Preparation of clones for further processing:

Example of further processings:

1) Transfer clones to a numbered master plate, in order to number the clones and analyse them further.
2) Transfer clones to sterile MQ for M13 PCR screening (check of insert size).

Additional options:

3) Transfer clones to liquid LB media with antiobiotics (for either preparation of plasmid DNA or for long term storage in 50 % glycerol solution at -80°C).

Comment: These steps can be performed separately or after each other (recommended because this saves time).

Requirements:

- LB-Plates with appropriate antibiotics (maximum weeks old, store at + 4° C). No X-Gal needed.
- Schematic lastic agar plate with numbers (e.g. 1-95).
- Sterile tooth sticks.
- Sterile glass tubes with 5-10 ml sterile LB medium with appropriate antibiotics.
- Sterile PCR tubes or 96 microwell plate with 10 µl sterile MQ, with lid.

Procedure:

1) Place a plastic agar lid with numberings (e.g. 1-95) under the bottom of a fresh LB agar plate with appropriate antibiotics. Mark with a pen and use a small piece of tape to align the empty agar plate to the agar plate with numberings, so that the location of the numbered clones can be easily identified afterwards.

2) Pick white clones with a sterile tooth stick from the original plate with the cloning reaction and transfer them to an empty LB agar plate with antibiotics according to the numbers on the numbered empty agar plate.

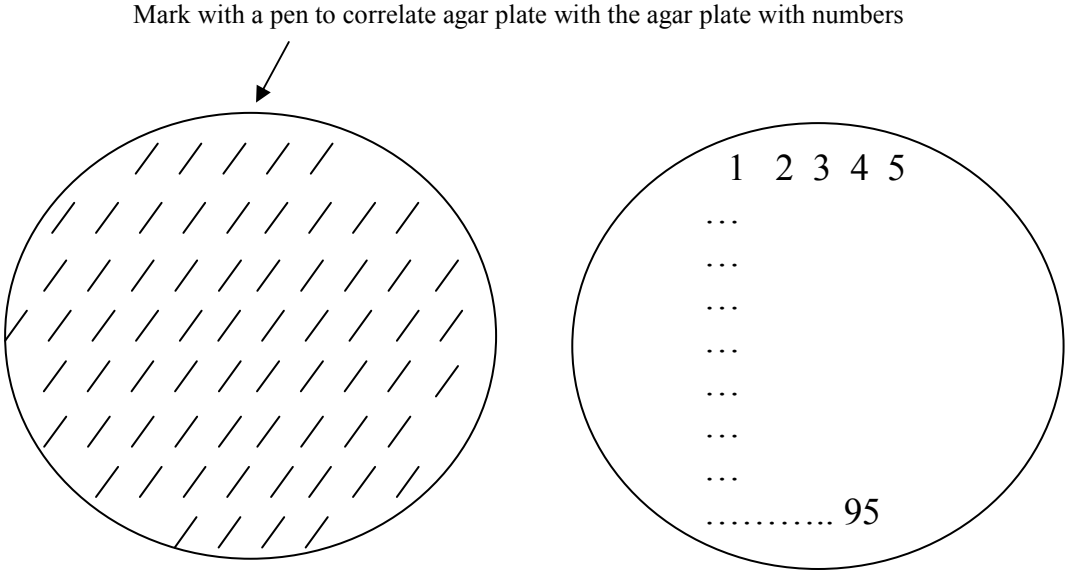

Plate is numbered, e.g. from 1-95.

4) After inocculating the master plate, the tooth stick can either be discarded or used to inocculate tubes/microplates with steril MQ, and then glass tubes with liquid LB media with antibiotics. Leave the tooth stick in the tubes. Use gloves or a sterile forceps.

5) Incubate the master plate and the tubes over night at 37° C. Freeze the tubes/the microtiter plate with the sterile MQ at -20° C (these can then be used for M13 PCR screening when ever appropriate).

6) Check that all clones on the master plate have grown up after 18-24 h. Store the master plate at +4° C up to 4 weeks. After this, the master plate must be reinocculated if glycerol stock cultures have not been prepared.