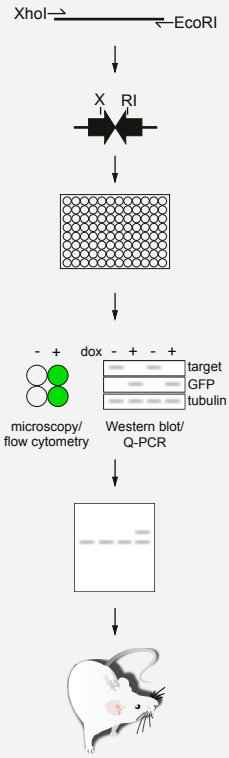
Table S1

Workflow	Step	Time	Details	Specific requirements	Key benefits (compared to CKO)
	PCR amplify shRNA Clone into miR30 backbone Electroporate ES cells Select with Hygromycin Pick indibidual clones Test target gene knockdown in 3-4 clones Southern blot to confirm single integration at the ColAL locus (GFP and ColA probes) Produce transgenic mice by tetraploid complementation or blastocyst injection	1 day 4 days 3-4 weeks 1 week 5-7 days 4 weeks	**Targeting vector:** Individual shRNAs are PCR amplified from single-stranded 97mer oligonucleotides using Pfx platinum polymerase (see Zuber et al., 2010). XhoI/EcoRI digested PCR fragments are cloned into any miR30 recipient vector, including pCol-TGM. **Electroporation:** ES cells are coelectroporated with the pCol-TGM targeting vector and FlpE plasmid and plated on irradiated feeder cells. 48 hours post electroporation cells are selected with Hygromycin (140µg/ml) and individual clones are picked after 8-14 days. **Testing clones:** Individual ES clones are tested *in vitro* by treating with 1µg/ml doxycycline in the culture media for 4 days (or longer depending on target stability). For testing knockdown ES cells are cultured in the absence of feeders. **Testing genomic integration:** Southern blot on gDNA from from ES cell clones confirms ColA targeting (ColA1 probe) and no off target integrations (GFP probe) GFP probe: EcoRI digest ColA1 probe: SpeI digest **Mouse production:** Founder animals can be produced by tetraploid embryo complementation. These founder animals are 100% ES cell derived and F1 mice do not need to be screened for transmission of the allele.	* 97mer shRNA oligo * miR30 For and Rev primers * Pfx platinum polymerase * PCR cycler * XhoI and EcoRI * miR30-based vector (pCol-TGM,LMP,LMS) * KH2 ES cells * pCol-TGM targeting vector * FlpE plasmid * Hygromycin * Hygro resistant feeder cells * Doxycycline * Flow cytometer * GFP and ColA1 probes * 32P-dATP * SpeI * EcoRI * Transgenic facility equipped for tetraploid embryo complementation or blastocyst injection.	**Vector cloning can be completed in less than 1 week.** (1-6 months for traditional vector cloning) **Vector design can be tested prior to ES cell production.** **Electroporations can be performed on a smaller scale as fewer clones need to be screened. Therefore more constructs can be handled in parallel.** **Easy to assess gene silencing before further screening and mouse production.** **Approximately 90% of GFP positive/hygromycin resistant clones have single correct integration.** Efficiency of traditional targeting ranges from 1-20% **No screening of F1 generation for germline transmission. Founder animals can be used directly for experiments.**