Results - June 2015
Total number of responses - 274

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Introduction

The development of increasingly sophisticated genetic techniques in Drosophila means the mutants and transgenic constructs being generated are extremely complex.

Genetic engineering techniques available in Drosophila include:

transposable-element based transformation
excisable cassettes *FRT, loxP*
targeted mutagenesis *phiC31, CRISPR, TALEN*
swappable cassettes *RMCE, MiMIC*
split systems *splitGAL4, GRASP*

As the complexity of these mutant increases it is important that FlyBase continues to curate these mutants and constructs in a way that makes them understandable and easily accessible to the user community.

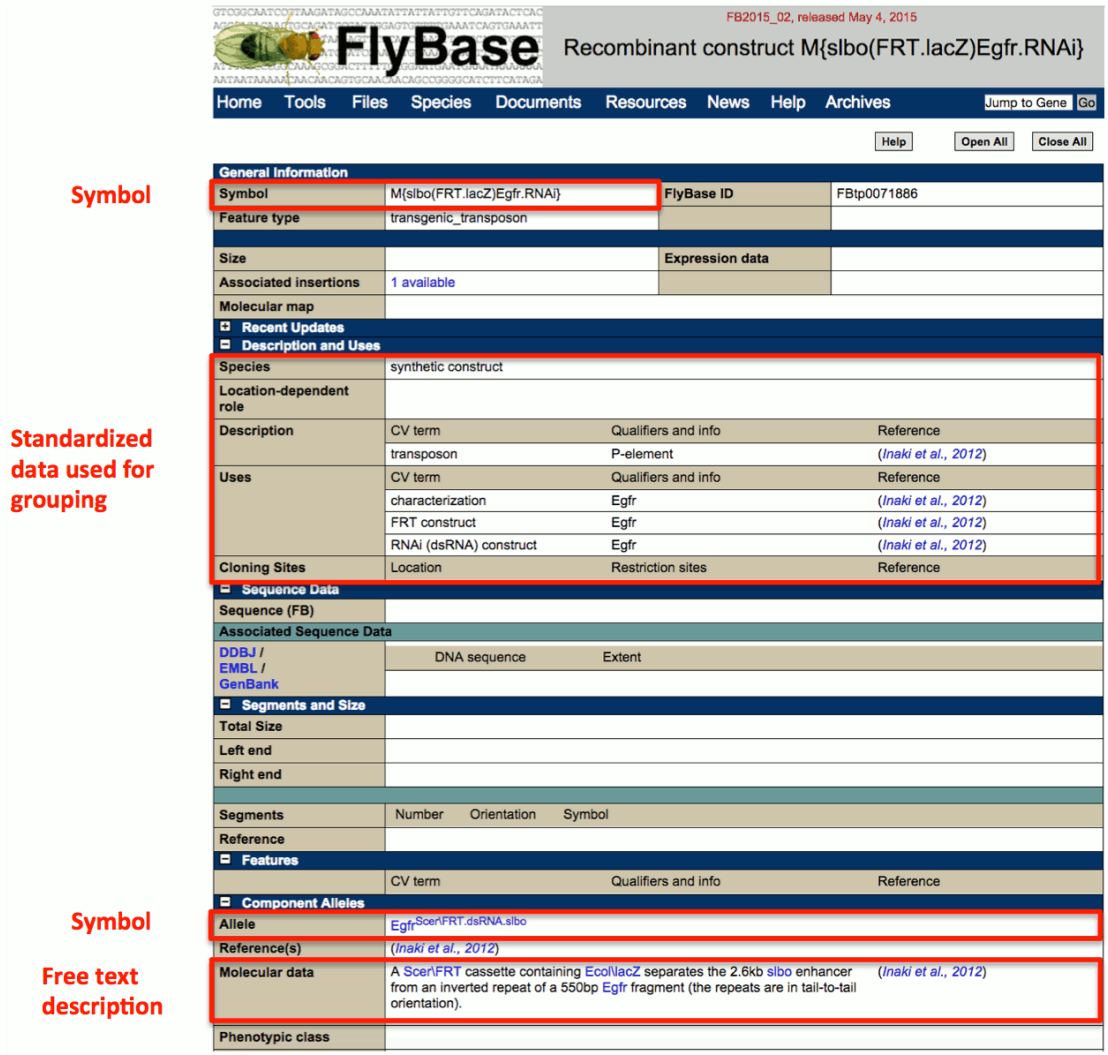
Example

The text below describes the generation of an RNAi line, taken from Inaki et al., 2012.

