

1 **Concise Title:**

2 **PhenoMIP: High Throughput Phenotyping of Diverse *C. elegans* Populations via**  
3 **Molecular Inversion Probes**

4

5 **Calvin Mok\***, **Gabriella Belmarez\***, **Mark L. Edgley†**, **Donald G. Moerman†**, **Robert H.**  
6 **Waterston\***

7 \* Department of Genome Sciences, University of Washington, Seattle, Washington, USA, 98195

8 † Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada,

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10 **Running Title:**

11 **Quantitative fitness analysis in *C. elegans* via PhenoMIP**

12

13 **Keywords:**

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20

21 **Corresponding Author:**

22 Calvin Mok

23 661 University Ave

24 MaRS Research Centre, West Tower, 16<sup>th</sup> Floor

25 Toronto, ON, Canada

26 M5G 1M1

27 **Abstract**

28 Whether generated within a lab setting or isolated from the wild, variant alleles continue to be an  
29 important resource for decoding gene function in model organisms such as *Caenorhabditis*  
30 *elegans*. With advances in massively parallel sequencing, multiple whole-genome sequenced  
31 (WGS) strain collections are now available to the research community. The Million Mutation  
32 Project (MMP) for instance, analysed 2007 N2-derived, mutagenized strains. Individually, each  
33 strain averages ~400 single nucleotide variants amounting to ~80 protein coding variants. The  
34 effects of these variants, however, remain largely uncharacterized and querying the breadth of  
35 these strains for phenotypic changes requires a method amenable to rapid and sensitive high-  
36 throughput analysis. Here we present a pooled competitive fitness approach to quantitatively  
37 phenotype subpopulations of sequenced collections via molecular inversion probes  
38 (PhenoMIP). We phenotyped the relative fitness of 217 mutant strains on multiple food sources  
39 and classified these into five categories. We also demonstrate on a subset of these strains, that  
40 their fitness defects can be genetically mapped. Overall, our results suggest that approximately  
41 80% of MMP mutant strains may have a decreased fitness relative to the lab reference, N2. The  
42 costs of generating this form of analysis through WGS methods would be prohibitive while  
43 PhenoMIP analysis in this manner is accomplished at less than 1% of projected WGS costs. We  
44 propose methods for applying PhenoMIP to a broad range of population selection experiments  
45 in a cost-efficient manner that would be useful to the community at large.

## 46 **Introduction**

47 The *C. elegans* haploid genome is compact, containing just over 100 Mb, and yet is capable of  
48 generating a complex organism with a defined cell lineage (Sulston et al., 1983). Despite our  
49 detailed knowledge of this organism, much of its biology remains unclear. At current, only 9,645  
50 Wormbase genes (Wormbase web site, 2019) have phenotype descriptions reported from either  
51 variant alleles or RNAi knockdown experiments, suggesting that the function of nearly half of *C.*  
52 *elegans* protein coding genes remain experimentally uncharacterized. Knowledge of where and  
53 when a gene is expressed can provide clues to function and many large data sets have  
54 elucidated gene expression patterns across embryonic, larval and adult timepoints.  
55 Furthermore, multiple techniques have begun to resolve tissue-specific and even cell-specific  
56 expression profiles (Boeck et al., 2016; Cao et al., 2017; Gracida and Calarco, 2017; Kaletsky et  
57 al., 2018; Warner et al., 2019). However, this information does not directly reveal gene function  
58 *per se*.

59 Forward genetics screens by methods such as chemical mutagenesis, provide a means  
60 of recovering alleles that result in a detectable phenotype of interest such as sterility, lethality, or  
61 altered reporter expression. These alleles can then be genetically mapped, sequenced, and  
62 functionally analysed. In this manner, a specific phenotype can be screened across hundreds of  
63 thousands of mutated genomes, thereby querying a very large search space (Brenner, 1974; De  
64 Stasio and Dorman, 2001; Kevin et al., 2006). The identification of causal variants across this  
65 space can be a laborious process although a variety of methods now exist to aid in the  
66 sequencing and mapping of mutant genomes (Doitsidou et al., 2010; Jaramillo-Lambert et al.,  
67 2015; Minevich et al., 2012; Mok et al., 2017). In contrast, a reverse genetics screen by RNAi,  
68 generates a smaller potential search space by querying a collection of specific gene knock-  
69 down targets for a detectable phenotype in a limited number of genetic backgrounds (Fraser et  
70 al., 2000; Kamath et al., 2003; Lehner et al., 2006). Consequently, the solution space is  
71 relatively well-defined since validated hits require no genetic mapping, although such screens

72 are generally confined to knocking down gene expression rather than necessarily exploring  
73 states of altered protein function. Depending upon assay format, an RNAi screen's throughput  
74 can be comparatively less than a mutagenesis screen. Furthermore its effects may be  
75 problematic, producing false negatives or weak hits due to incomplete knockdown or false  
76 positives from the knockdown of gene families (De-Souza et al., 2019; Fraser, 2000; Parrish et  
77 al., 2000). In both screening methods, the ability to score a detectable phenotype can be  
78 affected by the presence of redundant paralogs or entire parallel systems that can compensate  
79 for a reduced function (for review see (Jorgensen and Mango, 2002)).

80         Whether because of paralogs or other reasons, phenotypically weak alleles in both  
81 screens are potentially missed or simply disregarded. These weak alleles might be mistaken for  
82 stochastic variation in a cursory analysis but could provide important insights into function. For  
83 example, such weak alleles could produce small changes in developmental timing or fecundity  
84 that would affect population fitness (Diaz and Viney, 2014; Perez et al., 2017; Richards et al.,  
85 2013; Schnabel et al., 1997). Subtle population-wide shifts in phenotypic fitness require  
86 quantitative methods of analysis that go beyond low-resolution phenotype qualifiers such as  
87 slow-growth, sterile, or lethal. In recent years, strides have been made in the quantitative  
88 analysis of fitness (Crombie et al., 2018; Elvin et al., 2011; Ramani et al., 2012). Advances in  
89 next generation sequencing technologies have led to a number of quantitative approaches to  
90 population analysis of singular genetic backgrounds by comparing deeply-sequenced samples  
91 for changes to transcription, small RNA populations, and heterochromatin (Araya et al., 2014;  
92 Boeck et al., 2016; Daugherty et al., 2017; Warf et al., 2012). Leveraging current sequencing  
93 paradigms to analyse population fitness would contribute to the process of assigning function to  
94 poorly characterized genes or alleles.

95         To further expand our knowledge of *C. elegans* gene function, we sought to develop an  
96 assay that could 1) mimic the allelic diversity of a forward genetics screen but with a smaller  
97 solution space much like a reverse genetics screen and 2) generate quantitative data regarding

98 population fitness to assess potential gene function. We exploited the self-fertilizing  
99 hermaphroditic nature of *C. elegans* to grow multiple strains in pools without genetic mixing. We  
100 also realized that the distinct mutations in each strain could be treated as a barcode to identify  
101 and quantify the representation of the strain in the pool. To assay the mutations and thus the  
102 representation of each strain in these pools, rather than use whole genome sequencing, which  
103 would have been prohibitively expensive, we adapted molecular inversion probes (MIPs) to  
104 identify strain-specific variants (Hiatt et al., 2013). We previously used MIPs for the genetic  
105 mapping of temperature-sensitive alleles in a collection of *C. elegans* mutant strains (Mok et al.,  
106 2017); here we analyse population growth in a multi-generational competitive fitness assay to  
107 phenotype by MIPs (PhenoMIP) by quantifying the proportion of each strain in a pool. As a proof  
108 of principle, we utilized the Million Mutation Project (MMP) as a source for our strains. The MMP  
109 library of 2007 N2-derived mutant strains harbours a variety of coding alleles including potential  
110 null alleles across 8150 protein-coding genes, and coding or splice site-altering SNVs across  
111 19,666 genes (Thompson et al., 2013). The phenotypic consequences for many of these  
112 variants remain unexplored; we hypothesized that some may play a role in overall fitness.  
113 Therefore, we identified unique genetic markers suitable for detection by MIPs for each strain;  
114 using these strain-specific MIPs, we effectively generated barcodes for composition analysis of  
115 genotypes within a genetically heterogenous population – analogous to methods used in yeast  
116 (Hardenbol et al., 2003). We analysed population composition at multiple timepoints, thus  
117 determining the relative fitness for each individual strain within a pool, and thereby cataloging  
118 the potentially subtle phenotypes of this collection. Our observations suggest that PhenoMIP  
119 can identify strains with a range of population fitness phenotypes, including those that may  
120 ordinarily be overlooked. Overall, we show that PhenoMIP is a quantitative approach that  
121 combines mutagenized genomes that have been previously sequenced and assays them  
122 across multiple substrate conditions in a cost-efficient and high-throughput fashion.

## 123 **Results**

124 **Molecular inversion probes reliably track multiple strains within a mixed sample**

125 Previously, we demonstrated the usefulness of MIPs as a method to genetically map mutant  
126 alleles (Mok et al., 2017). In that study, our empirical analysis of MIP behaviour suggested that  
127 their accuracy and precision were highest when identifying smaller subpopulations of variants.  
128 Based on this observation, we recognized that the MIP assay could be applied in a large-scale  
129 analysis of diverse compositions of strains with complex mixtures of genomic DNA. The  
130 mutagenized strains of the MMP collection presented an excellent test set. The MMP strains  
131 have, on average, nearly 400 single nucleotide variants (SNVs) per strain, of which,  
132 approximately 80 are protein coding changes (Thompson et al., 2013). These strains represent  
133 a unique resource for analysing gene function on a large scale.

134 As a first step we designed a specific set of MIPs to track strain-specific variants (**Figure**  
135 **1a**). In order to avoid targeting closely spaced variants that might influence the effectiveness of  
136 individual MIP assays and because we wanted to preserve the ability to make pools from any  
137 combination of MMP and wild isolate strains, we first combined variants from the 2007 mutant  
138 and 40 wild isolates strains of the entire MMP project. We eliminated shared alleles, and then  
139 chose SNVs separated by a minimum distance of 300 bp. From this list of unique candidate  
140 sites, we generated candidate MIP sequences (Mok et al., 2017) and for each strain we  
141 identified the highest scoring MIP sequence on each linkage group. From these top six MIPs,  
142 we assigned four representative MIPs specific to each strain (**Figure 1b**, and **Supplemental**  
143 **Data SD1**) with the purposes of tracking chromosomal representation in the event of cross-  
144 progeny contamination while maintaining minimal reagent costs.

145 To ascertain the representation of each strain in a pool, the four MIPs representing each  
146 target strain within a desired composition of strains were combined into a single pool (**Figure**  
147 **1c**) and used in the generation of MIP sequencing libraries. The libraries were sequenced,  
148 demultiplexed and individual annealing events tracked by the unique molecular identifier (UMI)  
149 present on each oligo (**Figure 1d, e**). Probe sets were then combined to determine mean

150 relative abundance for each target strain within a pooled set of genomes (**Figure 1f**). To  
151 successfully analyse mixed populations in an efficient high-throughput manner the PhenoMIP  
152 approach would require 1) a relatively balanced distribution of reads for each probe; 2) a low  
153 false positive rate to determine a reasonable lower bound on probe accuracy; and 3) precision  
154 between strain-specific targets to ensure that subpopulation analysis was consistent.

