EUCOMM (ES cell clone HEPD0652_5_G10; targeting vector PG00127_X_1_G01; allele Fam120btm1a(EUCOMM)Hmgu)

http://www.knockoutmouse.org/martsearch/project/69690

ES cell injections performed by http://www.tigm.org/

Received two female and one male het from the original colony (born 4/26/12)

Maintaining original colony:
Mated male (fam120b/+) with female (+/+) to maintain colony
6 pups (4 female, 2 male) born 2/21/13 (3 female and 1 male are fam120b/+), sack other two (+/+)
4 pups (1 female, 3 male) born 3/17/13 (all are fam120b/+)

Mated female (F1) from litter 2 and male (M2) of litter 1
4 pups (2 female, 2 male) born 10/7/13 (1 female is fam120b/fam120b; 1 female and 1 male are fam120b/+)
other male is (+/+)
Shipping F1 (fam120b/+), F2 (fam120b/fam120b), M1 (fam120b/+)

Making floxed mice:
Mated male (fam120b/+) with female (FLPer/+) to make floxed allele
3 pups (3 male) born 10/2/12 (2 males are flox/+; FLPer/+)
8 pups (5 female, 2 male) born 11/11/12 (3 female are flox/+; FLPer/+)

To remove FLPer transgene:
Mated male (M1) of litter 1 (10/2/12) and female wild-type (+/+)
5 pups (2 female, 3 male) born 3/24/13 (1 male is (flox/+;FLPer/+) and 1 female is (flox/+; +/+)
rest are (+/+;Flper/+) or (+/+;+/+)
* female (F2) (flox/+;+/+) died
5 pups (1 female, 4 male) born 4/18/13 (1 female is (flox/+;FLPer/+)
rest are (+/+;Flper/+) or (+/+;+/+)

To remove FLPer transgene:
Mated male (M3) from litter 1 (flox/+;FLPer/+) and female (F1) from litter 2 (flox/+;FLPer/+)
5 pups (3 female, 2 male) born 7/29/13 (1 male and 2 female are (flox/+;+/+)
rest are: male (flox/+;FLPer/+) and female (+/+;FLPer/+)
5 pups (2 female, 2 male, 1 dead) born 9/13/13 (none are desirable)
rest are: males (flox/+;FLPer/FLPer), female (flox/flox;FLPer/FLPer), female (+/+;FLPer/+)

To make homozygous floxed allele mice:
Mated male (M2) of litter 1 (flox/+;+/+) with females (F1 & F2) of litter 1 (flox/+;+/+)
Shipping M2 (flox/+;+/+), F1 (flox/+;+/+), F2 (flox/+;+/+)

Primers used, location and PCR products formed:
14752 5' primer: TGCTCTTTATTTCCATGGGG (5' of targeting vector/Frt site, 3' of exon1) (in wildtype)
21608 5' primer: GCCATCACGAGATTTCGATT (3' of NeoR, 5' of SV40 polyA)
22425 3' primer: GGGGTGTTTTCAGTGGAAGA (3' of Frt/loxP site, 5' of exon2) (in wildtype)
19836 5' primer: CGGTCGCTACCATTACCAGT (lacZ 3' end)
24549 3' primer: CATGATGGCAGAGTTAGGCA (3' of last loxP) (in wildtype)

14752 + 24549 = 2767 bp if wildtype
21608 + 22425 = 818 bp if knockout-first vector
14752 + 22425 = 653 bp if wildtype
19836 + 24549 = 692 bp if loxP recombined
14752 + 22425 = 770 bp if Frt recombined
14752 + 24549 = 783 bp if Frt + loxP recombined

Information including primers for genotyping from the FLPer/+ mice:
http://jaxmice.jax.org/strain/009086.html

For all PCR reactions, I used Denville Choice-TAQ following manufacturer protocol for PCR reactions setup:
http://www.denvillescientific.com/sites/default/files/ChoiceTaq%20DNA%20Polymerase_1.pdf

For PCR cycling conditions:
94˚C, 2 min, 1 cycle
94˚C, 30 sec, 35 cycles
55˚C, 30 sec, 35 cycles
72˚C, 45 sec, 35 cycles
72˚C, 10 min, 1 cycle