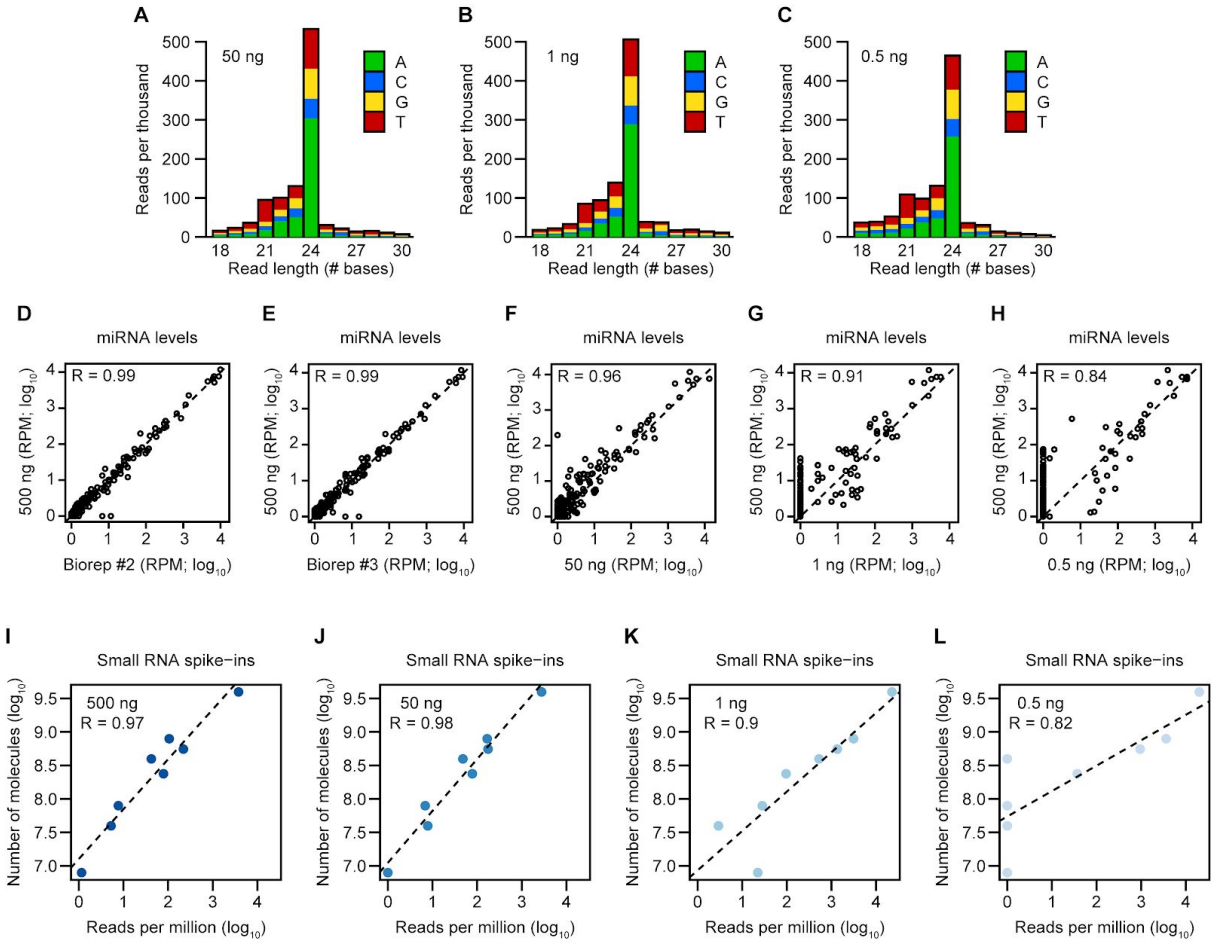

Figure S1. Establishment of Low-input Small RNA Sequencing Method, Related to Figure 1

(A to C) Stacked bar charts of normalized sRNA levels (reads per thousand genome-mapping reads) across different nucleotide (nt) lengths in libraries generated with either 50 ng (A), 1 ng (B) or 0.5 ng **(C)** of total RNA isolated from bent cotyledon stage embryos. Colors indicate proportions of sRNA-seq reads that begin with various bases as indicated in key.

