BAC preparation for zygote injection (B. Pelosi, with add-on from Y. Achouri protocol)

Day 1 :

- streak the glycerol stock (in this case, clone BP29=Vsx1iCre KANA-)
- grow O/N on a Chloramphenicol plate at 37°C

Day 2 :

- innoculate 1 isolated colony in 3ml LB + chloramphenicol
- grow 8h at 37°C
- innoculate 1 ml in 500 ml LB + chloramphenicol and grow O/N at 37°C (YJ lab ?)

Day 3 : Maxiprep with the Qiagen Large Construct kit (**never vortex or pipette BAC DNA !!!**)

Solutions : TE pH8
TE pH 7.4
isopropanol
70% ethanol
(all filtered on 0.2 um filter)

- centrifuge; if the pellet is very big, divide in 2 fractions for lysis and precipitation
- for each fraction, add 20 ml P1+**RNaseA** and resuspend the pellet
- add 20 ml P2 and roll slowly the tube to mix, incubate for 5' at RT
- add 20 ml **cold** P3 and very gently roll the tube to mix, incubate for 10' on ice
- filter the lysate through a folded filter premoistened with distilled water (if the sample has been processed in 2 fractions, they should be combined now)
- divide in 4 polyallomer tubes, and precipitate DNA by adding 0.6 volumes (approximately 15 ml) **room- temperature** isopropanol to the cleared lysate. Mix and centrifuge immediately for 30 min at 4°C. Carefully decant the supernatant.
- Wash DNA pellet with 5 ml room-temperature 70% ethanol, and centrifuge at ≥15,000 x *g* for 15 min. Carefully decant the supernatant without disturbing the pellet.
- Place the tube containing the DNA pellet upside down on a paper towel and allow the DNA to air-dry for **2–3 min**. Carefully remove any additional liquid visible on the tube opening and carefully redissolve (**avoid pipetting, shake genlty)** the **total DNA in the 4 tubes** in a total of **9.5 ml** Buffer EX, until the DNA is completely dissolved.
- Add 200 µl ATP-Dependent Exonuclease (! has to be completely dissolved !) and 300 µl ATP solution to the dissolved DNA, mix **gently** but thoroughly, and incubate in a water bath or heating block at 37°C for 60 min.
- Equilibrate a QIAGEN-tip 500 by applying 10 ml Buffer QBT, and allow the column to empty by gravity flow. Allow the QIAGEN- tip to drain completely.
- Add 10 ml Buffer QS to the DNA sample from step 12, apply the whole sample to the QIAGEN-tip, and allow it to enter the resin by gravity flow.
- Wash the QIAGEN-tip with 2 x 30 ml Buffer QC.
- Elute DNA with 15 ml Buffer QF, **prewarmed** to 65°C.

- Precipitate DNA by adding 10.5 ml (0.7 volumes) room-temperature isopropanol to the eluted DNA. Mix and centrifuge immediately at ≥15,000 x *g* for 30 min at 4°C. Carefully decant the supernatant.
- Wash DNA pellet with 5 ml room-temperature 70% ethanol, and centrifuge at ≥15,000 x *g* for 15 min. Carefully decant the supernatant without disturbing the pellet.
- Air-dry the pellet for 5–10 min, and redissolve the DNA in a suitable volume of **filtered** TE pH8 buffer (500 µl), store O/N at RT or 4°C to resuspend

Day 4 : further purification

- let the DNA at 55°C for a couple of hours to resuspend
- extract twice with **1 volume** of chloroform/isoamyl alcohol (500 µl)
- extract once with **1 volume** of chloroform only (500 µl)
- precipitate with 2 volumes 100% EtOH + 1/10 volume Sodium acetate 3M pH5.2
- centrifuge 30' at 4°C and carefully decant the supernatant
- wash with 1 vol. 70% EtOH, centrifuge 15' at 4°C, carefully decant the supernatant
- resuspend in TE pH 7.4 + NaCl 100 mM+spermine (amount ?)+spermidine (amount ?)
- store O/N at RT or 4°C to resuspend

Day 5 : digestion

- quantify at the Nanodrop and dilute to 1µg/µl
- digest 30 µg of BAC DNA with adequate enzyme : here PI-SceI
    - 30 µl BAC DNA
    - 10 µl BSA 10x
    - 10 µl PI-SceI buffer
    - 10 µl PI SceI enzyme
    - 40 µl milliQ water
    - incubate 3h (not more) at 37°C
- re-purify as on Day 4, resuspend in 30 µl of injection buffer (compo ?) and quantify
- PCR-/digest-control the BAC DNA

Day 6 : dilution and injection

- prepare 30 µl of non-linearized and of linearized BAC DNA at 75 ng/µl, 50 ng/µl and 25 ng/µl in injection buffer (compo ?)
- give to Younes and cross fingers !