGTCAAGTCCACTGCTGGAGACACTCATCTGGGTGGAGAAGACTTTGAC 702
X_maculatus_HSC70_AB062115          --CATCTTTGAGGTGAAGGCCACGGCGGGCGACACCCACCTGGGCGGCGAGGACTTCGAC 702
Human_HSPA8_HSC71_NP_006588         --AATCTTTGAGGTCAAGTCTACAGCTGGAGACACCCACTTGGGTGGAGAAGATTTTGAC 702
Rattus_HSC71_NM_024351              --AATTTTTGAAGTCAAATCAACAGCTGGAGACACCCACTTGGGCGGAGAAGACTTTGAC 702
Pelodiscus_HSC70_HQ219723           --CATCTTTGAAGTGAAGTCAACTGCTGGTGATACCCACTTAGGTGGGGAGGACTTTGAT 702
Bos_HSP70_U09861                    --CATCTTCGAGGTGAAGGCCACGGCCGGGGACACGCACCTGGGCGGGGAGGACTTCGAC 702
Human_HSPA1A_NM_005345              --CATCTTCGAGGTGAAGGCCACGGCCGGGGACACCCACCTGGGTGGGGAGGACTTTGAC 702
Rattus_HSP70_1A_NM_031971           --CATCTTCGAGGTGAAGGCCACGGCGGGCGACACGCACCTGGGCGGGGAGGACTTCGAC 702
X_maculatus_HSP70-1_AB062113        --CATATTTGAGGTGAAATCCACGGCCGGGGACACACATCTCGGCGGAGAGGATTTTGAC 708
X_maculatus_HSP70-2_AB062114        --CATCTTTGAGGTGAAGGCCACGGCCGGAGACACTCACTTGGGAGGAGAGGACTTTGAC 708
Human_HSPA1L_NM_005527              --GATTTTTGAGGTAAAGGCCACTGCTGGGGACACTCACCTGGGTGGGGAGGACTTTGAC 708
Human_HSPA6_NP_002146               --TGTCTTTGAGGTGAAAGCCACTGCTGGAGATACCCACCTGGGAGGAGAGGACTTCGAC 708
Alligator_HSP70_AB306279            --CATCTTCGAGGTGAAGTCGACGGCGGGCGACACGCACCTGGGCGGCGAGGACTTCGAC 711
                                        * ** ** **     * **  * ** ** ** **  * ** ** ** ** ** **
```

```
Drosophila_Bb_AF295957              AACCGGCTAGTCACCCACCTGGCGGAGGAGTTCAAGCGCAAGTACAAGAAGGATCTGCGC 756
Drosophila_Aa_AF295933              AACCGGCTAGTCACTCATCTGGCGGACGAGTTCAAGCGCAAGTACAAGAAGGATCTGCGC 756
Spodoptera_FJ754276                 AACAGATTGGTGAACCATCTCGCGGACGAGTTCAAGCGCAAGTACAAGAAGGACATGCGC 756
Bombyx_AB035326                     AACCGACTAGTCAACCATCTTGCGGAAGAGTTCAAGCGCAAGTACAAGAAGGATCTGCGC 756
Paralvinella_EF580992               AACAGACTGGTGAACCATTTCATCAAGGAATTCTTTAGAAAGTACAAGAAAGACATCAGC 771
B_plicatilis_HSP70-1_AB775784       AACCGACTGGTCAACCATTTTGTCGAGGAGTTCAAGCGCAAGAACAAAAAGGACATCACC 762
B_plicatilis_HSP70-2_AB775785       AACCGACTGGTCAACCATTTTGTCGAGGAGTTCAAGCGCAAGAACAAAAAGGACATCACC 762
A_brightwelli_HSP70_KX119429        AACAGACTCGTGAACCATTTCGTCGAAGAATTCAAGAGAAAACATAAAAAAGACATCACC 759
Wuchereria_AF167352                 AATCGCATGGTGAATCATTTCGTTGCGGAATTCAAGCGGAACGACAAAAAGGATCTTGCG 762
Setaria_AF079360                    AACCGCATGGTGAATCATTTCGTTGCGGAATTCAAGCGTAAACACAAGAAGGATTTGGCT 762
C_elegans_HSP70A_M18540             AACCGCATGGTGAACCACTTCTGTGCCGAGTTCAAGCGCAAGCACAAGAAGGATCTTGCT 765
C_elegans_HSP-1_NM_070667           AACCGCATGGTGAACCACTTCTGTGCCGAGTTCAAGCGCAAGCACAAGAAGGATCTTGCT 765
M_nipponense_KC460343               AACAGAACGGTTAACCATTTCATTCAAGAATTCAAGAGAAAATACAAGAAAGATCCTTCC 762
M_nipponense_DQ660140               AACAGAATGGTTAACCATTTCATTCAAGAATTCAAGAGAAAATACAAGAAAGATCCTTCC 762
Mytilus_HSP70_AY861684              AACAGAATGGTCAATCATTTCATTCAAGAATTCAAACGCAAGCACAAAAAAGACATTAGT 765
Perinereis_KU255783                 AACAGAATGGTCAACCACTTCATTCAGGAATTCAAGCGCAAGTTCAAGAAAGACATCTCT 759
Paralvinella_HSP70-2_EF580993       AACCGTATGGTGAACCACTTCATTCAGGAATTTAAGAGAAAGTTCAAGAAGGACATCTCC 762
Haliotis_FJ812176                   AATCGTATGGTGAACCACTTCATCCAAGAGTTCAAAAGGAAACACAAGAAGGATATCTCT 762
Oncorhynchus_HSC71_AAB21658         AACCGCATGGTCAACCACTTCATCGCGGAGTTCAAACGCAAGTACAAGAAAGACATCAGC 762
X_maculatus_HSC70_AB062115          AACCGCATGGTGAACCACTTCATCTCCGAGTTCAAGCGCAAGTACAAGAAGGACATCAGC 762
Human_HSPA8_HSC71_NP_006588         AACCGAATGGTCAACCATTTTATTGCTGAGTTTAAGCGCAAGCATAAGAAGGACATCAGT 762
Rattus_HSC71_NM_024351              AACCGAATGGTCAACCATTTCATTGCTGAGTTTAAGCGAAAGCACAAGAAGGACATCAGT 762
Pelodiscus_HSC70_HQ219723           AACCGTATGGTCAATCATTTCATTGCTGAATTCAAGCGTAAGCACAAGAAGGATATTACT 762
Bos_HSP70_U09861                    AACAGGCTGGTGAACCACTTCGTGGAGGAGTTCAAGAGGAAGCACAAGAAGGACATCAGC 762
Human_HSPA1A_NM_005345              AACAGGCTGGTGAACCACTTCGTGGAGGAGTTCAAGAGAAAACACAAGAAGGACATCAGC 762
Rattus_HSP70_1A_NM_031971           AACCGGCTGGTGAGCCACTTCGTGGAGGAGTTCAAGAGGAAGCACAAGAAGGACATCAGC 762
X_maculatus_HSP70-1_AB062113        AACCGAATGGTCAGCCACTTTGTGGAGGAGTTTAAAAGAAAGCACAAGAAGGACATCAGC 768
X_maculatus_HSP70-2_AB062114        AACCGCATGGTCAACCACTTTGTGGAGGAGTTCAAGAGGAAACACAAGAAGGACATCAGC 768
Human_HSPA1L_NM_005527              AACAGGCTTGTGAGCCACTTCGTGGAGGAGTTCAAGAGGAAACACAAAAAGGACATCAGC 768
Human_HSPA6_NP_002146               AACCGGCTCGTGAACCACTTCATGGAAGAATTCCGGCGGAAGCATGGGAAGGACCTGAGC 768
Alligator_HSP70_AB306279            AACCGCATGGTGACGCACTTCGTGGAGGAGTTCAAGCGCAAGCACAAGCGCGACATCGGC 771
                                    **  *    ** *  **  *       ** **     * **          **

Drosophila_Bb_AF295957              TCCAACCCTCGCGCCCTACGACGCCTCAGAACAGCAGCTGAACGGGCCAAGCGCACACTC 816
Drosophila_Aa_AF295933              TCCAACCCTCGCGCCCTACGACGCCTCAGAACAGCAGCTGAACGGGCCAAGCGCACACTC 816
Spodoptera_FJ754276                 ATGAATCCTCGCGCACTGCGCCGCCTCCGCACAGCCGCCGAGCGCGCTAAGCGCACGCTG 816
Bombyx_AB035326                     CTGAACTCTCGCGCACTCCGACGCCTCCGCACGGCCGCTGAGCGCGCCAAGAGGACACTC 816
Paralvinella_EF580992               AACAACCCTCGAGCCATCAGACGACTGAGAACGGCTTGTGAACGAGCCAAGAGGACATTA 831
B_plicatilis_HSP70-1_AB775784       TCGAACAAGCGAGCGCTGCGCCGACTCCGCACTGCTTGCGAGAGGGCCAAGCGCACTCTG 822
B_plicatilis_HSP70-2_AB775785       TCGAACAAGCGAGCGCTGCGCCGACTCCGCACTGCTTGCGAGAGGGCCAAGCGCACTCTG 822
A_brightwelli_HSP70_KX119429        GGAAACAAACGTGCCATTCGAAGATTGAGAACGGCCTGTGAAAGAGCAAAGAGAACTTTG 819
Wuchereria_AF167352                 TCGAATCCTCGTGCGCTTCGTCGTTTACGTACTGCATGCGAAAGAGCAAAACGAACATTG 822
Setaria_AF079360                    TCAAACCCTCGTGCCCTTCGTCGCTTACGTACTGCATGCGAAAGAGCAAAAAGAACACTC 822
C_elegans_HSP70A_M18540             TCCAACCCACGTGCTCTTCGTCGTCTTCGTACCGCCTGCGAGCGCGCAAACGAGACTCTT 825
C_elegans_HSP-1_NM_070667           TCCAACCCACGTGCTCTTCGTCGTCTTCGTACCGCCTGCGAGCGCGCAAAGAGAACTCTT 825
M_nipponense_KC460343               GAAAACAAGCGTGCTCTACGTCGTTTACGTACTGCTTGTGAGCGTGCCAAGCGTACTCTT 822
M_nipponense_DQ660140               GAAAACAAGCGTGCTCTACGTCGTTTACGTACTGCTTGTGAGCGCGCCAAGCGTACTCTT 822
Mytilus_HSP70_AY861684              GAAAACAAGCGTGCTGTCCGACGACTTAGAACTGCTTGTGAAAGGGCAAAGAGAACCCTT 825
Perinereis_KU255783                 GACAACAAGAGGGCTGTAAGACGTCTCCGAACAGCCTGTGAGCGTGCGAAGAGGACCCTG 819
Paralvinella_HSP70-2_EF580993       GACAACAAGCGTGCTGTTCGTCGTCTGCGTACAGCATGTGAACGCGCGAAACGTACCTTG 822
Haliotis_FJ812176                   GACAACAAGAGGGCTGTGAGACGTCTGAGAACCGCATGCGAGAGAGCAAAGAGGACACTT 822
Oncorhynchus_HSC71_AAB21658         GACAACAAGAGGGCTGTTCGCCGTCTCCGCACCGCATGTGAGAGGGCAAAGCGCACCCTG 822
X_maculatus_HSC70_AB062115          GACAACAAGAGGGCGGTGCGTCGCCTGCGCACGGCCTGCGAGCGGGCCAAGCGCACGCTG 822
Human_HSPA8_HSC71_NP_006588         GAGAACAAGAGAGCTGTAAGACGCCTCCGTACTGCTTGTGAACGTGCTAAGCGTACCCTC 822
Rattus_HSC71_NM_024351              GAGAACAAGAGAGCTGTCAGGCGTCTCCGCACTGCCTGTGAGCGGGCCAAGCGCACCCTC 822
Pelodiscus_HSC70_HQ219723           GAGAACAAGAGAGCAGTTCGCCGGCTACGCACAGCATGTGAACGTGCTAAGCGTACTCTC 822
Bos_HSP70_U09861                    CAGAACAAGCGGGCCGTGAGGCGGCTGCGCACCGCATGCGAGCGGGCCAAGAGAACCTTG 822
Human_HSPA1A_NM_005345              CAGAACAAGCGAGCCGTGAGGCGGCTGCGCACCGCCTGCGAGAGGGCCAAGAGGACCCTG 822
Rattus_HSP70_1A_NM_031971           CAGAACAAGCGCGCGGTGCGGCGACTGCGCACGGCGTGCGAGAGGGCCAAGAGGACGCTG 822
X_maculatus_HSP70-1_AB062113        CAGAACAAGAGAGCAGTGAGGAGACTGCGCACAGCTTGTGAGAGAGCAAAGAGGACCTTG 828
X_maculatus_HSP70-2_AB062114        CAGAACAAGAGAGCCTTGAGGAGGCTGCGCACAGCTTGCGAGAGGGCCAAGAGGACCCTG 828
Human_HSPA1L_NM_005527              CAGAACAAGCGAGCCGTGAGGCGGCTGCGCACCGCCTGCGAGAGGGCCAAGAGGACCCTG 828
Human_HSPA6_NP_002146               GGGAACAAGCGTGCCCTGCGCAGGCTGCGCACAGCCTGTGAGCGCGCCAAGCGCACCCTG 828
Alligator_HSP70_AB306279            GGCAACAAGCGGGCGGTGCGGCGGCTGCGCACGGCGTGCGAGCGGGCCAAGCGCACGCTG 831
                                       **     * **  *  *  *  *  * ** **    **  * ** **    **  *

Drosophila_Bb_AF295957              TCCTCTAGCACGGAGGCCACCATCGAGATCGACGCATTGTTTGAGGGCCAAGACTTCTAC 876
Drosophila_Aa_AF295933              TCCTCCAGCACGGAGGCCACCATCGAGATTGACGCACTGTTTGAGGGCCAAGACTTCTAC 876
Spodoptera_FJ754276                 TCGTCCAGCCCCGAAGCCACCATCGAGATCGACGCGCTCTACGAGGGAATCGACTTCTAC 876
Bombyx_AB035326                     TCATCCAGCACCGAGGCCACCATCGAGATTGACGCTCTGTATGAGGGCATCGACTTCTAC 876
Paralvinella_EF580992               TCGACCAGTACACAATCGAATATAGAAATCGACTCTCTCTACGAAGGAATCGACTTCTAT 891
B_plicatilis_HSP70-1_AB775784       TCCTCGTCGGCCCAGGCCAACATCGAAATAGACTCGTTGCACGAGGGCGTCGACTTCTAC 882
B_plicatilis_HSP70-2_AB775785       TCCTCGTCGGCCCAGGCCAACATCGAAATAGACTCGTTGCACGAGGGCGTCGACTTCTAC 882
A_brightwelli_HSP70_KX119429        TCATCGTCTGCCCAGGCCAACATTGAGATTGACTCGTTGCATGAGGGAGTTGACTTTTAC 879
Wuchereria_AF167352                 TCTAGTTCATCTCAAGCGAGTATCGAAATTGATTCGTTGTTCGAGGGGATCGATTTCTAC 882
Setaria_AF079360                    TCTAGTTCGTCACAAGCTAGTATCGAAATTGATTCGCTATTCGAGGGCATTGATTTCTAC 882
C_elegans_HSP70A_M18540             TCGTCGTCTTGCCAGGCTTCGATTGAGATCGATTCTCTCTTCGAAGGAATTGACTTCTAC 885
C_elegans_HSP-1_NM_070667           TCGTCGTCTTCCCAGGCTTCAATTGAGATCGATTCTCTCTTCGAGGGAATTGACTTCTAC 885
```

```
M_nipponense_KC460343              TCAGCCTCTGCTCAAGCTAGTGTTGAGATTGATTCTCTGTATGAAGGTATTGATTTTTAC 882
M_nipponense_DQ660140              TCAGCCTCTGCTCAAGCTAGTGTTGAGATTGATTCTCTGTATGAAGGTATTGATTTTTAC 882
Mytilus_HSP70_AY861684             TCTTCAAGCACACAAGCAAGTGTTGAGATTGACTCTCTGTTTGAAGGAGTTGACTTTTAT 885
Perinereis_KU255783                TCCTCCAGCACCCAAGCCAGTATTGAAATTGATTCCTTGTATGAAGGTGTCGACTTCTAC 879
Paralvinella_HSP70-2_EF580993      TCCAGCAGCACACAGGCAAGCATTGAAATAGATTCCCTCTATGAGGGCATAGACTATTAC 882
Haliotis_FJ812176                  TCCTCCAGCACCCAGGCCAGCATAGAGATCGACTCCCTGTTTGAAGGAGTAGATTACTAC 882
Oncorhynchus_HSC71_AAB21658        TCCTCCAGCACCCAGGCCAGCATCGAGATCGACTCTTTGTACGAGGGAATCGACTTCTAC 882
X_maculatus_HSC70_AB062115         TCTTCCAGCACGCAGGCCAGCATCGAGATCGACTCCCTGTACGAGGGCATCGACTTCTAC 882
Human_HSPA8_HSC71_NP_006588        TCTTCCAGCACCCAGGCCAGTATTGAGATCGATTCTCTCTATGAAGGAATCGACTTCTAT 882
Rattus_HSC71_NM_024351             TCCTCCAGCACCCAGGCCAGTATTGAGATTGATTCTCTCTATGAGGGAATTGACTTCTAC 882
Pelodiscus_HSC70_HQ219723          TCTTCTAGCACTCAGGCCAGTATTGAAATTGACTCGCTCTATGAGGGCATTGATTTTTAC 882
Bos_HSP70_U09861                   TCGTCCAGCACCCAGGCCAGCCTGGAGATCGACTCCCTGTTCGAGGGCATCGACTTCTAC 882
Human_HSPA1A_NM_005345             TCGTCCAGCACCCAGGCCAGCCTGGAGATCGACTCCCTGTTTGAGGGCATCGACTTCTAC 882
Rattus_HSP70_1A_NM_031971          TCGTCCAGCACCCAGGCCAGCCTGGAGATCGACTCTCTGTTCGAGGGCATCGACTTCTAC 882
X_maculatus_HSP70-1_AB062113       TCCTCCAGCACTCAGGCAAGCATTGAGATTGACTCACTCTTTGAGGGCATTGACTTTTAC 888
X_maculatus_HSP70-2_AB062114       TCCTCCAGCTCCCAGGCCAGCATCGAGATCGACTCTCTGTTCGAGGGTGTGGACCTGTAC 888
Human_HSPA1L_NM_005527             TCGTCCAGCACCCAGGCCAACCTAGAAATTGATTCACTTTATGAAGGCATTGACTTCTAT 888
Human_HSPA6_NP_002146              TCCTCCAGCACCCAGGCCACCCTGGAGATAGACTCCCTGTTCGAGGGCGTGGACTTCTAC 888
Alligator_HSP70_AB306279           AGCTCCTCCACGCAGGCCAGCATCGAGATCGACTCGCTGTTCGAGGGCATCGACTTCTAC 891
                                                 *  *     * ** ** **  *  *    ** **    **    **