155 To test the above parameters, we generated a pool of 192 MIPs designed to target SNV  
156 sites for 48 MMP strains (**Supplemental Data SD2**). We generated five different sets of  
157 genomic DNA mixtures composed of subsets of 46 of the 48 MMP target strains in different  
158 proportions (two strains failed to yield adequate amounts of DNA) and used these as template  
159 samples for the generation of MIP sequencing libraries (**Supplemental Data SD2**). From these  
160 libraries we observed the expected composition and proportion of genotypes for the original  
161 genomic templates, suggesting that overall cross-MIP interference from multiplexing was  
162 negligible (**Supplemental Figure S1a**) and that the variant information from sequencing was  
163 correct. We analysed the total number of UMIs for each MIP to gauge the efficiency of each  
164 probe. We observed eleven MIP targets that, across all libraries, consistently produced UMI  
165 counts below 20% of the mean number of UMIs per MIP in an individual library; these were  
166 removed from further analyses (**Supplemental Figure S1b**). To investigate the read distribution  
167 of this adjusted dataset, we normalized the UMI counts for each MIP against the minimum read  
168 number within its sequencing set. The normalized distribution of reads spanned across a ~9-fold  
169 range with an inter-quartile range of 2-fold to 6-fold suggesting that our distribution was  
170 relatively unimodal and ranged within a single order of magnitude (**Supplemental Figure S2a**  
171 **and S2b**).

172 Next, for each sequenced library, we analysed the MIP reads from target strains that  
173 were excluded from the genomic template, calculating a total false positive rate of  $1.6 \times 10^{-4}$   
174 across five MiSeq-generated data sets for which the mean UMI count per MIP was 1630 with  
175  $1.2 \times 10^6$  unique capture events across the total set. We also compared two sequencing runs of



176 the same PhenoMIP library with false positive rates of  $1.49 \times 10^{-4}$  at  $3.9 \times 10^5$  total capture events  
177 versus  $1.18 \times 10^{-4}$  at  $5.14 \times 10^6$  total capture events. Combining all data sets we confirmed a total  
178 false positive rate of  $1.25 \times 10^{-4}$  across all MIPs. We estimated the mean false positive rate per  
179 individual MIP to be  $1.29 \times 10^{-4} \pm 1.38 \times 10^{-4}$ , which compares well with our prior observations  
180 (Mok et al., 2017).

181 When initially planning experimental design, we chose to work with pools of  
182 approximately 50 strains per set, resulting in an expected average initial population abundance  
183 of  $2 \times 10^{-2}$ . With such a low starting abundance it was important to assess the precision between  
184 each set of strain-specific MIPs to ensure that the variation between these probes was low  
185 enough to consider their mean value a consistent assessment of strain abundance. We  
186 observed the mean standard deviation across all strain-specific MIP sets was  $2.33 \times 10^{-3} \pm$   
187  $6.88 \times 10^{-3}$ . Confirming prior observations, the absolute variance between strain-specific MIPs  
188 was dependent upon relative abundance within the sample. Subsetting the data, target strains  
189 above  $5 \times 10^{-2}$  abundance had a combined standard deviation between strain-specific MIPs of  
190  $1.62 \times 10^{-2}$ . Samples with abundance below  $2 \times 10^{-2}$ , however, had a combined standard deviation  
191 between MIPs of  $2.12 \times 10^{-4}$ , which is similar in magnitude to our false positive rate. These  
192 findings were in line with our expectations from prior modeling of MIP behaviour (Mok et al.,  
193 2017) (**Supplemental Figure S2c**).

194 From our analyses, we concluded that relatively consistent and balanced pools of MIPs  
195 could be generated for future analysis on complex populations; that our false positive rates  
196 remained in line with previous observations; and that overall variance among MIPs for a specific  
197 target strain was low, especially in the lower ranges of abundance. In combination with our MIP-  
198 MAP data (Mok et al., 2017), our analysis conservatively suggests that MIPs can accurately  
199 detect variant abundances as low as five standard deviations above the estimated false positive  
200 rate. We determined that relative abundances as low as  $8.2 \times 10^{-4}$  would have a high probability  
201 of being true signal as our largest false-positive value from the dataset was  $7.4 \times 10^{-4}$ . For

202 simplicity, we designated  $1 \times 10^{-3}$  as the minimum abundance required to be considered as  
203 biologically present within a given pooled population. Practically speaking, based on an average  
204 pooled experiment of 50 strains, this translates to detecting a 20-fold decrease from the  
205 expected initial abundance for a target strain. The cut-off value of  $1 \times 10^{-3}$  was the foundation for  
206 later analysis of our data sets with these and other MIP pools (**Methods**).

### 207 **MIPs identify strain fitness defects over multiple generations**

208 Confident of the estimation capabilities of the MIPs, we selected sets of MMP strains to pool for  
209 growth analysis. Each pool was made up of 45-60 different MMP strains and 8-10 independent  
210 replicates were grown for multiple generations to look for differences in fitness between the  
211 strains (**Table 1**). In addition, to investigate the effects of different propagation methods, three  
212 food sources (*E. coli* strains HT115, NA22 or OP50) were used in different experiments and in  
213 one experiment two different methods of transfer were used (see below). The proportion of each  
214 strain in the pool was assayed at the start, terminal and various intermediate points. To ensure  
215 that a similar number of animals was present at the start and in each of the replicates (and  
216 different conditions in experiments where more than one condition was assayed), we hand-  
217 picked 20 animals from each strain at either the L1 (pool M1, M3, M5) or L4 (pool M7, M8, M10,  
218 M11) stages to duplicate *E. coli* seeded plates. We grew these “starter” pools to starvation and  
219 combined uncontaminated plates for an estimated 300-700K animals. This population was  
220 collected and aliquots containing 5-10K animals were used to inoculate replicate cultures under  
221 their specific conditions. Cultures were grown to starvation (72-96 hours at 20-22°C; about a  
222 generation) and aliquots transferred to fresh plates. For all pools except M11, animals were  
223 transferred by chunking, while M11 replicates were split into two groups with transfer either by  
224 chunking or by washing (**Table 1, Methods**). This inoculation-to-starvation cycle was repeated  
225 4-9 times, depending on the experiment. At each cycle a fraction of the population was saved  
226 for later DNA analysis. (**Figure 2**).

227 *In toto*, we used 217 MMP strains across seven experimental pools (**Table 1,**  
228 **Supplemental Data SD3**) to assay their relative fitness. To check the reproducibility of the data  
229 and observe overall trends we applied principal component analysis to the datasets. For  
230 example, with the M11 dataset, replicate samples with the same food source and transfer  
231 method tended to cluster tightly, but with clusters from different generations separating well after  
232 the first generation, particularly along the axis of the first principal component (**Figure 3a, b and**  
233 **Supplemental Figure S3**). Samples also separated by the methods of transfer. PCA analysis  
234 on all the M11 samples at a single timepoint shows the effect of food source as well as method  
235 of transfer (**Figure 3c and Supplemental Figure S4**). The OP50 replicates were not as well-  
236 correlated, and it was observed that these populations starved more quickly than other food  
237 sources. Our observations suggest that under a given experimental condition, population  
238 composition was changing with each generation in a consistent manner that was detectable by  
239 PhenoMIP analysis.

240 Confident that the assay was behaving well overall, we next assessed each strain  
241 separately for relative changes in its abundance over multiple generations across multiple  
242 replicates. For each replicate condition within a pooling experiment, this effectively created a  
243 growth profile for each strain consisting of the total fold-change and the mean fold-change rate  
244 (FCR) per generation. For example, **Figure 4a** plots the relative abundance of strain VC20019  
245 in the M11 pools under various conditions. The log-fold change is modest, with the mean across  
246 all conditions almost zero, indicating that this strain is of average fitness. Closer inspection  
247 suggests that some of the variation is due to the different growth conditions used in M11, with  
248 replicates grown on NA22 and transferred by washing showing better than average growth,  
249 whereas growth on HT115 and chunk transfer grew less well. In agreement with the overall  
250 PCA analysis, growth on OP50 resulted in the most variable log-fold change. We combined  
251 results across replicates for all strains to analyse FCR as a distribution across conditions  
252 (**Figure 4b and Supplemental Figure S5**). We identified 15 strains that failed to thrive (class 0)

253 in the initial pool expansion steps (initial abundance  $< 2.5 \times 10^{-3}$ ) suggesting they harboured  
254 potentially strong deficits to population fitness (**Supplemental Table S1**). We classified the  
255 remaining 202 strains using 393 sequencing libraries across seven competitive fitness pooling  
256 experiments on 95 replicate conditions to generate profiles for 170 strains grown on the bacteria  
257 HT115, 149 strains grown on NA22, and 105 strains grown on OP50 (**Supplemental Figure**  
258 **S6a**). While we observed more subtle differences within some strains for growth on different  
259 bacteria and even for methods of transfer (**Supplemental Figure S6b,c**), we observed  
260 pronounced differences in growth profiles between strains and focused further analysis on this  
261 feature. We observed strains that exhibited poor growth with steep population decline  
262 suggesting fitness defects as well as strains with enhanced growth when compared to our  
263 reference strain VC20019. Based on these observations, we classified each strain into one of  
264 four classes as determined by its mean FCR across all experimental replicates (**Table 2**,  
265 **Supplemental Data SD3**). Classes were designated using a simple 10-generation growth  
266 model to calculate a final abundance ( $A_{10}$ ) based on the  $\log_2$ -transformed mean fold-change rate  
267 ( $\overline{FCR}$ ) such that

$$268 \quad A_{i+1} = A_i * 2^{\overline{FCR}}$$

269 From our initial modeling of MIP behaviour, we determined a lower limit of  $1 \times 10^{-3}$  on abundance  
270 within a pooled sample; we, therefore, used  $A_{10}$  cut-offs of  $1 \times 10^{-3}$ ,  $1 \times 10^{-2}$ ,  $1 \times 10^{-1}$  as boundaries  
271 for determining classes 1 through 4 (**Supplemental Figure S5**). In particular, we observed that  
272 the MMP strain VC20019, which we had previously observed as having a rate of growth similar  
273 to the lab reference strain N2, fell into class 3 with a  $\overline{FCR}$  of 0.135 or growth multiplier ( $2^{\overline{FCR}}$ ) of  
274 1.10 per generation (**Figure 4b**). Subdividing VC20019 data by experimental pool, however,  
275 suggested there was potential for pool-specific variation on a larger scale (**Supplement Figure**  
276 **S7**). The higher  $\overline{FCR}$  for pool M8 is likely a result of over-representation in the seeding  
277 population by double as VC20019 was also conspicuously absent from the M7 seeding

278 population, which was pooled in parallel to M8. Our analysis of the  $\overline{FCR}$  across all strains  
279 suggests a wide range of fitness phenotypes across the MMP collection (**Figure 4c**).

### 280 **The reduced fitness phenotypes of MMP strains were mapped to candidate mutations**

281 Based on the results of our growth analysis, we hypothesized that underlying mutations within  
282 some strains could account for the observed growth rates. We proceeded to genetically map a  
283 subset of class 0 and class 1 strains as they exhibited the greatest reduced fitness in  
284 comparison to our control strain VC20019. We used our MIP-MAP protocol (Mok et al., 2017) to  
285 competitively select against the reduced fitness phenotype and identify a small genomic region  
286 containing the associated causal variant. Briefly, mutant strains were crossed with males of the  
287 mapping strain VC20019 and the population was grown until starvation. A small portion of the  
288 population was then transferred to OP50-seeded 10cm NGM plates. This transfer was  
289 completed approximately once per generation for up to 6 generations. Samples were taken at  
290 each transfer step and used to prepare genomic DNA for MIP-MAP libraries and sequencing.