"To reduce Egfr expression in all border cells, slbo-flipout-EGFR-RNAi was constructed: a 550-bp fragment was amplified using primers TAGCTCTAGAGCGACTGGAGGTGTTCTC and TAGCTCTAGACTCCTGGCAGTGATCTG (extracellular) and cloned into pWIZ (36) tail to tail. The 2.6-kb slbo border cell enhancer (37) was inserted in HindIII and EcoRI of pUAS-attB, the EGFR inverted repeats cloned into AvrII site, and flip- out lacZ cassette (38) inserted into Asp718 site in forward direction."

The image below shows how we have curated this information in FlyBase. We can capture information in three ways:

- **Symbols** - key information is included in symbols (either the construct symbol or the associated allele symbols).
- **Free text description** - the information is captured in a free text sentence. We capture this information for all new constructs from what is written in the paper.
- **Standardized data used for grouping** - the information is captured in a standardized way that allows it to be used to group constructs with the same attributes. We currently only do this in a limited way, for example this construct has been categorised as an 'RNAi (dsRNA) construct' and an 'FRT construct', but we would like to extend it. More standardized capture of information would allow improved searching and better grouping of constructs on gene report pages.



The aim of this survey

In this survey we want to find out what information you think it is most important for us to capture about these complex mutants, and how that information should be presented.

Question 1 – Transgenic constructs

Below we have listed a number of different attributes we could capture about a transgenic construct and how it was generated. For each attribute, indicate whether you think we should capture this information and, if yes, how you think we should capture it. Tick as many options as apply

Examples are given for each attribute, and extracts from actual papers are included at the bottom to allow you to see the different types of data in context. The information is color-coded to make it easier to find the corresponding information.

Points to consider when answering the question:

- We will always capture the gene symbol and the reference in which the construct is described so these two attributes are not listed below.
- Sometimes overlapping information is present in the different categories, for example you can infer that UAS regulatory sequences are used from the fact that a pUAS-attB vector was used. In these cases please indicate which format you would prefer to see the information in.
- When considering what information should be captured in symbols, be aware that our symbols cannot be too long and should contain only the key information.
- FlyBase has a limited number of curators and can't capture everything!

Gene of interest

	Yes, capture in symbols	Yes, capture in a free text description	Yes, capture in a standardized way	No, do not capture
Region of gene used/deleted (full length cDNA, residues 1-346, 1131bp of Gga sequence (including 536bp of the third exon, the entire third intron and 526bp of the fourth exon))	☐	☐	☐	☐
Protein domains used/deleted (C-terminal half of the VHS domain, the entire GAT domain and the	☐	☐	☐	☐

N-terminal half of the hinge domain)				
Allele class (null, wild type, dominant negative)	☐	☐	☐	☐
Clone number (LD23292)	☐	☐	☐	☐

Additional features

	Yes, capture in symbols	Yes, capture in a free text description	Yes, capture in a standardized way	No, do not capture
Regulatory sequences (UAS, 2.6-kb slbo border cell enhancer)	☐	☐	☐	☐
Presence of epitope tag (GFP, EYFP, lacZ)	☐	☐	☐	☐
Presence of subcellular localization tag (nuclear localization sequence)	☐	☐	☐	☐
Location of tags (C-terminus)	☐	☐	☐	☐
Transgene marker (mini-white)	☐	☐	☐	☐
Presence of excisable cassette (flip- out cassette)	☐	☐	☐	☐
Vector (pUASTattB, pXH87, pWIZ)	☐	☐	☐	☐

Mutagenesis

	Yes, capture in symbols	Yes, capture in a free text description	Yes, capture in a standardized way	No, do not capture
Method used for mutagenesis (ends-out gene replacement, EMS, P-element insertion)	☐	☐	☐	☐
Enzymes used in method (FLP recombinase, I-sceI)	☐	☐	☐	☐
Source of the transposable element ends (P-element, PBac)	☐	☐	☐	☐

Examples from papers

Example 1 (from Batz et al., 2009)

UAS-Mcr was generated by amplifying the Mcr coding sequence from the Mcr full-length cDNA LD23292 (BDGP) using oligonucleotides (5'-3', restriction sites are underlined): EcoRI-Mcr-F, ATATGAATTCGAGCAATGATGTGGCACTTGC; and NotI-Mcr-R, TATAGCGGCCGCCCTGTGAGCAGTTGCATCATGT. The fragment was inserted between the EcoRI and NotI sites of UASTattB (Bischof et al., 2007). The construct was integrated into the attP2 site at 68A4 using ΦC31 integrase (Bischof et al., 2007).

Example 2 (from Inaki et al., 2012.)