Drosophila_Bb_AF295957             ACCAAAGTAAGCCGTGCCAGGTTTGAGGAGCTGTGCGCGGACCTCTTCCGCAACACCCTG 936
Drosophila_Aa_AF295933             ACCAAAGTGAGCCGCGCCAGGTTTGAGGAGCTGTGCGCGGACCTCTTCCGCAACACCCTG 936
Spodoptera_FJ754276                ACTCGCGTGTCCCGCGCGCGCTTCGAGGAGCTCAACGCTGACCTGTTCCGAGGCACCCTC 936
Bombyx_AB035326                    ACGCGAGTCTCCCGCGCCCGCTTCGAGGAACTGAACGCGGACCTGTTCAGGGGAACTCTG 936
Paralvinella_EF580992              ACAACTATTACCAGAGCCAGATTCGAAGAGCTCTGCTCCGATCTGTTCAGGTCAACTTTG 951
B_plicatilis_HSP70-1_AB775784      ACGAGCATCACCAGAGCTCGCTTTGAAGAGCTGTGCGCCGATTTGTTCCGCGGCACTCTC 942
B_plicatilis_HSP70-2_AB775785      ACGAGCATCACCAGAGCTCGCTTTGAAGAGCTGTGCGCCGATTTGTTCCGCGGCACCCTC 942
A_brightwelli_HSP70_KX119429       ACAAACATCACCAGAGCCAGATTCGAAGAGCTCAACGCTGATTTATTCCGCGGCTGTTTA 939
Wuchereria_AF167352                ACCAATATCACTCGTGCTCGCTTTGAGGAATTATGTGCTGATCTTTTCCGTTCAACTATG 942
Setaria_AF079360                   ACTAATATTACTCGTGCTCGCTTTGAAGAACTTTGTGCTGATCTTTTCCGCTCAACAATG 942
C_elegans_HSP70A_M18540            ACCAACATCACTCGTGCTCGTTTCGAGGAGCTCTGCGCTGATCTCTTCAGATCCACCATG 945
C_elegans_HSP-1_NM_070667          ACCAACATCACTCGTGCTCGTTTCGAGGAGCTCTGCGCTGATCTCTTCAGATCCACCATG 945
M_nipponense_KC460343              ACTTCTATTACTCGTGCCAGGTTTGAGGAACTTTGTGGTGACTTGTTCCGTGGCACCCTT 942
M_nipponense_DQ660140              ACTTCTATTACTCGTGCCAGGTTTGAGGAACTTTGTGGTGACTTGTTCCGTGGCACCCTG 942
Mytilus_HSP70_AY861684             ACAAGCATCACAAGAGCCAGGTTTGAGGAATTGAATGCAGATCTTTTCAGAGGAACCATG 945
Perinereis_KU255783                ACAACCATCACCAGGGCTCGTTTTGAGGAGCTGTGTGCCGATCTCTTCCGAGGCACTCTC 939
Paralvinella_HSP70-2_EF580993      ACAACAATCACTAGAGCTAGATTTGAGGAGCTGAATGCTGACCTATTCCGTGGTACGCTG 942
Haliotis_FJ812176                  ACCAGCATCACTAGAGCCAGGTTTGAGGAGCTCAATGCTGATCTTTTCCGTGGCACACTA 942
Oncorhynchus_HSC71_AAB21658        ACCTCCATCACCAGGGCTCGCTTTGAGGAGCTCAATGCAGACCTTTTCCGTGGCACCCTT 942
X_maculatus_HSC70_AB062115         ACGTCCATCACCAGGGCGCGCTTTGAGGAGCTGAACGCGGACCTGTTCCGCGGAACGCTG 942
Human_HSPA8_HSC71_NP_006588        ACCTCCATTACCCGTGCCCGATTTGAAGAACTGAATGCTGACCTGTTCCGTGGCACCCTG 942
Rattus_HSC71_NM_024351             ACCTCCATTACCCGTGCTCGATTTGAGGAGTTGAATGCTGACCTGTTCCGTGGCACACTG 942
Pelodiscus_HSC70_HQ219723          ACCTCAATTACCAGAGCTCGTTTTGAAGAGTTAAATGCTGATCTGTTCCGTGGCACTCTG 942
Bos_HSP70_U09861                   ACGTCCATCACCAGGGCGCGGTTCGAGGAGCTGTGCTCCGACCTGTTCCGGAGCACCCTA 942
Human_HSPA1A_NM_005345             ACGTCCATCACCAGGGCGAGGTTCGAGGAGCTGTGCTCCGACCTGTTCCGAAGCACCCTG 942
Rattus_HSP70_1A_NM_031971          ACGTCCATCACGCGGGCGCGGTTCGAGGAGCTGTGCTCGGACCTGTTCCGCGGCACGCTG 942
X_maculatus_HSP70-1_AB062113       ACCTCAATCACAAGGGCACGCTTCGAGGAGCTCAACTCAGACCTCTTTAGGGGAACACTG 948
X_maculatus_HSP70-2_AB062114       ACCTCCATCACCAGGGCTCGCTTTGAGGAGCTGTGCTCCGACCTGTTCAGGGGAACCTTG 948
Human_HSPA1L_NM_005527             ACATCCATCACCAGAGCTCGATTTGAAGAGTTGTGTGCAGACCTGTTTAGGGGTACCCTG 948
Human_HSPA6_NP_002146              ACGTCCATCACTCGTGCCCGCTTTGAGGAACTGTGCTCAGACCTCTTCCGCAGCACCCTG 948
Alligator_HSP70_AB306279           ACGTCCATCACGCGGGCGCGCTTCGAGGAGCTGAACGCCGACCTGTTCCGCGGCACGCTG 951
                                   **     *     * **  * ** ** **  *       **  * **  *        *

Drosophila_Bb_AF295957             CAGCCTGTGGAGAAGGCCCTCAACGATGCCAAGATGGACAAGGGTCAGATCCACGACATC 996
Drosophila_Aa_AF295933             CAGCCTGTGGAGAAGGCCCTCAACGATGCCAAGATGGATAAGGGTCAGATCCACGACATC 996
Spodoptera_FJ754276                GAGCCGGTCGAGAAAGCGCTCAAGGACGCCAAGATGGACAAGAGCCAGATCCACGACGTC 996
Bombyx_AB035326                    GAACCCGTCGAGAAGGCACTCAAGGATGCTAAACTCGACAAGAGTCAGATCCACGACGTG 996
Paralvinella_EF580992              GAGCCAGTAGAGAAAGCTCTCCGAGATGCCAAGATGGACAAGTCCAAGATCGACGAAGTG 1011
B_plicatilis_HSP70-1_AB775784      GACCCAGTGGAGAAGGCGCTGCGAGACGCCAAAATGGACAAGTCAAGTGTGAACGAGATT 1002
B_plicatilis_HSP70-2_AB775785      GACCCAGTGGAGAAGGCGCTGCGAGACGCCAAAATGGACAAGTCAAGTGTGAACGAGATT 1002
A_brightwelli_HSP70_KX119429       GACCCAGTCGAGAAATCTTTGAGAGACGCTAAACTCGACAAAGCCCAGATCCATGAAATT 999
Wuchereria_AF167352                GATCCAGTTGAGAAAGCACTCCGTGATGCTAAGATGGACAAGGCCCAAGTCCATGACATT 1002
Setaria_AF079360                   GATCCAGTTGAGAAAGCATTGCGTGATGCCAAGATGGATAAGGCACAAGTTCATGATATT 1002
C_elegans_HSP70A_M18540            GACCCAGTCGAGAAGTCTCTCCGTGACGCCAAGATGGACAAGAGCCAAGTTCATGACATC 1005
C_elegans_HSP-1_NM_070667          GACCCAGTCGAGAAGTCTCTCCGTGACGCCAAGATGGACAAGAGCCAAGTTCATGACATC 1005
M_nipponense_KC460343              GAGCCAGTTGAGAAGTCTCTGAGAGATGCAAAAATGGACAAAGCCCAAATCTATGACATT 1002
M_nipponense_DQ660140              GAGCCAGTTGAGAAGTCTCTGAGAGATGCAAAAATGGACAAAGCCCAAATCCATGACATT 1002
Mytilus_HSP70_AY861684             GAACCAGTTGAAAAAGCTCTACGCGATGCCAAACTAGACAAGGCTGCTGTCCATGAAATT 1005
Perinereis_KU255783                GAGCCAGTAGAGAAGTCCATCCGTGATGCCAAGATGGGAAAAGATGCCATCCACGACATC 999
Paralvinella_HSP70-2_EF580993      GAACCTGTAGAAAAGTCTCTGCGTGATGCCAAGCTGGACAAGTCCAGCATCCATGACATC 1002
Haliotis_FJ812176                  GAGCCAGTGGAGAAGGCTTTACGTGATGCCAAGGCAGACAAGGTCTCCATCCATGACATA 1002
Oncorhynchus_HSC71_AAB21658        GACCCAGTGGAGAAATCCCTCCGCGACGCCAAGATGGACAAAGCCCAGGTACACGACATC 1002
X_maculatus_HSC70_AB062115         GAGCCCGTGGAGAAGTCTCTCCGAGACGCCAAGATGGACAAGGCTCAGATCCACGAGATT 1002
Human_HSPA8_HSC71_NP_006588        GACCCAGTAGAGAAAGCCCTTCGAGATGCCAAACTAGACAAGTCACAGATTCATGATATT 1002
Rattus_HSC71_NM_024351             GACCCTGTAGAGAAGGCCCTTCGAGATGCCAAACTAGACAAGTCACAGATCCATGATATT 1002
Pelodiscus_HSC70_HQ219723          GATCCTGTGGAGAAGGCCCTGCGGGATGCCAAACTAGACAAATCACAGATCCATGACATT 1002
Bos_HSP70_U09861                   GAGCCCGTGGAGAAGGCGCTACGCGACGCCAAGCTGGACAAGGCGCAGATCCACGACCTG 1002
Human_HSPA1A_NM_005345             GAGCCCGTGGAGAAGGCTCTGCGCGACGCCAAGCTGGACAAGGCCCAGATTCACGACCTG 1002
```

```
Rattus_HSP70_1A_NM_031971          GAGCCCGTGGAGAAGGCCCTGCGCGACGCCAAGCTGGACAAGGCGCAGATCCACGACCTG 1002
X_maculatus_HSP70-1_AB062113       GAGCCAGTTGAGAAGGCCCTACAAGATGCTAAGCTGGATAAATCCAAGATCCACGAAGTT 1008
X_maculatus_HSP70-2_AB062114       GAGCCTGTGGAGAAGGCTTTGAGGGACGCCAAGATGGACAAGGGCCAGATCCATGACGCG 1008
Human_HSPA1L_NM_005527             GAGCCTGTAGAAAAAGCGCTTCGGGATGCCAAGATGGATAAGGCTAAAATCCATGACATT 1008
Human_HSPA6_NP_002146              GAGCCGGTGGAGAAGGCCCTGCGGGATGCCAAGCTGGACAAGGCCCAGATTCATGACGTC 1008
Alligator_HSP70_AB306279           GAGCCCGTGGAGAAGGCGCTGCGCGACGCCAAGCTGGACAAGGGGCAGGTGCAGGAGATC 1011
                                    * ** ** ** **  *  *    ** ** **    *  **        *  * **

Drosophila_Bb_AF295957             GTGCTCGTCGGCGGATCCACTCGCATTCCCAAGGTGCAAAGTCTGCTGCAGGAGTTCTTC 1056
Drosophila_Aa_AF295933             GTGCTCGTCGGCGGATCCACTCGCATTCCCAAGGTGCAAAGTCTGCTGCAGGACTTCTTC 1056
Spodoptera_FJ754276                GTTCTCGTCGGAGGCTCGACCAGAATCCCGAAGGTGCAGAGTCTGCTGCAAAACTTCTTC 1056
Bombyx_AB035326                    GTCCTCGTCGGAGGCTCCACCCGCATTCCGAAAGTACAAACTATGCTCCAAAACTTCTTC 1056
Paralvinella_EF580992              GTTCTGGTCGGAGGATCGACTAGAATCCCGAAGATCCAGAAACTGCTACAGGAGTTCATG 1071
B_plicatilis_HSP70-1_AB775784      GTGTTGGTGGGCGGCTCGACCCGCATTCCAAAAGTTCAAAAGCTGCTCCAGGACTTTTTC 1062
B_plicatilis_HSP70-2_AB775785      GTGTTGGTGGGCGGCTCGACCCGCATTCCAAAAGTTCAAAAGCTGCTCCAGGACTTTTTC 1062
A_brightwelli_HSP70_KX119429       GTTTTAGTCGGTGGATCGACCAGAATCCCGAAAGTGCAAAAATTATTACAAGACTTTTTC 1059
Wuchereria_AF167352                GTGCTAGTAGGTGGATCAACCCGTATCCCGAAAGTGCAGAAGCTCTTGTCAGATTTCTTT 1062
Setaria_AF079360                   GTACTGGTGGGTGGGTCAACTCGTATCCCGAAAGTGCAGAAACTGCTCTCGGATTTCTTC 1062
C_elegans_HSP70A_M18540            GTCCTTGTCGGAGGATCCACTCGTATCCCAAAGGTCCAGAAACTTTTGTCCGATCTCTTC 1065
C_elegans_HSP-1_NM_070667          GTCCTTGTCGGAGGATCCACTCGTATCCCAAAGGTCCAGAAACTTTTGTCCGATCTCTTC 1065
M_nipponense_KC460343              GTCCTTGTTGGAGGTTCCACCAGAATTCCAAAGATTCAGAAGCTGTTACAGGATTTCTTC 1062
M_nipponense_DQ660140              GTCCTTGTTGGAGGTTCCACCAGAATTCCAAAGATTCAGAAGCTGTTACAGGATTTCTTC 1062
Mytilus_HSP70_AY861684             GTCTTGGTAGGTGGATCAACCAGAATTCCAAAAATCCAGAAGTTACTTCAGGACTTTTTT 1065
Perinereis_KU255783                GTGTTGGTCGGTGGTTCCACCCGTATCCCCAAGATCCAGAAACTCCTTCAGGATTTCTTC 1059
Paralvinella_HSP70-2_EF580993      GTACTGGTTGGTGGCTCCACCAGAATTCCCAAGATCCAAAAACTGCTGCAGGACTTTTTC 1062
Haliotis_FJ812176                  GTGCTGGTTGGGGGATCTACACGTATCCCCAAGATCCAGAAACTCCTCCAAGACTTCTTC 1062
Oncorhynchus_HSC71_AAB21658        GTCCTGGTCGGAGGCTCCACTCGTATCCCCAAGATCCAGAAACTGCTCCAAGATTTCTTC 1062
X_maculatus_HSC70_AB062115         GTCTTGGTGGGAGGCTCGACCCGCATCCCAAAGATCCAAAAGCTCCTGCAGGACTTCTTT 1062
Human_HSPA8_HSC71_NP_006588        GTCCTGGTTGGTGGTTCTACTCGTATCCCCAAGATTCAGAAGCTTCTCCAAGACTTCTTC 1062
Rattus_HSC71_NM_024351             GTCCTGGTGGGTGGTTCTACCAGAATCCCCAAGATCCAGAAACTTCTGCAAGACTTCTTC 1062
Pelodiscus_HSC70_HQ219723          GTACTGGTTGGTGGATCAACCCGTATTCCCAAGATTCAGAAACTCCTGCAGGATTTCTTC 1062
Bos_HSP70_U09861                   GTCCTGGTGGGGGGCTCCACCCGCATCCCCAAGGTGCAGAAGCTGCTGCAGGACTTCTTC 1062
Human_HSPA1A_NM_005345             GTCCTGGTCGGGGGCTCCACCCGCATCCCCAAGGTGCAGAAGCTGCTGCAGGACTTCTTC 1062
Rattus_HSP70_1A_NM_031971          GTGCTGGTGGGCGGCTCGACGCGCATCCCCAAGGTGCAGAAGCTGCTGCAGGACTTCTTC 1062
X_maculatus_HSP70-1_AB062113       GTCCTAGTTGGTGGCTCTACAAGAATCCCCAAAATCCAAAAGCTCCTGCAGGACTTCTTC 1068
X_maculatus_HSP70-2_AB062114       GTCCTGGTGGGAGGCTCCACCCGAATCCCCAAGATCCAGAAGCTGCTGCAGGACTTCTTC 1068
Human_HSPA1L_NM_005527             GTTTTAGTAGGGGGCTCCACCCGCATCCCCAAGGTGCAGCGGCTGCTTCAGGACTACTTC 1068
Human_HSPA6_NP_002146              GTCCTGGTGGGGGGCTCCACACGCATCCCCAAGGTGCAGAAGTTGCTGCAGGACTTCTTC 1068
Alligator_HSP70_AB306279           GTGCTGGTGGGCGGCTCGACGCGCATCCCCAAGATCCAGAAGCTGCTGCAGGACTTCTTC 1071
                                   **  * ** ** ** ** **  * ** ** **  * **     *  *     *     *