291 We chose five class 0 and two class 1 strains to map, and successfully identified a  
292 single locus linked to a reduced population fitness for six strains (**Table 3 and Supplemental**  
293 **Figure S8**); a seventh strain appeared to have two loci. After phenotyping individual strains for  
294 possible causes of fitness defects, we were able to assign candidate alleles based on genes  
295 with shared phenotypes. In particular, we verified the mapping results of strain VC40788 by  
296 following a partially penetrant maternal-effect embryonic lethal phenotype (**Figure 4d**). From  
297 VC40788 and VC20019 cross progeny, we individually cultured 100 F2 animals and observed  
298 F3 and F4 progeny to specifically identify recombinant populations that failed to produce dead  
299 embryos or those that starved at the same rate as VC20019 controls. Positively identified  
300 populations were combined for MIP-MAP analysis (**Methods**). The primary candidate mutation  
301 for VC40788 is a G405R mutation in the mitochondrial protein B0303.3, which is predicted to  
302 have multiple functions including an acetyl-CoA C-acyltransferase activity. *B0303.3* has no  
303 reported hypomorphic or null mutant alleles but is reported to have an embryonic lethal

304 phenotype by RNAi (Gönczy et al., 2000; Sönnichsen et al., 2005) and its human ortholog  
305 *HADHB* is implicated in trifunctional protein deficiency phenotype (Purevsuren et al., 2009;  
306 Spiekerkoetter et al., 2003). The identification of a maternal hypomorphic allele of *B0303.3*  
307 provides a means with which to study this disease and its phenotypes in a nematode model.

## 308 **Discussion**

309 With advances in sequencing, genome-editing, and imaging, one remaining bottleneck in  
310 the characterization of the *C. elegans* genome is our ability to identify the phenotypes  
311 associated with gene function (Granier and Vile, 2014; Houle et al., 2010). The ability to quantify  
312 population fitness along a spectrum provides a window into gene functions that may otherwise  
313 be overlooked under current experimental paradigms. Dissecting the contribution of weaker  
314 alleles will help to generate new gene networks and build upon our understanding of worm  
315 development, reproduction, and overall fitness. With PhenoMIP, we analysed strains from the  
316 Million Mutation Project, which offers a unique library of mutagenized genomes with coding and  
317 non-coding elements that remain largely unexplored. We efficiently identified phenotypic traits  
318 related to population fitness in a high-throughput manner by pooling multiple MMP strains in a  
319 multi-generational experiment and sequencing these populations with molecular inversion  
320 probes.

321 To use MIPs as a means of barcoding strains for population analysis, we designed a  
322 series of probes for the 2007 MMP strains and tested a subset on the MMP collection. We  
323 observed that we could accurately gauge a strain's relative abundance within a sample. By  
324 sequencing multiple genomic mixtures, we confirmed a low false positive rate, suggesting we  
325 could use MIPs to accurately identify subpopulations with abundance as low as  $8 \times 10^{-4}$  which  
326 translates to better than 1 in 1000 genomes per sample.

327 As a demonstration of this method, we pooled MMP strains into groups and dissected  
328 population composition over multiple generations. Our observations suggest that this form of  
329 population barcoding is indeed capable of identifying specific Million Mutation Project strains

330 with differing levels of relative fitness. Our analysis shows that PhenoMIP identifies reproducible  
331 condition-dependent population stratification among populations that have been separated for  
332 multiple generations. Based on the strains tested thus far, we estimate upwards of 82% of MMP  
333 strains may harbour alleles contributing to fitness phenotypes in the range of class 0 to class 2.  
334 Given the mutagenized and inbred nature of the MMP strains (Thompson et al., 2013), it is not  
335 surprising to find such an array of fitness phenotypes. These strains, however, represent a  
336 valuable resource to study fitness as the causative alleles of these effects may be in putative  
337 essential genes, poorly characterized genes with only small effects on fitness, or even  
338 regulatory regions of the genome.

339 The observed population-level phenotypes presented in this work are a readout of  
340 relative fitness in a multi-strain competitive environment. Depending on selection and pooling  
341 method, weaker changes to relative fitness may be attributed to the population mixture rather  
342 than the selection variable itself. For instance, in our series of experiments, pools were initially  
343 generated by combining small numbers of larval animals as a seeding parental population that  
344 was expanded before aliquoting out to replicate experiments. During the initial expansion of the  
345 seed population, the stochastic loss of even a single parental animal could impact the  
346 abundance of a strain in the initial stages of the experiment. Conversely, we saw in our analysis  
347 of pool M8, that the doubling of VC20019 animals in the initial pooling also affected the  
348 population structure and mean fold-change rate of VC20019 itself. A potential solution to  
349 mitigate “seeding” variation would be to bleach synchronize (Stiernagle, 2006) all of the target  
350 strains to the L1 larval stage and then combine them in equal portions into a single population  
351 before aliquoting to replicate experiments. Another influence on population structure is the  
352 group of Class 4 strains identified in our study. Their rapid growth and expansion can lead to  
353 drastic population stratification and the premature loss of subpopulations. In these cases, the  
354 quantitative phenotyping of less fit strains may be hindered, less informative, or potentially less  
355 accurate when analysing a multi-generational experiment. Therefore, depending on the nature

356 of the experiment, it may be more advantageous to consider pooling strains of a similar fitness  
357 based on prior phenotype data. Our observations also suggest that food source can alter  
358 population growth with food scarcity contributing to greater variation between replicates. For  
359 example, our OP50 replicates may have experienced premature starvation or uneven food  
360 distribution amongst populations, leading to lower population sizes and possibly affecting the  
361 consistency of the OP50-grown replicates. For an auxotrophic food source such as OP50, it  
362 would be best to highly concentrate cultures in order to generate a thicker lawn for nematode  
363 populations to consume. Lastly, the method and timing of population transfer is a potential  
364 source of selective influence. Our data suggested that chunking versus washing populations to  
365 propagate them did introduce technical variation with some strains. A method of population  
366 transfer that was not addressed in this work is the bleach synchronization method (Stiernagle,  
367 2006), which would add the benefit of removing sporadic contamination while indirectly assaying  
368 developmental timing and fecundity. Some strains may also be differentially sensitive to  
369 bleaching, starvation or recovery from starvation (Baugh, 2013; Webster et al., 2019). Over  
370 many generations, the above technical variation can amplify within the population, potentially  
371 skewing the changes observed. Therefore, when applying specific selective pressures to a  
372 population (temperature, food source, RNAi, etc.), the proper use of control conditions and  
373 replicates can help to reduce the effects of technical variation with minimal impact to the  
374 sequencing burden of the experiment.

375 Looking to the future, given the wide range of sequenced strains available from the  
376 Million Mutation Project and *Caenorhabditis elegans* Natural Diversity Resource (Cook et al.,  
377 2017), a more extensive competitive fitness assessment by PhenoMIP would set the stage for  
378 generating balanced pools of strains based on similar growth rates. From similarly profiled  
379 strains, balanced pools could be generated randomly or based on parameters such as  
380 geographic distribution or specific genotypes or haplotypes of interest. These pools could be  
381 used to screen for phenotypic differences among any number of conditions from temperature or



382 food source (Dirksen et al., 2016; Zhang et al., 2017) to resource limitation, small molecule  
383 exposure, or pathogen infection. Recently, Webster et al., utilized RAD-seq techniques to  
384 assess starvation resistance on a multiplexed pool of 96 wild isolate strains (Webster et al.,  
385 2019). This form of competitive fitness selection is an ideal experimental context for PhenoMIP  
386 to increase potential throughput by addressing additional parameters or variables related to  
387 starvation response. Furthermore, the process of pooled competition facilitates screening on  
388 multiple strains in scenarios where the substrates or reagents to test have limited availability. In  
389 combination with GWAS and genetic mapping, PhenoMIP could prove useful in assembling a  
390 greater understanding of the many unexplored gene and regulatory sequence functions within  
391 the *C. elegans* genome.

392 To our knowledge these experiments are the first to use molecular inversion probes to  
393 analyse *C. elegans* populations for relative fitness. With PhenoMIP, we analysed 217 MMP  
394 strains across 95 replicate conditions and 29 timepoints for a total of 393 genomic samples. A  
395 similar analysis of our experimental data via whole genome sequencing across 393 genomic  
396 samples would be prohibitively expensive. In contrast, our data can be generated on the  
397 equivalent of a single Illumina NextSeq run. Targeted sequencing by PhenoMIP permits  
398 experimentation at a scale well beyond what is reasonably accomplished by standard WGS.  
399 PhenoMIP, however, is not without its caveats as the data generated is limited to assessing  
400 relative abundance and the variants assessed are limited to the population of strains in the  
401 experiment. We believe, however, that the initial processing steps and costs as well as the  
402 “limited” variant diversity of the data are outweighed by the increase in experimental throughput.

403 PhenoMIP has the potential to be applied beyond the MMP and wild isolate strains to the  
404 quantitative analyse of genomic variants in many contexts. Coupled with genome-level editing  
405 techniques, PhenoMIP could be useful in studying allelic series or mutants of entire pathways  
406 for subtle phenotypic effects. The assay format could be converted to look at selection of  
407 phenotypes occurring within a single event or generation, as in a bulk taxis assay or as a

408 method for targeted genome monitoring under selective conditions. The fundamental leverage  
409 of this method is the use of MIPs to reduce the sequencing burden while maintaining informative  
410 parity with WGS formats in identifying subpopulation frequency. In doing so, the throughput of  
411 experimentation can be increased without raising experimental sequencing costs.

## 412 **Methods**

### 413 ***MIP site selection and design***

414 MIP sites were selected in two rounds. Initially the entire MMP SNV data set was used to select  
415 for sites that were spaced a minimum of 300 bp apart to avoid potential collisions with  
416 neighbouring probes. Site selection and rejection was completed in a linear manner based on  
417 the first available SNV on each linkage group within the data set. Locations were not filtered or  
418 optimized to reduce the occurrence of neighbouring SNVs within the 300 bp window. The initial  
419 set of MMP mutant strain MIP sites was then used to remove candidate sites from the MMP wild  
420 isolate data set. Any wild isolate sites within a 350 bp window of mutant candidate sites was  
421 removed from selection. Of the remaining wild isolate SNV sites, a 350 bp selection window was  
422 used to identify potential MIP sites. The list of candidate MIP sites were used to design and  
423 score MIPs based on previously published criteria (Mok et al., 2017). The list of designed MIPS  
424 was subdivided into each individual strain where the highest-scoring MIP for each linkage group  
425 was identified. Of the six MIPs designed for each strain, four were randomly selected for use in  
426 population analysis (**Supplemental Data SD1**)

### 427 ***MIP library pooling, preparation and sequencing***

428 MIPs were pooled based on worm pools being tested and generated as previously published  
429 (Mok et al, 2017). Individual MIPs were normalized to a concentration of 100 uM and pooled to  
430 a maximum volume of 85 ul. 10 ul of 10X Polynucleotide Kinase (PNK) Buffer and 5 ul of PNK  
431 were added to a volume of 85 ul pooled MIPs before incubating for 45 minutes at 37°C and 20  
432 minutes at 80°C. This pool was then diluted to a working concentration of 330 nM. MIP libraries  
433 were generated with 500 ng genomic DNA and appropriate MIP pools as previously described  
434 in Mok et al., 2017. Libraries were sequenced on Illumina MiSeq or NextSeq systems. Libraries  
435 across pools ranged between  $8.3 \times 10^6$  and  $32.7 \times 10^6$  total reads with an average 1507 reads per  
436 probe.