(D to H) Scatter plots of miRNA family levels in sRNA-seq libraries generated from either 500 ng (biological replicate #2) (D), 500 ng (biological replicate #3) (E), 50 ng (F), 1 ng (G) or 0.5 ng (H) compared to 500 ng of total RNA (biological replicate #1). sRNA levels were normalized for reads per million genome-mapping reads (RPM) and $\log_{10}$-transformed. Pearson's *R* values are indicated, as well as a dashed line with an intercept of 0 and slope of 1.

(I to L) Scatter plots of relative sRNA spike-in levels (RPM; $\log_{10}$) compared to the absolute number of sRNA spike-in molecules ($\log_{10}$) added during RNA isolation for a sRNA-seq library generated from either 500 ng (I), 50 ng (J), 1 ng (K) or 0.5 ng (L) of total RNA. Pearson's *R* values are shown, and the dashed lines represent linear models derived from the plotted data points.

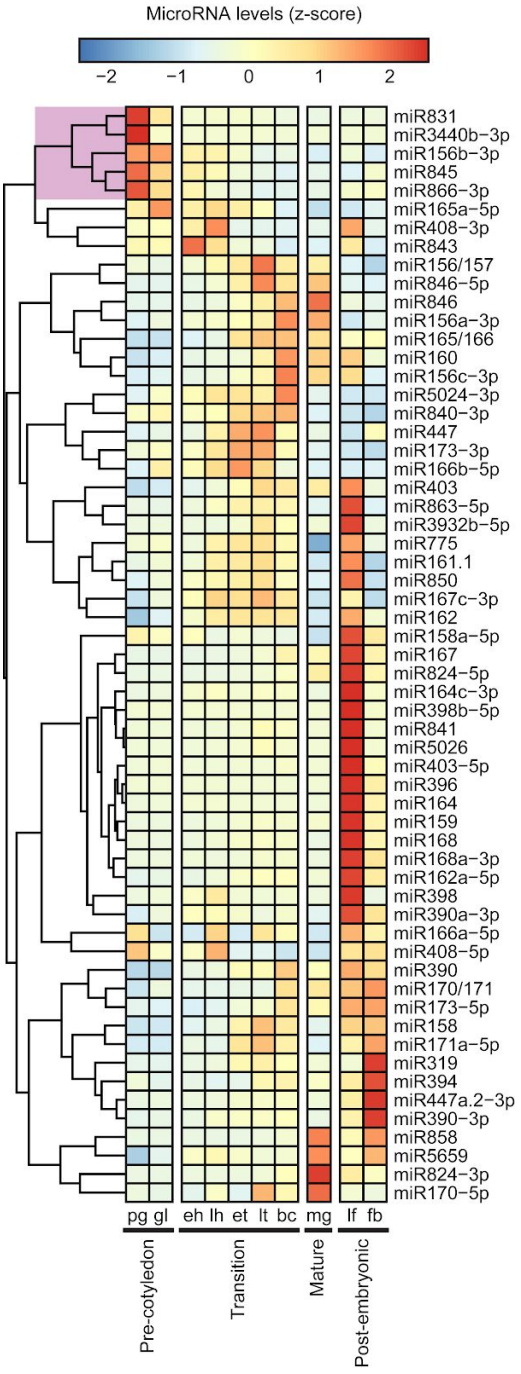

Figure S2. Embryo-Enriched miRNAs, Related to Figure 3

Heat map illustrating the relative levels of miRNA families across embryogenesis, leaves and floral buds. miRNA families with ≥10 mean RPM in at least one embryonic stage are shown, and colors represent z-scores for each individual miRNA family according to the key. Three major phases of embryo development are indicated at the bottom and individual columns are labelled according to stage: pg, preglobular; gl, globular; eh, early heart; lh, late heart; et, early torpedo; lt, late torpedo; bc, bent cotyledon; mg, mature green; lf, leaves; fb, unopened floral buds. The dendrogram clade color-coded in violet indicates the five miRNA families enriched in early embryos.