Drosophila_Bb_AF295957             CACGGCAAGAACCTCAACCTATCCATCAACCCAGACGAGGCAGTGGCATACGGAGCTGCT 1116
Drosophila_Aa_AF295933             CACGGCAAGAACCTCAACCTATCCATCAACCCAGACGAGGCAGTGGCATACGGAGCTGCT 1116
Spodoptera_FJ754276                TGCGGCAAGAAGCTGAATTTGTCCATCAACCCGGACGAGGCGGTCGCGTATGGCGCGGCC 1116
Bombyx_AB035326                    TGTGGGAAGAAACTGAACTTATCCATCAATCCGGACGAAGCCGTAGCCTACGGTGCAGCA 1116
Paralvinella_EF580992              AACGGGAAAGAGCTGAACAAGTCGATCAATCCCGATGAAGCAGTGGCCTATGGAGCGGCC 1131
B_plicatilis_HSP70-1_AB775784      AATGGCAAAGAGCTGAACAAGTCGATTAACCCGGACGAGGCGGTGGCGTACGGCGCGGCC 1122
B_plicatilis_HSP70-2_AB775785      AATGGCAAAGAGCTGAACAAGTCGATTAACCCGGACGAGGCGGTGGCGTACGGCGCGGCC 1122
A_brightwelli_HSP70_KX119429       AATGGAAAAGAGTTGAACAAGAGCATTAACCCAGACGAGGCCGTGGCCTACGGTGCTGCC 1119
Wuchereria_AF167352                TCTGGCAAAGAATTGAATAAAAGCATCAATCCGGACGAGGCCGTGGCGTATGGTGCAGCA 1122
Setaria_AF079360                   TCTGGCAAAGAATTGAACAAAAGTATCAACCCTGATGAGGCTGTGGCATATGGTGCTGCT 1122
C_elegans_HSP70A_M18540            TCAGGAAAGGAATTGAACAAGTCCATCAACCCAGATGAGGCGTTAGCCTACGGAGCTGCC 1125
C_elegans_HSP-1_NM_070667          TCAGGAAAGGAATTGAACAAGTCCATCAACCCAGATGAGGCCGTTGCCTACGGAGCTGCC 1125
M_nipponense_KC460343              AATGGAAAGGAGCTCAACAAATCCATCAATCCTGATGAAGCTGTAGCTTATGGTGCAGCT 1122
M_nipponense_DQ660140              AATGGAAAGGAGCTCAACAAATCCATCAATCCTGATGAAGCTGTAGCTTATGGTGCAGCT 1122
Mytilus_HSP70_AY861684             CAAGGCAAAGAATTGAACAAATCCATTAACCCTGATGAAGCTGTAGCATACGGTGCAGCT 1125
Perinereis_KU255783                AACGGCAAAGAATTGAACAAATCTATCAACCCTGATGAGGCTGTCGCATACGGAGCTGCC 1119
Paralvinella_HSP70-2_EF580993      AACGGCAAAGAACTAAACAAGTCGATCAACCCTGATGAAGCTGTTGCATATGGTGCAGCT 1122
Haliotis_FJ812176                  AACGGCAAGGAGCTATGCAAGAGCATCAACCCTGATGAGGCTGTTGCTTATGGTGCTGCC 1122
Oncorhynchus_HSC71_AAB21658        AACGGCAAAGAGCTCAACAAAAGCATCAACCCCGACGAAGCTGTGGCCTATGGCGCAGCT 1122
X_maculatus_HSC70_AB062115         AACGGGAAGGAACTCAACAAGAGCATTAACCCTGACGAGGCGGTGGCGTACGGTGCAGCT 1122
Human_HSPA8_HSC71_NP_006588        AATGGAAAAGAACTGAATAAGAGCATCAACCCTGATGAAGCTGTTGCTTATGGTGCAGCT 1122
Rattus_HSC71_NM_024351             AATGGAAAAGAGCTGAATAAGAGCATTAACCCCGATGAAGCTGTTGCCTATGGTGCAGCT 1122
Pelodiscus_HSC70_HQ219723          AATGGGAAAGAGCTGAACAAGAGCATCAATCCTGATGAAGCTGTGGCTTACGGTGCAGCT 1122
Bos_HSP70_U09861                   AACGGGCGCGACCTCAACAAGAGCATCAACCCCGACGAGGCGGTGGCGTACGGGGCGGCG 1122
Human_HSPA1A_NM_005345             AACGGGCGCGACCTGAACAAGAGCATCAACCCCGACGAGGCTGTGGCCTACGGGGCGGCG 1122
Rattus_HSP70_1A_NM_031971          AACGGGCGCGACCTGAACAAGAGCATCAATCCGGACGAGGCGGTGGCCTACGGGGCGGCG 1122
X_maculatus_HSP70-1_AB062113       AATGGTAGAGAACTAAACAAGAGCATCAACCCTGATGAAGCGGTGGCCTATGGTGCAGCA 1128
X_maculatus_HSP70-2_AB062114       AACGGCCGGGAGCTGAACAAGAGCATCAACCCGGACGAGGCGGTGGCTTACGGCGCCGCC 1128
Human_HSPA1L_NM_005527             AATGGACGTGATCTCAACAAGAGCATCAACCCTGATGAGGCCGTAGCATATGGGGCTGCG 1128
Human_HSPA6_NP_002146              AACGGCAAGGAGCTGAACAAGAGCATCAACCCTGATGAGGCTGTGGCCTATGGGGCTGCT 1128
Alligator_HSP70_AB306279           AATGGCAAGGAGCTGAACAAGAGCATCAACCCCGACGAGGCGGTGGCGTACGGCGCGGCG 1131
                                      **     *  *          ** ** ** ** ** **  * ** ** ** ** **

Drosophila_Bb_AF295957             GTGCAGGCCGCTATCCTCAGCGGAGACCAGAGCGGCAAGATCCAGGACGTGCTGCTGGTG 1176
Drosophila_Aa_AF295933             GTGCAGGCCGCTATCCTCAGCGGAGACCAGAGCGGCAAGATCCAGGACGTGCTGCTGGTG 1176
Spodoptera_FJ754276                GTCCAGGCGGCCATCCTGAGTGGCGAACAAGACTCGAAGATCCAGGACGTGTTGCTGGTG 1176
Bombyx_AB035326                    GTCCAGGCAGCCATCCTGAGCGGCGAGACCGACTCCAAGATACAGGACGTCTTGTTGGTC 1176
Paralvinella_EF580992              GTTCAAGCAGCTATCCTGACAGGTTCTGAACACTCGGCCGTTAAAGATCTACTCTTGGTA 1191
```

```
B_plicatilis_HSP70-1_AB775784      GTCCAGGCGGCCATTTTGACCGGCGACAAGTCCGAGGCCGTCCAGGACCTGCTGCTGCTT 1182
B_plicatilis_HSP70-2_AB775785      GTCCAGGCGGCCATTTTGACCGGCGACAAGTCCGAGGCCGTCCAGGACCTGCTGCTGCTT 1182
A_brightwelli_HSP70_KX119429       GTCCAAGCCGCTATTTTAACGGGTGATAAATCCGAAGCCGTTCAAGATTTATTGTTATTA 1179
Wuchereria_AF167352                GTGCAGGCAGCTATTCTTTCTGGTGATAAGTCGGAAGCCGTACAAGATTTGTTACTGCTT 1182
Setaria_AF079360                   GTCCAGGCAGCTATCCTCTCTGGTGATAAGTCAGAAGCCGTACAAGATTTGTTATTCGTC 1182
C_elegans_HSP70A_M18540            GTCCAAGCCGCTATCCTCTCCGGAGACAAGTCTGAGGCTGTCCAGGATCTTCTTCTTCTT 1185
C_elegans_HSP-1_NM_070667          GTCCAAGCCGCTATCCTCTCCGGAGACAAGTCTGAGGCTGTCCAGGATCTTCTTCTTCTT 1185
M_nipponense_KC460343              GTACAGGCAGCCATATTGTGTGGTGACAAGTCTGAAGCAGTTCAAGATTTGTTGCTTTTG 1182
M_nipponense_DQ660140              GTACAGGCAGCCATATTGTGTGGTGACAAGTCTGAAGCAGTTCAAGATTTGTTGCTTTTG 1182
Mytilus_HSP70_AY861684             GTGCAGGCAGCCATTTTGTCAGGTGACAAGTCAGAAGAGGTACAAGACTTGTTATTGTTA 1185
Perinereis_KU255783                GTTCAGGCTGCCATCTTGCACGGTGACAAGTCAGAAGAGGTCCAAGACTTGCTTTTGCTT 1179
Paralvinella_HSP70-2_EF580993      GTCCAGGCTGCCATCCTTCATGGAGACAAGTCCGAGGCAGTTCAGGATCTGCTGCTACTG 1182
Haliotis_FJ812176                  GTCCAGGCTGCTATCCTCCATGGTGACAAGTCCGAGGAAGTCCAAGACCTGCTACTGCTG 1182
Oncorhynchus_HSC71_AAB21658        GTCCAGGCAGCCATACTCTCAGGTGACAAGTCTGAGAATGTCCAGGACCTGCTTTTGCTG 1182
X_maculatus_HSC70_AB062115         GTCCAGGCGGCCATCTTGTGCGGAGACAAATCCGAGATTGTTCAGGACCTGCTTCTGTTG 1182
Human_HSPA8_HSC71_NP_006588        GTCCAGGCAGCCATCTTGTCTGGAGACAAGTCTGAGAATGTTCAAGATTTGCTGCTCTTG 1182
Rattus_HSC71_NM_024351             GTCCAGGCAGCCATTCTATCTGGAGACAAGTCTGAGAATGTTCAGGATTTGCTGCTCTTG 1182
Pelodiscus_HSC70_HQ219723          GTTCAGGCAGCAATCTTGTCTGGGGACAAGTCTGAGAATGTACAAGACCTGCTACTGTTG 1182
Bos_HSP70_U09861                   GTGCAGGCGGCCATCCTGATGGGGGACAAGTCGGAGAACGTGCAGGACCTGCTGTTGCTG 1182
Human_HSPA1A_NM_005345             GTGCAGGCGGCCATCCTGATGGGGGACAAGTCCGAGAACGTGCAGGACCTGCTGCTGCTG 1182
Rattus_HSP70_1A_NM_031971          GTGCAGGCGGCCATCCTGATGGGGGACAAGTCGGAGAACGTGCAGGACCTGCTGCTGCTG 1182
X_maculatus_HSP70-1_AB062113       GTCCAGGCTGCTATCCTCATGGGTGACACCTCAGAAAATGTCCAAGATTTACTGCTCCTG 1188
X_maculatus_HSP70-2_AB062114       GTCCAGGCCGCCATCCTGACGGGAGACACGTCGGGGAACGTCCAGGACCTGCTGCTGCTG 1188
Human_HSPA1L_NM_005527             GTACAAGCAGCCATCCTGATGGGGGACAAGTCTGAGAAGGTACAGGACCTGCTGCTGCTG 1188
Human_HSPA6_NP_002146              GTGCAGGCGGCCGTGTTGATGGGGGACAAATGTGAGAAAGTGCAGGATCTCCTGCTGCTG 1188
Alligator_HSP70_AB306279           GTGCAGGCCGCCATCCTCATGGGCGACAAGTCGGAGAACGTGCAGGACCTGCTGCTGCTG 1191
                                   ** ** ** **  *  *    **                 *  * **  *  *  *  *

Drosophila_Bb_AF295957             GACGTGGCCCCACTTTCATTGGGAATTGAGACCGCTGGAGGTGTAATGACCAAGCTGATC 1236
Drosophila_Aa_AF295933             GACGTGGCCCCACTTTCATTGGGAATTGAGACCGCTGGAGGTGTAATGACCAAGCTGATC 1236
Spodoptera_FJ754276                GACGTGGCCCCTCTGTCCCTCGGCATCGAAACCGCCGGCGGCGTGATGACGAAGATCATC 1236
Bombyx_AB035326                    GACGTGGCTCCTCTGTCCCTCGGTATTGAGACAGCCGGCGGCGTGATGACGAAGATCATC 1236
Paralvinella_EF580992              GACGTGACGCCATTATCTTTAGGTCTGGAGACGGCTGGAGGCGTGATGACGAAACTGGTG 1251
B_plicatilis_HSP70-1_AB775784      GACGTGGCGCCTCTATCGCTGGGCATTGAGACGGCCGGCGGCGTCATGACCGCCCTCATC 1242
B_plicatilis_HSP70-2_AB775785      GACGTGGCGCCTCTATCGCTGGGCATTGAGACCGCCGGCGGCGTCATGACCGCCCTCATC 1242
A_brightwelli_HSP70_KX119429       GACGTTGCTCCTTTGTCTCTTGGTATCGAGACTGCCGGCGGTGTTATGACATCATTGATC 1239
Wuchereria_AF167352                GACGTCGCACCGCTTTCACTTGGTATTGTAACAGCTGGGGGTGTTATGACTGCGCTTATC 1242
Setaria_AF079360                   GACGTCGCACCGCTTTCACTTGGTATCGAGACTGCTGGTGGTGTTATGACTGCTCTCATT 1242
C_elegans_HSP70A_M18540            GACGTTGCCCCACTTTCCCTTGGTATTGAGACCGCTGGAGGAGTCATGACTGCTCTCATC 1245
C_elegans_HSP-1_NM_070667          GACGTTGCCCCACTTTCCCTTGGTATTGAGACCGCTGGAGGAGTCATGACTGCTCTCATC 1245
M_nipponense_KC460343              GATGTAACACCACTGTCTCTTGGTATTGAAACTGCTGGTGGTGTCATGACTGCCCTTATA 1242
M_nipponense_DQ660140              GATGTAACACCTTTGTCTCTTGGTATTGAAACTGCTGGAGGTGTTATGACAGCACTTATT 1242
Mytilus_HSP70_AY861684             GATGTTACCCCACTGTCTCTTGGTATTGAAACAGCTGGAGGTGTGATGACAGCTCTAATC 1245
Perinereis_KU255783                GATGTGACCCCCTTGTCCCTTGGTATCGAGACCGCGGGTGGCGTCATGACCTCCTTGATC 1239
Paralvinella_HSP70-2_EF580993      GATGTGACACCTCTGTCTCTGGGTATAGAGACAGCCGGTGGTGTGATGACGTCGCTGATC 1242
Haliotis_FJ812176                  GATGTCACTCCACTGTCACTTGGTATCGAGACTGCTGGCGGTGTGATGACTGTCCTCATC 1242
Oncorhynchus_HSC71_AAB21658        GACGTCACACCCCTCTCCCTGGGTATTGAGACCGCTGGAGGTGTCATGACCGTCCTGATC 1242
X_maculatus_HSC70_AB062115         GACGTCACGCCCTTGTCTCTGGGCATCGAGACGGCAGGCGGAGTCATGACCGTGCTGATC 1242
Human_HSPA8_HSC71_NP_006588        GATGTCACTCCTCTTTCCCTTGGTATTGAAACTGCTGGTGGAGTCATGACTGTCCTCATC 1242
Rattus_HSC71_NM_024351             GATGTCACTCCTCTTTCCCTTGGGATTGAAACTGCTGGTGGAGTCATGACTGTCCTCATC 1242
Pelodiscus_HSC70_HQ219723          GATGTCACTCCTCTGTCTCTGGGTATTGAAACAGCAGGTGGTGTCATGACAGTCCTGATC 1242
Bos_HSP70_U09861                   GACGTGGCTCCCCTGTCGCTGGGACTGGAGACGGCCGGAGGCGTGATGACCGCCCTGATC 1242
Human_HSPA1A_NM_005345             GACGTGGCTCCCCTGTCGCTGGGGCTGGAGACGGCCGGAGGCGTGATGACTGCCCTGATC 1242
Rattus_HSP70_1A_NM_031971          GACGTGGCGCCGCTGTCGCTGGGTCTGGAGACCGCGGGCGGCGTGATGACGGCGCTCATC 1242
X_maculatus_HSP70-1_AB062113       GATGTGGCACCCTTGTCGCTGGGCATTGAGACAGCCGGTGGCGTTATGACACCTCTTATC 1248
X_maculatus_HSP70-2_AB062114       GACGTGGCTCCTCTGTCCCTGGGGATCGAGACGGCCGGAGGAGTGATGACTGCCCTGATC 1248
Human_HSPA1L_NM_005527             GACGTGGCTCCCCTGTCCCTGGGGCTGGAGACGGCTGGGGGCGTGATGACTGCCCTGATA 1248
Human_HSPA6_NP_002146              GATGTGGCTCCCCTGTCTCTGGGGCTGGAGACAGCAGGTGGGGTGATGACCACGCTGATC 1248
Alligator_HSP70_AB306279           GACGTGACGCCGCTGTCGCTCGGCATCGAGACGGCGGGCGGCGTCATGACGGCGCTCATC 1251
                                   ** **  * **  * **  * **  * *  ** ** ** ** ** *****     *  *

Drosophila_Bb_AF295957             GAGCGCAACTGTCGCATTCCGTGCAAGCAGACTAAGACGTTCTCCACGTACTCGGACAAC 1296
Drosophila_Aa_AF295933             GAGCGCAACTGCCGCATTCCGTGCAAGCAGACTAAGACATTCTCCACATACGCGGACAAC 1296
Spodoptera_FJ754276                GAGCGCAACTGTAAGATCCCGTGCAAGCAGTCGCAGACGTTCACCACGTACTCGGACAAC 1296
Bombyx_AB035326                    GAACGAAACTCAAAGATTCCGTGCAAACAGTCTCAGACATTCACGACGTACTCAGACAAC 1296
Paralvinella_EF580992              GAACGTAACACGACCATTCCTCACAAGACGTCTAAGACATTCACCACCTACTCTGATAAT 1311
B_plicatilis_HSP70-1_AB775784      AAACGCAACACCACCATACCCACCAAGCAGACACAGACGTTCACCACTTATGCGGACAAC 1302
B_plicatilis_HSP70-2_AB775785      AAACGCAACACCACCATACCCACCAAGCAGACACAGACGTTCACCACTTATGCGGACAAC 1302
A_brightwelli_HSP70_KX119429       AAGAGAAACACCACAATCCCGACTAAACAAACCCAAGTTTTCACTACTTATTCAGACAAC 1299
Wuchereria_AF167352                AAGCGAAATACCACTATTCCCACGAAAACCTCCCAAACTTTTACAACATACTCCGACAAC 1302
Setaria_AF079360                   AAGCGAAACACTACCATCCCGACGAAAACGTCGCAAACCTTTACCACCTACTCTGATAAT 1302
C_elegans_HSP70A_M18540            AAGAGAAACACCACCATCCCAACCAAGACCGCTCAGACCTTCACAACCTATTCTGATAAC 1305
C_elegans_HSP-1_NM_070667          AAGAGAAACACCACCATCCCAACCAAGACCGCTCAGACCTTCACAACCTATTCTGATAAC 1305
M_nipponense_KC460343              AAAAGAAACACCACCATTCCAACCAAACAGACCCAGACCTTTACCACCTATTCTGACAAC 1302
M_nipponense_DQ660140              AAGCGTAACACAACAATCCCAACCAAGCAGACTCAGACATTCACCACTTATTCAGACAAT 1302
Mytilus_HSP70_AY861684             AAACGTAATACAACCATTCCAACAAAACAGACACAAACCTTCACTACCTACTCTGACAAT 1305
Perinereis_KU255783                AAGAGGAACACAACTATCCCCACCAAGCAGACACAGACCTTCACAACCTATTCCGACAAC 1299
Paralvinella_HSP70-2_EF580993      AAGCGCAACACGACCATTCCAACAAAACAGACCCAAACCTTCACCACCTACTCGGACAAT 1302
Haliotis_FJ812176                  AAGAGGAACACAACCATCCCCACCAAACAGACCCAGACCTTCACAACATACTCAGACAAT 1302
```

