

Mini-Prep

DNA purification method. For isolating and collecting plasmid DNA from bacterial cells. Protocols for each kit can be found on suppliers websites. If urgent new mini-prep kits can be collected from vending machines next to stores, cards for vending machine in bottom drawer of little silver drawers). Mini- and maxi- preps can also be carried out by Bill in communal lab upstairs (needs name of plasmid, antibiotic resistance and grant code). (Do not need to do under sterile conditions).

Protocol for Promega WIZARD kit (ones in vending machines downstairs):

1. Produce cleared lysate:
 - a. Pellet 1-10ml of overnight culture for 5 minutes (mini-centrifuge).
 - b. Resuspend pellet in 250µl of cell resuspension solution.
 - c. Add 250µl of cell lysis solution, invert 4 times.
 - d. Add 10µl of alkaline protease solution, invert 4 times. Incubate 5 mins at room temp.
 - e. Add 350µl of neutralisation solution, invert 4 times.
 - f. Centrifuge full speed (mini-centrifuge) for 10 mins at room temp.
2. Binding Plasmid DNA:
 - a. Insert spin column into collection tube (found in kit).
 - b. Decant cleared lysate (from above step) into spin column.
 - c. Centrifuge at top speed for 1 min at room temp. Discard flowthrough.
3. Washing Plasmid DNA:
 - a. Add 750µl of wash solution (check ethanol has been added on label).
 - b. Centrifuge at top speed for 1 min. Discard flowthrough.
 - c. Repeat above step with 250µl of wash solution.
 - d. Centrifuge at top speed, 2 mins at room temp.
4. Eluting Plasmid DNA:
 - a. Transfer spin column to 1.5ml Eppendorf tube.
 - b. Add 100µl (Tanel uses just 50µl) of nuclease free water to spin column.
 - c. Centrifuge at top speed for 1 min at room temp.
 - d. Discard spin column.
 - e. Label Eppendorf tube with initials, date, DNA sample etc.
 - f. Store in -20°C freezer.