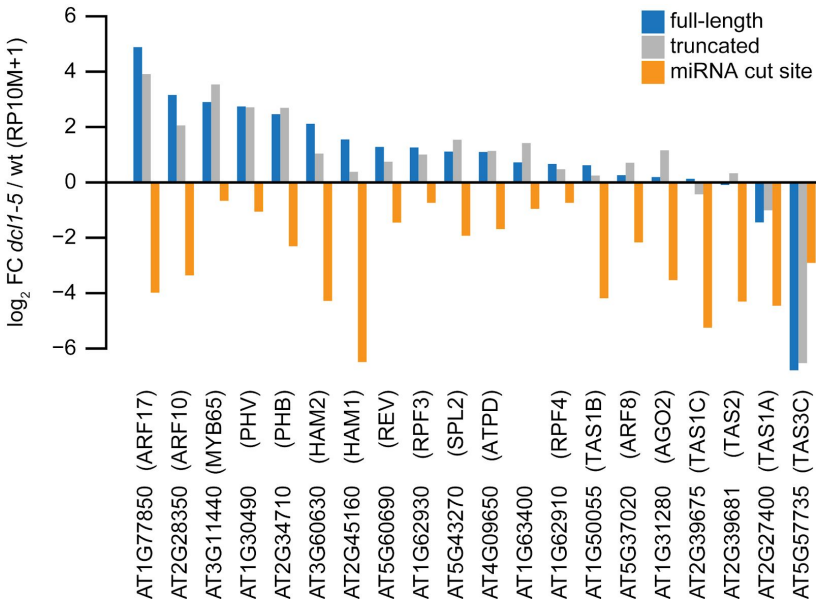

Figure S3. mRNA 5′ Ends of miRNA Targets in *dcl1-5* Mutant Embryos, Related to Figure 4
Comparison of the abundance of nanoPARE read 5′ ends mapping to different positions within all 20 high-confidence miRNA target transcripts identified in wild-type globular embryos. Each gene was subdivided into three regions: blue, annotated transcription start sites identified with nanoPARE (Schon et al. 2018); orange, positions 9 and 10 of the miRNA:target site; gray, all other exonic positions in the gene. Y-axis represents $\log_2$ fold change of the mean abundance of each gene feature in globular-stage *dcl1-5* mutant embryos compared to wild-type embryos of the same stage (reads per ten million genome-matching reads, RP10M).

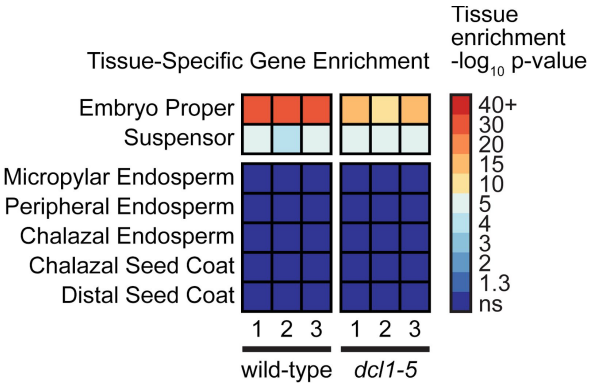

Figure S4. Tissue-Enrichment Test of Wild-Type and *dcl1-5* Mutant Embryo Transcriptomes, Related to Figure 5
Statistical enrichment of seven distinct seed tissue types inferred through the expression of tissue-enriched gene sets using the tissue-enrichment test (Schon and Nodine 2017) with default parameters. The three wild-type replicates are globular-stage mRNA-seq samples from GEO series GSE121236; *dcl1-5* replicates were generated for this study.