```
Oncorhynchus_HSC71_AAB21658         AAACGTAACACCACCATCCCAACCAAGCAGACTCAGACCTTCACCACCTACTCAGACAAC 1302
X_maculatus_HSC70_AB062115          AAGAGGAACACCACCATCCCCACCAAGCAGACCCAGACCTTCACAACTTACTCCGACAAC 1302
Human_HSPA8_HSC71_NP_006588         AAGCGTAATACCACCATTCCTACCAAGCAGACACAGACCTTCACTACCTATTCTGACAAC 1302
Rattus_HSC71_NM_024351              AAGCGCAATACCACCATTCCCACCAAGCAGACCCAGACTTTCACCACCTACTCTGACAAC 1302
Pelodiscus_HSC70_HQ219723           AAGCGGAACACTACAATTCCCACCAAGCAGACTCAGACATTCACCACATACTCTGACAAC 1302
Bos_HSP70_U09861                    AAGCGCAACTCCACCATCCCCACGAAGCAGACGCAGATCTTCACCACCTACTCGGACAAC 1302
Human_HSPA1A_NM_005345              AAGCGCAACTCCACCATCCCCACCAAGCAGACGCAGATCTTCACCACCTACTCCGACAAC 1302
Rattus_HSP70_1A_NM_031971           AAGCGCAACTCCACCATCCCCACCAAGCAGACGCAGACCTTCACCACCTACTCGGACAAC 1302
X_maculatus_HSP70-1_AB062113        AAGCGAAACACCACCATCCCCACAAAGCAGACTCAGATCTTCTCCACATACTCAGATAAC 1308
X_maculatus_HSP70-2_AB062114        AAACGCAACACCACCGTCCCCACCAAGCAGACCCAGGTGTTCAGCACCTACTCCGACAAC 1308
Human_HSPA1L_NM_005527              AAGCGCAACTCCACCATCCCCACCAAGCAGACACAGATTTTCACCACCTACTCTGACAAC 1308
Human_HSPA6_NP_002146               CAGAGGAACGCCACTATCCCCACCAAGCAGACCCAGACTTTCACCACCTACTCGGACAAC 1308
Alligator_HSP70_AB306279            AAGCGCAACACCACCATCCCCACCAAGCAGACGCAGACCTTCACCACCTACTCGGACAAC 1311
                                     *  * **        * **    **     *  *    **    ** **  * ** **

Drosophila_Bb_AF295957              CAGCCCGGAGTCTCCATCCAGGTGTATGAGGGCGAACGTGCGATGACGAAGGACAACAAT 1356
Drosophila_Aa_AF295933              CAGCCCGGAGTCTCCATCCAGGTGTATGAGGGCGAACGTGCGATGACGAAGGACAACAAT 1356
Spodoptera_FJ754276                 CAGCCAGCGGTCACGATCCAAGTGTACGAGGGCGAGCGAGCGATGACCAAGGACAACAAC 1356
Bombyx_AB035326                     CAGCCGGCCGTCACCATCCAGGTGTACGAGGGAGAGAGAGCGATGACAAAGGACAACAAC 1356
Paralvinella_EF580992               CAGCCTGCAGTGACCATCCAAGTGTACGAGGGCGAGCGAGCGATGACCAAAGACAACAAC 1371
B_plicatilis_HSP70-1_AB775784       CAGCCCGGCGTGTTGATTCAAGTGTACGAGGGCGAGAGGGCCATGACCAAGGACAATCAT 1362
B_plicatilis_HSP70-2_AB775785       CAGCCCGGCGTGTTGATTCAAGTGTACGAGGGCGAGAGGGCCATGACCAAGGACAATCAT 1362
A_brightwelli_HSP70_KX119429        CAGCCGGGAGTGTTGATTCAAGTCTACGAAGGCGAAAGAGCCATGACTAAAGACAACAAT 1359
Wuchereria_AF167352                 CAGCCTGGTGTCCTTATTCAGGTTTACGAAGGTGAACGTGCTTTGACCAAAGACAATAAC 1362
Setaria_AF079360                    CAGCCAGGTGTTCTTATTCAGGTTTATGAAGGCGAACGTGCTATGACCAAGGACAATAAT 1362
C_elegans_HSP70A_M18540             CAACCAGGAGTGTTGATCCAGGTTTACGAAGGAGAACGTGCCATGACCAAGGACAACAAC 1365
C_elegans_HSP-1_NM_070667           CAACCAGGAGTGTTGATCCAGGTTTACGAAGGAGAACGTGCCATGACCAAGGACAACAAC 1365
M_nipponense_KC460343               CAGCCTGGAGTGCTCATTCAAGTTTACGAAGGAGAACGTGCCATGACAAAAGACAATAAC 1362
M_nipponense_DQ660140               CAGCCTGGTGTGTTAATTCAAGTTTATGAAGGAGAGCGTGCAATGACAAAAGATAACAAC 1362
Mytilus_HSP70_AY861684              CAGCCTGGTGTATTAATCCAGGTTTATGAAGGAGAGAGAGCTATGACCAAGGACAACAAC 1365
Perinereis_KU255783                 CAGCCAGGTGTGTTGATCCAAGTTTACGAGGGTGAGCGTGCCATGACCAAGGACAACAAC 1359
Paralvinella_HSP70-2_EF580993       CAGCCCGGTGTCTTGATCCAGGTGTACGAGGGAGAGCGTGCCATGACCAAAGACAACAAC 1362
Haliotis_FJ812176                   CAACCTGGTGTGCTGATTCAGGTATTCGAAGGTGAGAGAGCCATGACAAAGGATAACAAC 1362
Oncorhynchus_HSC71_AAB21658         CAGCCTGGTGTGCTCATTCAGGTGTATGAGGGTGAGAGGGCCATGACCAAGGACAACAAC 1362
X_maculatus_HSC70_AB062115          CAGCCTGGCGTGCTCATTCAGGTGTTTGAGGGCGAAAGGGCAATGACCAAAGATAACAAC 1362
Human_HSPA8_HSC71_NP_006588         CAGCCTGGTGTGCTTATTCAGGTTTATGAAGGCGAGCGTGCCATGACAAAGGATAACAAC 1362
Rattus_HSC71_NM_024351              CAGCCAGGTGTACTCATCCAGGTGTATGAAGGTGAAAGGGCCATGACCAAGGACAACAAC 1362
Pelodiscus_HSC70_HQ219723           CAGCCTGGTGTACTCATTCAGGTCTACGAAGGGGAGAGAGCCATGACAAAAGACAACAAC 1362
Bos_HSP70_U09861                    CAGCCGGGCGTGCTGATCCAGGTGTACGAGGGCGAGAGGGCCATGACGCGGGACAACAAC 1362
Human_HSPA1A_NM_005345              CAACCCGGGGTGCTGATCCAGGTGTACGAGGGCGAGAGGGCCATGACGAAAGACAACAAT 1362
Rattus_HSP70_1A_NM_031971           CAGCCCGGGGTGCTGATCCAGGTGTACGAGGGCGAGAGGGCCATGACGCGCGACAACAAC 1362
X_maculatus_HSP70-1_AB062113        CAGCCAGGAGTCCTGATTCAGGTCTATGAAGGTGAGAGAGCCATGACAAAGGACAACAAT 1368
X_maculatus_HSP70-2_AB062114        CAGCCCGGGGTCCTGATCCAGGTCTACGAAGGGGAGAGAGCCATGACCAAGGACAACAAC 1368
Human_HSPA1L_NM_005527              CAACCCGGGGTGCTGATCCAGGTGTATGAGGGCGAGAGGGCCATGACAAAGGACAACAAC 1368
Human_HSPA6_NP_002146               CAGCCTGGGGTCTTCATCCAGGTGTATGAGGGTGAGAGGGCCATGACCAAGGACAACAAC 1368
Alligator_HSP70_AB306279            CAGAGCAGCGTGCTGGTGCAGGTGTACGAGGGCGAGCGCGCCATGACCAAGGACAACAAC 1371
                                    **       **     * ** ** *  ** ** **  * **  ****    ** **  *

Drosophila_Bb_AF295957              GCATTGGGCACCTTCGATCTGTCCGGCATTCCACCTGCACCAAGGGGTGTGCCCCAGATA 1416
Drosophila_Aa_AF295933              GCATTGGGCACCTTCGATCTGTCCGGCATTCCACCTGCACCAAGGGGTGTGCCCCAGATA 1416
Spodoptera_FJ754276                 CTGCTGGGCACGTTCGACTTGACCGGCATCCCGCCCGCGCCTCGCGGTGTCCCTAAGATC 1416
Bombyx_AB035326                     CTACTGGGCACGTTCGACCTGACGGGGATCCCGCCCGCGCCGCGAGGAGTGCCCAAGATC 1416
Paralvinella_EF580992               CTCCTCGGACGATTTGAGCTCAGCGGTATTCCGCCAGCTCCACGCGGAATTCCCAAGATC 1431
B_plicatilis_HSP70-1_AB775784       TTGTTGGGCAAGTTTGAGCTGTCGGGCATACCGCCGGCGCCCCGCGGCGTGCCCCAAATC 1422
B_plicatilis_HSP70-2_AB775785       TTGTTGGGCAAGTTTGAGCTGTCGGGCATACCGCCGGCGCCCCGCGGCGTGCCCCAAATC 1422
A_brightwelli_HSP70_KX119429        TTACTCGGTAAATTTGAGCTTACTGGCATTCCACCGGCGCCAAGAGGTGTTCCTCAAATC 1419
Wuchereria_AF167352                 TTGCTTGGTAAATTCGAGTTGTCTGGGATTCCGCCTGCTCCTCGCGGTGTGCCGCAGATT 1422
Setaria_AF079360                    TTGCTCGGTAAATTTGAGTTGTCTGGAATCCCGCCTGCTCCTCGTGGTGTGCCACAAATA 1422
C_elegans_HSP70A_M18540             TTGCTCGGAAAGTTCGAGCTCTCCGGAATCCCACCAGCACCACGCGGAGTCCCACAAATC 1425
C_elegans_HSP-1_NM_070667           TTGCTCGGAAAGTTCGAGCTCTCCGGAATCCCACCAGCACCACGCGGAGTCCCACAAATC 1425
M_nipponense_KC460343               ATTTTGGGTAAGTTTGAGCTGACTGGTATTCCCCCAGCGCCAAGGGGAGTGCCACAAATC 1422
M_nipponense_DQ660140               CTGCTAGGTAAATTTGAACTGTCTGGTATTCCTCCAGCTCCACGAGGAGTGCCACAAATT 1422
Mytilus_HSP70_AY861684              TTGCTTGGAAAGTTTGAATTAACTGGAATACCTCCAGCACCAAGAGGTGTGCCCCAGATT 1425
Perinereis_KU255783                 CTCTTGGGTAAATTCGAGCTGTCCGGCATTCCCCCTGCTCCTCGTGGCGTTCCCCAGATC 1419
Paralvinella_HSP70-2_EF580993       CTGCTGGGAAAGTTCGAGCTGACTGGCATTCCACCTGCTCCTCGTGGTGTGCCACAGATA 1422
Haliotis_FJ812176                   ATCTTGGGCAAGTTTGAGCTGACTGGAATTCCCCCAGCACCCAGAGGTGTCCCACAGATT 1422
Oncorhynchus_HSC71_AAB21658         CTGTTGGGCAAGTTTGAGCTGACTGGAATCCCCCCTGCACCTCGCGGTGTTCCTCAGATT 1422
X_maculatus_HSC70_AB062115          CTGCTCGGGAAATTTGAGCTGACGGGAATACCGCCAGCTCCTCGGGGGGTCCCTCAGATT 1422
Human_HSPA8_HSC71_NP_006588         CTGCTTGGCAAGTTTGAACTCACAGGCATACCTCCTGCACCCCGAGGTGTTCCTCAGATT 1422
Rattus_HSC71_NM_024351              CTGCTTGGGAAGTTTGAGCTCACAGGCATACCTCCAGCACCCCGTGGGGTTCCTCAGATT 1422
Pelodiscus_HSC70_HQ219723           TTGCTGGGCAAGTTTGAGCTGACTGGCATTCCTCCAGCTCCAAGAGGTGTACCTCAAATT 1422
Bos_HSP70_U09861                    CTGCTGGGGCGCTTCGAGCTGAGCGGCATCCCGCCGGCCCCGCGGGGGGTGCCCCAGATC 1422
Human_HSPA1A_NM_005345              CTGTTGGGGCGCTTCGAGCTGAGCGGCATCCCTCCGGCCCCCAGGGGCGTGCCCCAGATC 1422
Rattus_HSP70_1A_NM_031971           CTGCTGGGGCGCTTCGAGTTGAGCGGCATCCCGCCGGCTCCCAGGGGCGTGCCCCAGATC 1422
X_maculatus_HSP70-1_AB062113        CTCCTGGGGAAGTTTGAGCTCTCTGGTCTTCCTCCTGCTCCCAGAGGCGTGCCACAGATC 1428
X_maculatus_HSP70-2_AB062114        CTGCTGGGCAGGTTTGAGCTGACGGGAATCCCGCCCGCTCCACGAGGGGTCCCGCAGATC 1428
Human_HSPA1L_NM_005527              CTGCTGGGGCGGTTTGACCTGACTGGAATCCCTCCAGCACCCAGGGGAGTTCCTCAGATC 1428
Human_HSPA6_NP_002146               CTGCTGGGGCGTTTTGAACTCAGTGGCATCCCTCCTGCCCCACGTGGAGTCCCCCAGATA 1428
Alligator_HSP70_AB306279            CTGCTGGGCAAGTTCGACCTGACCGGCATCCCGCCCGCGCCGCGCGGCGTGCCGCAGATC 1431
```

```
                                                  * **    ** **  *    **  * ** ** ** **  * **  * **  * **
Drosophila_Bb_AF295957                GAAGTAACCTTCGACTTGGACGCCAATGGAATCCTGAACGTCAGCGCCAAGGAGATGAGC 1476
Drosophila_Aa_AF295933                GAAGTTACCTTCGACTTGGACGCCAATGGAATCCTGAACGTCAGCGCCAAGGAGATGAGC 1476
Spodoptera_FJ754276                   GACGTCACTTTCGACCTGGACGCGAACGGCATTCTCAACGTGTCGGCCAAAGAGAACAGC 1476
Bombyx_AB035326                       GACGTGACGTTCGACATGGACGCTAACGGCATCCTGAACGTGTCGGCCAAGGAGAACAGC 1476
Paralvinella_EF580992                 GACGTCACATTCGACATCGACGCCAACGGAATCCTACACGTGACGGCCAAAGACCAAAGT 1491
B_plicatilis_HSP70-1_AB775784         GAGGTCACATTTGACATTGATGCCAATGGTATTCTGAATGTGTCCGCCGCAGACAAATCG 1482
B_plicatilis_HSP70-2_AB775785         GAGGTCACATTTGACATTGATGCCAATGGTATTCTGAATGTGTCCGCTGCAGACAAATCG 1482
A_brightwelli_HSP70_KX119429          GAGGTCACTTTTGACATCGATGCCAACGGTATTTTGAACGTTAGCGCTTGTGATAAGTCA 1479
Wuchereria_AF167352                   GAAGTCGACTTTGATATTGATGCCAACGGTATTCTGAATGTTTCCGCCCAGGATAAATCC 1482
Setaria_AF079360                      GAAGTTACTTTTGATATTGATGCAAACGGTATCTTGAATGTTTCTGCGCAAGACAAATCT 1482
C_elegans_HSP70A_M18540               GAAGTCACTTTCGATATTGACGCCAACGGAATCTTGAACGTCTCTGCCACTGACAAGTCC 1485
C_elegans_HSP-1_NM_070667             GAAGTCACTTTCGATATTGACGCCAACGGAATCTTGAACGTCTCTGCCACTGACAAGTCC 1485
M_nipponense_KC460343                 GAAGTAACATTTGATATTGATGCTAATGGTATTCTAAATGTATCTGCTGCTGACAAGTCC 1482
M_nipponense_DQ660140                 GAAGTAACTTTTGATATTGATGCCAATGGTATCTTGAATGTATCTGCTGCAGACAAGTCA 1482
Mytilus_HSP70_AY861684                GAAGTGACCTTTGACATTGATGCCAATGGTATCCTGAATGTATCTGCAGTAGATAAGAGT 1485
Perinereis_KU255783                   GAGGTTACCTTCGATATTGATGCCAACGGTATCCTGAATGTCACTGCTGCGGACAAGAGC 1479
Paralvinella_HSP70-2_EF580993         GAGGTCACATTCGATATCGATGCCAATGGTATCCTTAACGTGTCTGCTGTAGACAAGAGT 1482
Haliotis_FJ812176                     GAAGTAACCTTTGATATTGATGCCAACGGTATCCTCAATGTATCAGCTGTAGACAAGAGC 1482
Oncorhynchus_HSC71_AAB21658           GAGGTCACATTTGACATTGATGCTAACGGCATCATGAACGTGTCTGCTGCTGACAAGAGC 1482
X_maculatus_HSC70_AB062115            GAGGTGACATTCGATATCGACGCTAACGGCATCATGAACGTCTCCGCTGTCGACAAGAGC 1482
Human_HSPA8_HSC71_NP_006588           GAAGTCACTTTTGACATTGATGCCAATGGTATACTCAATGTCTCTGCTGTGGACAAGAGT 1482
Rattus_HSC71_NM_024351                GAGGTTACTTTTGACATTGATGCCAATGGCATCCTCAATGTTTCTGCTGTAGATAAGAGC 1482
Pelodiscus_HSC70_HQ219723             GAAGTAACATTTGATATTGATGCCAATGGCATCCTGAATGTGTCTGCTGTGGACAAGAGC 1482
Bos_HSP70_U09861                      GAGGTGACCTTCGACATCGACGCCAATGGCATCCTGAACGTCACGGCCACGGACAAGAGC 1482
Human_HSPA1A_NM_005345                GAGGTGACCTTCGACATCGATGCCAACGGCATCCTGAACGTCACGGCCACGGACAAGAGC 1482
Rattus_HSP70_1A_NM_031971             GAGGTGACCTTCGACATCGACGCCAACGGCATCCTGAACGTCACGGCCACTGACAAGAGC 1482
X_maculatus_HSP70-1_AB062113          GAAGTTACCTTTGATATCGACGCCAACGGCATCCTAAATGTGTCTGCCGTCGACAAAAGC 1488
X_maculatus_HSP70-2_AB062114          GAGGTCACCTTTGACGTGGACGCCAACGGCATCCTGAACGTGTCCGCGGTGGACAAAAGC 1488
Human_HSPA1L_NM_005527                GAGGTGACGTTTGACATTGATGCCAATGGTATTCTCAATGTCACAGCCACGGACAAGAGC 1488
Human_HSPA6_NP_002146                 GAGGTGACTTTTGACATTGATGCTAATGGCATCCTGAGCGTGACAGCCACTGACAGGAGC 1488
Alligator_HSP70_AB306279              GAGGTCACTTTCGACATCGACGCCAACGGCATCCTCAACGTGAGCGCGGTCGACAAGAGC 1491
                                      ** **    ** **  * ** ** ** ** **  *    **    **    **