To reduce Egfr expression in all border cells, slbo-flipout-EGFR-RNAi was constructed: a 550-bp fragment was amplified using primers TAGCTCTAGAGCGACTGGAGGTGTTCTC and TAGCTC- TAGACTCCTGGCAGTGATCTG (extracellular) and cloned into pWIZ (36) tail to tail. The 2.6-kb slbo border cell enhancer (37) was inserted in HindIII and EcoRI ofpUAS-attB, the EGFR inverted repeats cloned into AvrII site, and flip- out lacZcassette (38) inserted into Asp718 site in forward direction. For transgenics, the attP landing site at 51D was used.

Example 3 (from Ding et al., 2009)

For Chro-NTD, cDNA sequence corresponding to Chromator N-terminal residues 1–346 was inserted into the pUASP vector (Rorth, 1998) with a C-terminal GFPtag. Three tandemly arrayed nuclear localization sequences (NLS) excised from the pECFP vector (Clontech) were added to the C-terminus.

Example 4 (from Luan et al., 2012.)

To generate a dGGA null mutation, we turned to site-directed mutagenesis using an ends-out gene replacement strategy adapted from Chen et al. (2009). A 3 kb fragment extending 5′ from the *dGGA* coding region and a 2.7 kb fragment extending 3′ from the dGGA coding region were separately amplified by PCR and cloned into the pXH87 vector [35] (Figure 3A). We included the *FM7* balancer chromosome during the knockout mobilization steps in order to (1) rescue the*dGGA* null mutant flies in case knocking out GGA causes lethality and (2) increase the efficiency of recovering targeted events on the X chromosome [35] We used a scheme (Figure 3B) in which the *p{GGA knockout transgene}* targeting cassette is mobilized by FLP recombinase and linearized by I-SceI provided by *P{70 FLP} P{70-Sce-I}* after heat shock. We collected ~3000 mosaic- or white-eyed virgin females and mass-crossed these with males of a stock carrying *P{70 FLP}* to eliminate any residual autosomal copies of *p{GGA knockout transgene}*. From the progeny, 300 flies carried the w^+ transgene marker and seven of these carried the transgene. From the progeny, 300 flies carried the w^+ transgene marker and seven of these carried the transgene marker linked to the X chromosome. Using PCR primers flanking outside the targeted region and inside the EYFP-mini-*white*knockout cassette, as well as Western blot analysis, we were able to confirm six lines in which GGA was knocked out (Figure 4 and data not shown). The targeting rate was calculated as 0.23%, which is at the low end of the targeting frequency range reported by Chen et al. (2009) for autosomal knockouts using this approach. GGA^{Δ} was the result of replacing a total of 1131 bp, including 536 bp of the third exon, the entire third intron and 526 bp of the fourth exon, with the EYFP and mini-*white* cassette of pXH87. This results in the loss of 353 codons, including the C-terminal half of the VHS domain, the entire GAT domain and the N-terminal half of the hinge domain.

Results

	Yes, capture in symbols	Yes, capture in a free text description	Yes, capture in a standardized way	No, do not capture	No answer
Gene of interest					
Region of gene used/deleted	29%	71%	38%	1%	1%
Protein domains used/deleted	23%	69%	33%	4%	1%
Allele class	43%	34%	52%	0%	1%
Clone number	26%	31%	45%	9%	3%
Additional features					
Regulatory sequences	47%	51%	43%	1%	3%
Presence of epitope tag	64%	34%	49%	0%	2%
Presence of subcellular localization tag	38%	47%	44%	2%	3%
Location of tags	24%	49%	43%	5%	1%
Transgene marker	42%	33%	51%	3%	1%
Presence of excisable cassette	43%	43%	50%	1%	2%
Vector	38%	41%	48%	3%	3%
Mutagenesis					
Method used for mutagenesis	18%	54%	47%	4%	1%
Enzymes used in method	17%	46%	36%	17%	2%
Source of the transposable element ends	29%	40%	46%	9%	2%

Question 2 - Insertions

In the previous question we asked you about attributes of the transgenic construct. For some constructs we make a specific insertion for where that construct has been inserted into the genome.

