Generation of a transgenic ORFeome library in Drosophila
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Vector Preparation & Gateway ORF Cloning

Barcode the pGW-HA.attB vector

Clone and sequence ORFs into pDONR221

Transfer ORFs into pGW-HA.attB

Determine barcodes of pGW-HA.attB plasmids

Inject pooled plasmids

Establish fly strains

Identify strains via barcode determination

Confirm ORFs by single-fly PCR

Figure 1
Figure 2

(a) Gateway destination vector pGW-HA.attB

(b) Integration of a UAS-ORF transgene into an attP site
Entry clones

100 µl of each o/n culture

Pool

Well A1

Pooled culture

Miniprep

End repair and A tailing

Adapter ligation

Library amplification

Sequencing

Plasmid fragmentation

0.5-1 µg DNA

10-45 min

“beads-in” magnetic bead purification

Illumina sequencing library preparation

Vector and insert DNA (300-800 bp) with short random overhangs

End repair (NEBNext End Repair Module) (blunt ends)

A tailing (NEBNext dA-Tailing Module)

Adapter ligation (T4 ligase)

Library amplification by PCR

sequencing barcode (optional)

Gene specific primer F

AAAAAGCAGGCTTCAACATGTGTGACGAAGAAGTTGCTGCT

Gene specific primer R (rev. complement)

CCATTGTCACCAGCAAGTGCTTCGACCCAGCTTTCT

Act5C ORF

Stop codon omitted

GGGGACAAGTTTGTACAAAAAAGCAGGCT ACCCAGCTTTCTTGTACAAAGTGGTCCCC

Gene specific primer F

AAAAAGCAGGCTTCAACATGTGTGACGAAGAAGTTGCTGCT

Gene specific primer R (rev. complement)

CCATTGTCACCAGCAAGTGCTTCGACCCAGCTTTCT

attB1 primer

GGGGACAAGTTTGTACAAAAAAGCAGGCT

attB2 primer (rev. complement)

CCATTGTCACCAGCAAGTGCTTCGACCCAGCTTTCTTGTACAAAGTGGTCCCC

attB2 primer

GGGGACAAGTTTGTACAAAAAAGCAGGCT

500 bp

Gene specific primer R (rev. complement)

CCATTGTCACCAGCAAGTGCTTCGACCCAGCTTTCT

attB1 primer

GGGGACAAGTTTGTACAAAAAAGCAGGCT

attB2 primer (rev. complement)

CCATTGTCACCAGCAAGTGCTTCGACCCAGCTTTCTTGTACAAAGTGGTCCCC

attB1

Act5C ORF

attB2

Figure 3
**Figure 4**

**a**  
N-terminal *in vivo* exchange

- `white^+`  
- loxP  
- UAS-hsp70  
- ORF  
- FL  
- 3xHA  
- 3'UTR

+ FLP

- yellow^+  
- actin5C  
- white^+

↓

- yellow^+  
- actin5C  
- ORF  
- FL  
- 3xHA  
- 3'UTR  
- eGFP

**b**  
C-terminal *in vivo* exchange

- `white^+`  
- loxP  
- UAS-hsp70  
- ORF  
- FL  
- 3xHA  
- 3'UTR

+ FLP

- eGFP  
- 3'UTR  
- yellow^+

↓

- `white^+`  
- loxP  
- UAS-hsp70  
- ORF  
- FL  
- 3xHA  
- 3'UTR  
- eGFP

**c**  
Additional N- and C-terminal swapping lines

- lexB  

Exchange to *lexO* promoter

- 3xSTOP

Elimination of 3xHA tag

- VNm9  
- 3'UTR

Bimolecular fluorescence complementation

- VC155  
- 3'UTR

- FL  
- TEV  
- 2xTY1  
- 3'UTR

Exchange to 2xTY1 tag
Figure 5

G0: 2 inj. yw $\Phi$C31 x yw

F1: 1 yw ; $\frac{UAS-ORF(w^+)}{+}$ x yw ; $\frac{D gl3}{TM3 Sb Ser}$

F2: yw ; $\frac{UAS-ORF(w^+)}{TM3 Sb Ser}$