### 437 ***Worm maintenance and pooling***

438 Worms were maintained on standard nematode growth media (NGM) seeded with OP50. Worm  
439 pools were generated from well-fed source plates using exclusively twenty L1 or L4 animals for  
440 each strain. Starting pools were grown on 15cm NGM made with 8X peptone and seeded with  
441 NA22 or HT115. Pools were grown at 20°C to starvation as mostly L1 animals (96-120 hours)  
442 before washing off with 10-15 mL M9. Worms were pelleted and aspirated to 5-6 mL before  
443 population density was assessed. 50-100 ul of pellet was frozen as a representative sample of  
444 the initial pooled population. Pools were then redistributed in equal-sized populations between  
445 5000 and 10000 animals on 15 cm NGM plates that were prepared based on experimental  
446 conditions and grown for 4 days before being transferred to replicate condition plates either by  
447 chunking or washing again. Any remaining animals were washed from plates with double-  
448 distilled water, pelleted, and frozen as samples for later analysis. Each cycle of transfer  
449 approximately followed a single generation and pooling experiments were propagated for 6-10  
450 generations. Heavily contaminated plates/conditions were terminated from propagation and  
451 removed from analysis.

#### 452 ***Mapping of mutant strains***

453 Mutant strains were mapped using either the VC20019 mapping strain or DM7448 (VC20019;  
454 Ex[pmyo-3::YFP]). Briefly, mapping strain males were crossed with mutant hermaphrodites. 15-  
455 20 cross progeny L4 hermaphrodites were selected to a single 10 cm OP50-seeded NGM plate  
456 and grown to starvation before propagating a subpopulation to a replicate 10 cm plate. Slow  
457 growth mutants were mapped on 10 cm NGM plates seeded with OP50 and grown at 20°C.  
458 Mapping populations were propagated under selection for four to seven generations.  
459 Representative samples were chosen to extract genomic DNA as template for MIP-MAP  
460 libraries and then sequenced on Illumina MiSeq or NextSeq instruments. MIP-MAP analysis  
461 was completed as previously described (Mok et al., 2017).

#### 462 ***Competitive Fitness MIP library data analysis***

463 For each specific MIP pool, reads were initially analysed as previously described (Mok et al.,  
464 2017) with the exclusion of the normalization step for each MIP. After abundance of each MIP  
465 was calculated, an average abundance was calculated for each strain as well as a standard  
466 deviation across this average. These values were used in downstream analysis of population  
467 structure across multiple timepoints.

468 Population structure and fold-change analysis was calculated across each experiment  
469 using the amalgamated data from above. Strains with a starting abundance value below  $2.5 \times 10^{-3}$   
470 were eliminated from downstream population analysis. Remaining data were further  
471 transformed with any values below  $1.0 \times 10^{-3}$  being converted to this value to accommodate log  
472 growth analysis. Total fold-change and mean fold change are calculated based on starting and  
473 end-point changes in abundance versus total generations (one generation per expansion). In  
474 samples with negative trajectories, however, the final generation of growth was calculated as  
475 the first instance of abundance at or below the lower limit of  $1.0 \times 10^{-3}$ . Mean fold-change rate  
476 was calculated based on the total fold-change abundance in the final generation of growth  
477 divided by the expected number of generations passed.

#### 478 ***Data Availability***

479 File SD1 contains molecular inversion probe sequences and data for all 2007 MMP strains and  
480 40 wild isolates of the Million Mutation Project. Four candidate probes for each strain were  
481 designed and listed in this file. File SD2 contains all information used in the false positive and  
482 precision analysis of PhenoMIP. File SD3 contains all mean FCR data for each strain on each  
483 replicate in each experimental pool. Custom scripts used to analyse sequencing data are  
484 available upon request. Raw sequence files for each pool are available upon request.

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492 **Literature Cited**

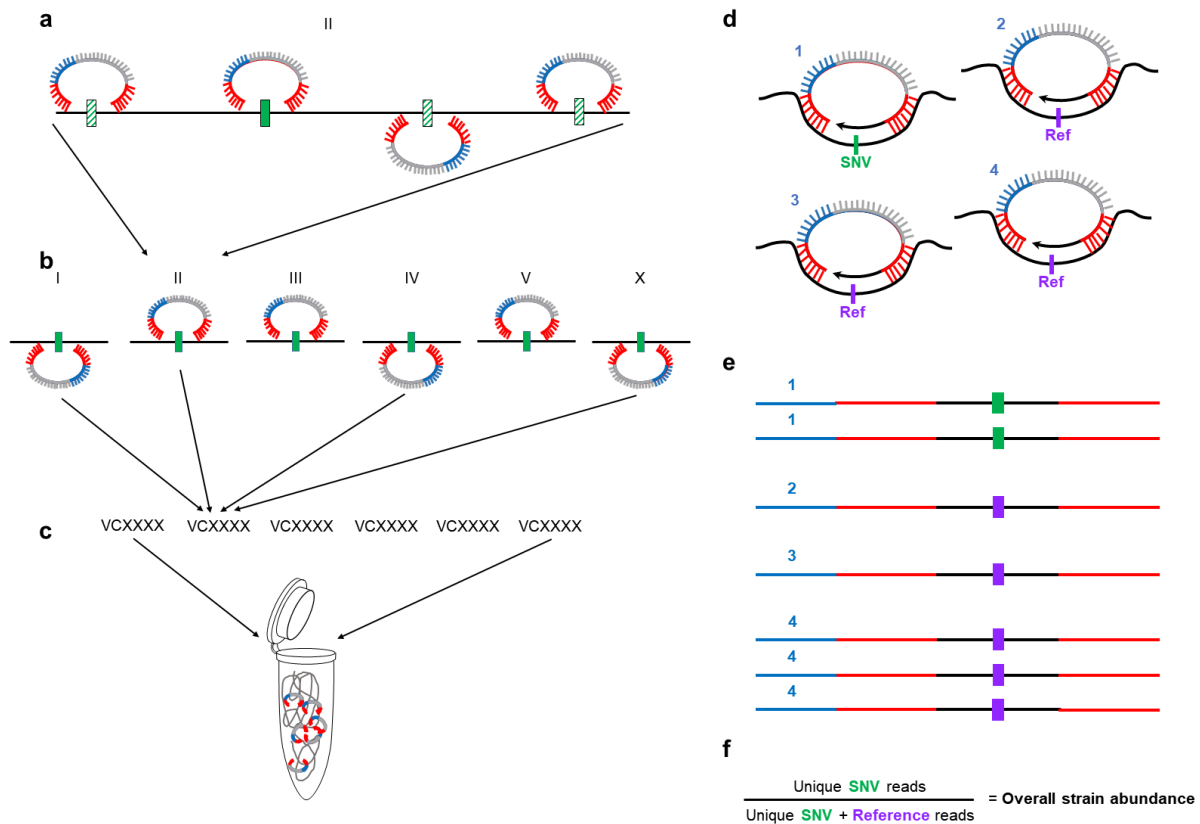
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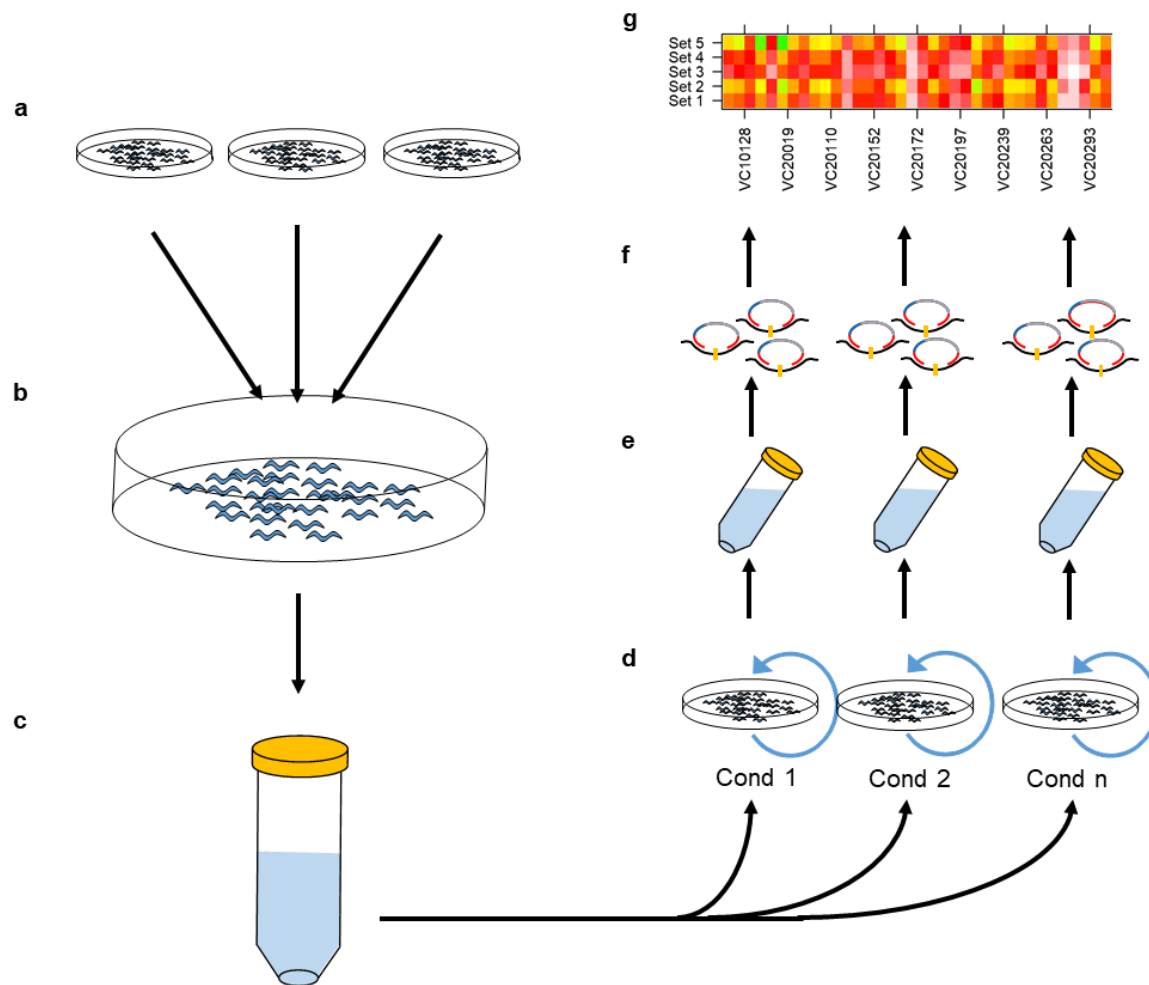
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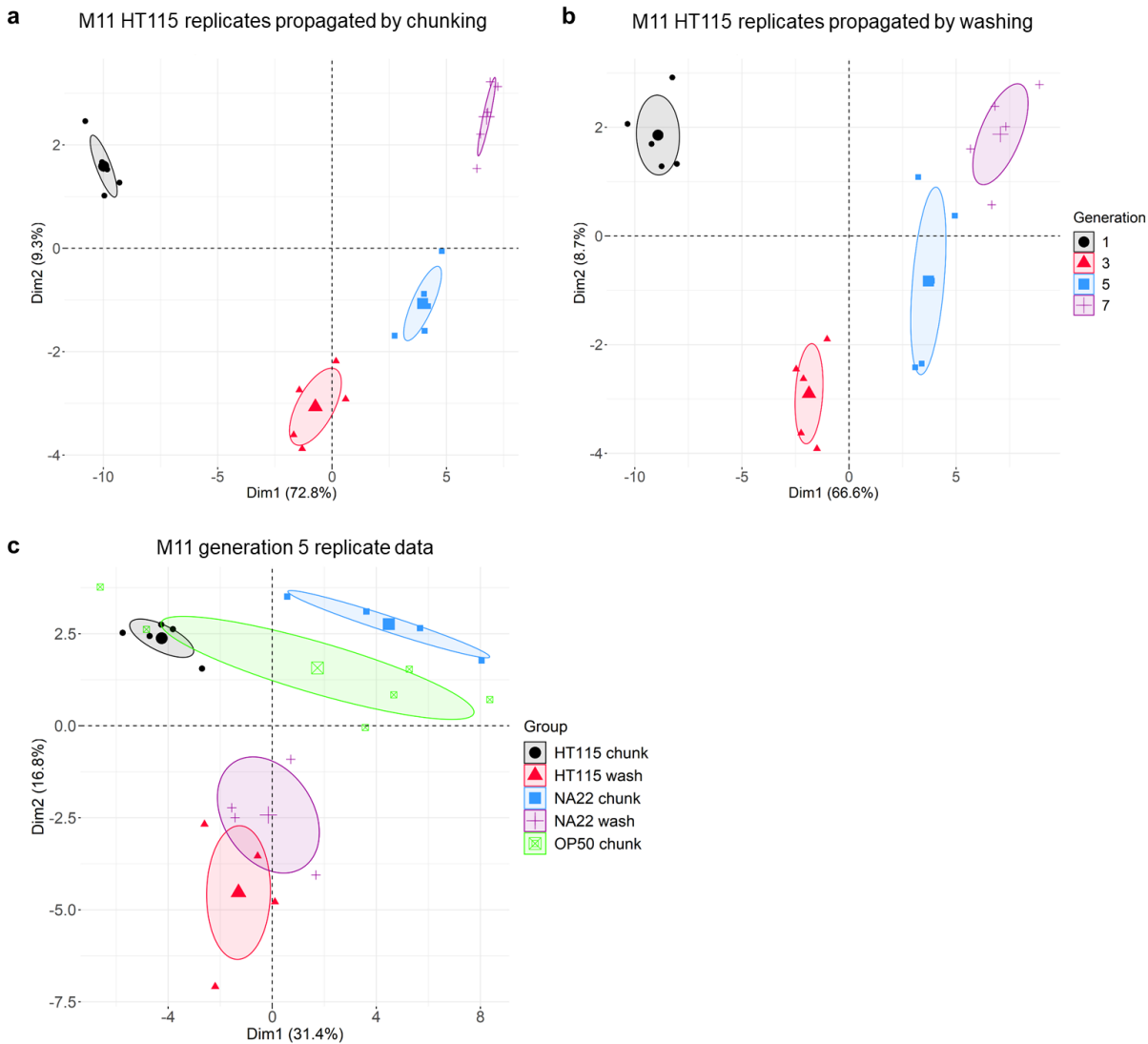


