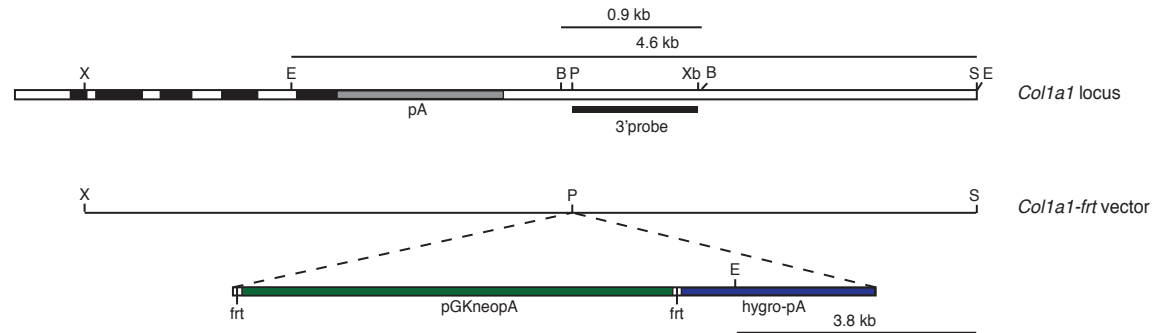


Col1a1-frt



Introduction of *Col1a1-frt* construct in the *Col1a1* locus by homologues recombination as described by Beard et al., 2006. Schematic representation of the *Col1A1* locus and the *Col1a1-frt* targeting construct. The latter contains a Neomycin expression cassette flanked by frt sites, followed by an ATG-less Hygromycin resistance gene. The 3' internal probe is indicated. Exons are shown as black boxes and 3' UTR as a gray box. pA, polyadenylation signal. X, XhoI; E, EcoRI; B, BglIII; P, PstI; Xb, XbaI; S, SpeI.

Original vector from Beard et al., 2006 *Genesis*. Schematic representation from Huijbers et al., 2013 *EMBO Molecular Medicine*

Southern blot analysis:

Detection of 3' end of Col1a1-frt construct

Digest: EcoRI
Probe: 3' fragment PstI-XbaI (842 bp)
Germline band: 4.6 kb
Targeted band: 3.8 kb

PCR analysis Col1a1-frt targeted locus:

Primers:

Forward forHyg1: gCC CCA gCA CTC gTC CgA ggg C
Reverse revIvoCol1b: CCC Agg TCC TgC CTC CTC CgT gC

DNA	1 µl
Phusion Flash HF PCR master mix (2x)	10 µl
forHyg1 (100uM)	0.15 µl
revIvoCol1b (100uM)	0.15 µl
H2O	8.7 µl +
	20 µl

PCR program:

3 min 98C
3 sec 94C \
5 sec 71C 32 cycles
60 sec 72C /
60 sec 72C

Bands:

Targeted band: 3 kb