

Fig. S1. Gene expression trajectories from short-day to long-day conditions for (A) E-strain Wnt signaling genes, (B) Z-strain Wnt signaling genes, (C) E-strain ecdysone-related genes, (D) Z-strain ecdysone-related genes, (E) E-strain insulin signaling genes, and (F) Z-strain insulin signaling genes.

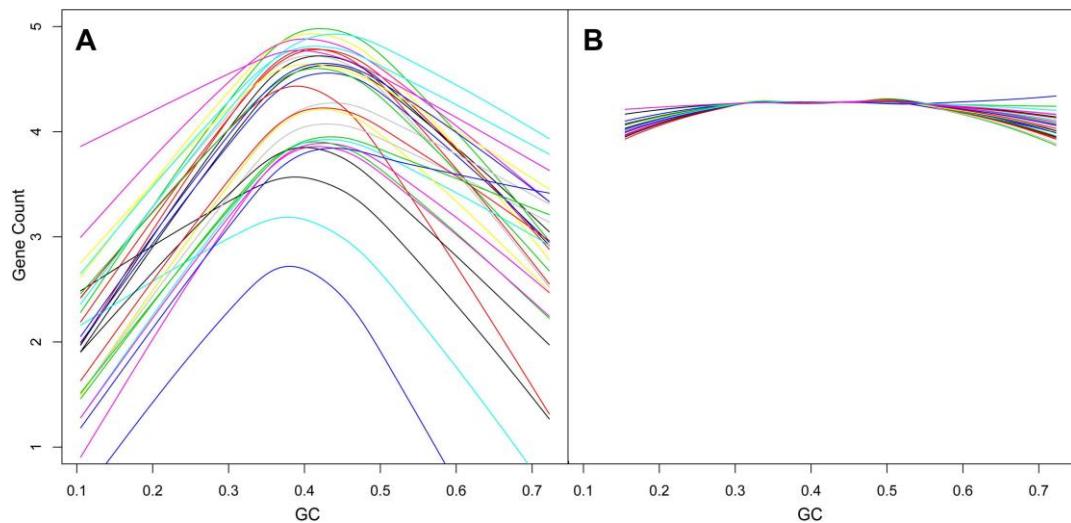


Fig. S2. GC normalization in EDAs. (A) Lowess regression of log gene-level counts on GC content for each library. (B) Full quantile within and between lane normalization for each library.