Drosophila_Bb_AF295957                ACGGGCAAGGCCAAGAACATCACGATCAAGAACGACAAGGGACGCCTCTCGCAGGCCGAG 1536
Drosophila_Aa_AF295933                ACGGGCAAGGCCAAGAACATCACGATCAAGAACGACAAGGGACGGCTCTCGCAGGCCGAG 1536
Spodoptera_FJ754276                   ACCGGCCGAAGCAAGAACATCGTGATCAAGAACGACAAGGGACGCCTGTCGCAGGCCGAG 1536
Bombyx_AB035326                       ACCGGCCGCAGCAAGAACATCGTGATCAAGAACGACAAGGGTCGTCTCTCGCAAGCGGAG 1536
Paralvinella_EF580992                 ACTGGTAGGTCAAGTGATATCCACATCAAGAACGAGAAAGGTCGTTTGTCACAGGCCGAA 1551
B_plicatilis_HSP70-1_AB775784         ACTGGCAAGACGAACAAAATCACCATCACCAATGACAAGGGAAGACTTAGCAAAGAGGAG 1542
B_plicatilis_HSP70-2_AB775785         ACTGGCAAGACGAACAAAATCACCATCACCAATGACAAGGGAAGACTTAGCAAAGAGGAC 1542
A_brightwelli_HSP70_KX119429          ACTGGCAAACAGAACAAGATCACGATCACGAACGACAAAGGAAGACTCAGCAAAGAGGAC 1539
Wuchereria_AF167352                   ACAGGCAAACAAAACAAAATTACCATTACCAATGATAAAGGACGTTTGTCAAAGGATGAG 1542
Setaria_AF079360                      ACTGGTAAACAAAATAAGATAACTATTACTAATGACAAGGGAAGGTTATCGAAGGATGAG 1542
C_elegans_HSP70A_M18540               ACCGGAAAGGCAAAACAGATCACCATCACCAACGACAAGGATCGCTTTTCCAAGGATGAC 1545
C_elegans_HSP-1_NM_070667             ACCGGAAAGCAAAACAAGATCACCATCACCAACGACAAGGGACGTCTTTCCAAGGATGAC 1545
M_nipponense_KC460343                 ACAGGCAAAGAGAACAAGATCACCATCACTAATGATAAGGGCCGCTTGAGCAAGGAAGAG 1542
M_nipponense_DQ660140                 ACTGGTAAGGAGAACAAAATCACAATCACAAATGACAAGGGTCGTCTCAGCAAAGAAGAA 1542
Mytilus_HSP70_AY861684                ACTGGTAAAGAGAACAAAATCACCATCACTAACGACAAAGGTCGTTTGAGCAAAGAAGAA 1545
Perinereis_KU255783                   ACCGGCAAGGAGAACAAGATTACCATCACCAACGACAAAGGCCGTCTCAGTAAGGAAGAT 1539
Paralvinella_HSP70-2_EF580993         ACTGGTAGAGAGAACAAGATCACTATCACCAATGATAAGGGTCGTCTAAGCAAAGAGGAG 1542
Haliotis_FJ812176                     ACTATGAAAGAGAACAAGATCACCATCACAAACGACAAAGGTCGCTTGTCGAAGGAAGAG 1542
Oncorhynchus_HSC71_AAB21658           ACTGGGAAGGAGAACAAGATCACCATCACAAATGACAAGGGTCGCCTGAGCAAGGAGGAC 1542
X_maculatus_HSC70_AB062115            ACCGGGAAGGAGAACAAAATCACCATCACAAACGACAAGGGCCGCTTGAGCAAAGAGGAT 1542
Human_HSPA8_HSC71_NP_006588           ACGGGAAAAGAGAACAAGATTACTATCACTAATGACAAGGGCCGTTTGAGCAAGGAAGAC 1542
Rattus_HSC71_NM_024351                ACAGGAAAGGAGAACAAGATCACCATCACCAATGACAAGGGCCGCTTGAGTAAGGAGGAT 1542
Pelodiscus_HSC70_HQ219723             ACTGGCAAGGAGAACAAGATCACCATTACCAATGACAAAGGGCGATTAAGCAAGGAAGAC 1542
Bos_HSP70_U09861                      ACGGGCAAGGCCAACAAGATCACCATCACCAACGACAAGGGCCGGCTGAGCAAGGAGGAG 1542
Human_HSPA1A_NM_005345                ACCGGCAAGGCCAACAAGATCACCATCACCAACGACAAGGGCCGCCTGAGCAAGGAGGAG 1542
Rattus_HSP70_1A_NM_031971             ACCGGCAAGGCCAACAAGATCACCATCACCAACGACAAGGGCCGCCTGAGCAAGGAGGAG 1542
X_maculatus_HSP70-1_AB062113          ACGGGCAAAGAAAACAAAATCACAATCACCAACGACAAGGGCCGACTCAGCAAAGATGAG 1548
X_maculatus_HSP70-2_AB062114          ACCGGCAAAGAGAACAAGATCACCATCGCCAACGACAAGGGCCGACTGAGCAAAGACGAG 1548
Human_HSPA1L_NM_005527                ACCGGCAAGGTGAACAAGATCACCATCACCAATGACAAGGGCCGCCTGAGCAAGGAGGAG 1548
Human_HSPA6_NP_002146                 ACAGGTAAGGCTAACAAGATCACCATCACCAATGACAAGGGCCGGCTGAGCAAGGAGGAG 1548
Alligator_HSP70_AB306279              ACGGGCAAGGAGAACAAGATCACCATCACCAACGACAAGGGCCGGCTCAGCAAGGACGAC 1551
                                      **          *   * **    **    ** ** ** *   *  *     * *  **

Drosophila_Bb_AF295957                ATTGATCGCATGGTGAACGAGGCTGAGAAGTACGCCGACGAGGACGAAAAGCATCGCCAG 1596
Drosophila_Aa_AF295933                ATTGATCGCATGGTGAACGAGGCTGAAAAGTACGCCGACGAGGACGAGAAGCATCGCCAG 1596
Spodoptera_FJ754276                   ATCGAGCGCATGCTCGCCGAGGCCGAGAAGTACAAGGACGAGGACGAGAAGCAGAGGCAG 1596
Bombyx_AB035326                       ATCGATCGCATGCTGTCCGAGGCAGAGCGGTACAAGGAGGAAGACGAGAAGCAGAGACAG 1596
Paralvinella_EF580992                 ATCGACAGGATGTTGGCCGAAGCTGAGAAATATCGCGAAGAAGACGAGAAACAACGAGAA 1611
B_plicatilis_HSP70-1_AB775784         ATCGACCGAATGGTCAACGAGGCCGAGAAGTACAAGAAGGATGACGAGGAGCAGAGAGAT 1602
B_plicatilis_HSP70-2_AB775785         ATCGATCGAATGGTCAATGAGGCCGAGAAGTACAAGAAAGATGACGAGGAGCAGAGAGAT 1602
A_brightwelli_HSP70_KX119429          ATTGAGAGAATGGTCAACGATGCCGAGAAGTATAAAAAAGACGACGAGCAACAAAGAGAG 1599
Wuchereria_AF167352                   ATCGAGCGGATGGTACAGGAAGCTGAGAAATACAAGGCGGATGATGAAGCACAGAAGGAT 1602
Setaria_AF079360                      ATCGAACGGATGGTGCAAGAAGCTGAGAAATATAAGGCAGATGATGAAGCTCAGAAAGAC 1602
C_elegans_HSP70A_M18540               ATTGAACGCATGGTCAACGAAGCTGAGAAATACAAGGCTGACGATGAGGCCCAAAAGGAC 1605
```

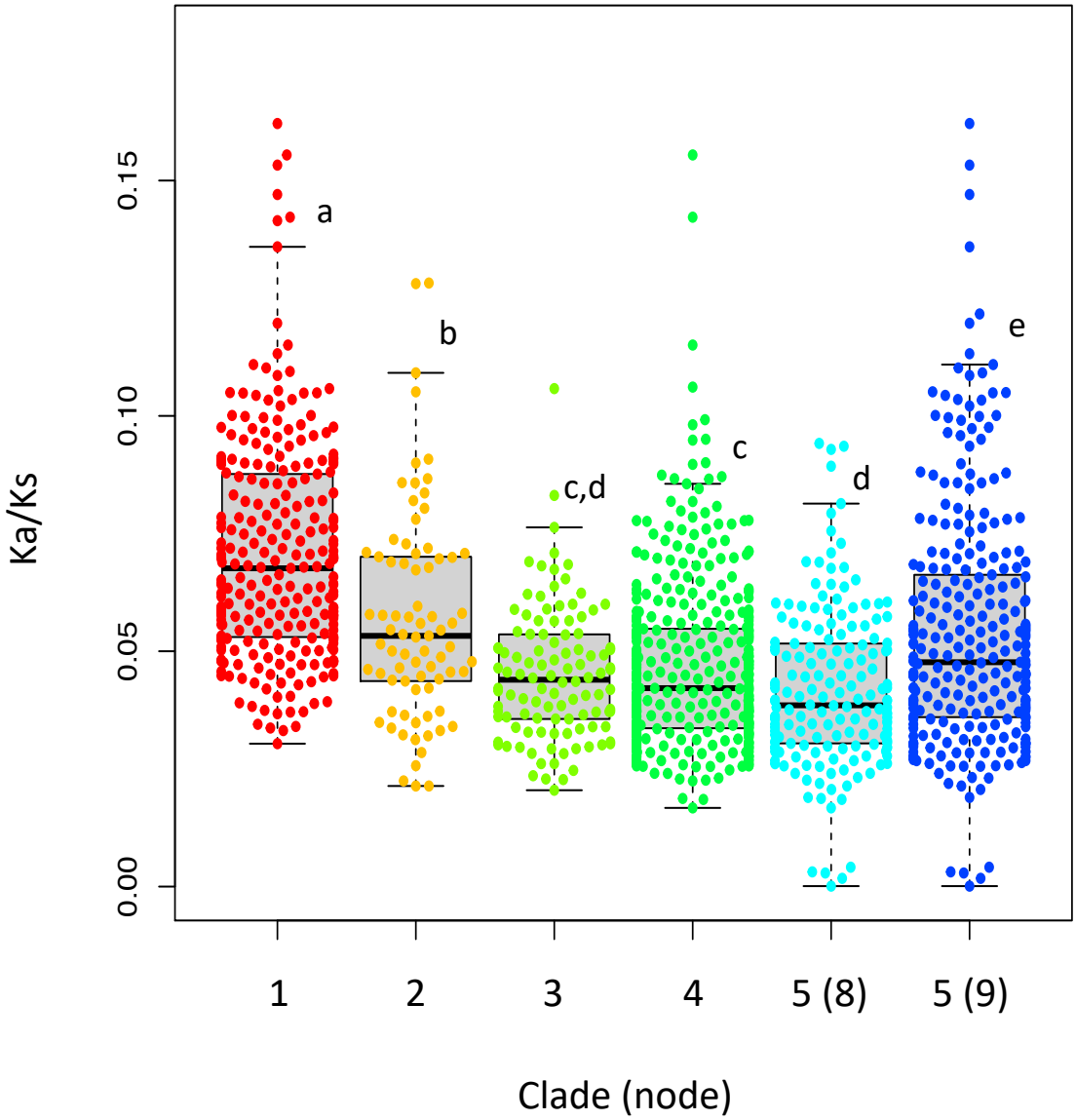
```
C_elegans_HSP-1_NM_070667          ATTGAACGCATGGTCAACGAAGCTGAGAAATACAAGGCTGACGATGAGGCCCAAAAGGAC 1605
M_nipponense_KC460343              ATTGAGCGGATGGTGCAAGAGGCCGAGAAGTACAAAGCTGACGATGAGAAACAGCGGGAG 1602
M_nipponense_DQ660140              ATTGAACGTATGGTTCAAGAAGCTGAAAAGTACAAAGCTGATGATGAGAAGCAACGTGAT 1602
Mytilus_HSP70_AY861684             ATTGAACGCATGGTCAATGATGCTGAGAAATACAAGGCAGAAGACGAGAAACAGAAGGAC 1605
Perinereis_KU255783                ATTGACCGCATGGTTAACGAAGCCGAGCGTCTCAAGGCTGAAGATGATGCGCAGAGAGAA 1599
Paralvinella_HSP70-2_EF580993      ATTGAACGTATGGTCAAGGATGCTGAGAGGTACAAGCAAGAAGATGAAAGTCAGAAGGAT 1602
Haliotis_FJ812176                  ATTGAGCGAATGGTTAATGAGGCAGAGAACTACAAGGCCGAGGATGAGAAGCAGAAGGAT 1602
Oncorhynchus_HSC71_AAB21658        ATTGAGCGCATGGTCCAGGAGGCTGAGAAGTACAAGTGTGAGGATGATGTGCAGCGTGAC 1602
X_maculatus_HSC70_AB062115         ATTGAGCGCATGGTTCACGAAGCGGAGACATACAGGGCTGAAGACGACTTGCAGCGAGAA 1602
Human_HSPA8_HSC71_NP_006588        ATTGAACGTATGGTCCAGGAAGCTGAGAAGTACAAAGCTGAAGATGAGAAGCAGAGGGAC 1602
Rattus_HSC71_NM_024351             ATTGAGCGCATGGTCCAAGAAGCTGAGAAGTACAAAGCTGAGGATGAGAAGCAGAGAGAT 1602
Pelodiscus_HSC70_HQ219723          ATTGAACGCATGGTGCAGGAAGCAGAAAAATACAAAGCAGAAGATGAGAAACAGCGTGAC 1602
Bos_HSP70_U09861                   ATCGAGCGCATGGTGCAGGAGGCGGAAAAGTACAAGGCGGAGGACGAGGTGCAGCGCGAG 1602
Human_HSPA1A_NM_005345             ATCGAGCGCATGGTGCAGGAGGCGGAGAAGTACAAAGCGGAGGACGAGGTGCAGCGCGAG 1602
Rattus_HSP70_1A_NM_031971          ATCGAGCGCATGGTGCAGGAGGCCGAGCGCTACAAGGCGGAGGACGAGGTGCAGCGCGAG 1602
X_maculatus_HSP70-1_AB062113       ATCGAGAAGATGGTGCAGGATGCAGACAAGTACAAGGCGGAGGATGACCAGCAGAGAGAA 1608
X_maculatus_HSP70-2_AB062114       ATCGAGAGGATGGTGCAGGACGCCGAGAAGTACAAAGCCGAGGACGAGCTGCAGAGGGAC 1608
Human_HSPA1L_NM_005527             ATTGAGCGCATGGTTCTGGATGCTGAGAAATATAAAGCTGAAGATGAGGTCCAGAGGGAG 1608
Human_HSPA6_NP_002146              GTGGAGAGGATGGTTCATGAAGCCGAGCAGTACAAGGCTGAGGATGAGGCCCAGAGGGAC 1608
Alligator_HSP70_AB306279           ATCGACCGCATGGTGCAGGAGGCCGAGAAGTACAAGGCGGAGGACGACGCCAACCGCGAG 1611
                                    * **    *** *    ** ** **             ** ** **     *     *

Drosophila_Bb_AF295957             CGCATAACCTCTAGAAATGCTCTGGAGAGCTACGTATTCAACGTAAAGCAGTCCGTGGAG 1656
Drosophila_Aa_AF295933             CGAATAACCTCTAGAAATGCCCTGGAGAGCTACGTCTTCAATGTGAAGCAGGCCGTGGAA 1656
Spodoptera_FJ754276                CGCGTAGCGTCTCGGAACCAGCTCGAGTCGTACGTGTTCAGCGTGAAGCAGGCGCTGGAC 1656
Bombyx_AB035326                    CGCGTGGCCGCTCGGAACCAGCTCGAATTGTATTTGTTCAGCGTGAAGCAGGCGCTCGAC 1656
Paralvinella_EF580992              CGTATCTCGGCCAGAAACAGCTTGGAACAATACATCTACTCATACAAAGAGGCCGCTACT 1671
B_plicatilis_HSP70-1_AB775784      AAGGTGGCCGCCAAGAACTCGCTTGAGTCCTACTGCTTTAACATGAAACAAACGGTGGAG 1662
B_plicatilis_HSP70-2_AB775785      AAGGTGGCCGCCAAGAACTCGCTTGAGTCCTACTGCTTTAACATGAAACAAACGGTGGAG 1662
A_brightwelli_HSP70_KX119429       AGAATCACTGCCAAGAACTCGTTGGAATCCTACTGCTTCAACATGAAACAAACCATCGAG 1659
Wuchereria_AF167352                CGTATTGCGGCAAAAAATGCTCTGGAATCGTACGCATTCAATATGAAACAAACGATCGAG 1662
Setaria_AF079360                   CGTATTGCGGCAAAGAATGCTCTTGAATCATATGCGTTCAATATGAAGCAAACAATTGAG 1662
C_elegans_HSP70A_M18540            CGTATTGGAGCCAAGAACGGACTCGAGTCATACGCCTTCAACCTTAAGCAGACCATTGAG 1665
C_elegans_HSP-1_NM_070667          CGTATTGGAGCCAAGAACGGACTCGAGTCATACGCCTTCAACCTTAAGCAGACCATTGAG 1665
M_nipponense_KC460343              CGTATCTCTGCCAAGAACAACCTGGAGTCTTACTGTTTCAATATGAAGTCCACTGTTGAA 1662
M_nipponense_DQ660140              CGCATTGCAGCAAAGAATAGTTTGGAATCTTACTGCTTCAACATGAAATCCACAGTGGAA 1662
Mytilus_HSP70_AY861684             CGTATCACCGCCAAAAATAGTCTAGAAAGCTACTCATTTAACATGAAACAAACAGTTGAA 1665
Perinereis_KU255783                CGTATCACAGCCAAGAACCAGCTGGAGAGCTATGCGTTCAACATGAAATCAACTGTCGAG 1659
Paralvinella_HSP70-2_EF580993      CGCATCCAAGCCAAGAATGCCCTGGAGAGCTATGCCTTTAACATGAAGTCGACTGTCGAG 1662
Haliotis_FJ812176                  CGCATCCAGGCCAAGAACGGTTTGGAAAGCTATGCCTTCAACATGAAGTCGACTGTAGAG 1662
Oncorhynchus_HSC71_AAB21658        AAGGTCTCTTCTAAGAACTCCCTAGAGTCCTACGCTTTCAACATGAAGTCTACTGTGGAG 1662
X_maculatus_HSC70_AB062115         AAGGTGGTGGCCAAGAACAGCCTGGAGTCCTACACTTTCAACATGAGGTCCACCATAGAG 1662
Human_HSPA8_HSC71_NP_006588        AAGGTGTCATCCAAGAATTCACTTGAGTCCTATGCCTTCAACATGAAAGCAACTGTTGAA 1662
Rattus_HSC71_NM_024351             AAGGTTTCCTCTAAGAACTCGCTGGAGTCTTATGCTTTCAACATGAAAGCAACTGTTGAG 1662
Pelodiscus_HSC70_HQ219723          AAAGTTTCCTCTAAGAATTCTCTAGAATCATATGCCTTTAATATGAAGGCTACAGTTGAA 1662
Bos_HSP70_U09861                   AGGGTGTCTGCCAAGAACGCGCTGGAGTCGTACGCCTTCAACATGAAGAGCGCCGTGGAG 1662
Human_HSPA1A_NM_005345             AGGGTGTCAGCCAAGAACGCCCTGGAGTCCTACGCCTTCAACATGAAGAGCGCCGTGGAG 1662
Rattus_HSP70_1A_NM_031971          AGGGTGGCTGCCAAGAATGCGCTCGAGTCCTATGCCTTCAACATGAAGAGCGCCGTGGAG 1662
X_maculatus_HSP70-1_AB062113       AAGATCGCGGCAAAGAATTCCCTGGAGTCCTACGCTTACCACATGAAGAGCAGCGTCGAA 1668
X_maculatus_HSP70-2_AB062114       AAGATAGCTGCCAAGAACTCCCTGGAGTCCTACGCTTTCACCGTGAAGAGCAGCGTGGAG 1668
Human_HSPA1L_NM_005527             AAAATTGCTGCAAAGAATGCCTTAGAATCCTATGCTTTTAACATGAAGAGTGTTGTGAGT 1668
Human_HSPA6_NP_002146              AGAGTGGCTGCCAAAAACTCGCTGGAGGCCCATGTCTTCCATGTGAAAGGTTCTTTGCAA 1668
Alligator_HSP70_AB306279           CGGGTGGCGGCCAAGAACTCGCTCGAGTCCTACGCCTACAACATGAAGCAGACGGTGGAG 1671
                                       *     *    **     * **     *    *        *

