台灣生物資訊核心設施
Taiwan Bioinformatics Institute
Core Facility

國家衛生研究院
National Health Research Institutes

The MamPhEA *and* DroPhEA Tutorials

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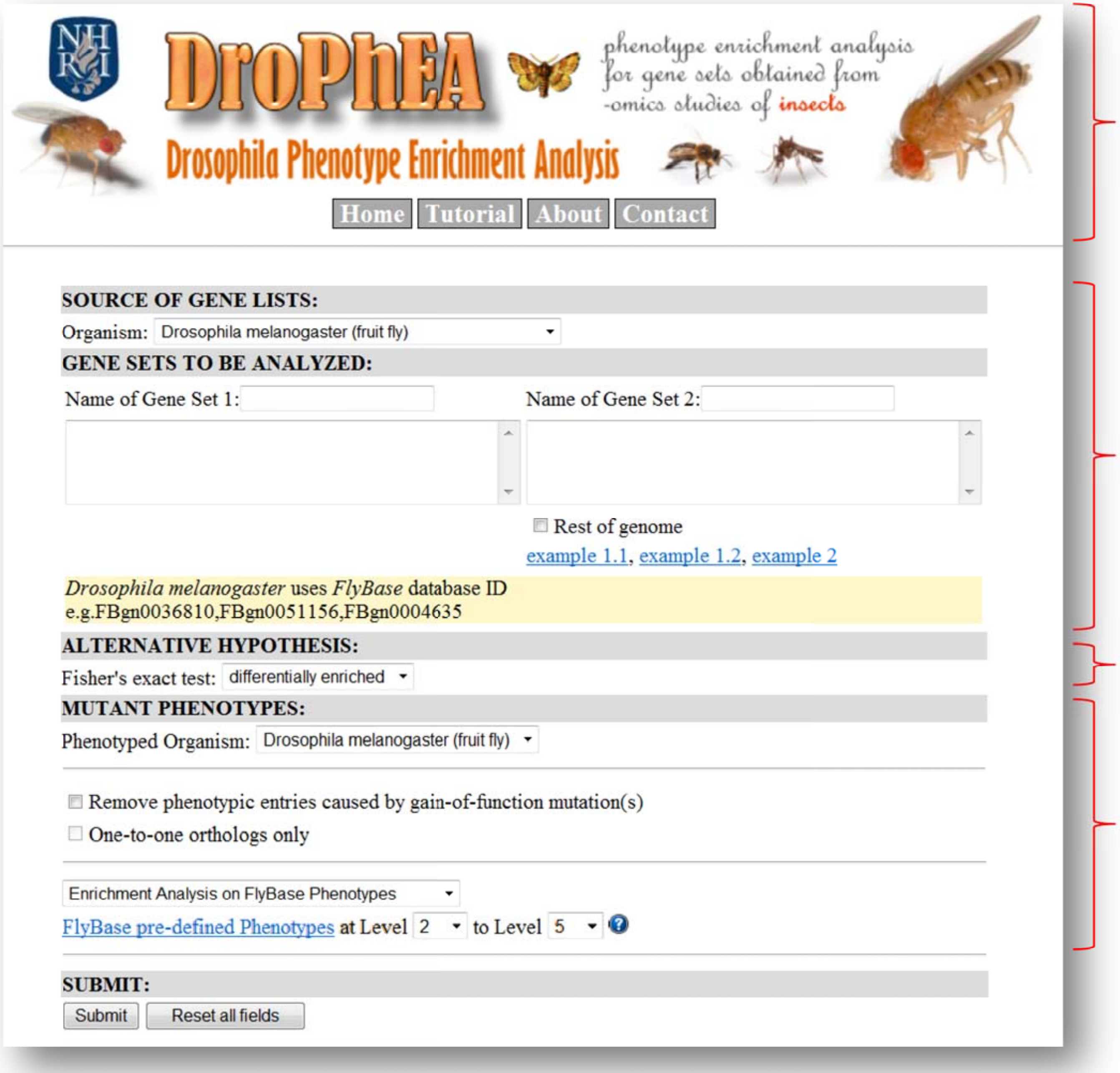
Outline

1. Interface introduction	
2. Data input	Input gene set Select parameter Input phenotype
3. Result	Result display option Hierarchical result Listing result

Interface introduction



SOURCE OF GENE LISTS:

Organism: Drosophila melanogaster (fruit fly)

GENE SETS TO BE ANALYZED:

Name of Gene Set 1:

Name of Gene Set 2:

☐ Rest of genome

example 1.1, example 1.2, example 2

Drosophila melanogaster uses *FlyBase* database ID
e.g.FBgn0036810,FBgn0051156,FBgn0004635

ALTERNATIVE HYPOTHESIS:

Fisher's exact test: differentially enriched

MUTANT PHENOTYPES:

Phenotyped Organism: Drosophila melanogaster (fruit fly)

☐ Remove phenotypic entries caused by gain-of-function mutation(s)

☐ One-to-one orthologs only

Enrichment Analysis on FlyBase Phenotypes

FlyBase pre-defined Phenotypes at Level 2 to Level 5

SUBMIT:

