Generation of BAC-driven miRNA knockdown transgenic mice

- Identify driver gene
- Select BAC using MapViewer
- Obtain driver BAC from www.chori.org
- Identify silenced gene
- Select miRNA of interest (RNAi Codex)
- Bioinformatic testing of miRNA specificity



- Transform BAC into recombineering bacteria
- Confirm BAC integrity by restriction digest and PFGE



- PCR targeting arms with intervening Ncol site
- Clone into pSTBlue vector



- Amplify miRNA oligonucleotide
- ullet Clone miRNA into the intron of GFP/eta-globin intermediate construct



- Transfer GFP/βglobin/miRNA cassette to homology arm vector
- Add FRT-Neomycin resistance cassette
- Isolate targeting fragment



- Electroporate competent bacteria with the targeting construct
- Select for recombinants



- Confirm recombinants with RE map/PFGE
 - Remove FRT-Neo cassette using arabinose induction



• Test intermediate construct knock-down efficacy in vitro using cell lines transfected with the target gene (qPCR and Western)

- Grow and purify final construct
- Perform pronuclear injections
- Screen for founders
- Expand colonies and characterize TG mouse lines