Drosophila_Bb_AF295957             CAGGCGCC---CGCTGGCAAACTGGACGAGGCCGACAAGAACTCCGTCCTGGACAAGTGC 1713
Drosophila_Aa_AF295933             CAGGCACC---TGCTGGCAAATTGGACGAGGCTGACAAGAACTCCGTCTTGGACAAGTGC 1713
Spodoptera_FJ754276                GAGGCCGG------AGACAAGCTCTCGGAACAGGACAAGAGCACGGCGAGGGACGCGTGC 1710
Bombyx_AB035326                    GAGGCCGG------CGACAAACTGAGCGACGCGGACAAGAGCACGGCGCGCGACGCGTGT 1710
Paralvinella_EF580992              GAGGC---CGATAGTTCTAAACTGTCACAGACTGATAAGGATCAAGTGATCAACAAATGT 1728
B_plicatilis_HSP70-1_AB775784      GACGAAAAGCTGGCTGCTAAGATTAGTGCCGACGACAAGAAGAAGATTTTGGACGCGTGT 1722
B_plicatilis_HSP70-2_AB775785      GACGAAAAGCTGGCTGCTAAGATTAGTGCAGATGACAAGAAGAAGATTTTGGACGCGTGT 1722
A_brightwelli_HSP70_KX119429       GACGAGAAATTGGCAGCTAAAGTCTCGGCTGATGAAAAGAAGAAGATTTTGGACGCGTGC 1719
Wuchereria_AF167352                GACGAGAAGTTAAAAGATAAAATTTCGGAAGAGGATAAGAAAAAATTCAAGAAAAATGC 1722
Setaria_AF079360                   GATGAAAAGCTGAGGGACAAACTATCTGAGGAGGATAAGAAGAAGATCCAGGAGAAGTGT 1722
C_elegans_HSP70A_M18540            GACGAGAAGCTCAAGGATAAGATCAGCCCAGAAGACAAGAAGAAGATCGAGGACAAGTGC 1725
C_elegans_HSP-1_NM_070667          GACGAGAAGCTCAAGGATAAGATCAGCCCAGAAGACAAGAAGAAGATCGAGGACAAGTGC 1725
M_nipponense_KC460343              GATGACAAATTCAAGGATAAGGTTCCTGAGGATGATCGCAACAAGATCATGGAAGCCTGT 1722
M_nipponense_DQ660140              GATGACAAGTTTAAGGACAAGGTGCCAGAGGAGGATCGCAACAAGATAATGGAAGCTTGC 1722
Mytilus_HSP70_AY861684             GATGAAAAACTCAAGGATAAAATCAGTGAAAGTGACAAAAAGGAAATCATGGACAAATGT 1725
Perinereis_KU255783                GACGAGAAACTGAAGGACAAGATTTCCGACGAGGACAAGACCAAGATCACTGAGAAATGC 1719
Paralvinella_HSP70-2_EF580993      GACGAGAAATTGAAGGACAAGATCAGTGATGCTGATAAGAAGACCATTACAGACAAATGT 1722
Haliotis_FJ812176                  GATGAGAAACTGAAGGACAAGATCAGTGAAGATGACAAGAAAACCATCACTGACAAGTGC 1722
Oncorhynchus_HSC71_AAB21658        GATGAGAAACTGCAGGGGAAAATCAGTGACGAGGACAAGACTAAGATTCTGGAGAAGTGC 1722
X_maculatus_HSC70_AB062115         GACGAGAAGCTGAAGGATAAGATCAGCGAATCGGACAAGAAGAAGGTCCTGGACAAATGC 1722
Human_HSPA8_HSC71_NP_006588        GATGAGAAACTTCAAGGCAAGATTAACGATGAGGACAAACAGAAGATTCTGGACAAGTGT 1722
Rattus_HSC71_NM_024351             GATGAGAAACTTCAAGGCAAGATCAATGATGAAGACAAACAGAAGATTCTTGACAAGTGC 1722
Pelodiscus_HSC70_HQ219723          GATGAGAAGCTCCAGGGCAAGATCAGCAGTGAAGACAAGCAGAAAATCCTAGACAAATGT 1722
Bos_HSP70_U09861                   GATGAGGGGCTGAAGGGCAAGATCAGCGAGGCGGACAAGAAGAAGGTGCTGGACAAGTGC 1722
```

```
Human_HSPA1A_NM_005345            GATGAGGGGCTCAAGGGCAAGATCAGCGAGGCGGACAAGAAGAAGGTGCTGGACAAGTGT 1722
Rattus_HSP70_1A_NM_031971         GACGAGGGTCTCAAGGGCAAGATCAGCGAGGCTGACAAGAAGAAGGTGCTGGACAAGTGC 1722
X_maculatus_HSP70-1_AB062113      GACGACGCCTTGAAAGGAAAAATCAGTGAGGAGGAGAAAAAGCTGGTTATTGACAAATGC 1728
X_maculatus_HSP70-2_AB062114      GACGAGAGCTTGAAGGGCAAGATCAGCCTTAGAGGACAAGAAGAAGGTGTGAACAAGTGC 1728
Human_HSPA1L_NM_005527            GATGAAGGTTTGAAGGGCAAGATTAGTGAGTCTGATAAAAATAAAATATTGGATAAATGC 1728
Human_HSPA6_NP_002146             GAGGAAAGCCTTAGGGACAAGATTCCCGAAGAGGACAGGCGCAAAATGCAAGACAAGTGT 1728
Alligator_HSP70_AB306279          GACGAGAAGCTGGCGGGCAAGATCGGCGACCAGGACAAGCAGCGTGTGCTCGACAAGTGC 1731
                                   * *              **  *          *                  *    **

Drosophila_Bb_AF295957            AACGAAACTATTCGATGGCTGGACAGCAACACCACCGCCGAGAAGGAGGAGTTCGACCAC 1773
Drosophila_Aa_AF295933            AACGACACTATCCGGTGGCTGGACAGCAACACCACCGCCGAGAAGGAGGAGTTCGACCAC 1773
Spodoptera_FJ754276               GACGACGCGCTCAAGTGGCTCGACAACAACACGCTGGCGGAGCAGGAGGAGTACGAGCAC 1770
Bombyx_AB035326                   GACGAGGCGCTGCGGTGGCTGGACAACAACACTCTCGCTGACCAGGATGAATACGAGCAC 1770
Paralvinella_EF580992             GAGCAGACTGTTAAGTGGTTGGATAACAACACTTTGGCAGAAAAAGAGGAATACGAACAT 1788
B_plicatilis_HSP70-1_AB775784     GAAGAGGCCTTGAAATGGCTGGACTCGAACCAGACCGCTGAGAAGGACGAGTTCGAGCAC 1782
B_plicatilis_HSP70-2_AB775785     GAAGAGGCCTTGAAATGGCTGGACTCGAACCAGACCGCTGAGAAGGACGAGTTCGAGCAC 1782
A_brightwelli_HSP70_KX119429      GAGAACGCGCTCAAATGGCTGGACGCGAATCAAACGGCCGAGAAGGATGAGTTTGAGCAT 1779
Wuchereria_AF167352               GACGAAACAGTTAGGTGGTTGGATGGAAATCAAACAGCTGAAAAAGATGAATTTGAGCAT 1782
Setaria_AF079360                  GATGAGACGGTCAGGTGGTTGGATGGGAATCAGACAGCTGAGAAGGATGAGTTTGAGCAT 1782
C_elegans_HSP70A_M18540           GACGAGATCTTGAAGTGGCTCGACAGCAACCAGACCGCAGAGAAGGAGGAGTTCGAGTCA 1785
C_elegans_HSP-1_NM_070667         GACGAGATCTTGAAGTGGCTCGACAGCAACCAGACCGCAGAGAAGGAGGAGTTCGAGCAC 1785
M_nipponense_KC460343             AATGATGCCATCAAGTGGCTCGACACTAATCAGCTTGGGGAGAAGGAAGAATATGAACAC 1782
M_nipponense_DQ660140             AATGATGCCATCAAATGGCTGGATACTAACCAGCTGGGAGAGAAGGAAGAATATGAACAT 1782
Mytilus_HSP70_AY861684            GACGAAATCATTAAATGGTTGGATGCCAACAATCTGGCTGAGAAGGAAGAATTTGAACAC 1785
Perinereis_KU255783               AACGAGGTCATCAGGTGGTTGGACTCCAACCAGACCGCAGAGAAGGACGAGTTTGAACAC 1779
Paralvinella_HSP70-2_EF580993     AACGATATCATAGCTTGGCTGGATGCTAACCAGCTCGCTGAAAAAGATGAATTTGAACAC 1782
Haliotis_FJ812176                 AACGATGTTATCAGCTGGTTAGACTCTAACCAGTTGGCTGAGAAGGATGAGTTCGAACAC 1782
Oncorhynchus_HSC71_AAB21658       AACGAGGTCATCGGGTGGCTGGACAAGAACCAGACTGCTGAGAAGGAAGAGTATGAGCAC 1782
X_maculatus_HSC70_AB062115        AACGAGGTCATCAGCTGGCTCGACAAAAATCAGAGTGCAGAGAAAGAGGAGTTTGAGCAC 1782
Human_HSPA8_HSC71_NP_006588       AATGAAATTATCAACTGGCTTGATAAGAATCAGACTGCTGAGAAGGAAGAATTTGAACAT 1782
Rattus_HSC71_NM_024351            AACGAAATCATCAGCTGGCTGGATAAGAACCAGACTGCGGAGAAGGAAGAATTTGAGCAT 1782
Pelodiscus_HSC70_HQ219723         AACGAAATTATCAACTGGCTGGATAAGAATCAGACGGCTGAAAAAGAAGAGTTTGAACAT 1782
Bos_HSP70_U09861                  CAGGAGGTGATTTCCTGGCTGGACGCCAACACCTTGGCGGAGAAGGACGAGTTTGAGCAC 1782
Human_HSPA1A_NM_005345            CAAGAGGTCATCTCGTGGCTGGACGCCAACACCTTGGCCGAGAAGGACGAGTTTGAGCAC 1782
Rattus_HSP70_1A_NM_031971         CAGGAGGTCATCTCCTGGCTGGACTCTAACACGCTGGCTGAGAAAGAGGAGTTCGTGCAC 1782
X_maculatus_HSP70-1_AB062113      AACCAGACAATTTCCTGGCTGGAGAACAACCAGCTGGCAGAGAAGGAGGAGTATGAACAT 1788
X_maculatus_HSP70-2_AB062114      CAGGAGACCATCAGCTGGCTGGAGAACAACCAGCTGGCTGAGAAAGACGAGTACCAACAC 1788
Human_HSPA1L_NM_005527            AACGAGCTCCTTTCGTGGCTGGAGGTCAATCAACTGGCAGAGAAAGATGAGTTTGATCAT 1788
Human_HSPA6_NP_002146             CGGGAAGTCCTTGCCTGGCTGGAGCACAACCAGCTGGCAGAGAAGGAGGAGTATGAGCAT 1788
Alligator_HSP70_AB306279          CAGGAGCTGATCGCCTGGCTGGACCGCAACCAGATGGCCGAGAAGGACGAGTACGAGCAC 1791
                                      *     *    *** * **    **       *  **  * ** ** *

Drosophila_Bb_AF295957            AAGATGGAGGAGCTCACTCGCCACTGTTCCCCTATCATGACCAAGATGCATCAGCAGGGA 1833
Drosophila_Aa_AF295933            AAGATGGAGGAGCTCACTCGCCACTGCTCCCCTATCATGACCAAGATGCATCAGCAGGGA 1833
Spodoptera_FJ754276               AAGCTGAAGGACGTGCAGCGCGTCTGCTCGCCCGTCATGGCCAAGATGCATGGGGCGGGC 1830
Bombyx_AB035326                   AAGCTAAAGGATGTGCAGCGAGTGTGTTCGCCGGTCATGAGCAAGATGCACGGTGCGGCG 1830
Paralvinella_EF580992             CATTTGGAGGAGTTACACAAGGTCTGTTCTCCGATAATGGCCAAGCTACATGGATCACAG 1848
B_plicatilis_HSP70-1_AB775784     AAGATGAAAGAAGTGGAGAAAATTTGCTCGCCGATTATTACTCAGTTGTATCAGGGCGCT 1842
B_plicatilis_HSP70-2_AB775785     AAGATGAAAGAAGTGGAGAAAATTTGCTCGCCGATTATTACTCAGTTGTATCAGGGCGCT 1842
A_brightwelli_HSP70_KX119429      AAGTTGAAGGAGATCGAGAAGACTTGTTCGCCGATCATTACCAAGTTGTATCAGGGTGGT 1839
Wuchereria_AF167352               CGCCAAAAAGAATTGGAATCTGTTTGCAATCCGATTATTACAAAACTCTACCAGAGCGCT 1842
Setaria_AF079360                  CGTCAGAAAGAGTTGGAAGCAGTTTCGAATCCAATCATTACGAAACTGTACCAGAGTGCT 1842
C_elegans_HSP70A_M18540           CAACAGAAGGATTTGGAAGGATTGGCCAAGCCCGATCTTTCCAAGCTTTACCAGAGTGCC 1845
C_elegans_HSP-1_NM_070667         CAACAGAAGGATTTGGAAGGATTGGCCAACCCAATCATTTCCAAGCTTTACCAGAGTGCC 1845
M_nipponense_KC460343             AAACTGAAGGAAATTGAACAGATATGTAATCCTATCATCACCAAAATGTACCAAGCAGCT 1842
M_nipponense_DQ660140             AAGTTGAAGGAAATTGAGCAAATATGTAATCCTATCATCACCAAAATGTACCAAGCAGCT 1842
Mytilus_HSP70_AY861684            AAACAGAAAGAACTTGAGGGAGTGTGTAATCCAATCATCACTAAACTGTACCAGTCTGCT 1845
Perinereis_KU255783               CAACAGAAGGAATTGGAAGGCATCTGCATGCCTATCATCACCAAGTTGTACCAGGCAGGT 1839
Paralvinella_HSP70-2_EF580993     CAACAAAAGGAACTGGAGAAGATCTGTATGCCGATCATTACAAAGCTGTACCAGGCAGGT 1842
Haliotis_FJ812176                 AAGCAGAAGGAGCTGGAGGGTGTGTGTAATCCAATCATCACCAAGCTGTACCAGGCAGCT 1842
Oncorhynchus_HSC71_AAB21658       CACCAGAAGGAGTTGGAGAAGGTGTGCAACCCCATCATCACCAAGCTGTACCAGGGTGCT 1842
X_maculatus_HSC70_AB062115        CAGCAGAAGGAGCTTGAGAAGTTGTGCAACCCGATTATGACCAAACTGTACCAGAGTGAA 1842
Human_HSPA8_HSC71_NP_006588       CAACAGAAAGAGCTGGAGAAAGTTTGCAACCCCATCATCACCAAGCTGTACCAGAGTGCA 1842
Rattus_HSC71_NM_024351            CAGCAGAAAGAACTGGAGAAGGTCTGCAACCCTATCATCACCAAGCTGTACCAGAGTGCT 1842
Pelodiscus_HSC70_HQ219723         CAGCAGAAGGAGCTGGAGAAGGTCTGCAATCCCATAATCACAAAGCTGTACCAGAGCGCT 1842
Bos_HSP70_U09861                  AAGAGGAAGGAGCTGGAGCAGGTGTGTAACCCCATCATCAGCAGACTGTACCAGGGGGCG 1842
Human_HSPA1A_NM_005345            AAGAGGAAGGAGCTGGAGCAGGTGTGTAACCCCATCATCAGCGGACTGTACCAGGGTGCC 1842
Rattus_HSP70_1A_NM_031971         AAGCGGGAGGAGCTGGAGCGGGTGTGCAACCCGATCATCAGCGGGCTGTATCAGGGTGCG 1842
X_maculatus_HSP70-1_AB062113      CAGCAGAATGAGCTGGAGAGGGTGTGCAAACCGGTCGTGGCAAAGTTGTATCAAGGTGCA 1848
X_maculatus_HSP70-2_AB062114      CAGCAGAAAGAGCTGGAGAAGGTGTGCAACCCCATCATCAGCAACTTGTACCAGGGAGGG 1848
Human_HSPA1L_NM_005527            AAGAGAAAGGAATTGGAGCAGATGTGTAACCCTATCATCACAAAACTCTACCAAGGAGGA 1848
Human_HSPA6_NP_002146             CAGAAGAGGGAGCTGGAGCAAATCTGTCGCCCCATCTTCTCCAGGCTCTATGGGGGGCCT 1848
Alligator_HSP70_AB306279          AAGCAGAAGGAGCTGGAGAAGATTTGCAACCCCATCATCACCAAGCTATACCAAGGCGGC 1851
                                           **  *                **     *        *  *