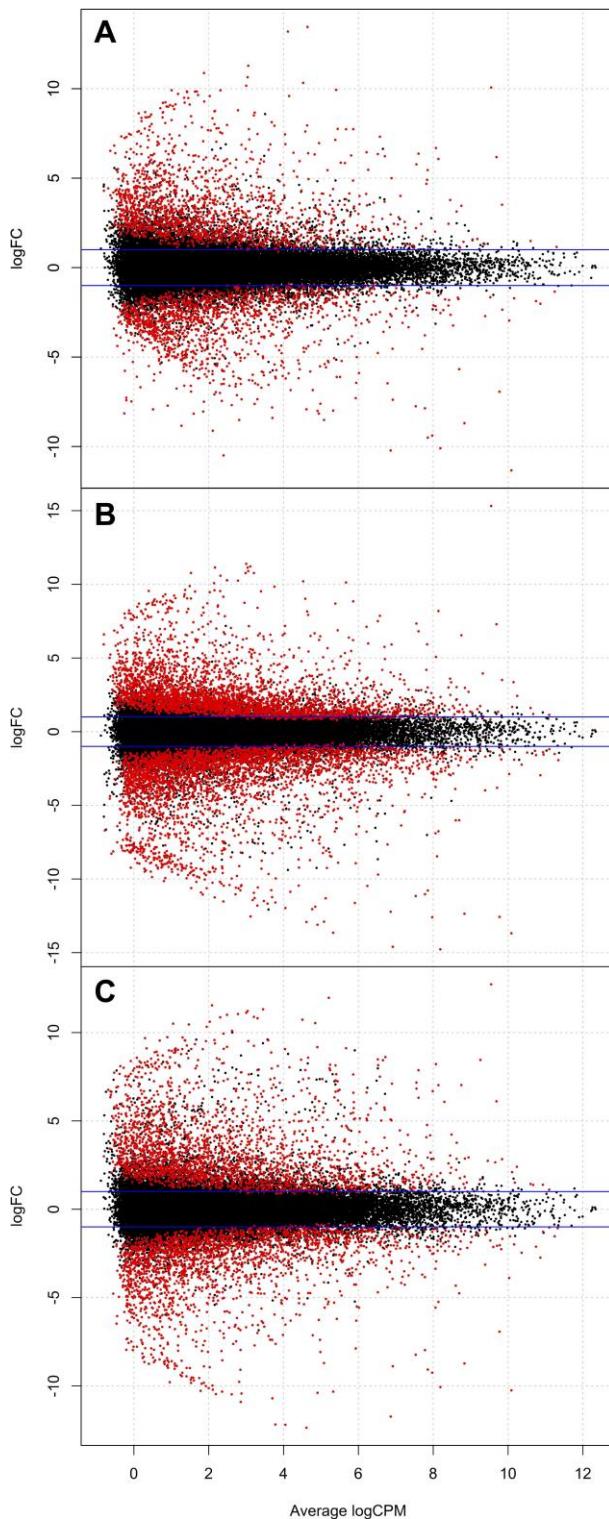


Fig. S3. MA plot of differential expression within strain shown as log-fold change of expression versus average log counts per million. (A) E-strain diapause maintenance to day 1, (B) E-strain diapause maintenance to day 7, (C) Z-strain diapause maintenance to day 1, (D) Z-strain diapause maintenance to day 7. Red dots are significantly differentially expressed at FDR < 0.01.

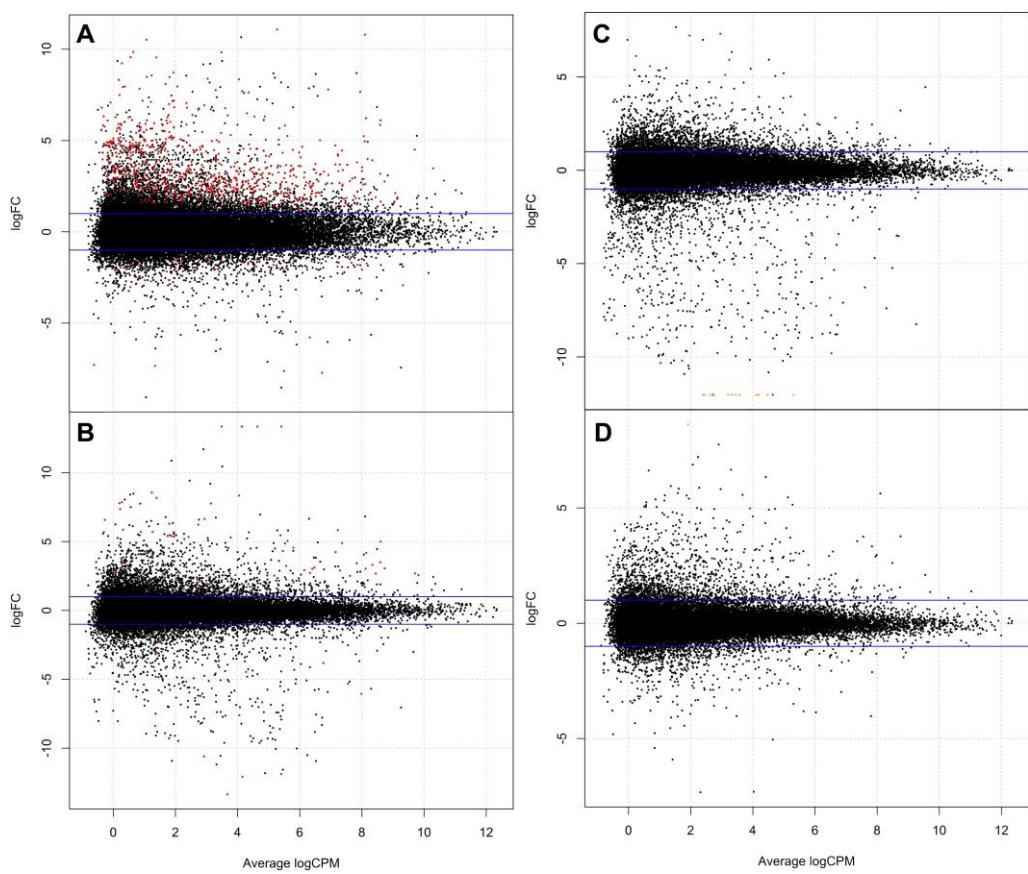


Fig. S4. MA plot of differential expression between strains (A) in diapause maintenance, (B) on day 1, and (C) on day 7, shown as log-fold change of expression versus average log counts per million. Red dots are significantly differentially expressed at FDR < 0.01.