632

633 **Figure 1. Molecular inversion probes as a system of barcoding *C. elegans* strains.** MIP  
 634 sequences include two annealing arms complementary to target sites (red), a unique molecular  
 635 identifier (UMI, blue) and a common backbone used for library amplification and barcoding  
 636 (grey). MIP sites were selected for each of 2047 MMP strains across each chromosome by  
 637 excluding shared variants from all strains and then choosing sites (regardless of strain) across  
 638 the genome that were separated by a minimum of 300-350bp. (a) For each strain, MIP  
 639 candidate sequences were scored (solid and hatched variants). (b) The highest-scoring MIP on  
 640 each chromosome (solid green) was identified. (c) Four of the six MIPs were then selected to  
 641 identify a target strain amongst a pool of strain-specific MIPs. The MIPs would therefore have  
 642 two identifiable states from the gap-fill segment of a sequencing read (d); either the strain-  
 643 specific single nucleotide variant (SNV, green), or a sequence identical to the reference genome  
 644 (purple). (e) After sequencing, each sample was demultiplexed by MIP target and further by the  
 645 UMI to count the total number of unique annealing events specific to the SNV or reference  
 646 sequences. (f) Values were compared to estimate the percentage of SNV events versus the  
 647 total annealing events.



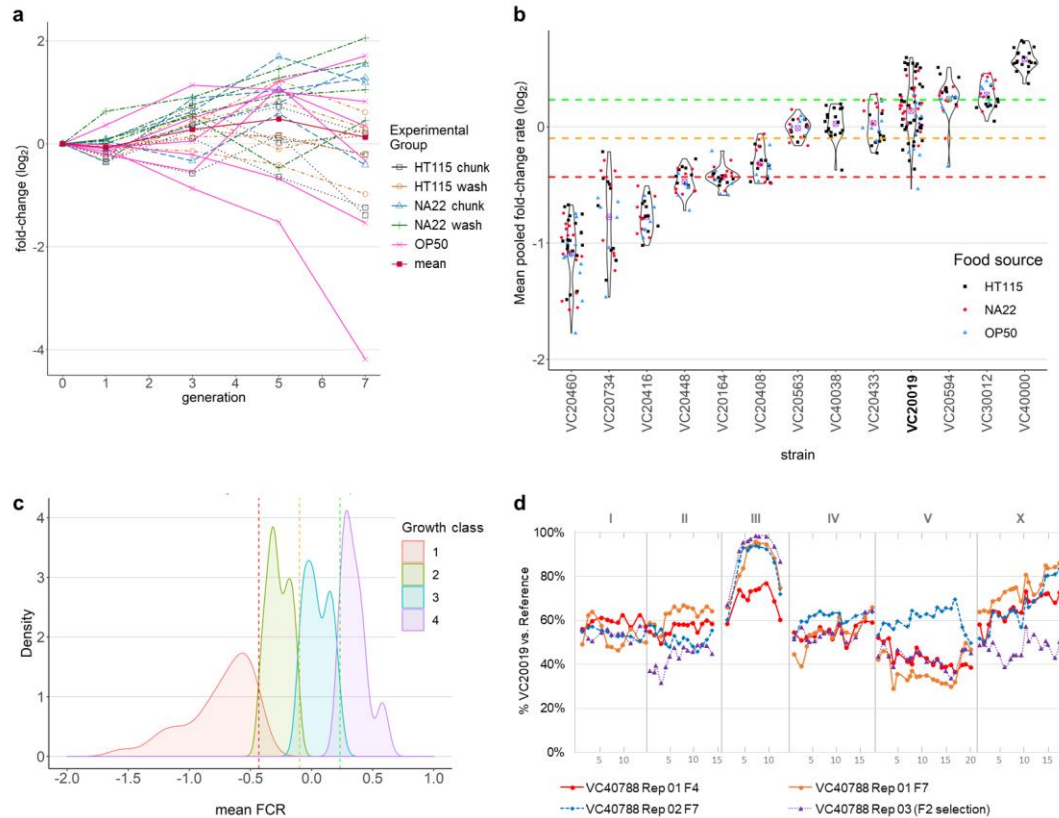
648 **Figure 2. Workflow of PhenoMIP multigeneration competitive fitness assay.** (a) MMP  
 649 strains were selected and grown as separate populations in relative synchronization before 20  
 650 animals of each strain at the L1 (pools M1, M3, M5) or L4 stage (M7, M8, M10, M11) are  
 651 transferred (b) to a communal NGM plate seeded with a bacterial lawn. The communal plates  
 652 are grown in duplicate until the population has starved. (c) Uncontaminated plates are then  
 653 washed and combined into a single starting population and counted for population density  
 654 before being redistributed (d) onto multiple 150 mm NGM plates of varying conditions. Every 72-  
 655 96 hours, the plates reach starvation and a subpopulation of animals is transferred to a new  
 656 plate of the same experimental condition. (e) The remaining animals are collected for extraction  
 657 of genomic DNA to generate MIP libraries for sequencing (f) and data analysis (g) of strain  
 658 abundance and relative fitness.



659

660 **Figure 3. Principal component analysis of PhenoMIP data suggests consistent**  
661 **population stratification related to growth conditions.** (a) PCA of M11 HT115 population  
662 replicates propagated by chunking and (b) M11 HT115 population replicates propagated by  
663 washing are projected along principal component 1 and 2 with samples coloured by generation.  
664 PCA of all M11 replicates from generation 5 projected along principal component 1 and 2 with  
665 samples coloured by combined food source and transfer method.

666



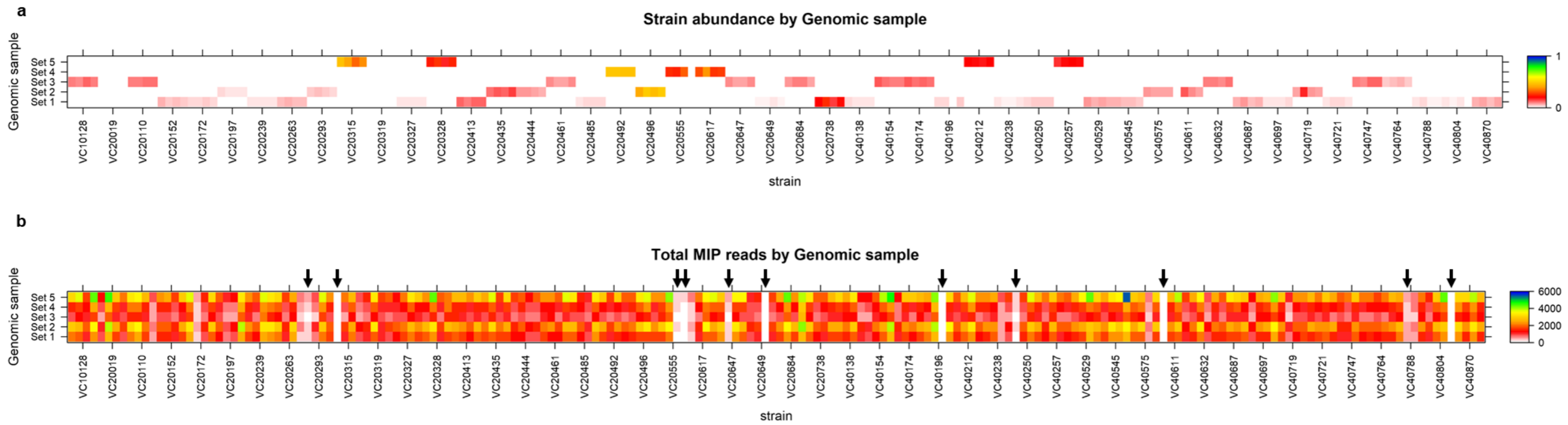
667

668 **Figure 4. Relative fitness can be quantified by PhenoMIP and classified into subgroups.**

669 (a) Line graph of VC20019 growth rate from pool M11 with y-axis showing fold-change ( $\log_2$ ) in  
670 abundance relative to initial abundance at generation 0 (starting population) across multiple  
671 generations (x-axis). Replicates are coloured by experimental food source and transfer method:  
672 HT115 chunk (black squares), HT115 wash (orange circles), NA22 chunk (blue triangles), NA22  
673 wash (green cross), OP50 chunk (pink x) and mean (mean fold change abundance across all  
674 replicates, red square). (b) Violin plots of mean fold-change per generation for a representative  
675 panel of strains. Each point represents the mean fold-change rate calculated from multiple  
676 timepoints for an experimental replicate across one or more pooling experiments. Dots are  
677 colour-coded by experimental condition for growth on either HT115 (black squares), NA22 (red  
678 circles), OP50 (blue triangles) *E. coli* as a food source with overall mean fold change rate (FCR,  
679 purple cross). Coloured dotted lines represent category boundaries using an FCR of -0.4315  
680 (red), -0.0.985 (yellow), and 0.2327 (green). VC20019 (bold) is provided as a reference for  
681 comparison to growth rates shown in (a). (c) 202 strains were assigned a mean FCR and  
682 subdivided into one of four growth classes with kernel density plots for each class. (d) Mapping  
683 data for VC40788, a strain observed to have poor growth rate, identified an interval of interest at  
684 III:7.6-10.8 Mb. Mapping was accomplished using two replicates by competitive fitness for wild  
685 type growth (orange circle and blue diamond) as well as by identifying F2 homozygous wild-type  
686 F2 recombinants in a bulk segregant assay (purple triangle). X-axis units are in megabases  
687 across each chromosome.