Information

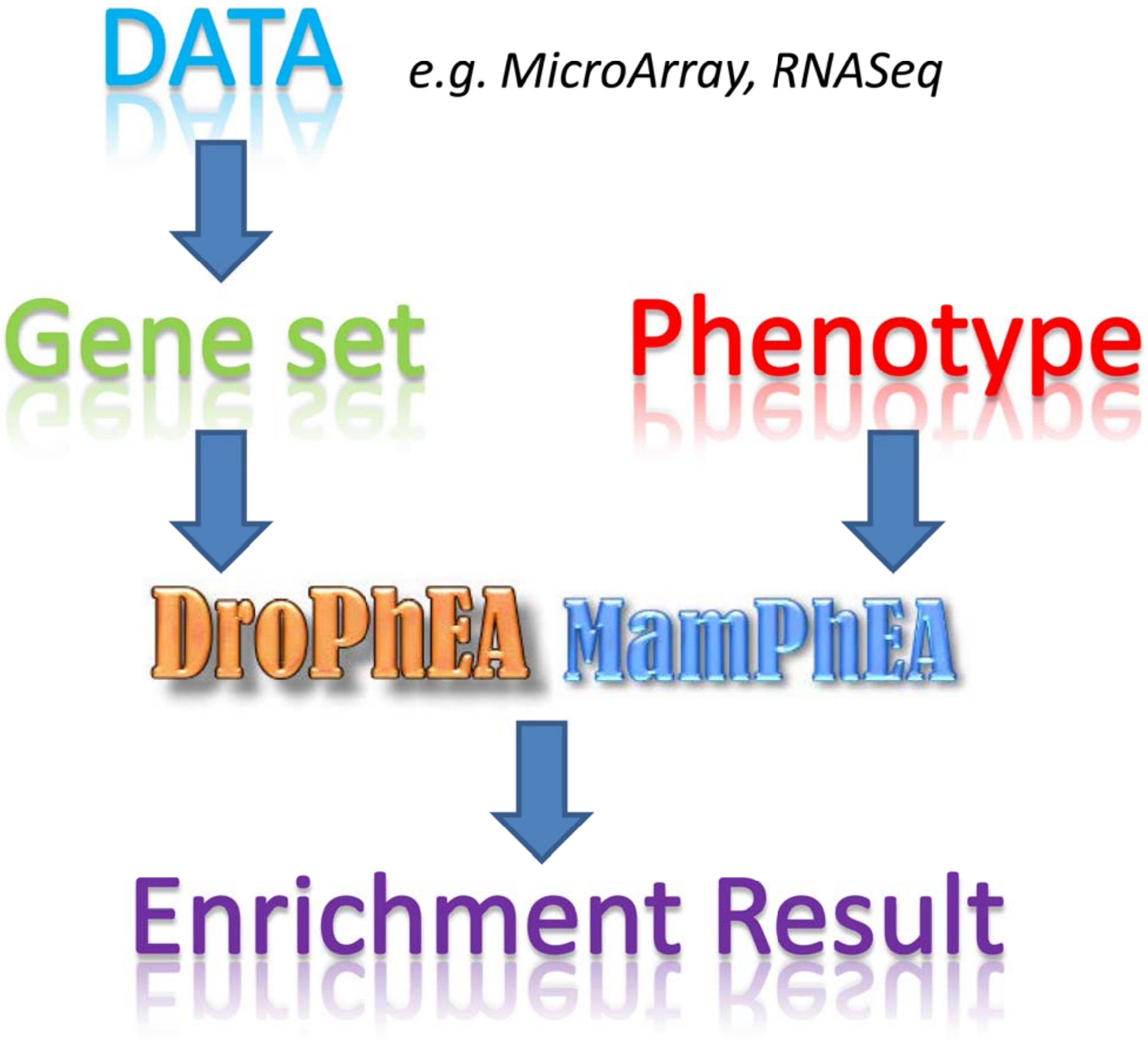
Data input

Test hypothesis

Phenotype

http://evol.nhri.org.tw/phenome/

*Dro*PhEA/*Mam*PhEA working flow chart



DATA

e.g. MicroArray, RNASeq

Gene set

Phenotype

DroPhEA MamPhEA

Enrichment Result

A real case apply to *Dro*PhEA (example 2 of *Dro*PhEA)

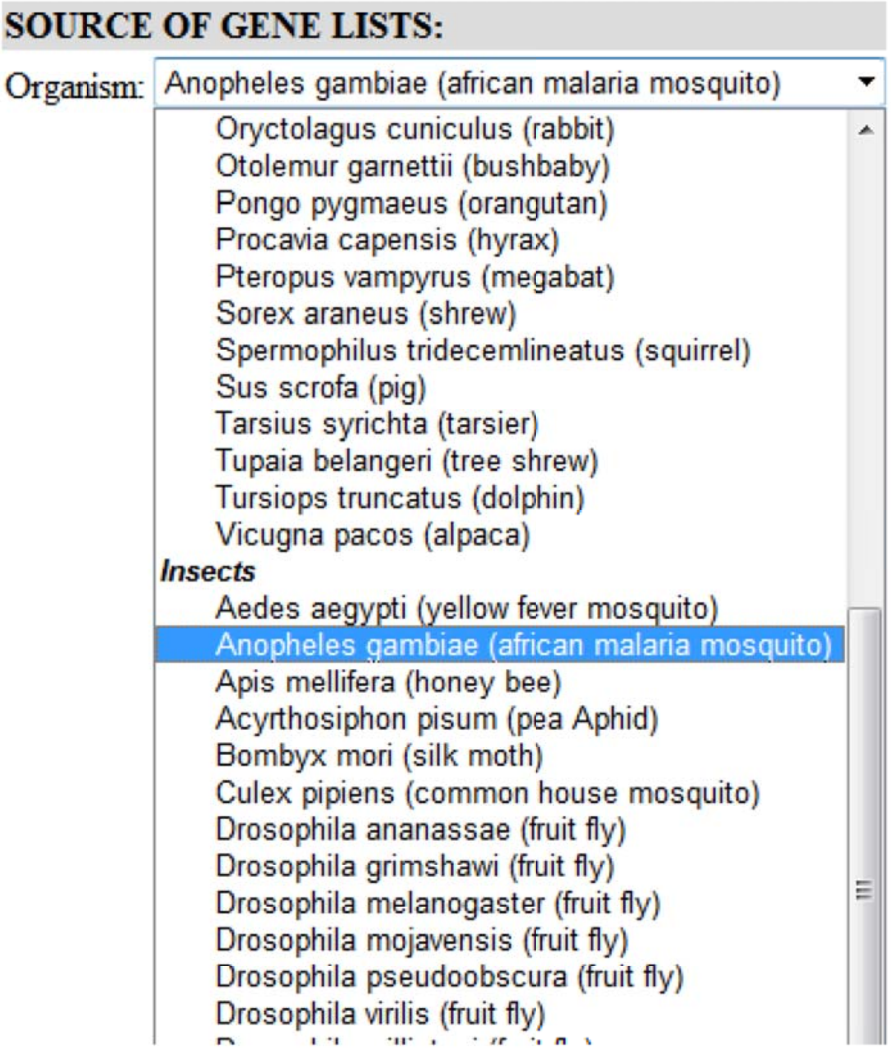
Example: Gene expression difference after a Blood-meal in 24hr

