

### Ear Notches

- 12 days old
- Write down the notches and the numbers, make sure you have the right cage number and DOB
- Can already prepare the spread sheet for the cages notched

### gDNA prep

- Add 150  $\mu$ L of 50 mM NaOH
- Incubate 1hr on heat block at 95°C
- Let cool down at room temp 5 min
- Add 150  $\mu$ L of 100 mM Tris-HCl pH 7.5, mix well then spin down 5 min, 13000 rpm
- Dilute supernatant 1 to 5 (100  $\mu$ L of gDNA + 400  $\mu$ L H<sub>2</sub>O)
- Freeze the rest of concentrated gDNA at -20 and proceed to PCR right away or in a short amount of time (2-3 days max).

### PCR

- Use 5  $\mu$ L of diluted gDNA to run the PCR in a 15  $\mu$ L total reaction.
- Add a water only and WT B6 mice as PCR controls
- Put the pictures in the spread sheet right away and identify the wells and the primers used
- Enter genotyping in spread sheet, print.
- Write down future date of wean

### PCR Program

- See PCR excel sheet for specific genotyping

### Wean

- Decide which mice to keep for planned experiment
- Prepare cage cards, write down cage card numbers on spread sheet