Rapid screening for plasmids with inserts

Bacteria are lysed and the plasmids separated on an agarose gel withour enzyme digestion. Plasmids with inserts will travel slower than the "no insert" control. See Law, D. and Crickmore, N. (1997) Use of a simplified rapid size screen protocol for the detection of recombinant plasmids. Elsevier Trends Technical Tips

Materials:

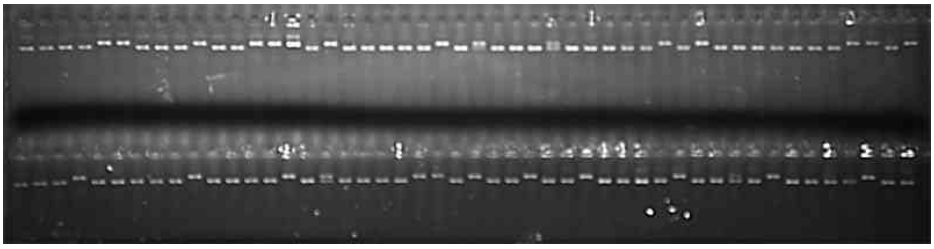
2 x Lysis Buffer : (20 % Sucrose w/v, 200 mM NaOH, 120 mM KCl, 10 mM EDTA, 0.5 % SDS, 0.1 % Bromophenol Blue). It is not necessary to measure the Bromophenol Blue, just add a pinch. Store at -20°C; the dye will fade during prolonged storage; If that happens, add more Bromophenol Blue.

96-well plates:
Polypropylene, with conical bottom (e.g. NUNC cat # 249944). Can be reused: wash, wrap individually in aluminum foil and autoclave.

Procedure:

1. Dilute the lysis buffer to **1x**, prewarm at 37 ° C
2. Pick transformant colony and lightly streak on master plate. Also include colonies from self-ligated vector control.
3. Transfer remainder of colony to 30 µl warmed lysis buffer in a microwell plate
4. Incubate at 37 - 45 C for 5 minutes
5. Place on ice for 5 minutes.
6. Cover with Parafilm and secure it with rubber bands. Spin 10 minutes @ max speed in a centrifuge accomodating 96-well plates.
7. Using a multichannel pipette, load 15 ul of supernatant onto 0.8% agarose gel and run the gel.
8. Recombinant plasmids will travel slower than the re-ligated vector. Start liquid cultures from selected clones on the master plate for DNA minipreps and restriction analysis.

Figure 1: EtBr-stained agarose gel. The entire gel had 4 rows, 50 wells each, allowing thus loading samples from two 96-well plates). The "no-insert" control was loaded in lanes 1 and 2 of each row. Recombinant plasmids are detected as higher bands e.g. in lanes 5 and 6 in the top row.



Notes: If the amount of plasmid is too low to be reliably detected in gel, use an alternative protocol, where in step 3, the remainder of the colony is used to inoculate 30 ul growth medium (LB, ..), the 96-well plate is then covered with parafilm or aluminum foil and incubated at 37 °C for few hours. Then add 30 ul double-concentrated lysis buffer and proceed with step 4 of the protocol.