Species: African malaria mosquito
Data set 1: Top 20% highly expressed gene after a Blood-meal
Data set 2: Bottom 80% lowly expressed gene after a Blood-meal

Wu et. al,. (2009) Molecular Biology and Evolution

** You can find this case in DroPhEA example 2*

Data input: select species of input gene



SOURCE OF GENE LISTS:

Organism: Anopheles gambiae (african malaria mosquito)

- Oryctolagus cuniculus (rabbit)
- Otolemur garnettii (bushbaby)
- Pongo pygmaeus (orangutan)
- Procavia capensis (hyrax)
- Pteropus vampyrus (megabat)
- Sorex araneus (shrew)
- Spermophilus tridecemlineatus (squirrel)
- Sus scrofa (pig)
- Tarsius syrichta (tarsier)
- Tupaia belangeri (tree shrew)
- Tursiops truncatus (dolphin)
- Vicugna pacos (alpaca)

Insects

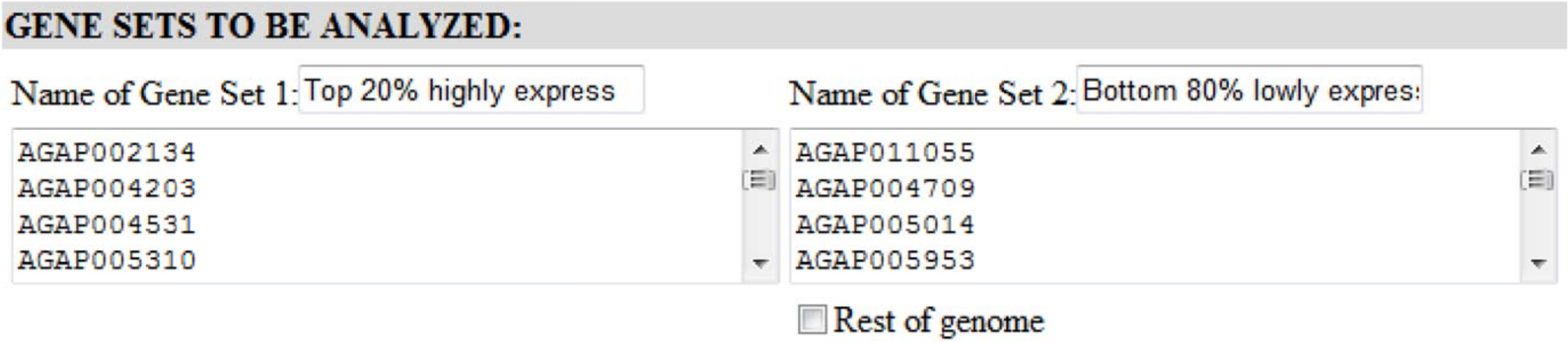
- Aedes aegypti (yellow fever mosquito)
- Anopheles gambiae (african malaria mosquito)
- Apis mellifera (honey bee)
- Acyrthosiphon pisum (pea Aphid)
- Bombyx mori (silk moth)
- Culex pipiens (common house mosquito)
- Drosophila ananassae (fruit fly)
- Drosophila grimshawi (fruit fly)
- Drosophila melanogaster (fruit fly)
- Drosophila mojavensis (fruit fly)
- Drosophila pseudoobscura (fruit fly)
- Drosophila virilis (fruit fly)

Specify the organism of gene set(s) to be analyzed

In total,
35 mammalian species
17 insect species

http://evol.nhri.org.tw/phenome/

Data input: input gene set and assign data name



GENE SETS TO BE ANALYZED:

Name of Gene Set 1: Top 20% highly express

```
AGAP002134
AGAP004203
AGAP004531
AGAP005310
```

Name of Gene Set 2: Bottom 80% lowly expres:

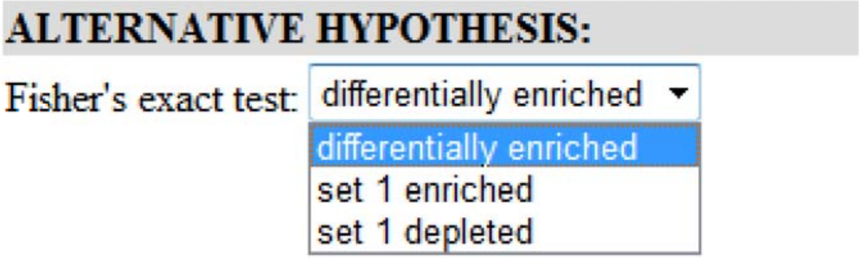
```
AGAP011055
AGAP004709
AGAP005014
AGAP005953
```

☐ Rest of genome

Use (a) a tab, (b) a return, (c) a comma, (d) a semicolon, or (e) a single space to separate genes.