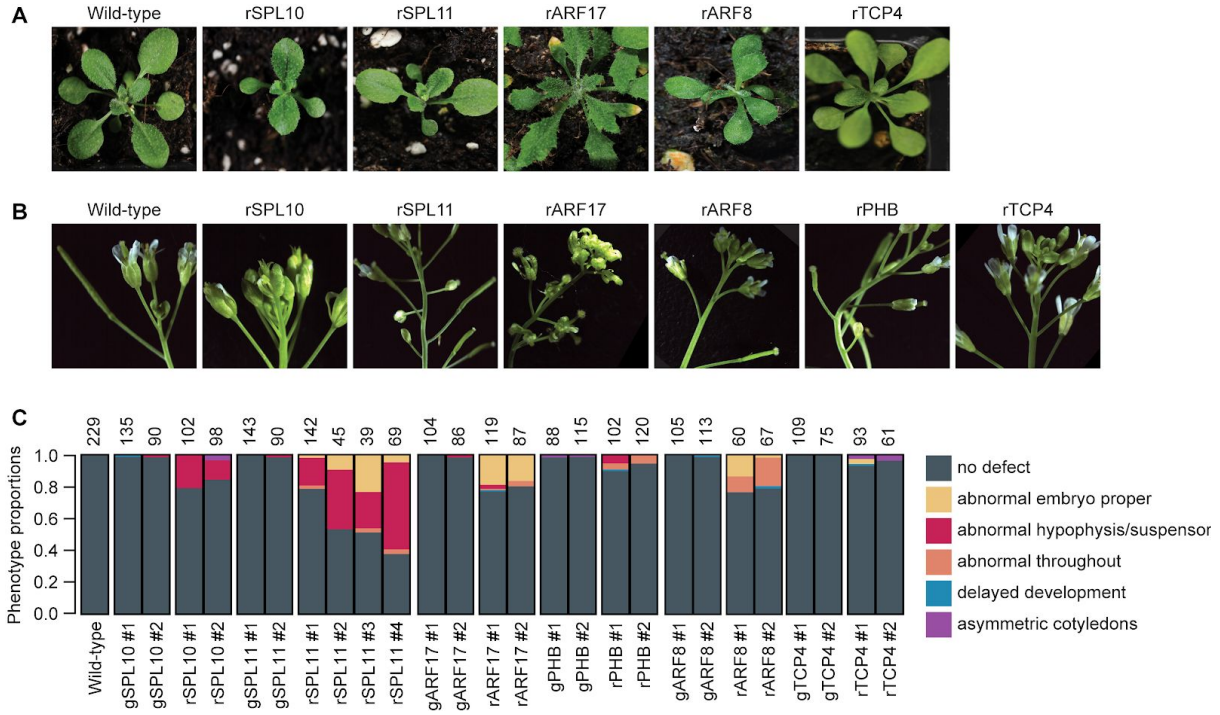

Figure S5. Post-Embryonic Phenotypes of Plants Expressing miRNA-Resistant Targets and Quantification Details, Related to Figure 7

(A-B) Representative images of vegetative (A) or flowering (B) plants (A) expressing miRNA-resistant targets. Genotypes are indicated above each panel.

(C) Stacked bar plot illustrating the proportions of phenotypes observed for embryos derived from crosses between wild-type mothers and fathers that were either wild-type, or expressed transgenic copies of target transcripts containing wild-type (genomic; gTARGET) or abolished (resistant; rTARGET) miRNA binding sites 120 hours after pollination. Paternal genotypes used in the crosses, including transgenic line numbers, are labelled below. Numbers above each bar denote how many embryos were examined. Phenotypes are color-coded according to the legend.

