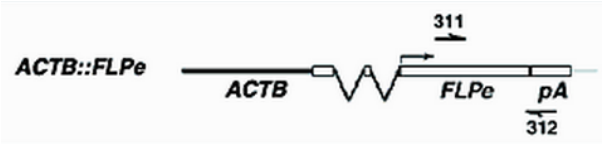
Genotyping Tg*(ACTB-FlpE)9205*^{Dym}

(ACT-FLPe; ACTB::FLPe; ACTB:FLPe; ACTFlpe; CAGG-Flpr; CAGGS-flp; FLPe; Flp; Flp deleter; TgaFlpe; TgpCAG-flpe; actin-FLPe; beta-actinFLPe; hACTB-FLPe; hACTB::FLPe)

JDW 6/14

MGI: 2448985

Reference: High-efficiency deleter mice show that FLPe is an alternative to Cre-loxP. Rodriguez CI, Bucholz F, Galloway J, Sequerra R, Kasper J, Ayala R, Stewart AF, Dymecki SM. 2000. Nature Genetics 25(2):139-140



S. cerevisiae FLP1 recombinase is driven by the human ACTB promoter (3kb flanking sequence, 78-bp 5' untranslated region, 832-bp of exon 1). Transgenic, non-targeted, enhancer FLP1 variant (FLPe) version of FLP1 recombinase.

Gene Specific Primers:
JDW 205 (B-actin pro FWD): 5' CGACCAGTGTTTGCCTTTTA
JDW 206 (FLPe Rev): 5' GCAAACTACTTACAATATCAGTG (not tested yet)
B-actin FWD is ~250 bp from the TSS. FLPe rev is 370 from ATG

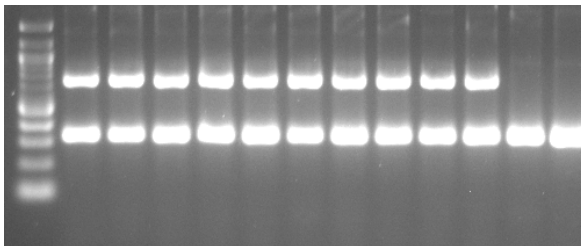
Transgene = ~ 650 bp

Primers from Jax*:
JDW XXX (oIMR1348): 5' CAC TGA TAT TGT AAG TAG TTT GC
JDW XXX (oIMR1349): 5' CTA GTG CGA AGT AGT GAT CAG G

*These primers do not distinguish between Tg/0 and Tg/Tg.
FWD is 370 bp in

New primers from 10/2013 from Jax, =100 bp
Tg REV (oIMR 17772) TGC CGG TCC TAT TTA CTC GT
Tg FWD (oIMR 17773) TAC TTC TTT AGC GCA AGG GGT AG

**Internal control primers (can multiplex with above primers):
JDW XXX (oIMR7338): 5' CTA GGC CAC AGA ATT GAA AGA TCT
JDW XXX (oIMR7339): 5' GTA GGT GGA AAT TCT AGC ATC ATC C



Transgene= ~725 bp
Internal Control (in IL2) = ~324 bp

Reaction Conditions:

10x CL buffer (Qiagen)	2.5µl
Q solution (QIagen)	2.5µl
dNTPs (10mM each stock)	0.5µl
Tg FWD (20mM stock)	0.5µl
Tg REV (20mM stock)	0.5µl
Con FWD (20 mM stock)	0.5µl
Con REV (20 mM stock)	0.5µl
DNA	1µl
Taq (Qiagen)	0.25µl
H2O	17.25µl

PCR Program:

94°C – 3 minutes	
94°C – 30 seconds	
58°C – 30 seconds	**X** 35 Cycles
72°C – 45 seconds	
72°C – 7 minutes	
16°C – forever	