**Gene ID can be any, please see our online document to find our supported gene ID*

Test hypothesis:

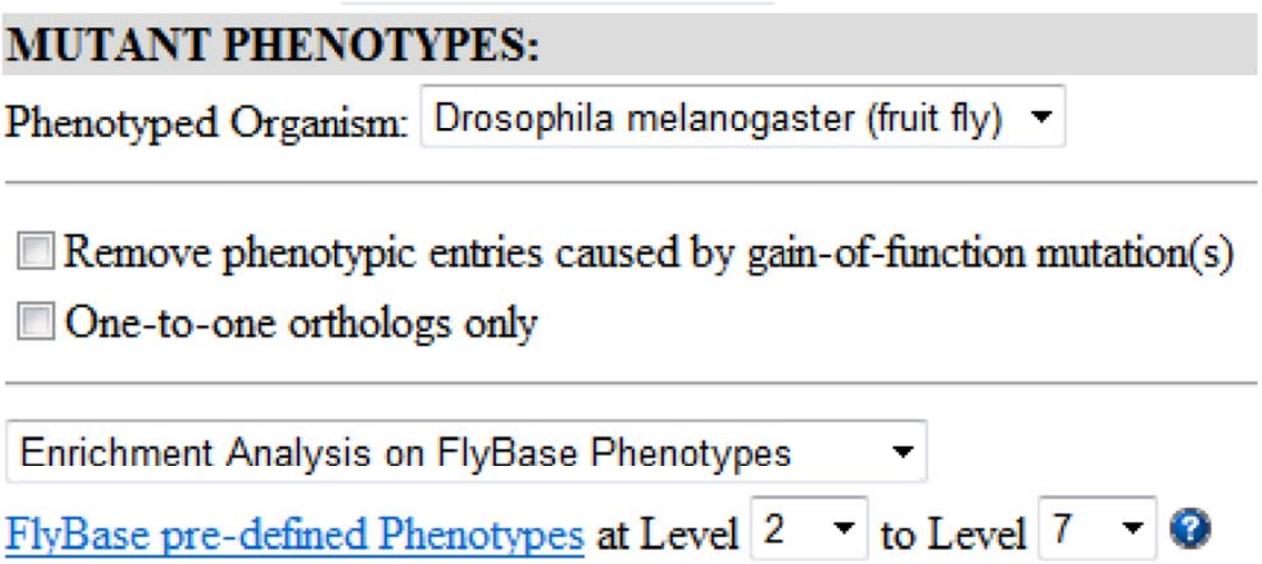


ALTERNATIVE HYPOTHESIS:

Fisher's exact test: differentially enriched

Differentially enriched – genes differently distribute in phenotype
Set1 enriched – gene set1 significantly enrich in phenotype
Set1 depleted – gene set1 significantly depleted in phenotype

Input phenotype: (1) hierarchical structure



MUTANT PHENOTYPES:

Phenotyped Organism: Drosophila melanogaster (fruit fly)

☐ Remove phenotypic entries caused by gain-of-function mutation(s)

