1. First Generation: Heterozygous *cre* male is mated to a homozygous floxed female in order to generate mice that are heterozygous for the flox and heterozygous for the *cre*.

$$\text{Cre}^{mut/wt} \text{ Gene}^{wt/wt} \text{ Male x Cre}^{wt/wt} \text{ Gene}^{fl/fl} \text{ Female}$$

Offspring:

$$\text{Cre}^{mut/wt} \text{ Gene}^{fl/wt} \quad \text{and} \quad \text{Cre}^{wt/wt} \text{ Gene}^{fl/wt}$$

Second Generation: Heterozygous *cre* male heterozygous floxed male is mated back to a homozygous floxed female.

$$\text{Cre}^{mut/wt} \text{ Gene}^{fl/wt} \text{ Male x Cre}^{wt/wt} \text{ Gene}^{fl/fl} \text{ Female}$$

Offspring:

$$\text{Cre}^{wt/wt} \text{ Gene}^{fl/wt}, \text{Cre}^{wt/wt} \text{ Gene}^{fl/fl} \text{ (control flox mice which lack } cre\text{),}$$
$$\text{Cre}^{mut/wt} \text{ Gene}^{fl/wt} \text{ , and } \boxed{\text{Cre}^{mut/wt} \text{ Gene}^{fl/fl}} \text{ (Goal)}$$

2. Third Generation and Maintenance: Heterozygous *cre* male homozygous floxed is mated to a homozygous floxed female.

$$\text{Cre}^{mut/wt} \text{ Gene}^{fl/fl} \text{ Male x Cre}^{wt/wt} \text{ Gene}^{fl/fl} \text{ Female}$$

Offspring:

$$\text{Cre}^{wt/wt} \text{ Gene}^{fl/fl} \text{ (control flox mice which lack } cre\text{), and } \text{Cre}^{mut/wt} \text{ Gene}^{fl/fl} \text{ (Specific KO)}$$

Control Line Maintenance:

Cre: Heterozygous cre Male x Wild-type female

$$\text{Cre}^{mut/wt} \text{ Male x Cre}^{wt/wt} \text{ Female}$$

Offspring:

$$\text{Cre}^{mut/wt} \text{ and } \text{Cre}^{wt/wt}$$

Flox: Homozygous Male x Homozygous Female

$$\text{Gene}^{fl/fl} \text{ Male x } \text{Gene}^{fl/fl} \text{ Female}$$

Offspring:
$$\text{Gene}^{fl/fl}$$

3. First generation: Wild-type female impregnated with $Cre^{mut/wt}$ $Gene^{fl/fl}$ sperm

Offspring:

$Cre^{mut/wt}$ $Gene^{fl/wt}$ and $Cre^{wt/wt}$ $Gene^{fl/wt}$

Second Generation: Heterozygous cre heterozygous floxed male is mated to a heterozygous floxed female which lacks *cre*.

$Cre^{mut/wt}$ $Gene^{fl/wt}$ Male x $Cre^{wt/wt}$ $Gene^{fl/wt}$ Female

Offspring:

$Cre^{mut/wt}$ $Gene^{wt/wt}$, $Cre^{wt/wt}$ $Gene^{wt/wt}$
$Cre^{mut/wt}$ $Gene^{fl/wt}$, $Cre^{wt/wt}$ $Gene^{fl/wt}$, $Cre^{mut/wt}$ $Gene^{fl/fl}$ and $Cre^{wt/wt}$ $Gene^{fl/fl}$ (Goal)

Third Generation and Maintenance: Heterozygous *cre* male homozygous floxed is mated to a homozygous floxed female.

$Cre^{mut/wt}$ $Gene^{fl/fl}$ Male x $Cre^{wt/wt}$ $Gene^{fl/fl}$ Female

Offspring:

$Cre^{wt/wt}$ $Gene^{fl/fl}$ (control flox mice which lack *cre*), and $Cre^{mut/wt}$ $Gene^{fl/fl}$ (Specific KO)

Estimation: 9 months to a year, but could probably be decreased if multiple breeding cages are ongoing. About a year if only 1 cage is breeding.

4. Mice are weaned at 4 weeks/ 28 days. Mice are genotyped at about 6-8 weeks via PCR. Only the S100A8 Cre lines can be genotyped by flow cytometry, but it can also be typed by PCR.