Table S1. Enriched GO Terms and KEGG Pathways for Differentially Expressed Genes Between E and Z Strains (*P*-value < 0.001)

Category	Term	Count	Size	<i>P</i> -value
Day1				
GO: biological process	proteasome-mediated ubiquitin-dependent protein catabolic process	30	83	8.39E-06
GO: biological process	cellular protein catabolic process	43	146	4.24E-05
GO: biological process	protein-DNA complex subunit organization	17	39	5.12E-05
GO: biological process	modification-dependent protein catabolic process	42	145	7.95E-05
GO: biological process	macromolecule catabolic process	54	210	2.44E-04
GO: biological process	nucleosome assembly	8	13	2.92E-04
GO: biological process	DNA endoreduplication	8	13	2.92E-04
GO: biological process	mitotic spindle assembly checkpoint	6	8	3.88E-04
GO: biological process	spindle checkpoint	6	8	3.88E-04
GO: biological process	pyrimidine-containing compound metabolic process	10	20	4.92E-04
GO: biological process	DNA packaging	19	54	5.57E-04
GO: biological process	regulation of imaginal disc-derived wing size	8	14	5.85E-04
GO: biological process	regulation of cell cycle process	39	146	8.57E-04
GO: biological process	regulation of tube size	13	32	8.64E-04
GO: biological process	canonical Wnt signaling pathway	8	21	1.35E-02*
GO: biological process	regulation of Wnt signaling pathway	15	54	2.29E-02*
GO: molecular function	glucose transmembrane transporter activity	8	11	5.78E-05
GO: molecular function	copper ion binding	10	17	9.61E-05
GO: molecular function	monosaccharide transmembrane transporter activity	9	17	6.31E-04
GO: molecular function	calcium ion binding	37	135	9.43E-04
KEGG Pathway	Proteasome	23	33	6.69E-12
KEGG Pathway	Ascorbate and aldarate metabolism	9	13	2.82E-05
KEGG Pathway	Retinol metabolism	8	13	2.76E-04
KEGG Pathway	DNA replication	12	26	3.13E-04
KEGG Pathway	Non-homologous end-joining	4	4	6.94E-04
Day7				
GO: biological process	amino sugar metabolic process	18	55	2.27E-07
GO: biological process	chitin-based cuticle development	22	79	2.62E-07
GO: biological process	chitin metabolic process	15	46	2.48E-06
GO: biological process	cuticle chitin catabolic process	3	3	5.82E-04
GO: biological process	regulation of hormone levels	10	43	2.34E-03
GO: biological process	regulation of tube size	10	44	2.81E-03
GO: biological process	regulation of tube architecture, open tracheal system	13	67	3.17E-03
GO: biological process	homophilic cell adhesion	7	25	3.48E-03
GO: biological process	cuticle pattern formation	7	25	3.48E-03
GO: biological process	aminoglycan catabolic process	5	14	4.25E-03
GO: biological process	pyrimidine nucleotide biosynthetic process	4	9	4.33E-03
GO: biological process	glucosamine-containing compound catabolic process	4	9	4.33E-03

GO: biological process	pyrimidine nucleoside monophosphate biosynthetic process	3	5	5.12E-03
GO: biological process	intercellular transport	3	5	5.12E-03
GO: biological process	septate junction assembly	7	28	6.88E-03
GO: biological process	negative regulation of hormone metabolic process	2	2	6.98E-03
GO: biological process	negative regulation of sequestering of calcium ion	2	2	6.98E-03
GO: biological process	detoxification of arsenic-containing substance	2	2	6.98E-03
GO: biological process	organonitrogen compound metabolic process	64	572	7.93E-03
GO: biological process	cellular hormone metabolic process	7	29	8.43E-03
GO: biological process	system process	35	279	9.17E-03
GO: biological process	activation of immune response	3	6	9.60E-03
GO: biological process	'de novo' pyrimidine nucleobase biosynthetic process	3	6	9.60E-03
GO: biological process	pyrimidine nucleoside biosynthetic process	3	6	9.60E-03
GO: molecular function	structural constituent of chitin-based cuticle	6	7	2.32E-06
GO: molecular function	chitin binding	13	39	1.22E-05
GO: molecular function	extracellular matrix structural constituent	7	13	3.64E-05
GO: molecular function	structural constituent of chitin-based larval cuticle	11	32	4.16E-05
GO: molecular function	serine-type endopeptidase activity	17	69	5.16E-05
GO: molecular function	serine hydrolase activity	20	91	6.74E-05
GO: molecular function	iron ion binding	16	67	1.28E-04
GO: molecular function	monosaccharide transmembrane transporter activity	7	17	3.04E-04
GO: molecular function	molybdenum ion binding	3	3	6.34E-04
KEGG Pathway	Drug metabolism - other enzymes	8	29	4.35E-04