Drosophila_Bb_AF295957            GCGGGAGCAGCTGGGGGTCCGGGA---GCCAACTGTGGCCAACAGGCCGGAGGATTTGGC 1890
Drosophila_Aa_AF295933            GCGGGAGCAGCTGGGGGTCCGGGA---GCCAACTGTGGCCAACAGGCCGGAGGATTTGGC 1890
Spodoptera_FJ754276               GCGCAAGGTGCGGGCGGGATGCCCGGAGGTATGCCTGGTGGCATGCCCGGAGGAATGCCT 1890
Bombyx_AB035326                   CCCGGCGGTATG---------CCAGGAGGAATGCCGGGCGGTATGCCGGGAGGATACCAA 1881
```

```
Paralvinella_EF580992              TCTGGATCTTGTGGTGGTTCCGGATCACAACAAGGTTGTTACGGATCACAGACTGG---A 1905
B_plicatilis_HSP70-1_AB775784      GGTGGTGCGCCTGGTGGCATGCCTGGTGGTATGC------------CTGGCGGTATGCCT 1890
B_plicatilis_HSP70-2_AB775785      GGTGGTGCGCCTGGTGGTATGCCTGGTGGTATGCCTGGTGGTATGCCGGGCGGTATGCCT 1902
A_brightwelli_HSP70_KX119429       GCGCCGGGCGGTATGCCGGGTGGTATGCCGGGCGGTGCTGGTGC---------------T 1884
Wuchereria_AF167352                GGTGGTATGCCTGGAGGGATGCCGGGTGGCATGCCTAGTGGAGCTCCAGGTGCTGGTTCA 1902
Setaria_AF079360                   GGTGGTATGCCCGGAGGAATGCCTGGTGGTATGCCTGGTGGAGCTCCAGGTGGAGGTTCG 1902
C_elegans_HSP70A_M18540            GGAGGAGCCCCACCAGGTGCTGCTCCAGGAGGAGCCGCCGGAGGAGC-------------- 1892
C_elegans_HSP-1_NM_070667          GGAGGAGCCCCACCAGGTGCTGCTCCAGGAGGAGCCGCCGGAGGAGC-------------- 1892
M_nipponense_KC460343              GGTGGTGCTCCTCCAGGTGGTATGCCAGGAGGTTTTCCAGGTGCCCCAGGGGCTGGTGCT 1902
M_nipponense_DQ660140              GGTGGTGCTCCTCCAGGTGGTATGCCAGGAGGTTTTCCAGGTGCCCCAGGGGCTGGTGCT 1902
Mytilus_HSP70_AY861684             GGTGGAGCCCCAGGTGGTGGTATGCCAAACTTTGGTGGAGCTGGTGGAGCCCCAGGAGGT 1905
Perinereis_KU255783                GGCGCCCCTGCTGGTGGAATGCCCGGTGGAATGCCCGGTGGAATGCCCGGAGGCTTCCCA 1899
Paralvinella_HSP70-2_EF580993      GGTGCTCCACCAGGCAGTATGCCAGGTGGCATGCCCGGTGGTATGCCAGGAGGCGCTGGT 1902
Haliotis_FJ812176                  GGCGGTGCTGGTGGTATGCCCAACTTCAACCCAGGTGCTGCTGGTGCTGGCGGTGCAGGA 1902
Oncorhynchus_HSC71_AAB21658        GGTGGGATGCCCGGCGGTATGCCTGAGGGCATGGCTGGCGGATTCCCTGGAGCTGGTGGT 1902
X_maculatus_HSC70_AB062115         GGACAGATGCCTAGAGCTGGTGCGACCGGAACAGGAGGGGCCTCGGGCCCCACCA----- 1897
Human_HSPA8_HSC71_NP_006588        GGAGGCATGCCAGGAGGAATGCCTGGGGGATTTCCTGGTGGTGGAGCTCCTCCCTCTG-- 1900
Rattus_HSC71_NM_024351             GGTGGCATGCCTGGAGGAATGCCTGGTGGCTTCCCTGGTGGAGGAGCTCCTCCATCTG-- 1900
Pelodiscus_HSC70_HQ219723          GGAGGAATGCCTGGTGGCATGCCTGGGGGATTCCCTGGAGGTGGAGCTGCCCCATCTG-- 1900
Bos_HSP70_U09861                   GGCGGCCCCGGGGCTGGCGGCTTTGGGGCTCAGGGCCCTAAAGGGGGC---TCTGGGTCT 1899
Human_HSPA1A_NM_005345             GGTGGTCCCGGGCCTGGGGGCTTCGGGGCTCAGGGTCCCAAGGGAGGG---TCTGGGTCA 1899
Rattus_HSP70_1A_NM_031971          GGTGCTCCCGGGGCTGGGGGCTTCGGGGCCCAGGCGCCCAAGGGAGGC---TCTGGGTCG 1899
X_maculatus_HSP70-1_AB062113       GCGGCAGGAAGCTCCAGCAGTCAGGCTGGAGGAGCCACTC------------------AG 1890
X_maculatus_HSP70-2_AB062114       AGGCCCACTGGGAGCTGCAGAGAGCAGGCAGGAGCCCACA---------------GCCAG 1893
Human_HSPA1L_NM_005527             TGCACTGGGCCTGCCTGCGGAACAGGGTATGTGCCTG------GAAGG---CCTGCCACA 1899
Human_HSPA6_NP_002146              GGTGTCCCTGGGGGCAGCAGTTGTGGCACTCAAGCCCGCCAGGGGGAC---CCCAGCACC 1905
Alligator_HSP70_AB306279           GCCCCGCCCGGCGGCCCCGGTCCCGCGTCCGCCGGCT------------------CCGGC 1893


Drosophila_Bb_AF295957             GG------CTACTCTGGACCCACAGTCGAGGAGGTCGACTAA------------------ 1926
Drosophila_Aa_AF295933             GG------CTACTCTGGACCCACAGTCGAGGAGGTCGACTAA------------------ 1926
Spodoptera_FJ754276                GGAGGAATGCCGGGCGGAATGCCTGGTGGCATGCCCGGCGGTATGCCAGGAGGAATGCCT 1950
Bombyx_AB035326                    CAAGCGAGGAGTGATGGACCCACAGTTGAGGAAGTCGACTAA------------------ 1923
Paralvinella_EF580992              TATGGAAGT---------------------------------------------------- 1914
B_plicatilis_HSP70-1_AB775784      GGTGGAATG---CCTGGTGCTGGACCTGAGAGTGC-------------------------- 1922
B_plicatilis_HSP70-2_AB775785      GGTGGAATG---CCTGGTGCTGGACCTGAGAGCGC-------------------------- 1934
A_brightwelli_HSP70_KX119429       GGTGGCGCC---TCCGGAGCCGGA------------------------------------- 1905
Wuchereria_AF167352                ACGGGAGGTGGACC----------------------------------------------- 1916
Setaria_AF079360                   GGTGGCAGTGGACC----------------------------------------------- 1916
C_elegans_HSP70A_M18540            -----TGGAGGACC----------------------------------------------- 1901
C_elegans_HSP-1_NM_070667          -----TGGAGGACC----------------------------------------------- 1901
M_nipponense_KC460343              GCTCCTG------GT------------------GG-------------------------- 1913
M_nipponense_DQ660140              GCTCCTG------GT------------------GG-------------------------- 1913
Mytilus_HSP70_AY861684             GCACCTGGAG---CA---------GGAGGCACTGG-------------------------- 1928
Perinereis_KU255783                GGTGGCGCTGCTCCCGGTGGAGACTCCGGTGCTGC-------------------------- 1934
Paralvinella_HSP70-2_EF580993      GGTGCTGGCGGGGCAGCTGG------------TTC-------------------------- 1925
Haliotis_FJ812176                  GGAGCTC---AGACAGG---------------CGG-------------------------- 1919
Oncorhynchus_HSC71_AAB21658        GCTGCTCCTGGAGGTGGTGGATC-------------------------------------- 1925
X_maculatus_HSC70_AB062115         ------------------------------------------------------------ 1897
Human_HSPA8_HSC71_NP_006588        -------------GTGGTGCTTC-------------------------------------- 1910
Rattus_HSC71_NM_024351             -------------GTGGTGCTTC-------------------------------------- 1910
Pelodiscus_HSC70_HQ219723          -------------GAGGGGCCTC-------------------------------------- 1910
Bos_HSP70_U09861                   GGC---------------------------------------------------------- 1902
Human_HSPA1A_NM_005345             GGC---------------------------------------------------------- 1902
Rattus_HSP70_1A_NM_031971          GGG---------------------------------------------------------- 1902
X_maculatus_HSP70-1_AB062113       GGT---------------------------------------------------------- 1893
X_maculatus_HSP70-2_AB062114       GGG---------------------------------------------------------- 1896
Human_HSPA1L_NM_005527             GGC---------------------------------------------------------- 1902
Human_HSPA6_NP_002146              GGC---------------------------------------------------------- 1908
Alligator_HSP70_AB306279           GGC---------------------------------------------------------- 1896


Drosophila_Bb_AF295957             ------------------------------------------------------      1926
Drosophila_Aa_AF295933             ------------------------------------------------------      1926
Spodoptera_FJ754276                GGCGGGTACGGAGGAAACCACAACAGCGGACCCACCATTGAAGAAGTTGATTAA-     2004
Bombyx_AB035326                    ------------------------------------------------------      1923
Paralvinella_EF580992              ---------------------CAACAAGGACCAACAGTGGAAGAGGTGGATTAA-     1947
B_plicatilis_HSP70-1_AB775784      -----------------GGGACGCAGTGGACCTACCATTGATGAGGTCGATTAA-     1959
B_plicatilis_HSP70-2_AB775785      -----------------GGGACGCAGTGGACCTACCATTGATGAGGTCGATTAA-     1971
A_brightwelli_HSP70_KX119429       ------------------------------CCTAAAATCGAAGAGGTCGACTAA-     1929
Wuchereria_AF167352                ---------------------------------AACAATTGAAGAAGTTGATTAG-     1938
Setaria_AF079360                   ---------------------------------AACAATTGAAGAAGTTGATTAG-     1938
C_elegans_HSP70A_M18540            ---------------------------------AACGATCGAGGAGGTCGACTAA-     1923
C_elegans_HSP-1_NM_070667          ---------------------------------AACGATCGAGGAGGTCGACTAA-     1923
M_nipponense_KC460343              -----------------TGGTTCATCAGGCCCCACAATTGAAGAGGTCGATTAA-     1950
M_nipponense_DQ660140              -----------------TGGTTCATCAGGCCCCACAATTGAAGAGGTCGATTAA-     1950
Mytilus_HSP70_AY861684             -----------------TGGAAGTGGTGGACCAACCATTGAAGAGGTCGACTAA-     1965
Perinereis_KU255783                -----------------CCCAGGTGGTGGCCCCACAATCGAAGAGGTCGACTAA-     1971
Paralvinella_HSP70-2_EF580993      -----------------CGGTGGCAGTGGACCCACCATTGAGGAGGTTGATTAAs     1963
```

