Genotyping

Procedure:

- Kelly obtains samples by ear punch and sends e-mail to notify that new samples are in the freezer by the CAF entrance (after you enter through the first set of doors, to the left, samples are located in the freezer door in a plastic box with blue lid)
- Pick up samples there and put in our -20°C freezer until ready for genotyping

Preparations:

- Make sure all reagents & materials are present:
  - ddH_2O
  - Lysis buffer A (-20°C – box “genotyping Antje”)
  - Lysis buffer B (-20°C – box “genotyping Antje”)
  - Red TAQ Mix (-20°C – box “genotyping Antje”)
  - Primers (-20°C – box “genotyping Antje”)
  - RedSafe (+4°C box “genotyping Antje”)
  - DNA ladder (+4°C box “genotyping Antje”)
  - Agarose (chemicals shelf)
  - small Erlenmeyer flask (shelf by Antje’s bench)
  - 100ml cylinder (shelf by Antje’s bench)
  - Pipettes + tips
- Write down specific protocol & make calculations beforehand:
  - Which samples # need to be processed
  - Which primer to use for each sample
  - Count number of samples for each primer + total number of samples
  - Think about how to lay out samples on gel
  - Keep in mind to include 1 ladder for each side of the gel + 1 positive control & 1 negative control for each primer
  - Gel has 20 spots per side = 1 ladder + 19 samples/controls

- Make a schedule about when to perform each step:
  - a) Lysis of samples: 1h
  - b) Set up PCR: 30min.
  - c) PCR is running for ~ 2hrs
  - d) Make gel: 40min.
  - e) Run gel + take picture + enter results: 1h

  Day 1: a) + b) = total of 1.5hrs → then run c) overnight
  Day 2: d) + e) = total of 2hrs

Step 1 – Lysis

- get samples out from -20°C freezer and line up on rack in numerical order
- get out Buffer A + Buffer B from -20°C freezer & thaw
- get 50ml tube with ddH2O
- add **70µl of ddH_20** to each sample – make sure sample is not stuck to the tube
- add **20µl of Buffer A** to each sample
- add **10µl of Buffer B** to each sample
- incubate on shaker for 5min. at 75°C (open caps a few times + vortex 2 times)
- increase temperature to 95°C and incubate for another 10min.
- put samples back onto rack and add **900µl of ddH_2O**
- put in centrifuge and spin for 2min. at 12500
- put samples back onto rack

Step 2 – PCR (set up one primer per PCR machine)

- label PCR tubes for samples + pos/neg controls & set aside
- label 1 Eppendorf tube for each Master Mix & put on ice
- get out Red TAQ Mix + primers from -20°C freezer & thaw (on ice)
- make up Master Mix for each primer (on ice):
    - 5µl **Red TAQ Mix**/sample
      → multiply by number of samples + 1 pos control + 1 neg control + 1-2 extra
    - 3.6µl **ddH_2O**/sample
      → multiply by number of samples + 1 pos control + 1 neg control + 1-2 extra
    - 0.4µl **primer mix**/sample
      → multiply by number of samples + 1 pos control + 1 neg control + 1-2 extra
- Set labeled PCR tubes onto purple "isofreeze" box (in bottom of -20°C freezer)
- Add 9µl of Master Mix to each PCR tube
- Add 1µl of sample to each PCR tube (get pos control from +4°C box "genotyping Antje")
- Close lid of Eppendorf tubes tightly
- Vortex
- Spin down in mini-centrifuge
- Put PCR tubes into PCR machine and start program
    - For Lox-P: login on **right** machine under Tony
        - Enter – program - "LoP-NEW" - start
    - For Cre login on **right** machine under Felix
        - Enter – program - "genotyping" - start
          or on **left** machine under Antje
        - Enter – program - "cre genotyping" - start
    - For β-gal login on **left** machine under Antje

- Enter – program - “Shroom3/β-gal genotyping” – start

→ Let run overnight or wait ~ 2hrs

Step 3 – run gel

- Take Erlenmeyer flask
- Measure **100ml of TAE buffer** and add to flask
- Weight out **1g of agarose** and add to flask
- Microwave 30sec – 30sec – 20sec (stop when it starts to boil) – CAUTION: HOT
- Add **5µl of RedSafe** to flask & swirl around
- Put gel chamber into holding apparatus (make sure it is tight)
- Put in 1.0 combs
- Pour TAE buffer solution from flask into gel chamber (make sure it is not leaking)
- Wait ~ 30min.
- Take out the combs
- Move gel chamber over to “Owl”
- Check that TAE buffer is up to the fill line
- Place paper towel underneath “Owl” so that the wells are easier to see
- Take samples out from PCR machine (press stop program – then turn off power)
- Load **10µl of 1kb ladder** in well #1 on each side of the gel
- Load **10µl of sample** from each PCR tube into corresponding well
  (when loading, be careful not to damage gel, release just above the well)
- Close lid and turn on BioRad
- Run at 130V for 40min.
- Take a picture of the gel:
  - Take out gel and bring over to West-May’s lab using the glass pyrex dish
  - Put gel in the machine and click on **gene sys**
  - Then click on **fluorodye**
  - Then modify zoom/brightness as needed
  - Invert picture
  - Print
  - “x” to close program
  - Take out gel and clean the machine
- Back in our lab….
  - Throw gel into biohazardous garbage
  - Close gel box and wrap tightly with plastic wrap
  - Make sure all the reagents & samples are put back into fridge/freezer
  - Wash & clean up everything
- Enter genotyping results in google drive for Kelly
  https://drive.google.com/drive/recent
  - For CRE + LoxP we use: “Cre pos” or “neg”
  - For β-gal (SHR3) we use: “WT” or “het”