* Asterix indicates P-value <0.05

Table S2. Gene enrichment analysis table for the seven significant STEM profiles (Bonferroni corrected P-value cutoff ≤ 0.0001)

Strain	Profile	GO Category Name	#Genes Category	#Genes Assigned	#Genes Expected	#Genes Enriched	p-value	Corrected p-value	Fold
E	1	single-organism developmental process	1967	83	30.8	52.2	2.70E-15	1.10E-11	2.7
E	1	macromolecule metabolic process	1960	82	30.6	51.4	6.30E-15	2.70E-11	2.7
E	1	regulation of biological process	2220	84	34.7	49.3	6.10E-13	2.60E-09	2.4
E	1	regulation of cellular process	2066	79	32.3	46.7	1.70E-12	7.30E-09	2.4
E	1	organic substance metabolic process	2606	91	40.7	50.3	5.40E-12	2.30E-08	2.2
E	1	endopeptidase activity	168	19	2.6	16.4	2.90E-11	1.20E-07	7.2
E	1	proteolysis	330	26	5.2	20.8	3.10E-11	1.30E-07	5
E	1	anatomical structure development	1064	49	16.6	32.4	6.00E-11	2.50E-07	2.9
E	1	homophilic cell adhesion via plasma membrane adhesion molecules	29	9	0.5	8.5	4.20E-10	1.80E-06	19.8
E	1	developmental process involved in reproduction	520	31	8.1	22.9	4.70E-10	2.00E-06	3.8
E	1	anatomical structure morphogenesis	900	42	14.1	27.9	9.00E-10	3.80E-06	3
E	1	proteasome regulatory particle	22	8	0.3	7.7	9.40E-10	4.00E-06	23.3
E	1	plasma membrane	417	27	6.5	20.5	1.00E-09	4.30E-06	4.1
E	1	cellular macromolecule metabolic process	1726	63	27	36	1.60E-09	6.90E-06	2.3
E	1	single organism reproductive process	548	31	8.6	22.4	1.70E-09	7.00E-06	3.6
E	1	macromolecule catabolic process	214	19	3.3	15.7	1.80E-09	7.70E-06	5.7
E	1	negative regulation of cellular process	756	37	11.8	25.2	2.50E-09	1.00E-05	3.1
E	1	peptidase activity, acting on L-amino acid peptides	268	21	4.2	16.8	2.50E-09	1.10E-05	5
E	1	primary metabolic process	2469	80	38.6	41.4	2.70E-09	1.10E-05	2.1
E	1	protein binding	1272	51	19.9	31.1	2.80E-09	1.20E-05	2.6
E	1	ubiquitin-dependent protein catabolic process	131	15	2	13	2.80E-09	1.20E-05	7.3
E	1	peptidase activity	274	21	4.3	16.7	3.80E-09	1.60E-05	4.9
E	1	modification-dependent macromolecule catabolic process	136	15	2.1	12.9	4.80E-09	2.00E-05	7.1
E	1	modification-dependent protein catabolic process	136	15	2.1	12.9	4.80E-09	2.00E-05	7.1
E	1	proteolysis involved in cellular protein catabolic process	136	15	2.1	12.9	4.80E-09	2.00E-05	7.1
E	1	cellular component organization	1534	57	24	33	5.10E-09	2.10E-05	2.4
E	1	protein catabolic process	99	13	1.5	11.5	5.90E-09	2.50E-05	8.4
E	1	proteasome-mediated ubiquitin-dependent protein catabolic process	83	12	1.3	10.7	7.40E-09	3.10E-05	9.2
E	1	proteasomal protein catabolic process	84	12	1.3	10.7	8.50E-09	3.60E-05	9.1
E	1	cellular developmental process	1249	49	19.5	29.5	1.10E-08	4.60E-05	2.5
E	1	intracellular part	3372	98	52.7	45.3	1.20E-08	5.00E-05	1.9
E	1	negative regulation of biological process	844	38	13.2	24.8	1.40E-08	5.90E-05	2.9
E	1	calcium-dependent cell-cell adhesion via plasma membrane cell adhesion molecules	21	7	0.3	6.7	2.20E-08	9.30E-05	21.3
E	2	nucleus	1056	48	17.1	30.9	4.80E-10	2.00E-06	2.8
E	2	protein catabolic process	99	14	1.6	12.4	9.20E-10	3.90E-06	8.7
E	2	single-organism developmental process	1967	71	31.9	39.1	1.20E-09	5.10E-06	2.2
E	2	modification-dependent macromolecule catabolic process	136	15	2.2	12.8	7.80E-09	3.30E-05	6.8
E	2	modification-dependent protein catabolic process	136	15	2.2	12.8	7.80E-09	3.30E-05	6.8
E	2	proteolysis involved in cellular protein catabolic process	136	15	2.2	12.8	7.80E-09	3.30E-05	6.8
E	2	proteasome-mediated ubiquitin-dependent protein catabolic process	83	12	1.3	10.7	1.10E-08	4.70E-05	8.9
E	2	proteasomal protein catabolic process	84	12	1.4	10.6	1.30E-08	5.40E-05	8.8
E	2	anatomical structure development	1064	45	17.3	27.7	1.40E-08	5.90E-05	2.6
E	2	cellular response to DNA damage stimulus	234	19	3.8	15.2	1.40E-08	6.10E-05	5
E	2	macromolecule catabolic process	214	18	3.5	14.5	2.00E-08	8.40E-05	5.2