☐ One-to-one orthologs only

Enrichment Analysis on FlyBase Phenotypes

FlyBase pre-defined Phenotypes at Level 2 to Level 7

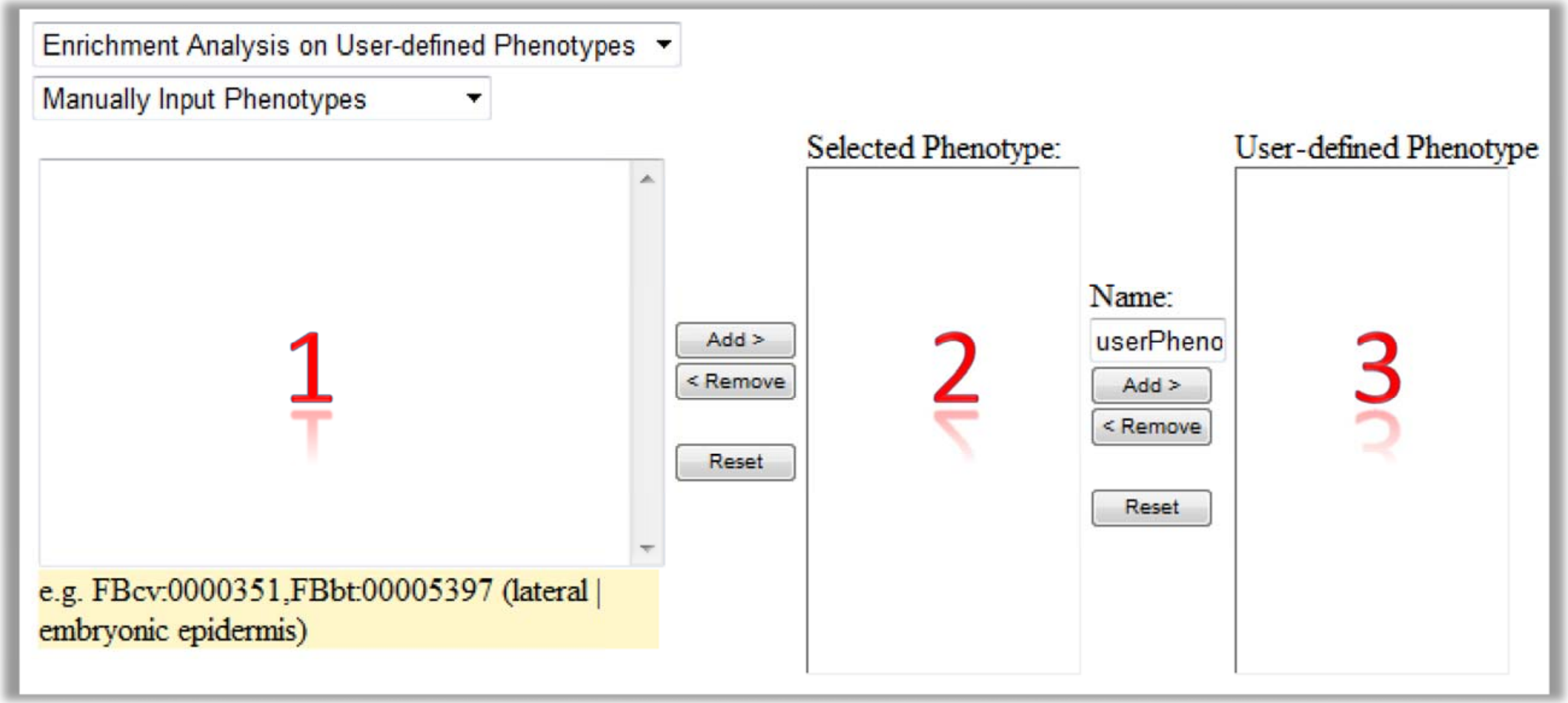
Switch *Dro*PhEA / *Mam*PhEA

Mapping method

Select analysis level

**Dro*PhEA, can specifically exclude gain of function mutation
*Mam*PhEA, can focus on loss-of-function phenotypes only

Input phenotype: (2) User-defined phenotypes



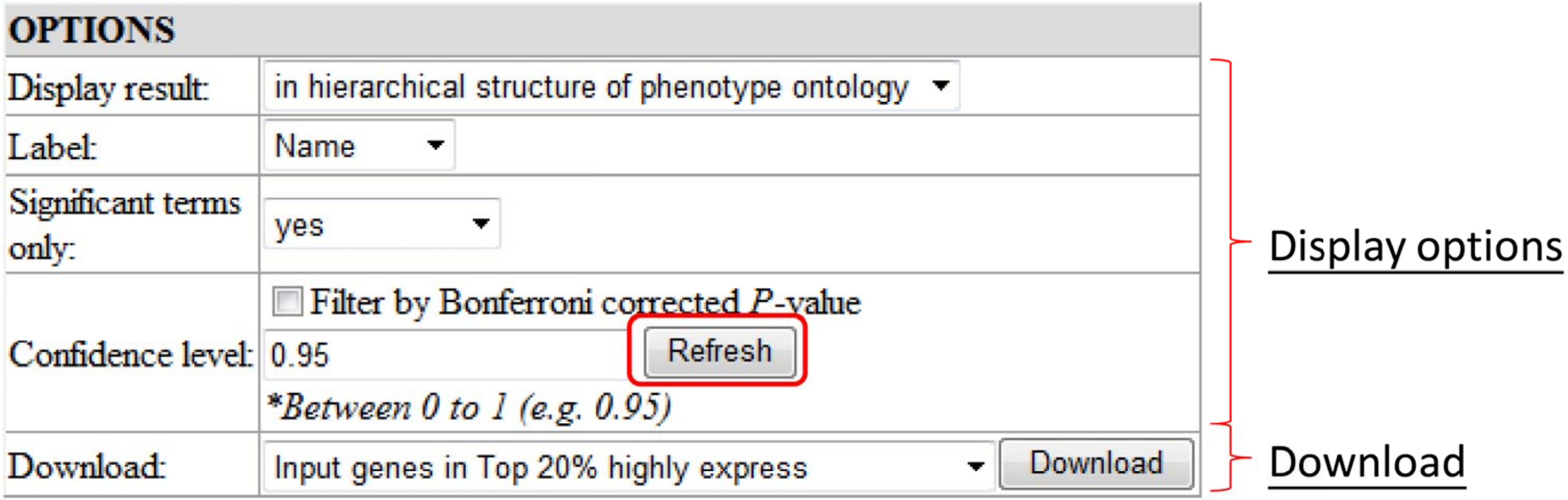
userPheno

e.g. FBcv:0000351,FBbt:00005397 (lateral | embryonic epidermis)

3 steps to customize a phenotype:

(1) search phenotype you want to combine
(2) add to selected phenotype in the middle
(3) give a name and make a customized phenotype