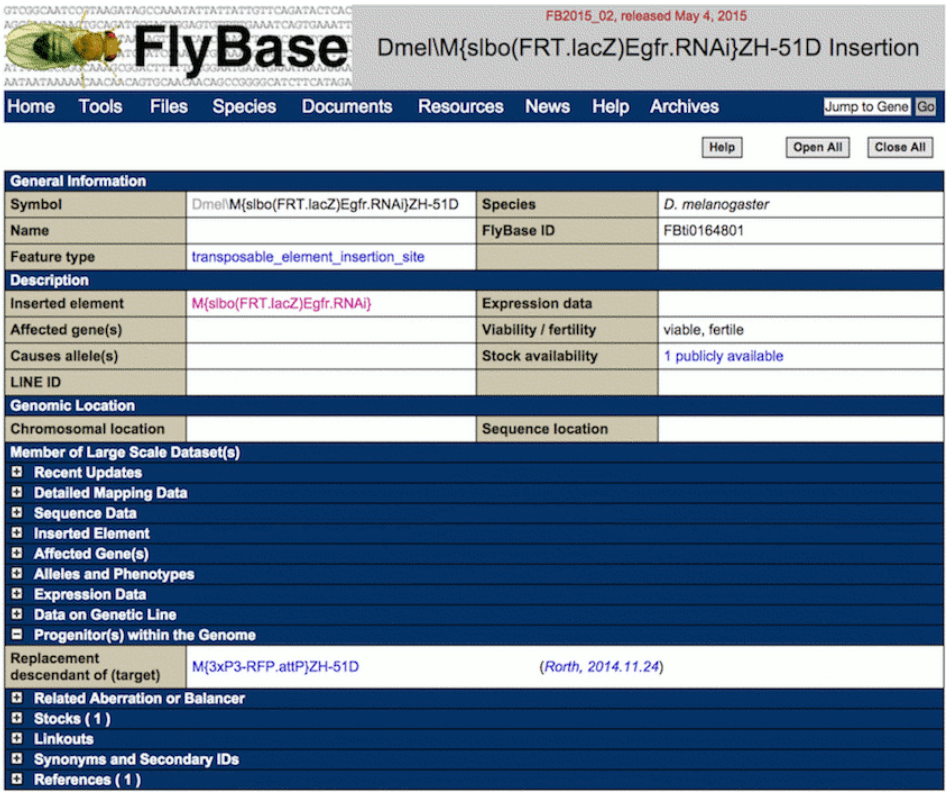
For the RNAi example given earlier, the relevant text from the paper read

"For transgenics, the attP landing site at 51D was used."

The insertion report containing this information is shown in the image. As before, indicate whether you think we should capture this information and, if yes, how you think we should capture it. The same options (symbol, free text description and standardised data) are given as before and color-coded examples are shown at the bottom of the page.

Symbol

Standardized data used for grouping



Insertion-related attributes

	Yes, capture in symbols	Yes, capture in a free text description	Yes, capture in a standardized way	No, do not capture
Method of genome integration (phi-C31 integrase-mediated, P-element mediated)	☐	☐	☐	☐
Location of transgene insertion -chromosome (X, 2R)	☐	☐	☐	☐
Location of transgene insertion - sequence location (3L:11070538, 2L:5108448)	☐	☐	☐	☐
Docking site (for phi-C31 integrase-mediated genome integration) (attP2 at 68A4, the attP landing site at 51D, P-element transformation)	☐	☐	☐	☐

Examples from papers

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Example 3 (from Ding et al., 2009)

Chromator construct pUAST or pUASP transgenic lines were generated by standard P-element transformation (BestGene, Inc.).

Results

	Yes, capture in symbols	Yes, capture in a free text description	Yes, capture in a standardized way	No, do not capture	No answer
Insertion					
Method of genome integration	30%	44%	53%	4%	1%
Location of transgene insertion - chromosome	42%	28%	53%	4%	1%
Location of transgene insertion - sequence location	25%	30%	57%	5%	1%
Docking site (for phi-C31 integrase-mediated genome integration)	34%	40%	55%	2%	1%

References

Bätz, T., Förster, D., Luschnig, S. (2014). The transmembrane protein Macroglobulin complement-related is essential for septate junction formation and epithelial barrier function in Drosophila. Development **141(4)**: 899--908.

Ding, Y., Yao, C., Lince-Faria, M., Rath, U., Cai, W., Maiato, H., Girton, J., Johansen, K.M., Johansen, J. (2009). Chromator is required for proper microtubule spindle formation and mitosis in Drosophila. Dev. Biol. **334(1)**: 253--263.

Inaki, M., Vishnu, S., Cliffe, A., Rørth, P. (2012). Effective guidance of collective migration based on differences in cell states. Proc. Natl. Acad. Sci. U.S.A. **109(6)**: 2027--2032.

Luan, S., Ilvarsonn, A.M., Eissenberg, J.C. (2012). The Unique GGA Clathrin Adaptor of Drosophila melanogaster Is Not Essential. PLoS ONE 7(9): e45163.