688

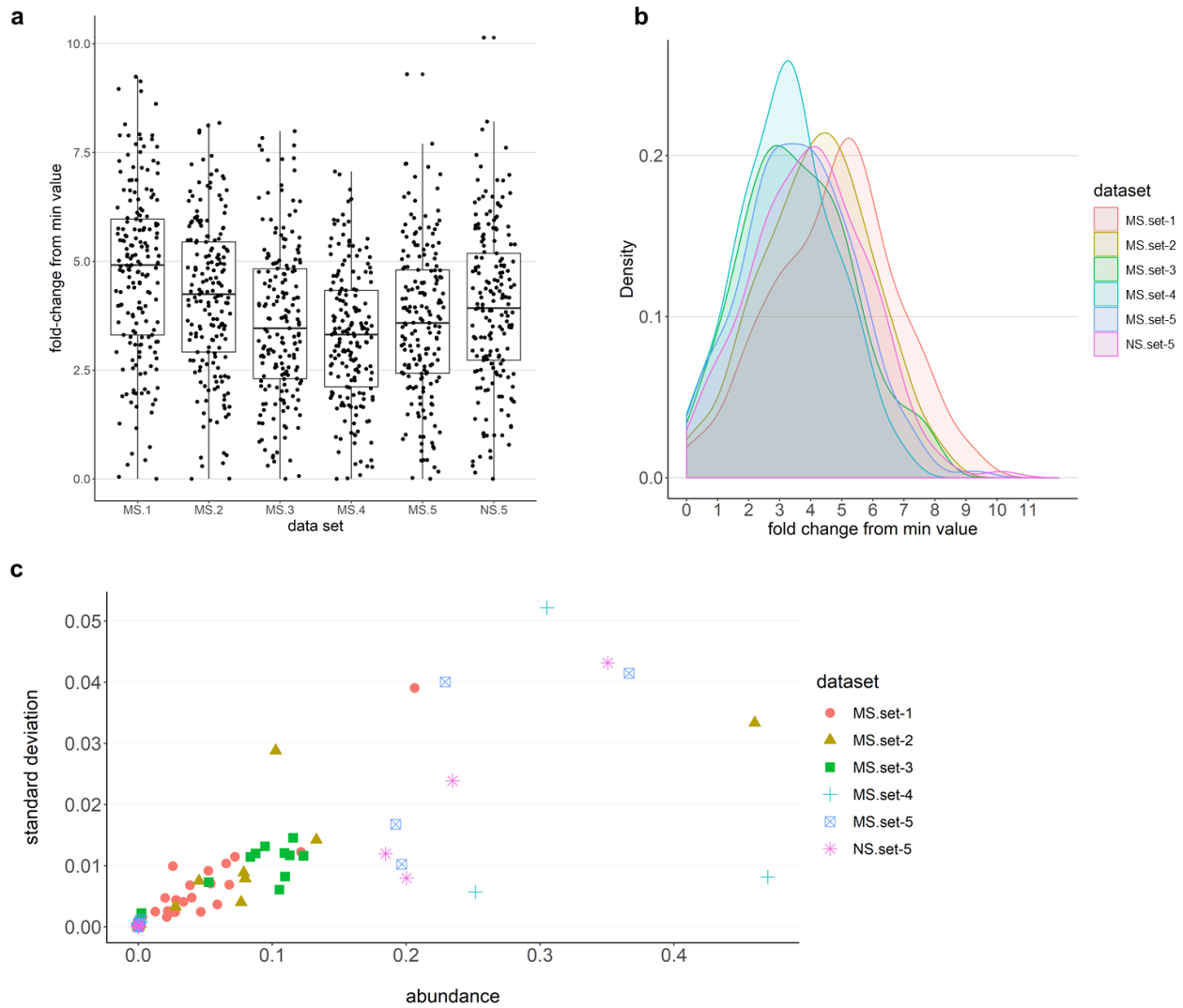
689 Supplemental Figure Legends



690

691 **Supplemental Figure S1. MIPs provide sufficient read depth to specific subpopulations of strain abundance in complex compositions of genomic DNA.** (a) Strains from non-overlapping sets of mixed genomic samples are identified using a multiplexed  
692 pool of MIPs. Strain abundance for each set is indicated by the heatmap legend. (b) A heatmap of total reads per MIP per set broken down by specific strain with black arrows indicating probes with total reads below 20% of the mean read depth across the set.

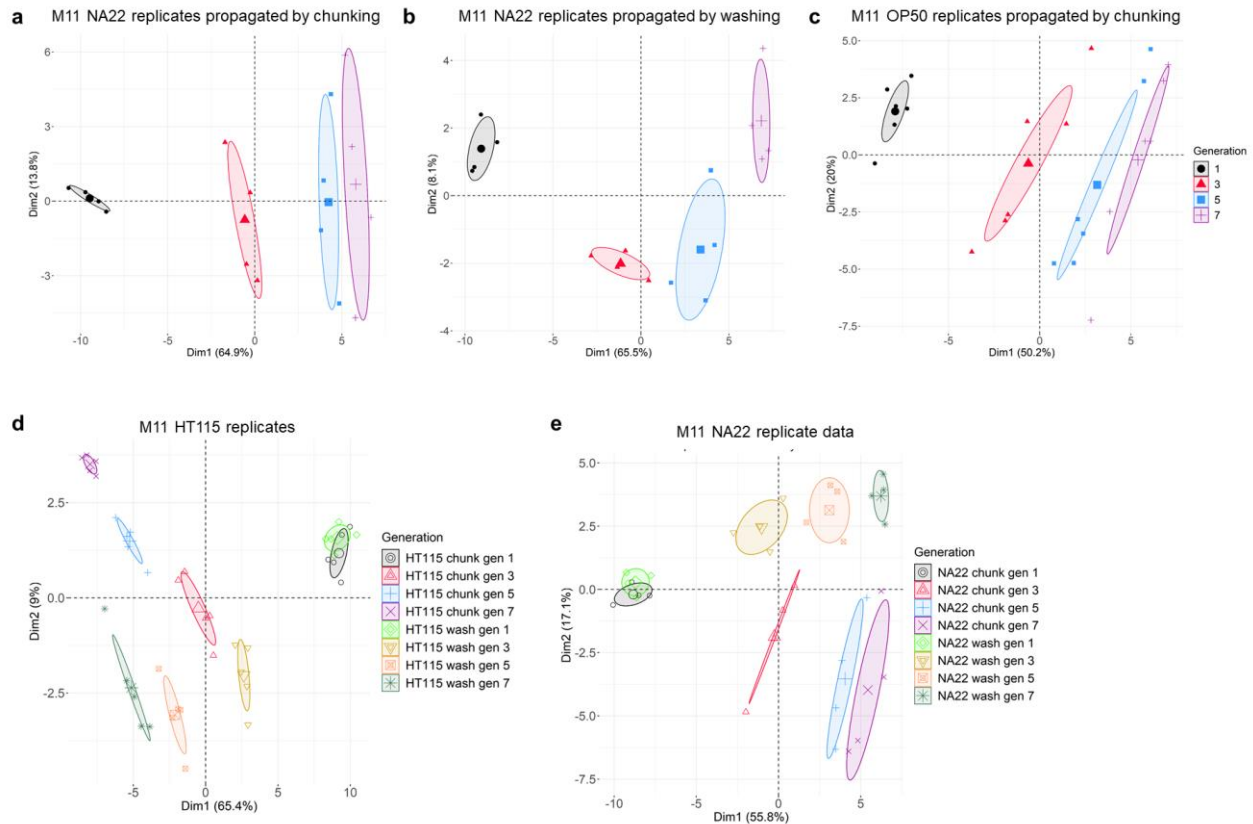
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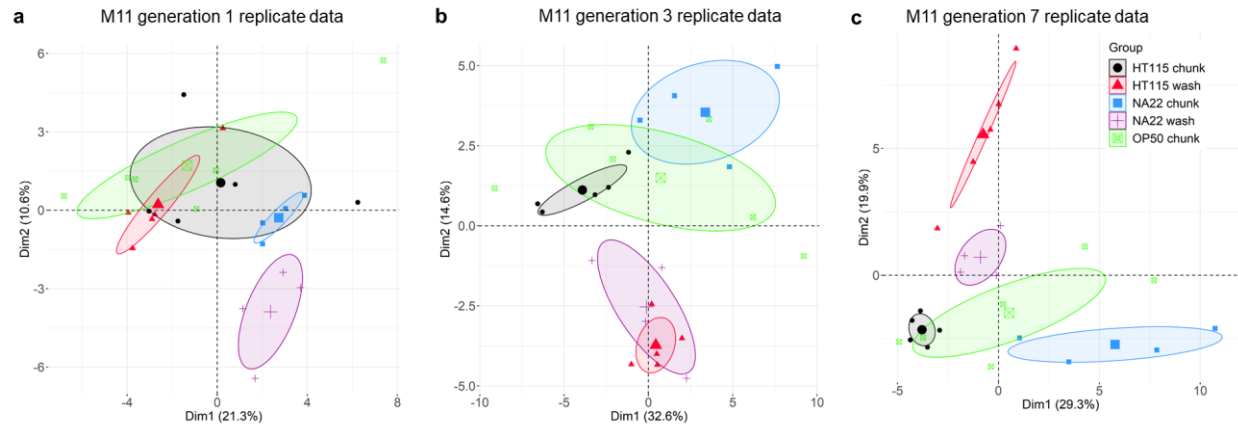
695 **Supplemental Figure S2. MIP pooling across multiple targets remains balanced and**  
696 **precise.** (a) Boxplot of sequencing libraries for the same set of probes across 5 separate  
697 genomic templates overlaid with the fold-change for each probe based on the probe with the  
698 fewest reads in each set. (b) a kernel density plot of each dataset based on the fold-change in  
699 read depth of each probe (MS = MiSeq-generated data; NS = NextSeq-generated data). (c) A  
700 scatterplot of abundance for all strains within each sequenced set versus the standard deviation  
701 of the 3 to 4 probes used to calculate that abundance.