E	3	oxidoreductase activity	380	42	6.1	35.9	3.00E-22	1.30E-18	6.9
E	3	oxidation-reduction process	325	33	5.2	27.8	1.00E-16	4.30E-13	6.3
E	3	single-organism metabolic process	1304	61	20.9	40.1	4.60E-13	1.90E-09	2.9
E	3	integral component of membrane	613	36	9.8	26.2	6.00E-11	2.50E-07	3.7
E	3	organic acid metabolic process	264	23	4.2	18.8	9.10E-11	3.80E-07	5.4
E	3	oxoacid metabolic process	264	23	4.2	18.8	9.10E-11	3.80E-07	5.4
E	3	carboxylic acid metabolic process	249	22	4	18	1.80E-10	7.40E-07	5.5
E	3	small molecule metabolic process	498	30	8	22	1.20E-09	5.20E-06	3.8
E	3	carbohydrate metabolic process	207	18	3.3	14.7	9.80E-09	4.10E-05	5.4
E	3	organonitrogen compound metabolic process	431	26	6.9	19.1	1.50E-08	6.20E-05	3.8
E	4	oxidoreductase activity	380	22	3.8	18.2	7.60E-11	3.20E-07	5.8
E	4	oxidation-reduction process	325	20	3.2	16.8	1.80E-10	7.80E-07	6.2
E	4	single-organism catabolic process	190	14	1.9	12.1	9.80E-09	4.10E-05	7.4

Table S3. Significance (FDR) values for differentially expressed genes between E and Z strain moths in long-day conditions

Gene Symbol	Contigs	FDR Day 1	FDR Day 7
Ecdysone			
<i>Usp</i>	comp37290_c0_seq1*; comp10851_c0_seq1; comp108932_c0_seq1	8.26E-03	5.41E-03
<i>Spo</i>	comp30567_c0_seq1	4.63E-07	9.21E-10
<i>Phm</i>	comp24584_c0_seq1	3.76E-06	2.69E-05
<i>Eip75B</i>	comp18210_c0_seq1; comp11010_c0_seq2*	6.48E-03	-
<i>Eip78C</i>	comp122877_c0_seq1; comp151979_c0_seq1	-	-
<i>EcR</i>	comp34369_c0_seq1	1.01E-03	-
<i>Nvd</i>	comp8057_c0_seq2	8.88E-03	2.62E-03
Circadian			
<i>Clk</i>	comp120765_c0_seq1	-	-
<i>Cpo</i>	comp89983_c0_seq1	-	-
<i>Cyc</i>	comp120765_c0_seq1	-	-
<i>Pdp1</i>	comp36140_c0_seq1*; comp15799_c0_seq1*	9.33E-03; 8.35E-04	-
<i>Tim</i>	comp27153_c0_seq1	-	-
<i>Vri</i>	comp23405_c0_seq1	-	-
<i>Per</i>	comp7426_c0_seq1; comp8184_c0_seq1; comp97089_c0_seq1	-	-
Cell Cycle			
<i>Cdk4</i>	comp127995_c0_seq1*; comp233925_c0_seq1*	2.61E-03; 1.22E-04	-
<i>CycB</i>	comp21605_c0_seq1	1.32E-07	5.73E-04
<i>Pcna</i>	comp9566_c0_seq1	2.38E-06	-
<i>Polo</i>	comp21903_c0_seq1*; comp10877_c0_seq1*	2.22E-03; 1.56E-03	-
<i>CycA</i>	comp29198_c0_seq1	-	-
<i>Trbl</i>	comp20015_c0_seq1; comp25406_c0_seq1	-	-
Heat Shock			
<i>Hsp83</i>	comp22537_c0_seq1	5.03E-04	-

