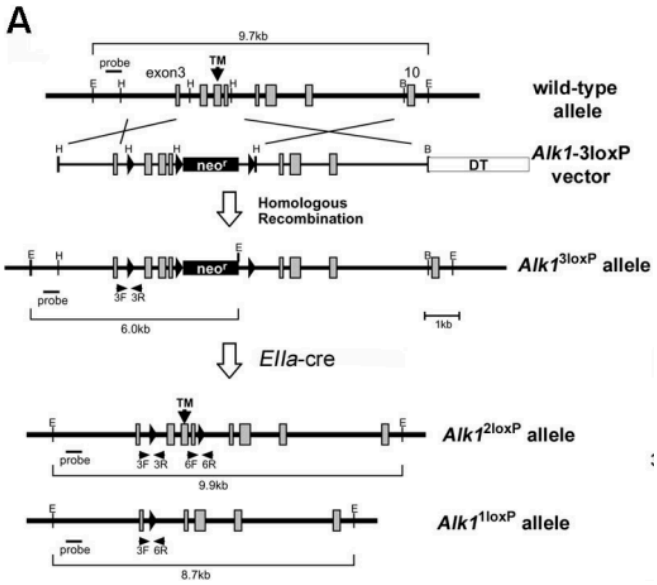
Genotyping *Alk1*$^{tm2.2Spo}$

(*Acvrl1*$^{tm2.2Spo}$*; Alk1*^{1f} or *Alk1*^{1loxP} = KO*; Alk1*^{2f} = floxed, no neo; *Alk1*^{2f} = floxed, with neo)

MGI: 4398902

Reference: *Park SO, et al., ALK5- and TGFBR2-independent role of ALK1 in the pathogenesis of hereditary hemorrhagic telangiectasia type 2. Blood. 2008 Jan 15;111(2):633-42*



Schematic diagram of the Alk1 wild-type allele, Alk1-conditional targeting vector, and Alk13loxP, Alk12loxP, and Alk11loxP alleles. Exons and loxP sequences are indicated by boxes and arrowheads, respectively. Locations of primer pairs used for amplifying specific regions containing a loxP sequence are also indicated. we generated a conditional Alk1 knockout allele in which 3 loxP sequences were inserted into intronic regions of the Alk1 gene. The 5 most loxP sequence and the neomycin cassette flanked by loxP sequences were inserted into the third and the sixth introns, respectively; thus, exons 4-6 could be deleted by Cre–mediated recombinations (Figure 2A). Because exon 5 encodes the transmembrane (TM) domain, the deletion of exons 4-6 would produce a null allele.

PRIMERS:

Alk1 (2f): - conditional allele

oJDW 703 (mAlk1-ex5FWD): 5'-cctggacagcgactgtactac
oJDW 704 (mAlk1-in6REV): 5'-gccccattgctctcctcaaac
WT = 350 bp
FLOX = 400 bp

Alk1 (1f) -after Cre mediated deletion (aka Null or KO allele)

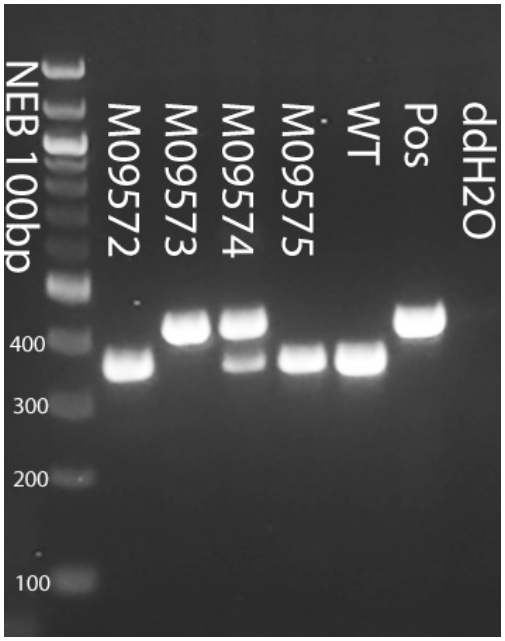
oJDW 705 (mAlk1-in3FWD4): 5'-CAGCACCTACATCTTGGGTGGAGA
oJDW 706 (mAlk1-in6REV, same as oJDW 704!): 5'-GCCCCATTGCTCTCCTCAAAC
KO=390bp

REACTION RECIPE:

9.375 ul ddH_2O
1.25 µl 10x Buffer
0.25 µl 10mM dNTPs
0.25 µl JDW 703 (@20µM)
0.25 µl JDW 704 (@20µM)
0.125 µl Homemade TAQ
1.0 ul genomic DNA

PCR PROGRAM:

Step 1:	95°C – 3 minutes
Step 2:	94°C – 30 seconds
Step 3:	63°C – 45 seconds
Step 4:	72°C – 45 seconds
Step 5:	GO TO STEP 2, x 35 cyles
Step 6:	72°C X 5 minutes
Step 7:	16°C FOREVER



*Run on a 2.5% gel

From the paper:

Primer sequences for Alk1 alleles are as follows: 3F cagcacctacatcttgggtggaga; 3R actgttcttcctcggagccttgtc; 6F cctggacagcgactgtactac; and 6R gccccattgctctcctcaaac. Combinations of 3F and 3R primers for the 3flox allele detection, 6F and 6R primers for the 2flox allele detection, and 3F and 6R primers for the 1flox (null) allele detection.