702

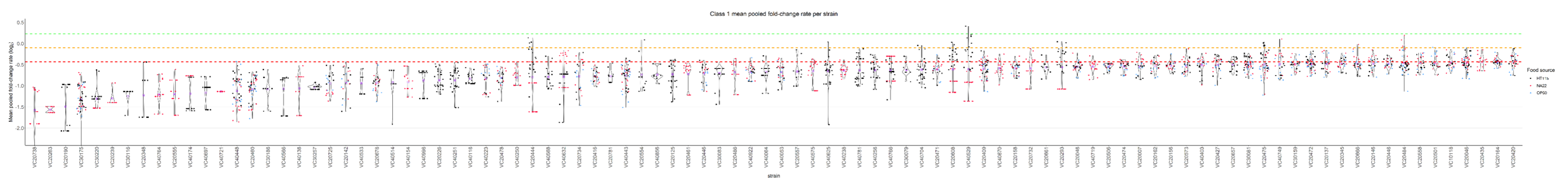
703 **Supplemental Figure S3. Principal component analysis of M11 samples suggest**  
704 **consistent changes to population structure at each generation.** PCA of M11 datasets  
705 separated by combined food source and transfer method into (a) NA22 replicates propagated by  
706 chunking, (b) NA22 replicates propagated by washing and (c) OP50 replicates propagated by  
707 chunking. PCA of M11 HT115 replicate (d) and NA22 replicate (e) data projected along principal  
708 components 1 and 2 with samples identified by combination of transfer method (chunking or  
709 washing) and sample generation (1, 3, 5, or 7).



710

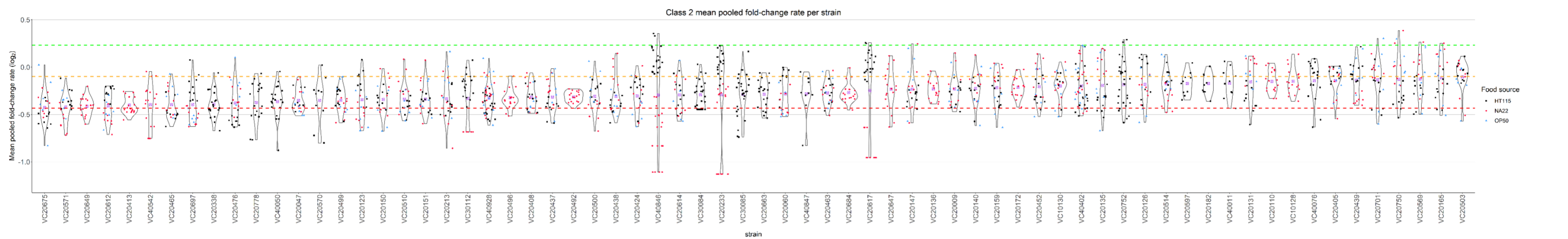
711 **Supplemental Figure S4. Principal component analysis of M11 samples suggest**  
712 **condition-dependent population structure.** PCA of M11 replicate datasets separated into (a)  
713 generation 1, (b) generation 3, and (c) generation 7. Samples are projected along principal  
714 components 1 and 2 for each individual data set and identified by combination of food source  
715 and transfer method.

716



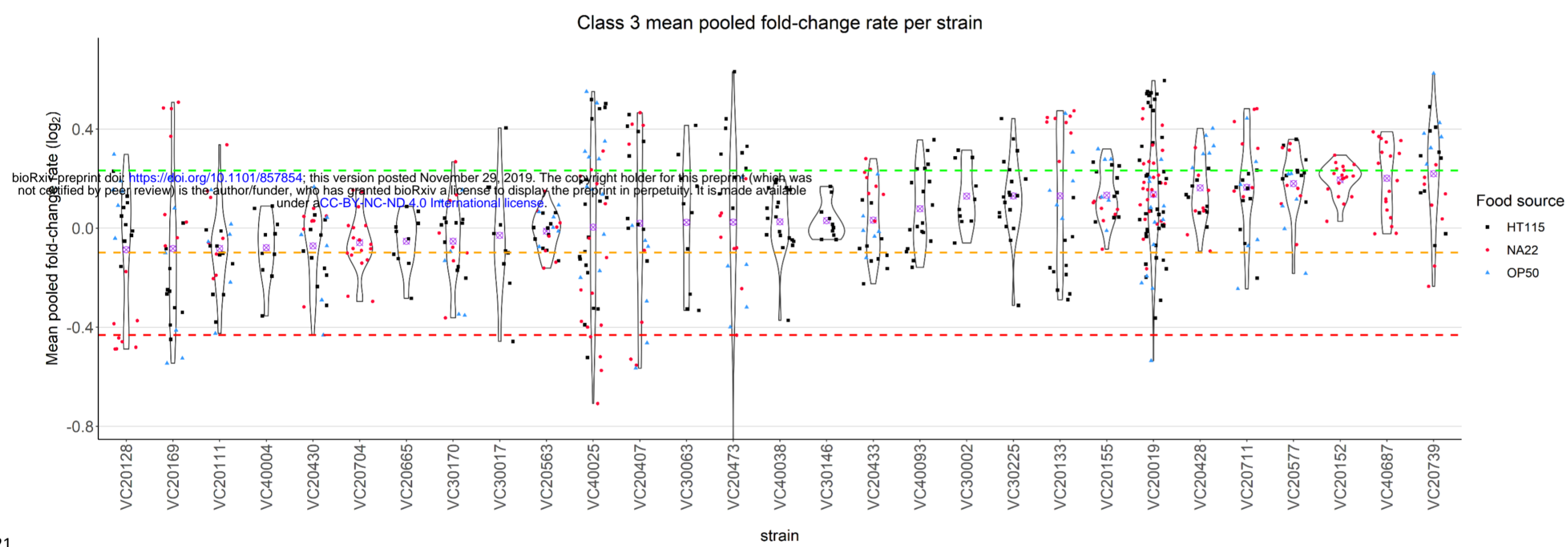
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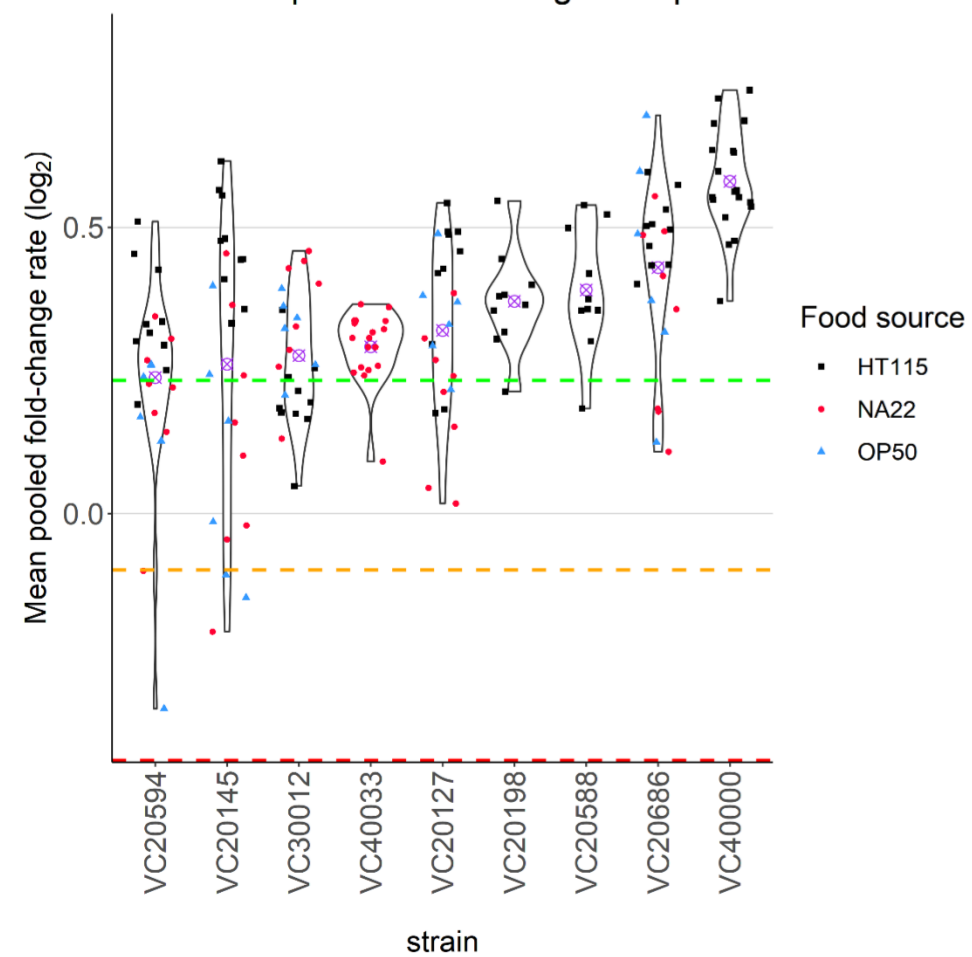
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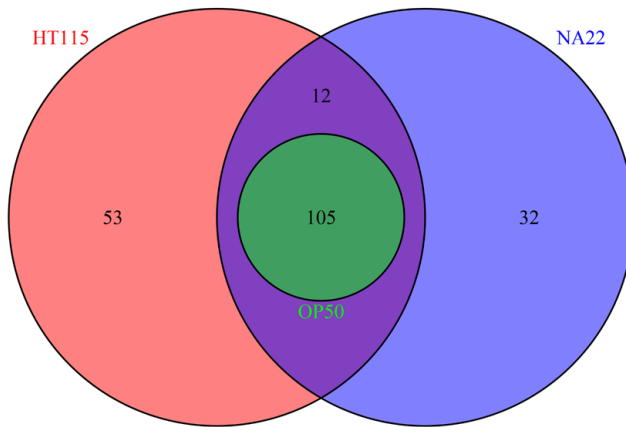
722

**Class 4 mean pooled fold-change rate per strain**

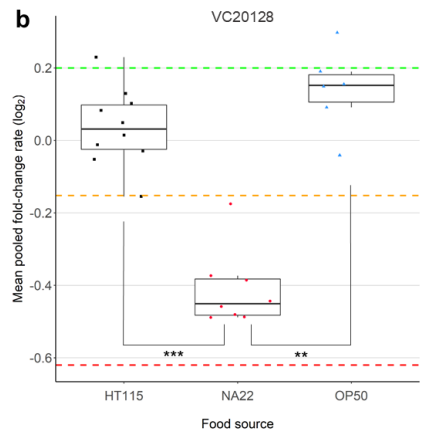


**Supplemental Figure S5. PhenoMIP can generate a gradient of fitness phenotypes from severe to subtle.** Violin plots of mean fold change rate per replicate for 202 MMP strains across the 4 defined classes 1 (a), class 2 (b), class 3 (c), and class 4 (d). Each violin plot discriminates between food sources HT115 (black squares), NA22 (red circles) and OP50 (blue triangles). Strains are sorted within class by the mean FCR (log<sub>2</sub>, purple cross) of all replicate conditions for that strain. Coloured dotted lines represent category boundaries using an FCR of -0.4315 (red), -0.0985 (yellow), and 0.2327 (green).

