Genotyping Tg *(Apj_BAC_CreER)*^{Krh}

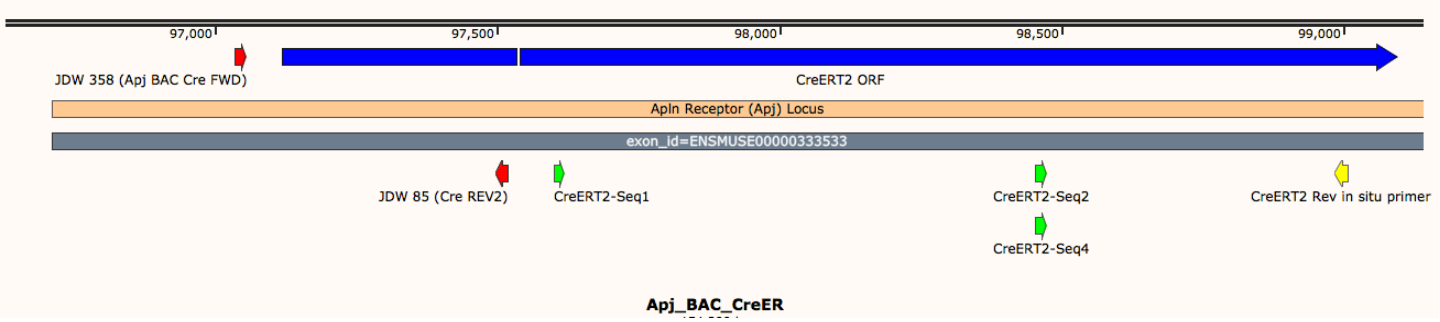
(aka AplnR-CreER,)

JDW 12/15

MGI: 5689869

Reference: Chen HI, Sharma B, Akerberg BN, Numi HJ, Kivela R, Saharinen P, Aghajanian H, McKay AS, Bogard PE, Chang AH, Jacobs AH, Epstein JA, Stankunas K, Alitalo K, Red-Horse K. 2014. The sinus venosus contributes to coronary vasculature through VEGFC-stimulated angiogenesis. Development 131, 4500-4512. [PubMed: 25377552][MGI Ref ID J:223081]

Note: ApjCreER was created through recombineering (Sharan et al., 2009; Warming et al., 2005). CreERT2 was inserted at the Apj start site of a BAC procured from the Children's Hospital Oakland Research Institute (CHORI) (clone RP24-301A16, 153,553 bp in length). Pronuclear injection (Cyagen) resulted in two founder lines, which exhibited similar expression patterns

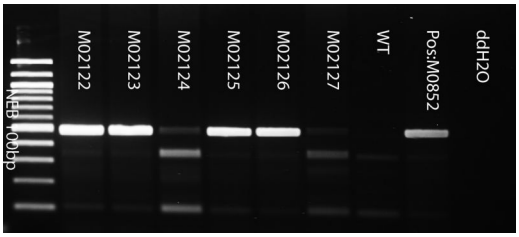


Primers:
JDW 358 (Apj_BAC_Cre FWD): 5': TTCAGGGTGCTTGCTGAGTTGG
JDW 83 (Cre REV2): 5': CCTGTTTTGCACGTTCACCG

MT= 484 bp

Reaction Recipe:

10x CL buffer (Qiagen)	1.25µl
Q solution (QIagen)	1.25µl
dNTPs (10mM each stock)	0.25µl
JDW 358 (20mM stock)	0.25µl
JDW 83 (20mM stock)	0.25µl
genomic DNA (hotshot)	2.0µl
Taq (Qiagen)	0.125µl
ddH_2O	7.125µl



PCR Conditions:

Step 1:	95°C – 2 minutes
Step 2:	95°C – 30 seconds
Step 3:	60°C – 30 seconds
Step 4:	72°C – 45 seconds
Step 5:	Go to step 2, **X** 35 Cycles
Step 6:	72°C – 2 minutes
Step 7:	16°C – forever

Note: We <u>often</u> observe germline recombination with this allele in the absence of tamoxifen administration, so genotype reporter mice accordingly (use the R26-ai14 "rec check PCR" for determining if they've recombined) in each generation to avoid a nasty surprise. The pups will appear "pink" on all skin surfaces if you have germline recombination in the Ai14/Ai14 background. As always with any BAC or PAC transgene of unknown copy number, do NOT breed the transgene to homozygosity, as it may contain other loci, and increasing their copy number may have an unanticipated effect.