Result: filtering result



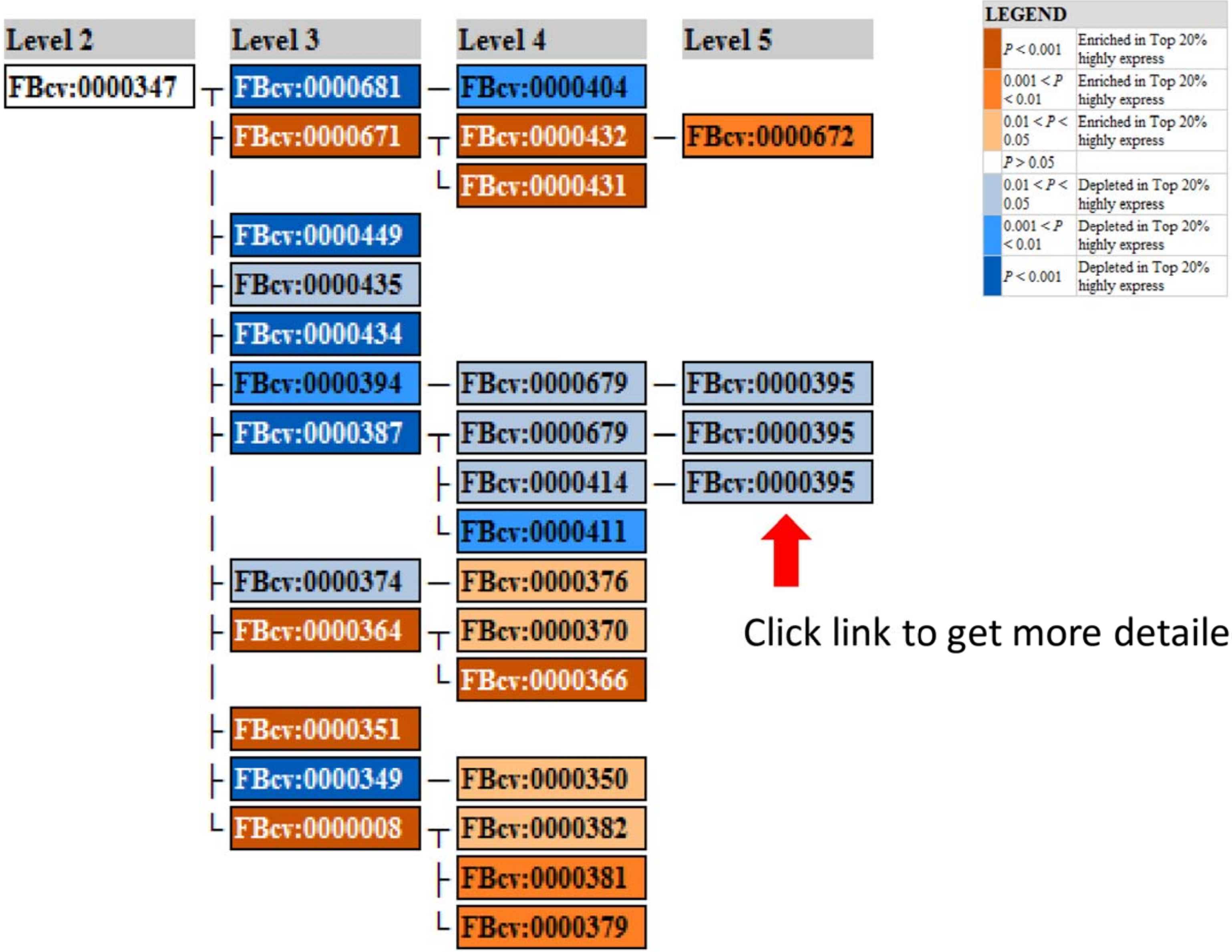
OPTIONS	
Display result:	in hierarchical structure of phenotype ontology
Label:	Name
Significant terms only:	yes
Confidence level:	☐ Filter by Bonferroni corrected *P*-value 0.95 Refresh _*Between 0 to 1 (e.g. 0.95)_
Download:	Input genes in Top 20% highly express Download

2 steps :

(1) Modify your filtering parameter or display style

(2) Hit **REFRESH** !