<i>Hsp60</i>	comp19138_c0_seq1	8.82E-03	-
<i>Hsp70Bc</i>	comp418731_c0_seq1; comp427912_c0_seq1	-	-
<i>Hsp70Ab</i>	comp9675_c0_seq1	-	-
Canonical Wnt Signaling			
<i>Fz3</i>	comp15297_c0_seq1	3.93E-03	-
<i>Arm</i>	comp17057_c0_seq1	9.38E-03	-
<i>Smo</i>	comp14234_c0_seq1	7.71E-03	-
<i>Wg</i>	comp13981_c0_seq1	1.91E-03	-
<i>Wnt6</i>	comp25466_c0_seq1	-	-
<i>Hs6st</i>	comp78453_c0_seq1	-	-
<i>Gro</i>	comp8218_c0_seq1	-	-
<i>Pan</i>	-	7.04E-03	-
Insulin			
<i>Akt1</i>	comp18543_c0_seq1; comp38567_c0_seq1; comp53008_c0_seq1; comp10465_c0_seq1	-	-
<i>Foxo</i>	comp19046_c0_seq1 comp25612_c0_seq1; comp14287_c0_seq1; comp16806_c0_seq1; comp22132_c0_seq1;	-	-
<i>InR</i>	comp223642_c0_seq1	-	-
<i>Pi3k</i>	comp190582_c0_seq1; comp28365_c0_seq1; comp31462_c0_seq1	-	-
<i>Pten</i>	comp6369_c0_seq1; comp7648_c0_seq1	-	-
<i>SNF4y</i>	comp26437_c0_seq1	-	3.11E-03
<i>Tor</i>	comp16536_c0_seq1; comp17899_c0_seq1; comp19143_c0_seq1; comp26489_c0_seq1	-	-

*Some genes had multiple transcripts, asterix denotes which transcript shows differential expression

Table S4. Short read archive accession numbers

Strain	Days After Long-day Exposure	Sample Name	SRA Accession
E	0	Shortday_E_biologicalrep1	SRX1206058
E	0	Shortday_E_biologicalrep2	SRX1206872
E	0	Shortday_E_biologicalrep3	SRX1211504
E	0	Shortday_E_biologicalrep4	SRX1211546
E	0	Shortday_E_biologicalrep5	SRX1211572
Z	0	Shortday_Z_biologicalrep1	SRX1211612
Z	0	Shortday_Z_biologicalrep2	SRX1211648
Z	0	Shortday_Z_biologicalrep3	SRX1211686
Z	0	Shortday_Z_biologicalrep4	SRX1211701
Z	0	Shortday_Z_biologicalrep5	SRX1211703
E	1	Longday1_E_biologicalrep1	SRX1211704
E	1	Longday1_E_biologicalrep2	SRX1211706
E	1	Longday1_E_biologicalrep3	SRX1211707
E	1	Longday1_E_biologicalrep4	SRX1211711
E	1	Longday1_E_biologicalrep5	SRX1211709
Z	1	Longday1_Z_biologicalrep1	SRX1211714
Z	1	Longday1_Z_biologicalrep2	SRX1211718
Z	1	Longday1_Z_biologicalrep3	SRX1211716
Z	1	Longday1_Z_biologicalrep4	SRX1211720
Z	1	Longday1_Z_biologicalrep5	SRX1211723
E	7	Longday7_E_biologicalrep1	SRX1211725
E	7	Longday7_E_biologicalrep2	SRX1211741
E	7	Longday7_E_biologicalrep3	SRX1211744
E	7	Longday7_E_biologicalrep4	SRX1211746
E	7	Longday7_E_biologicalrep5	SRX1211748
Z	7	Longday7_Z_biologicalrep1	SRX1211750
Z	7	Longday7_Z_biologicalrep2	SRX1211752
Z	7	Longday7_Z_biologicalrep3	SRX1211782
Z	7	Longday7_Z_biologicalrep4	SRX1211784
Z	7	Longday7_Z_biologicalrep5	SRX1211786