Table S5. Oligonucleotides Used in This Study

Name	Sequence	General purpose	Specific purpose
miR124-AS-LNA	/5DIGN/ACTGATA+TC+AG+CTC+AGTAG GCAC/3DIG_N/	miRNA in situs	Negative control; antisense to animal-specific miR124 (+ indicates LNA position, /5DIGN/ and /5DIGN/ represents digoxigenin labels on the 5′ and 3′ ends)
miR156-AS-LNA	/5DIGN/GTGCT+CACT+CT+CTTCTG+TCA /3DIG_N/	miRNA in situs	Antisense to miR156a-f isoforms (+ indicates LNA position, /5DIGN/ and /5DIGN/ represents digoxigenin labels on the 5′ and 3′ ends)
miR159-AS-LNA	/5DIGN/TAGAG+CT+CC+CTT+CAATC+CA AA/3DIG_N/	miRNA in situs	Antisense to miR159a isoform (+ indicates LNA position, /5DIGN/ and /5DIGN/ represents digoxigenin labels on the 5′ and 3′ ends)
miR160-AS-LNA	/5DIGN/GGCATA+CAGG+GAG+CCAGG+ CAC/3DIG_N/	miRNA in situs	Antisense to miR160a-c isoforms (+ indicates LNA position, /5DIGN/ and /5DIGN/ represents digoxigenin labels on the 5′ and 3′ ends)
miR166-AS-LNA	/5DIGN/GGGGAA+TGAA+GC+CTGGTC+C GA/3DIG_N/	miRNA in situs	Antisense to miR166a-f isoforms (+ indicates LNA position, /5DIGN/ and /5DIGN/ represents digoxigenin labels on the 5′ and 3′ ends)
PHB F10	GTAGCGATGGTGCAGAGGATGT	mRNA in situs	Forward primer for amplifying PHB amplicon from cDNA
PHB R10	CGAACGACCAATTCACGAACAT	mRNA in situs	Reverse primer for amplifying PHB amplicon from cDNA
PHB F12	GTAGCGATGGTGCAGAGGATGTTACTG	mRNA in situs	Forward primer for incorporation of T7 site for PHB antisense probe generation
PHB R12T7	CCAAGCTTCTAATACGACTCACTATAGG GAGACGAACGACCAATTCACGAAC	mRNA in situs	Reverse primer for incorporation of T7 site for PHB antisense probe generation
PHB F11T7	CCAAGCTTCTAATACGACTCACTATAGG GAGAGTAGCGATGGTGCAGAGGAT	mRNA in situs	Forward primer for incorporation of T7 site for PHB sense probe generation
PHB R11	CGAACGACCAATTCACGAAC	mRNA in situs	Reverse primer for incorporation of T7 site for PHB sense probe generation
CNA_F1	TTCAAAGGCAACTGGAACCG	mRNA in situs	Forward primer for amplifying CNA amplicon from cDNA and for CNA antisense probe generation
CNA_R1	CCGCACAGGTTCCTACAGCA	mRNA in situs	Reverse primer for amplifying CNA amplicon from cDNA
CNA_AS_R1T7	CCAAGCTTCTAATACGACTCACTATAGG GAGAAGCAAGTGGAAGTATAACCT	mRNA in situs	Reverse primer for incorporation of T7 site for CNA antisense probe generation
PHV_F1	GGTCGCTGAAATCCTCAAAG	mRNA in situs	Forward primer for amplifying PHV amplicon from cDNA
PHV_R1	TTCGGATTTGTTTTTTGGTC	mRNA in situs	Reverse primer for amplifying PHV amplicon from cDNA
PHV_AS_F1	TCGTCCATCTTGGTTCCGTG	mRNA in situs	Forward primer for incorporation of T7 site for PHV antisense probe generation
PHV_AS_R1T7	CCAAGCTTCTAATACGACTCACTATAGG GAGAATTTTCATCAACGCCGCTAC	mRNA in situs	Reverse primer for incorporation of T7 site for PHV antisense probe generation
pAlligatorR/G43-F1	CTGCAGATCGTTCAAACATTTG	Cloning	Forward primer for generating the backbone of pAlligatorG43/R43 (use for g/mARF17,CAN, PHB, TCP4)
pAlligatorR/G43-R1	CTGCAGGTCGACCATAGTG	Cloning	Reverse primer for generating the backbone of pAlligatorG43/R43 (use for g/mARF17,CAN, PHB, TCP4)
pAlligatorR/G43-R2	ATAGCTTGGCGTAATCATGG	Cloning	Alternative reverse primer for generating the backbone of pAlligatorG43/R43 (use for g/mPHB)
g/mARF17-F1	ACACAACATATCCAGTCACTATGGTCGA CCTGCAGTGTCTTTTGTTTTTAGGTTTTT TTTTTAAC	Cloning	Forward primer for genomic ARF17 amplification and gibson cloning into pAlligatorG43 /pAlligatorR43 destination vector
g/mARF17-R1	GAAACTTTATTGCCAAATGTTTGAACGAT CTGCAGTTTATTTAGTATTATTTGCTCTG TTTG	Cloning	Reverse primer for genomic ARF17 amplification and gibson cloning into pAlligatorG43 /pAlligatorR43 destination vector
rARF17-F2	CTGGAATGCAAGGTGCACGGCAATATGA TTTTGGGTC	Cloning	Forward primer for amplification of rARF17 gibson piece 2 (with g/mARF17-R1)
rARF17-R2	ACCCAAAATCATATTGCCGTGCACCTTG CATTCCAG	Cloning	Reverse primer for amplification of rARF17 gibson piece 1 (with g/mARF17-F1)
gARF8-TOPO-F	CACCTCTCCAAGTGATACACTC	Cloning	Forward primer for genomic ARF8 amplification and cloning into pENTR/D-TOPO
gARF8-TOPO-R	TAAGTCTGATGTGTGTGCA	Cloning	Reverse primer for genomic ARF8 amplification and cloning into pENTR/D-TOPO
ARF8-SDM-F	CCGGTTGTTACGGAAAATACAAAAACAA C	Cloning	Forward site-directed mutagenesis primer to generate rARF8
ARF8-SDM-R	GGCCTGATTCCATTGGAATCATCG	Cloning	Reverse site-directed mutagenesis primer to generate rARF8
g/mPHB-F1	AAACAGCTATGACCATGATTACGCCAAG CTATTGGAGGGAAGAGGCTACAAAG	Cloning	Forward primer for genomic PHB amplification and gibson cloning into pAlligatorG43 /pAlligatorR43 destination vector
g/mPHB-R1	ACTTTATTGCCAAATGTTTGAACGATCTG CAGTTGTCCGAGCATTGATTTTGTAC	Cloning	Reverse primer for genomic PHB amplification and gibson cloning into pAlligatorG43 /pAlligatorR43 destination vector
rPHB-F2	AATAGAATCTGGTCCAGGCTACACCAGC AATGAAG	Cloning	Forward primer for amplification of rPHB gibson piece 2 (with g/mPHB-R1)
rPHB-R2	CATTGCTGGTGTAGCCTGGACCAGATTC TATTGGC	Cloning	Reverse primer for amplification of rPHB gibson piece 1 (with g/mPHB-F1)
g/mTCP4-F1	ACACAACATATCCAGTCACTATGGTCGA CCTGCAGCATTTTGATGAGGCGTATATA TATACATTTAATTAATATTG	Cloning	Forward primer for genomic TCP4 amplification and gibson cloning into pAlligatorG43 /pAlligatorR43 destination vector
g/mTCP4-R1	GAAACTTTATTGCCAAATGTTTGAACGAT CTGCAGATATGATCTTTGTGTCATGACT	Cloning	Reverse primer for genomic TCP4 amplification and gibson cloning into pAlligatorG43 /pAlligatorR43 destination vector