Result: (1) Hierarchical structure result

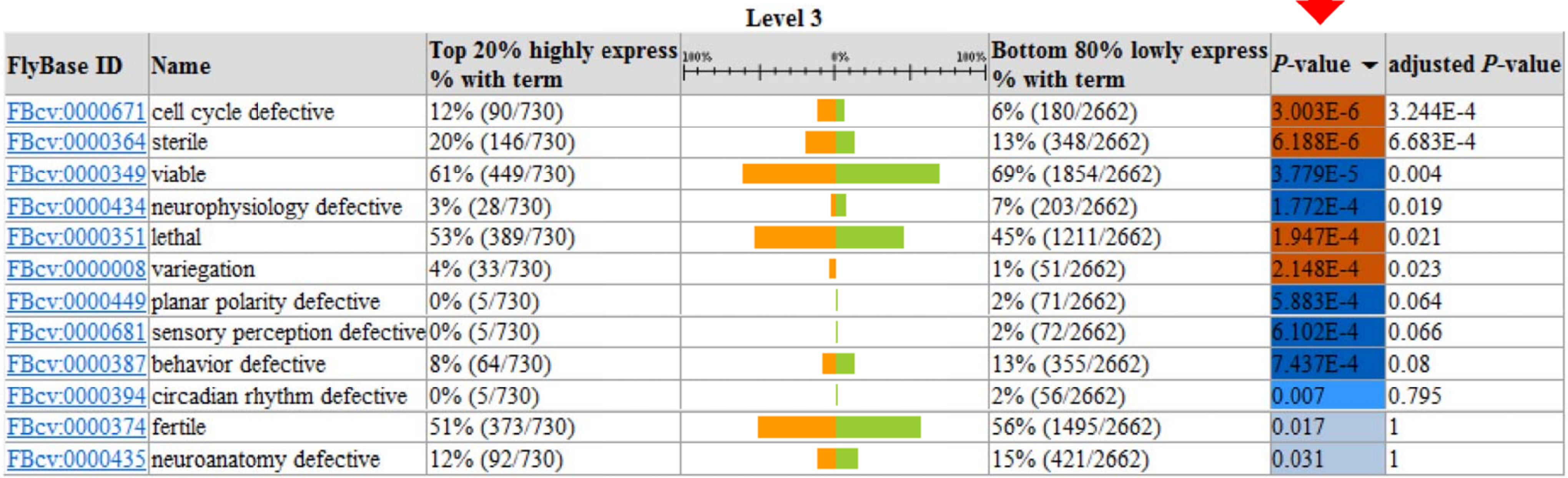


LEGEND		
🟧	$P < 0.001$	Enriched in Top 20% highly express
🟧	$0.001 < P < 0.01$	Enriched in Top 20% highly express
🟧	$0.01 < P < 0.05$	Enriched in Top 20% highly express
⬜	$P > 0.05$	
🟦	$0.01 < P < 0.05$	Depleted in Top 20% highly express
🟦	$0.001 < P < 0.01$	Depleted in Top 20% highly express
🟦	$P < 0.001$	Depleted in Top 20% highly express