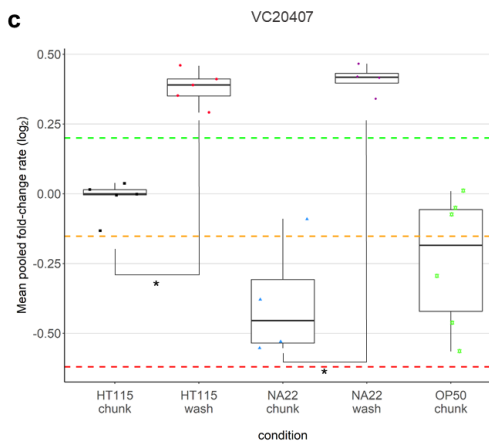
a



b

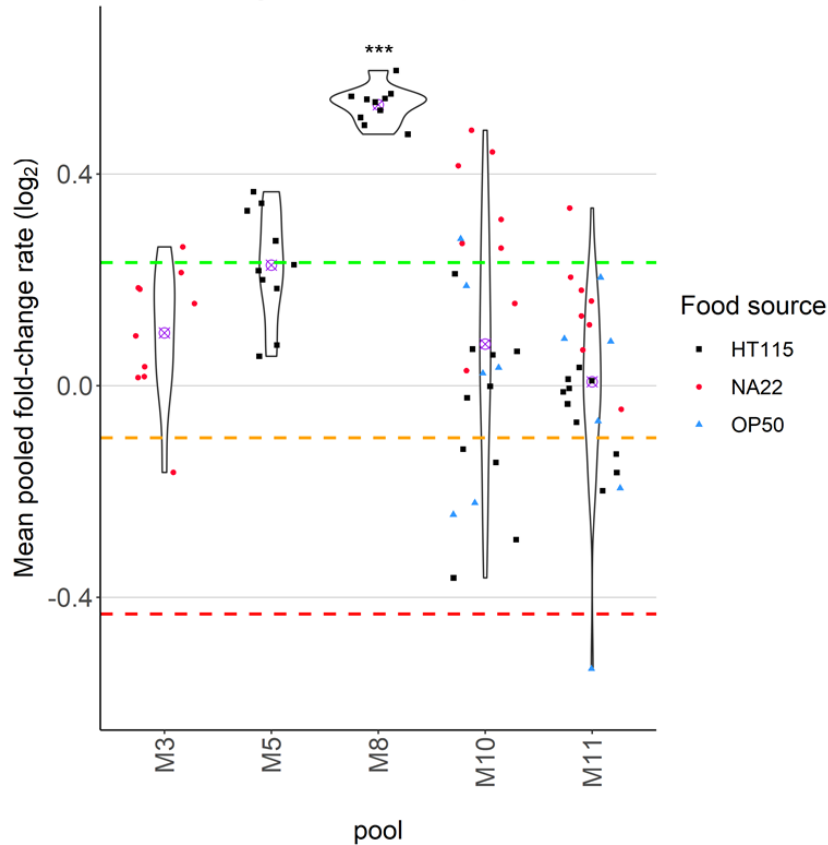


c



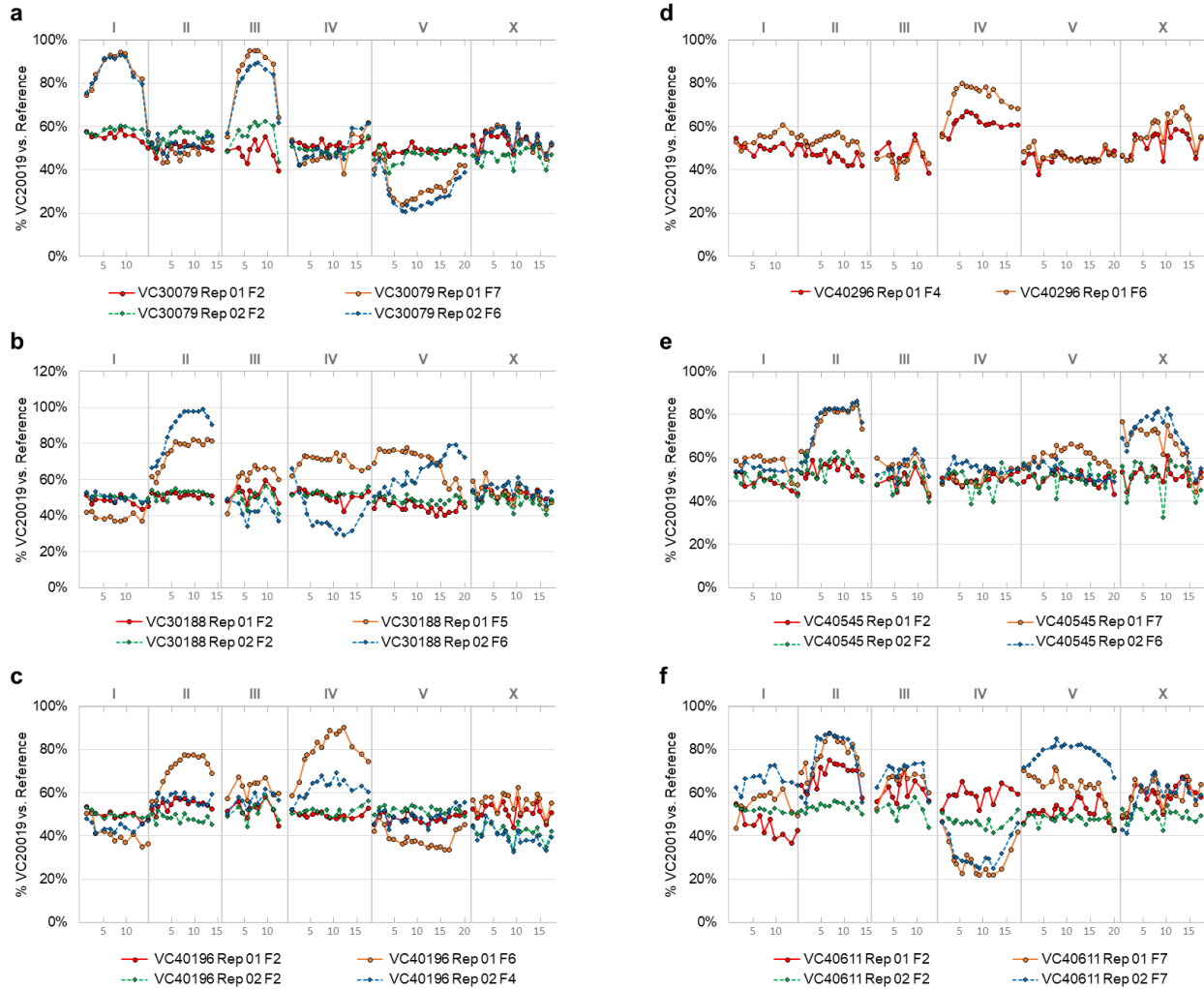
727

728 **Supplemental figure S6. MMP strains were tested across a trio of food sources.** (a) Venn  
729 diagram of each food source used in the PhenoMIP assays and the number of strains tested  
730 with HT115 (pink), NA22 (purple) and OP50 (green). 105 strains were tested on all three food  
731 conditions. (b) VC20128 data from the same pool (M10) suggests specific fitness differences  
732 between growth on NA22 versus growth on HT115 and OP50. (c) VC20407 data from the same  
733 pool (M11) suggests significant changes to growth when comparing samples transferred by  
734 chunking versus washing – regardless of food source. Coloured dotted lines represent category  
735 boundaries using an FCR of -0.4315 (red), -0.0985 (yellow), and 0.2327 (green). \*  $p < 0.05$ ; \*\*  
736  $p < 0.01$ ; \*\*\*  $p < 0.001$  by Kruskal-Wallis with p-values adjusted for multiple testing by  
737 Benjamini-Hochberg method



738

739 **Supplemental Figure S8. Violin plots of VC20019 mean FCR for all replicates grouped by**  
740 **pool.** Violin plots for VC20019 replicates in each pool were generated with M11 represented by  
741 combining datasets based on food source (HT115 = HT115 chunk + HT115 wash; NA22 =  
742 NA22 chunk + NA22 wash). M8 replicate data is significantly different compared to M3, M5,  
743 M10 and M11 which are not significantly different from each other. Each violin plot discriminates  
744 between food sources HT115 (black squares), Na22 (red circles) and OP50 (blue triangles) and  
745 mean FCR (purple cross). Coloured dotted lines represent category boundaries using an FCR  
746 of -0.4315 (red), -0.0985 (yellow), and 0.2327 (green). \*\*\* p < 0.001 by Kruskal-Wallis with p-  
747 values adjusted for multiple testing by Benjamini-Hochberg method.



748

749 **Supplemental Figure S9. MIP-MAP data for 6 strains categorized as class 0 or class 1 by**  
750 **mean FCR.** Strains were mapped using VC20019 with the y-axis representing the proportion of  
751 VC20019 present versus all reads for a MIP target at each locus across the genome. Strains  
752 were mapped in replicate (solid versus dotted lines) and sequenced at two timepoints each (ie.  
753 F2 vs F4). The strains mapped in this fashion were (a) VC30079, (b) VC30188, (c) VC40196,  
754 (d) VC40296, (e) VC40545, and (f) VC40611. X-axis units are in megabases across each  
755 chromosome.

756 **Table 1. Summary of pooled strains**

757

Pool name	Strains	Final				
		sequenced generation	HT115 replicates	NA22 replicates	OP50 replicates	Combined replicates
M1	56	7	0	8	0	8
M3	57	9	0	10	0	10
M5	45	4	10	0	0	10
M7	41	4	9	0	0	9
M8	42	4	10	0	0	10
M10	60	7	10	8	6	24
M11	59	7	10 (5+5)*	8 (4+4)*	6	24
<b>Combined</b>	<b>Unique strains</b>	<b>Timepoints Sequenced</b>	<b>Total HT115</b>	<b>Total NA22</b>	<b>Total OP50</b>	<b>Total Replicates</b>
Total	217	29	49	34	12	95

758 \* Two different methods of transfer were used for replicates

759 **Table 2. Mean fold-change rate summary**

760

Class	Lower bound FCR	Upper bound FCR	Total strains	% of strains
0	NA	NA	15	6.9
1	-8.64	< -0.4315	96	44.2
2	≥ -0.4315	< -0.0985	68	31.3
3	≥ -0.0985	< 0.2327	29	13.4
4	≥ 0.2327		9	4.1

761

762 **Table 3. Mapping data summary**

763

Strain	Pools	Mean FCR	Class	Mapping Interval	Coding alleles	Likely Candidate
VC20019	All but M1	0.136	3	--	--	--
VC30079	M5, M6	-0.740	1	II:7.49-11.5 Mb III:5.8-7.6 Mb	3 3	<i>hpo-35</i> <i>dig-1</i>
VC30188	M5, M6	-1.038	1	II:6.2-12.1 Mb	1	<i>mel-11</i>
VC40196	M1, M3	--	0	IV:8.4-13.9 Mb	13	--
VC40296	M5, M6	--	0	IV:4.2-6.4 Mb	2	<i>rme-2</i>
VC40545	M1, M3	--	0	II:4.4-8.1 Mb	12	<i>tsn-1</i>
VC40611	M1, M3	--	0	II:6.3-8.1 Mb	7	--
VC40788	M1, M3	--	0	III:7.6-10.8 Mb	2	<i>B0303.3</i>

764

765 **Supplemental Table S1. Class 0 mutants, not analysed due to low abundance at**  
766 **experimental start**

767

<b>Strain</b>	<b>Pool(s)</b>	<b>Initial abundance</b>
VC20190	M5	0.00180 / 0.00077
VC20245	M1 / M3	0.00027 / 0.00026
VC20262	M5	0.00074 / 0.00128
VC20315	M1 / M3	0.00000 / 0.00059
VC20328	M1 / M3	0.00136 / 0.00216
VC20338	M5	0.00166 / 0.00209
VC40291	M1 / M3	0.00114 / 0.00000
VC40296	M5	0.00118 / 0.00017
VC40545	M1 / M3	0.00064 / 0.00017
VC40611	M1 / M3	0.00000 / 0.00034
VC40697	M1 / M3	0.00176 / 0.00219
VC40745	M5	0.00098 / 0.00118
VC40747	M1 / M3	0.00151 / 0.00147
VC40788	M1 / M3	0.00038 / 0.00029
VC40804	M1 / M3	0.00054 / 0.00036

768