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rTCP4-F2	GGTCCCTTGCAAAGTAGCTACAGTCCCATGATCCGTG	Cloning	Forward primer for amplification of rTCP4 gibson piece 2 (with g/mTCP4-R1)
rTCP4-R2	ACGGATCATGGGACTGTAGCTACTTTGCAAGGGACC	Cloning	Reverse primer for amplification of rTCP4 gibson piece 1 (with g/mTCP4-F1)
eIF4A1 RTF	TGCAAGGCACTCTTTGATCTGATTT	qRT-PCR	Forward primer for detection of the housekeeping gene eIF4A1
eIF4A1 RTR	GAGATATGTTCGTAGCTGGGAGAGAGAG	qRT-PCR	Reverse primer for detection of the housekeeping gene eIF4A1
SPL10 RTF	TCAGGAGGCCTCCATGAATCTCA	qRT-PCR	Forward primer for SPL10 detection
SPL10 RTR	GGCCACGGGAGTGTGTTTGAT	qRT-PCR	Reverse primer for SPL10 detection
SPL11 RTF	CCAACCACATGTGCAGCCATTT	qRT-PCR	Forward primer for SPL11 detection
SPL11 RTR	GAACAGAGTAGAGAAAATGGCTGCA	qRT-PCR	Reverse primer for SPL11 detection
PHB RTF	GCTAGACAAGACCCTTGACGAACCT	qRT-PCR	Forward primer for PHB detection
PHB RTR	TCCCATGCTTGACGCACATACTC	qRT-PCR	Reverse primer for PHB detection
ARF8 RTF	CATGCAGATGTTGAGACGGATGAAG	qRT-PCR	Forward primer for ARF8 detection
ARF8 RTR	TTACTCGGTATCCCCAACTCAATCG	qRT-PCR	Reverse primer for ARF8 detection
ARF17 RTF	GTGCAGCAGCACCTGATCCAAG	qRT-PCR	Forward primer for ARF17 detection
ARF17 RTR	GGAGGATTTCCTCCAATGAATCCGG	qRT-PCR	Reverse primer for ARF17 detection
TCP4 RTF	CCAGTTCTTGGCCAAAGCCAAC	qRT-PCR	Forward primer for TCP4 detection
TCP4 RTR	ATGGTGGTGGTTGAGATCGTCG	qRT-PCR	Reverse primer for TCP4 detection