Click link to get more detailed result

Result: (2) List of phenotype enrichment

Hit the header to sort

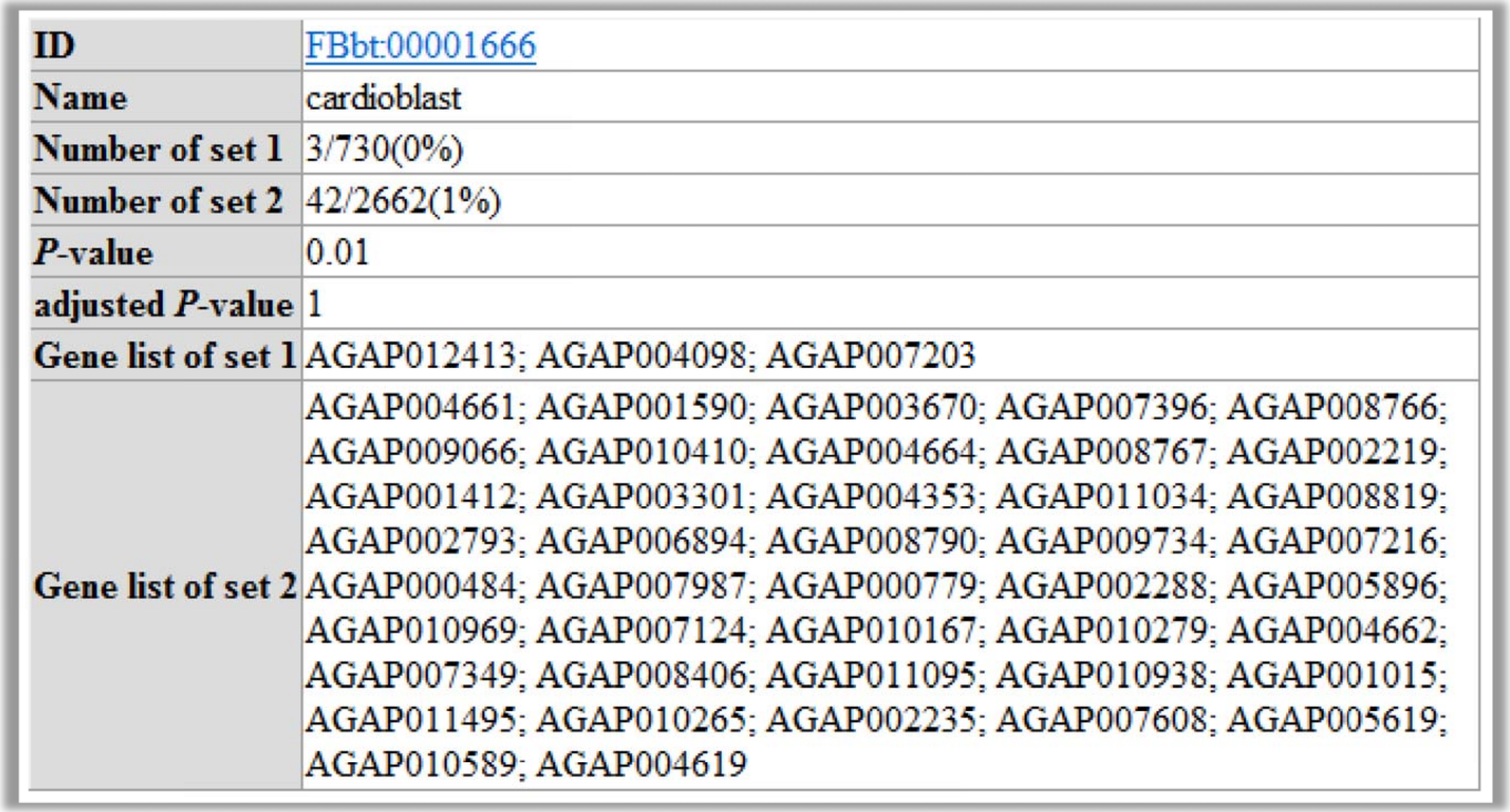


Level 3

FlyBase ID	Name	Top 20% highly express % with term	100% 0% 100%	Bottom 80% lowly express % with term	*P*-value ▾	adjusted *P*-value
FBcv:0000671	cell cycle defective	12% (90/730)		6% (180/2662)	3.003E-6	3.244E-4
FBcv:0000364	sterile	20% (146/730)		13% (348/2662)	6.188E-6	6.683E-4
FBcv:0000349	viable	61% (449/730)		69% (1854/2662)	3.779E-5	0.004
FBcv:0000434	neurophysiology defective	3% (28/730)		7% (203/2662)	1.772E-4	0.019
FBcv:0000351	lethal	53% (389/730)		45% (1211/2662)	1.947E-4	0.021
FBcv:0000008	variegation	4% (33/730)		1% (51/2662)	2.148E-4	0.023
FBcv:0000449	planar polarity defective	0% (5/730)		2% (71/2662)	5.883E-4	0.064
FBcv:0000681	sensory perception defective	0% (5/730)		2% (72/2662)	6.102E-4	0.066
FBcv:0000387	behavior defective	8% (64/730)		13% (355/2662)	7.437E-4	0.08
FBcv:0000394	circadian rhythm defective	0% (5/730)		2% (56/2662)	0.007	0.795
FBcv:0000374	fertile	51% (373/730)		56% (1495/2662)	0.017	1
FBcv:0000435	neuroanatomy defective	12% (92/730)		15% (421/2662)	0.031	1

Click link to get more detailed result

Result: (3) Click the link and get more detailed result



ID	FBbt:00001666
Name	cardioblast
Number of set 1	3/730(0%)
Number of set 2	42/2662(1%)
***P*-value**	0.01
adjusted *P*-value	1
Gene list of set 1	AGAP012413; AGAP004098; AGAP007203
Gene list of set 2	AGAP004661; AGAP001590; AGAP003670; AGAP007396; AGAP008766; AGAP009066; AGAP010410; AGAP004664; AGAP008767; AGAP002219; AGAP001412; AGAP003301; AGAP004353; AGAP011034; AGAP008819; AGAP002793; AGAP006894; AGAP008790; AGAP009734; AGAP007216; AGAP000484; AGAP007987; AGAP000779; AGAP002288; AGAP005896; AGAP010969; AGAP007124; AGAP010167; AGAP010279; AGAP004662; AGAP007349; AGAP008406; AGAP011095; AGAP010938; AGAP001015; AGAP011495; AGAP010265; AGAP002235; AGAP007608; AGAP005619; AGAP010589; AGAP004619

Result: (4) Blood-meal related phenotypes

phenotype_name	phenotype_id	Top 20% highly express	background	p_value	adj_p_value	
sterile	FBcv0000364	145	371	6.88E-06	3.44E-04	+
female sterile	FBcv0000366	110	269	2.75E-05	0.002	+
male germline cell	FBbt00057011	45	83	7.94E-05	0.114	+
male fusome	FBbt00007139	5	1	3.56E-04	0.28	+
spermatocyte fusome	FBbt00004937	5	1	3.56E-04	0.51	+
polar body nucleus	FBbt00000051	7	2	3.59E-04	0.282	+
pronucleus	FBbt00000048	7	2	3.59E-04	0.101	+
germline cell	FBbt00004860	101	266	6.19E-04	0.486	+
spermatocyte ring canal	FBbt00004939	5	1	0.002	1	+
fertile	FBcv0000374	371	1596	0.008	0.425	-
fusome	FBbt00004862	9	10	0.008	1	+
male fertile	FBcv0000376	29	61	0.008	0.682	+
egg	FBbt00000034	47	116	0.009	1	+
cell cycle defective	FBcv0000671	89	196	7.85E-06	3.92E-04	+
mitotic cell cycle defective	FBcv0000432	71	148	1.92E-05	0.002	+
meiotic cell cycle defective	FBcv0000431	32	57	7.27E-04	0.064	+
primordium	FBbt00005495	23	184	3.55E-04	0.1	-
planar polarity defective	FBcv0000449	5	72	9.21E-04	0.046	-
tergum	FBbt00004552	3	58	0.001	1	-
germ layer	FBbt00000110	3	50	0.005	1	-
viable	FBcv0000349	448	1983	1.19E-05	5.95E-04	-
lethal	FBcv0000351	387	1268	6.39E-05	0.003	+
embryonic neuron	FBbt00001446	8	114	2.93E-05	0.023	-
segmental nerve	FBbt00002037	2	55	3.90E-04	0.559	-
motor neuron	FBbt00005123	7	89	4.82E-04	0.378	-
neurophysiology defective	FBcv0000434	28	201	9.29E-04	0.046	-
neuron	FBbt00005106	75	417	0.002	0.486	-
sensory neuron	FBbt00005124	55	319	0.003	1	-
abdominal nerve	FBbt00002179	3	48	0.008	1	-
nerve	FBbt00005105	22	151	0.009	1	-
embryonic myoblast	FBbt00000463	1	33	7.82E-04	1	-
myoblast	FBbt00005083	1	42	9.54E-04	0.749	-
embryonic/larval somatic muscle	FBbt00000464	16	125	0.005	1	-
muscle cell	FBbt00005074	29	189	0.006	1	-
somatic muscle	FBbt00005073	28	179	0.01	1	-
antenna	FBbt00004511	6	74	0.002	1	-
inter-ommatidial cell	FBbt00007250	1	39	0.002	1	-

🟦 reproduction
🟪 cell cycle
🟥 development
🟩 mortality
🟧 neural
🟦 muscle
🟧 sensory
🟪 behavior
🟨 cell-line related
🟩 variegation related
⬜ other category

Thanks for your attention!

Question is welcome!