```
Haliotis_FJ812176                 -----------------CAGCTCCGGAGGCCCCACCATCGAGGAGGTTGATTAA-     1956
Oncorhynchus_HSC71_AAB21658       -----------------------ATCTGGACCAACCATTGAGGAAGTCGACTAA-     1956
X_maculatus_HSC70_AB062115        -------------------------------------TAGAGGAGGTCGACTAA-     1914
Human_HSPA8_HSC71_NP_006588       -----------------------CTCAGGGCCCACCATTGAAGAGGTTGATTAA-     1941
Rattus_HSC71_NM_024351            -----------------------TTCAGGCCCCACCATTGAAGAGGTCGATTAA-     1941
Pelodiscus_HSC70_HQ219723         -----------------------ATCTGGACCAACCATTGAGGAAGTAGATTAA-     1941
Bos_HSP70_U09861                  ------------------------------CCCACCATTGAGGAGGTGGATTAG-     1926
Human_HSPA1A_NM_005345            ------------------------------CCCACCATTGAGGAGGTAGATTAG-     1926
Rattus_HSP70_1A_NM_031971         ------------------------------CCCACCATCGAGGAGGTGGATTAG-     1926
X_maculatus_HSP70-1_AB062113      ------------------------------CCCACTGTCGAGGAGGTCGACTAA-     1917
X_maculatus_HSP70-2_AB062114      ------------------------------CCCACTGTGGAGGAGGTGGACTGA-     1920
Human_HSPA1L_NM_005527            ------------------------------CCCACAATTGAAGAAGTAGATTAA-     1926
Human_HSPA6_NP_002146             ------------------------------CCCATCATTGAGGAGGTTGATTGA-     1932
Alligator_HSP70_AB306279          ------------------------------CCCACCATCGAGGAGGTGGACTAG-     1920
```

Supplementary fig. S5. Comparison of HSP70 nucleotide sequences used for the calculation of synonymous and nonsynonymous substitution rates. Regions shown in bold were used for the calculation.



1: Invertebrate HSP70s (Lineage A)
2: Rotifera HSP70s (Lineage B)
3: Nematode HSP70s (Lineage B)
4: Invertebrate HSP/HSC70s (Lineage B)
5 (8): Vertebrate HSC70s (Lineage B)
5 (9): Vertebrate HSP70s (Lineage B)

Supplementary fig. S6. Bee swarm boxplots of Ka/Ks values for each clade. Only Ka/Ks values calculated from inter-cluster pairs were averaged. Statistical differences were calculated by Kruskal-Wallis test (chi-squared = 200.45, df = 5, p-value < 2.2e-16) followed by the non-parametric post-hoc tests (pairwise Wilcox test with P value adjustment by the Holm method). Clades sharing same letters are not significantly different at the 5% level of significance. One outlier in clade 3 (0.53) is not included in the plot although this value was used for all statistical analyses.

Ka

			Clade 1					Clade 2				Clade 3				Clade 4					Clade 5 (node 8)						Clade 5 (node 9)						
	Gene No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Clade 1	2	*D. melanogaster* HSP70Aa (AF295933)	0.00																													
	3	*Spodoptera* HSP70 (FJ754276)	0.09	0.09																												
	4	*Bombyx* HSP70 (AB035326)	0.10	0.10	0.03																											
	5	*Paralvinella* HSP70-1 (EF580992)	0.16	0.16	0.14	0.14																										
Clade 2	6	*B. plicatilis* _HSP70-1 (AB775784)	0.15	0.15	0.14	0.14	0.15																									
	7	*B. plicatilis* _HSP70-2 (AB775785)	0.15	0.15	0.14	0.14	0.15	0.00																								
	8	*A. brightwelli* _HSP70 (KX119429)	0.16	0.15	0.15	0.16	0.15	0.06	0.06																							
Clade 3	9	*Wuchereria* (AF167352)	0.16	0.15	0.15	0.16	0.16	0.08	0.09	0.09																						
	10	*Setaria* (AF079360)	0.15	0.15	0.15	0.15	0.16	0.08	0.08	0.09	0.01																					
	11	*C. elegans* HSP70A (M18540)	0.16	0.16	0.16	0.16	0.18	0.09	0.09	0.11	0.05	0.05																				
	12	*C. elegans* HSP-1 (NM_070667)	0.15	0.15	0.15	0.15	0.17	0.08	0.08	0.09	0.04	0.03	0.01																			
	13	*M. nipponense* HSP70-1 (KC460343)	0.16	0.15	0.15	0.15	0.15	0.07	0.07	0.08	0.08	0.08	0.09	0.08																		
	14	*M. nipponense* HSP70-2 (DQ660140)	0.15	0.15	0.15	0.15	0.15	0.07	0.07	0.07	0.08	0.08	0.09	0.08	0.01																	
Clade 4	15	*Mytilus* HSP70 (AY861684)	0.16	0.16	0.16	0.16	0.15	0.09	0.09	0.07	0.09	0.09	0.11	0.10	0.07	0.07																
	16	*Perinereis* HSP70-1 (KU255783)	0.15	0.15	0.17	0.16	0.15	0.09	0.09	0.08	0.10	0.10	0.11	0.10	0.07	0.07	0.06															
	17	*Paralvinella* HSP70-2 (EF580993)	0.17	0.17	0.17	0.17	0.16	0.10	0.10	0.09	0.11	0.11	0.12	0.11	0.08	0.08	0.07	0.06														
	18	*Haliotis* HSP70c (FJ812176)	0.15	0.15	0.16	0.16	0.16	0.10	0.10	0.09	0.10	0.10	0.12	0.11	0.08	0.08	0.06	0.06	0.07													
Clade 5 (node 8)	19	*Oncorhynchus* HSC71 (AAB21658)	0.16	0.16	0.16	0.16	0.16	0.08	0.08	0.08	0.09	0.09	0.11	0.09	0.06	0.06	0.07	0.06	0.07	0.08												
	20	*X. maculatus* HSC70 (AB062115)	0.18	0.17	0.16	0.16	0.15	0.09	0.09	0.08	0.11	0.11	0.12	0.11	0.07	0.07	0.07	0.08	0.09	0.08	0.04											
	21	Human_HSPA8 (NP_006588)	0.15	0.15	0.14	0.15	0.15	0.07	0.07	0.07	0.09	0.09	0.10	0.09	0.07	0.06	0.06	0.07	0.07	0.07	0.03	0.05										
	22	*Rattus* HSC71 (NM_024351)	0.15	0.15	0.14	0.15	0.15	0.07	0.07	0.07	0.09	0.09	0.10	0.09	0.06	0.06	0.06	0.07	0.07	0.07	0.03	0.05	0.00									
	23	*Pelodiscus* HSC70 (HQ219723)	0.15	0.15	0.14	0.15	0.14	0.07	0.07	0.07	0.09	0.08	0.10	0.09	0.06	0.06	0.06	0.07	0.07	0.06	0.03	0.04	0.00	0.00								
	24	*Bos* HSP70 (U09861)	0.15	0.15	0.15	0.15	0.16	0.10	0.10	0.09	0.10	0.10	0.12	0.11	0.09	0.09	0.09	0.10	0.11	0.10	0.08	0.09	0.07	0.07	0.07							
Clade 5 (node 9)	25	Human_HSPA1A (NM_005345)	0.15	0.15	0.15	0.15	0.15	0.10	0.09	0.09	0.10	0.09	0.11	0.10	0.08	0.08	0.09	0.09	0.11	0.10	0.07	0.09	0.06	0.06	0.06	0.00						
	26	*Rattus* HSP70_1A (NM_031971)	0.15	0.15	0.15	0.15	0.16	0.10	0.10	0.09	0.10	0.10	0.12	0.11	0.09	0.09	0.09	0.09	0.11	0.10	0.08	0.09	0.07	0.07	0.07	0.01	0.01					
	27	*X. maculatus* HSP70-1 (AB062113)	0.17	0.17	0.17	0.16	0.16	0.10	0.10	0.09	0.11	0.10	0.12	0.11	0.09	0.09	0.09	0.10	0.09	0.10	0.07	0.08	0.06	0.06	0.06	0.07	0.07	0.07				
	28	*X. maculatus* HSP70-2 (AB062114)	0.16	0.16	0.16	0.17	0.16	0.10	0.10	0.09	0.11	0.10	0.12	0.11	0.09	0.09	0.09	0.10	0.11	0.10	0.08	0.09	0.07	0.07	0.07	0.07	0.07	0.07	0.06			
	29	Human_HSPA1L (NM_005527)	0.15	0.15	0.15	0.16	0.16	0.10	0.10	0.10	0.10	0.10	0.12	0.11	0.09	0.09	0.10	0.10	0.11	0.11	0.09	0.10	0.08	0.08	0.08	0.04	0.04	0.04	0.07	0.07		
	30	Human_HSPA6 (NP_002146)	0.16	0.16	0.16	0.17	0.17	0.12	0.12	0.11	0.12	0.12	0.14	0.13	0.12	0.11	0.11	0.11	0.13	0.12	0.12	0.12	0.10	0.10	0.10	0.07	0.07	0.07	0.12	0.11	0.09	
	31	Alligator_HSP70 (AB306279)	0.16	0.16	0.16	0.16	0.17	0.09	0.09	0.08	0.10	0.10	0.12	0.10	0.09	0.09	0.09	0.09	0.10	0.10	0.08	0.09	0.07	0.07	0.07	0.09	0.09	0.09	0.08	0.09	0.10	0.11

Ks

			Clade 1					Clade 2				Clade 3				Clade 4					Clade 5 (node 8)						Clade 5 (node 9)						
	Gene No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Clade 1	2	*D. melanogaster* HSP70Aa (AF295933)	0.09																													
	3	*Spodoptera* HSP70 (FJ754276)	1.48	1.48																												
	4	*Bombyx* HSP70 (AB035326)	1.44	1.44	0.84																											
	5	*Paralvinella* HSP70-1 (EF580992)			2.83																											
Clade 2	6	*B. plicatilis* _HSP70-1 (AB775784)	2.23	1.80	1.50	2.48																										
	7	*B. plicatilis* _HSP70-2 (AB775785)	2.11	1.75	1.48	2.98		0.01																								
	8	*A. brightwelli* _HSP70 (KX119429)				4.43	2.41	1.93	1.90																							
Clade 3	9	*Wuchereria* (AF167352)																														
	10	*Setaria* (AF079360)									0.82																					
	11	*C. elegans* HSP70A (M18540)																														
	12	*C. elegans* HSP-1 (NM_070667)										2.53	0.02																			
	13	*M. nipponense* HSP70-1 (KC460343)									2.45	2.64	1.94	2.08																		
	14	*M. nipponense* HSP70-2 (DQ660140)											2.30	2.40	0.25																	
Clade 4	15	*Mytilus* HSP70 (AY861684)									2.56				2.41	2.74																
	16	*Perinereis* HSP70-1 (KU255783)			2.24	3.09		1.91	1.92				1.61	1.60	3.29		3.88															

	17	*Paralvinella* HSP70-2 (EF580993)	1.91	1.94	2.17	2.64		1.99	1.95		3.34	2.35			1.69	1.72	2.13	1.61														
	18	*Haliotis* HSP70c (FJ812176)											1.84	1.88	1.85	2.06		1.60	1.70													
Clade 5 (node 8)	19	*Oncorhynchus* HSC71 (AAB21658)	1.75	1.86				1.40	1.35				1.52	1.56	1.64	1.90	2.53	1.51	1.49	1.54												
	20	*X. maculatus* HSC70 (AB062115)			2.63	2.34		1.55	1.59				2.51					1.97	1.65	2.63	1.25											
	21	Human_HSPA8 (NP_006588)							3.36				1.78	1.85	1.51	1.59	2.00	1.71	1.76	1.72	1.29											
	22	*Rattus* HSC71 (NM_024351)			2.77			2.26	2.22				1.81	1.82	1.80	2.36	2.48	1.84	1.93	1.88	1.25	1.80	0.52									
	23	*Pelodiscus* HSC70 (HQ219723)		3.28								3.40			1.69	1.78	2.28	2.48	1.72		1.58	2.07	1.11	1.23								
	24	*Bos* HSP70 (U09861)	1.55	1.48				1.38	1.42										2.17		1.90	1.57										
	25	Human_HSPA1A (NM_005345)	1.46	1.42			2.48	1.28	1.31									2.47	1.65	2.56	1.47	1.27	2.86	2.27	2.45	0.22						
Clade 5 (node 9)	26	*Rattus* HSP70_1A (NM_031971)	1.43	1.39				1.10	1.09										2.08			1.46				0.35	0.35					
	27	*X. maculatus* HSP70-1 (AB062113)		2.43									3.59		2.75	2.98		2.32	1.87	2.12	2.98				2.59	2.33	1.87	2.88				
	28	*X. maculatus* HSP70-2 (AB062114)	2.02	1.71			2.33	1.22	1.24	3.00								1.85	1.65	2.32	1.37	1.27	2.30	1.81	2.40	0.86	0.73	1.21	1.37			
	29	Human_HSPA1L (NM_005527)	2.64	2.50	2.64			1.87	1.92						2.22		3.46	2.18	1.76	2.21	1.44	1.66	1.67	1.43	2.14	0.96	0.81	1.25		1.09		
	30	Human_HSPA6 (NP_002146)	1.62	1.44	1.86			1.55	1.57						2.41	3.15		2.20	1.57		1.44	1.34	2.19	1.61	2.27	0.80	0.71	0.79	3.10	0.92	1.12	
	31	Alligator_HSP70 (AB306279)	1.18	1.08	1.28			0.82	0.84									2.36	1.74		2.01	1.29				0.89	0.90	0.52	2.19	1.11	1.92	1.04

Ka/Ks

			Clade 1					Clade 2				Clade 3				Clade 4						Clade 5 (node 8)					Clade 5 (node 9)						
	Gene No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
	2	*D. melanogaster* HSP70Aa (AF295933)	0.04																													
Clade 1	3	*Spodoptera* HSP70 (FJ754276)	0.06	0.06																												
	4	*Bombyx* HSP70 (AB035326)	0.07	0.07	0.03																											
	5	*Paralvinella* HSP70-1 (EF580992)			0.05																											
Clade 2	6	*B. plicatilis* _HSP70-1 (AB775784)	0.07	0.08	0.09	0.06																										
	7	*B. plicatilis* _HSP70-2 (AB775785)	0.07	0.08	0.09	0.05		0.13																								
	8	*A. brightwelli* _HSP70 (KX119429)				0.04	0.06	0.03	0.03																							
	9	*Wuchereria* (AF167352)																														
Clade 3	10	*Setaria* (AF079360)									0.01																					
	11	*C. elegans* HSP70A (M18540)																														
	12	*C. elegans* HSP-1 (NM_070667)										0.01	0.53																			
	13	*M. nipponense* HSP70-1 (KC460343)									0.03	0.03	0.05	0.04																		
	14	*M. nipponense* HSP70-2 (DQ660140)											0.04	0.03	0.03																	
Clade4	15	*Mytilus* HSP70 (AY861684)									0.04				0.03	0.02																
	16	*Perinereis* HSP70-1 (KU255783)			0.07	0.05		0.05	0.05				0.07	0.06	0.02		0.02															
	17	*Paralvinella* HSP70-2 (EF580993)	0.09	0.09	0.08	0.06		0.05	0.05		0.03	0.04			0.05	0.05	0.03	0.04														
	18	*Haliotis* HSP70c (FJ812176)											0.06	0.06	0.04	0.04		0.04	0.04													
Clade 5 (node 8)	19	*Oncorhynchus* HSC71 (AAB21658)	0.09	0.09				0.06	0.06				0.07	0.06	0.04	0.03	0.03	0.04	0.05	0.05												
	20	*X. maculatus* HSC70 (AB062115)			0.06	0.07		0.06	0.06				0.05					0.04	0.05	0.03	0.03											
	21	Human_HSPA8 (NP_006588)							0.02				0.06	0.05	0.04	0.04	0.03	0.04	0.04	0.04	0.02											
	22	*Rattus* HSC71 (NM_024351)			0.05			0.03	0.03				0.06	0.05	0.04	0.03	0.02	0.04	0.04	0.03	0.02	0.03	0.00									
	23	*Pelodiscus* HSC70 (HQ219723)		0.05								0.02			0.04	0.03	0.03	0.03	0.04		0.02	0.02	0.00	0.00								
	24	*Bos* HSP70 (U09861)	0.10	0.10				0.07	0.07										0.05		0.04	0.06										
	25	Human_HSPA1A (NM_005345)	0.10	0.10			0.06	0.07	0.07									0.04	0.07	0.04	0.05	0.07	0.02	0.03	0.03	0.02						
Clade 5 (node 9)	26	*Rattus* HSP70_1A (NM_031971)	0.11	0.11				0.09	0.09										0.05			0.06				0.04	0.04					
	27	*X. maculatus* HSP70-1 (AB062113)		0.07									0.03		0.03	0.03		0.04	0.05	0.05	0.02				0.02	0.03	0.04	0.02				
	28	*X. maculatus* HSP70-2 (AB062114)	0.08	0.09			0.07	0.08	0.08	0.03								0.05	0.07	0.05	0.06	0.07	0.03	0.04	0.03	0.08	0.09	0.06	0.04			
	29	Human_HSPA1L (NM_005527)	0.06	0.06	0.06			0.05	0.05						0.04		0.03	0.04	0.06	0.05	0.06	0.06	0.05	0.06	0.04	0.04	0.05	0.03		0.06		
	30	Human_HSPA6 (NP_002146)	0.10	0.11	0.09			0.08	0.07						0.05	0.04		0.05	0.08		0.08	0.09	0.05	0.06	0.05	0.09	0.10	0.09	0.04	0.12	0.08	
	31	Alligator_HSP70 (AB306279)	0.13	0.15	0.13			0.11	0.11									0.04	0.06		0.04	0.07				0.10	0.10	0.16	0.04	0.08	0.05	0.11

Supplementary material 2

Heat Treatment

For semi-quantitative RT-PCR, rotifers were batch-cultured in 400 mL at 25˚C. In the exponential growth phase, approximately 6×10^4 individuals were randomly divided into two groups and concentrated to about 10 mL by filtration through 20 µm mesh filters. One group (heat-treated group) was transferred to culture medium at 30˚C for 10 min, collected with a 20 µm mesh filter, and stored in ISOGEN (Nippon Gene, Tokyo, Japan) at -80˚C. The control group was prepared by the same procedure at 25˚C.

For quantitative real-time PCR, 8 rotifers bearing 2 or 3 eggs were selected from a batch culture population and inoculated into a well of a 12-well plate (Nunc, Rochester, NY) containing 1 mL of culture medium at 25˚C. The 12-well plate was put on a water bath at 40˚C for 10 min, and then transferred to a water bath at 25˚C. Rotifers were sampled before heat treatment (control), just after heat treatment (time 0), and 2, 3, 4, 6, and 8 h after heat treatment (n = 3, each group contained 8 individuals). Rotifer samples were stored in 500 µL ISOGEN (Nippon Gene) and stored at -80˚C until use. Rotifers used for *in situ* hybridization were prepared by the same method except the heat shock temperature was 30˚C.

cDNA Cloning of HSP70

5' RACE for the partial sequence of *B. plicatilis* HSP70 gene [AB076052; (Kaneko et al. 2002)] was carried out using a GeneRacer kit (Invitrogen, Carlsbad, CA) with gene-specific primers rHSP70_5RACE1, rHSP70_5RACE2, and rHSP70_5RACE3 (supplementary table S1). Total RNA was extracted from a rotifer population in an exponential growth phase and used for 1st strand cDNA synthesis using the rHSP70_5RACE1 primer and the GeneRacer kit (Invitrogen). First round PCR was performed at 94˚C for 3 min followed by 30 cycles of 94˚C for 30 s, 55˚C for 30 s and 72 ˚C for 1 min. The final extension step was performed at 72˚C for 5 min. The 20 µL-reaction mixture contained about 1 µg of the 1st strand cDNA, 2 µL of Ex *Taq* buffer (Takara, Shiga, Japan), 1 U of Ex *Taq* DNA polymerase, 4 nmol of dNTP mixture, 10 pmol of rHSP70_5RACE2 primer and GeneRacer 5' primer (Table 1). The amplified products were diluted 10-fold with sterile distilled water and used as the template for the nested PCR with rHSP70_5RACE3 and GeneRacer 5' nested primers (supplementary table S1). The PCR conditions were same as the first round PCR except for the template and primers. The amplified products were subcloned into pGEM-T vector (Promega, Madison, WI) and sequenced as reported previously (Yoon et al. 2008).

3’ RACE for rotifer HSP70 gene was carried out as follows. An aliquot of first strand cDNA was synthesized from rotifers in an exponential phase using an oligo-dT adapter primer (supplementary table S1). PCR was performed with the first strand cDNA as a template and with rHSP70_3RACE1 and AUAP primers. PCR was carried out with 30 cycles of 94˚C for 30 s, 60˚C for 30 s, and 72˚C for 2 min. The final extension step was performed at 72˚C for 5 min. The 20 µl of reaction mixture contained 5 pmol of forward and reverse primers, approximately 1 µg of first strand cDNA, 4 nmol of dNTP mixture, 2 µL of 10 × PCR buffer [100 mM Tris-HCl, pH8.3, 500 mM KCl, 15 mM $MgCl_2$, 0.01% (w/v) gelatin] and 0.2 U of Ex *Taq* DNA polymerase (Takara). Nested PCR was performed under the same conditions using the PCR product diluted 100-fold and the rHSP70_3RACE2 primer. The amplified products were subcloned into pGEM-T vecter (Promega) and sequenced as described above. The nucleotide sequence of the open reading frame was confirmed by a single PCR using rHSP70_full_f1 and rHSP70_full_r1 primers

(supplementary table S1). Conditions of the single PCR were same as described in 5' RACE.

Semi-Quantitative RT-PCR

RNA extraction and 1st strand cDNA synthesis were performed as described above. Primers rHSP70_gapF and rHSP70_gapR were designed to amplify the region encoding the GGMP repeat of *B. plicatilis* HSP70cB1i and HSP70cB2i genes (supplementary table S1). The β-actin gene was used as the internal control with specific primers rActinF and rActinR reported previously (Kaneko et al. 2005). PCR was carried out at 94˚C for 3 min followed by 20 – 40 cycles of 94˚C for 30 s, 58˚C for 30 s and 72˚C for 30 s. A 20-µL reaction mixture contained approximately 1 µg of 1st strand cDNA, 10 pmol of rHSP70_gapF and rHSP70_gapR, 2 µL of Ampli *Taq* buffer (Applied Biosystems, Foster City, CA), 4 nmol of dNTP mixture, and 0.2 U of Ampli *Taq* DNA polymerase (Applied Biosystems). The PCR products were separated with an 8% acrylamide gel and visualized by ethidium bromide staining.

Quantitative Real-Time PCR

Rotifers were collected 0, 2, 4, 6, and 8 h after the heat treatment, and stored in ISOGEN (Takara) at -80˚C until use (three replicates for each time point, one replicate contained eight individuals). Rotifers collected before the heat treatment were used as the control. Quantitative real-time PCR was performed as reported previously (Kaneko et al. 2011). Primers for the β-actin gene were described previously (Kaneko et al. 2005); primers for HSP70 genes were designed using the Primer Express software ver. 2 (Applied Biosystems) (supplementary table S1). R version 3.5.1. and the multcomp package (Hothorn et al. 2008) was used for one-way analysis of variance (ANOVA) followed by the Dunnett's test on the Macintosh platform.

In situ Hybridization

Rotifers were fixed in PBS containing 4% paraformaldehyde 4 h after the heat treatment. *In situ* hybridization was performed as reported previously (Kaneko et al. 2011), using DIG-labeled RNA probes synthesized as follows. Primers HSP70_insituF and HSP70_insituR were designed to amplify the DNA fragment of 2026 - 2103 nt of *B. plicatilis* HSP70cB1i gene (supplementary table S1). PCR was performed in a 20 µL reaction mixture containing approximately 1 µg of the genomic DNA of *B. plicatilis,* 10 pmol each of the HSP70_insituF and HSP70_insituR primers, 2 µL of 10 × PCR buffer, 4 nmol dNTP mixture, and 0.2 U of Ex *Taq* DNA polymerase (Takara). The amplification was carried out with the initial denaturation at 94˚C for 3 min and 40 cycles of 94˚C for 30 s, 60˚C for 30 s, and 72˚C for 1.5 min, followed by the final extension step at 72˚C for 5 min. The amplified products were subcloned into pGEM-T easy vector (Promega). DIG-labeled RNA probes were synthesized with a DIG RNA labeling kit (Roche) according to the manufacturer's instructions.

Hothorn T, Bretz F, Westfall P. 2008. Simultaneous inference in general parametric models. *Biom J* 50:346-363.
Kaneko G, Kinoshita S, Yoshinaga T, Tsukamoto K, Watabe S. 2002. Changes in expression patterns of stress protein genes during population growth of the rotifer *Brachionus plicatilis*. *Fish Sci* 68:1317-1323.
Kaneko G, Yoshinaga T, Yanagawa Y, Kinoshita S, Tsukamoto K, Watabe S. 2005. Molecular characterization of Mn-superoxide dismutase and gene expression studies in dietary restricted *Brachionus plicatilis* rotifers. *Hydrobiologia* 546:117-123.
Kaneko G, Yoshinaga T, Yanagawa Y, Ozaki Y, Tsukamoto K, Watabe S. 2011. Calorie restriction-induced

maternal longevity is transmitted to their daughters in a rotifer. *Funct Ecol* 25:209-216.
Yoon SH, Itoh Y, Kaneko G, Nakaniwa M, Ohta M, Watabe S. 2008. Molecular characterization of Japanese sillago vitellogenin and changes in its expression levels on exposure to 17 beta-estradiol and 4-tert-octylphenol. *Mar Biotechnol* 10:19-30.