

## Time from DNA to knockout mouse

<b>Time 0</b>	<b>DNA to Core</b>
Week 3	Receive DNA from $\geq 200$ ES cell clones to analyze
Week 5	Give preliminary results to Core
Week 7	Receive 500ug of DNA from possible targets to verify
Week 9	Inform Core of best clones to karyotype
Week 10	Receive results from karyotyping
Week 11	Core will inject 2 best ES clones into blastocysts
Week 14	Pups born
Week 15	Chimerism analyzed
Week 19	Chimeric mice mated
Week 23	Chimeric offspring born
Week 24	Agouti pups identified
Week 25	Earclips given to PI to genotype
<b>Week 26</b>	<b>Weaned positive heterozygous pups sent to PI</b>
Week 28	Intercross heterozygous male and female
<b>Week 31</b>	<b>Homozygous knockout mice born—analyze phenotype</b>

### If conditional knockout, continue below:

Week 28	Mate heterozygous mouse to flip to get rid of neo
Week 28	Intercross heterozygous mice to check for hypomorphism
Week 28	Mate heterozygous mouse to cre mouse
Week 32	Flipped out heterozygous mice born—should have no phenotype
Week 32	Homozygous targeted mice born—should have no phenotype
Week 32	Heterozygous targeted, heterozygous cre mice born
Week 36	Intercross flipped out hets to maintain mice as homozygotes
Week 36	Backcross flipped out hets to C57Bl6 to get on pure background
Week 36	Intercross flipped out hets; cre hets to get homozygotes
<b>Week 39</b>	<b>Pups born with tissue specific knockout of your gene—analyze phenotype</b>

### If inducible knockout, continue below:

Week 28	Mate heterozygous mouse to flip to get rid of neo
Week 28	Intercross heterozygous mice to check for hypomorphism
Week 28	Mate heterozygous mouse to tTA mouse
Week 32	Flipped out heterozygous mice born—should have no phenotype
Week 32	Homozygous targeted mice born—should have no phenotype
Week 32	Heterozygous targeted, heterozygous tTA mice born
Week 36	Intercross flipped out hets to maintain mice as homozygotes

Week 36	Backcross flipped out hets to C57Bl6 to get on pure background
Week 36	Intercross flipped out hets;tTA hets to get homozygotes
	Feed doxycycline at appropriate time
<b>Week 39</b>	<b>Pups born with homozygous mutation of your gene that can be induced any time—analyze phenotype</b>