Mini-Prep

Purpose

To extract and purify plasmid DNA from a small-scale overnight culture, typically using a silica column-based kit

Materials:

- bacterial overnight culture
- miniprep kit
    - QIAGEN Buffers:
    - Buffer P1
    - Buffer P2
    - Buffer N3
    - Buffer PE
    - Buffer EB
- QIAprep 2.0 spin column

Procedure:

1) Pellet cells: Pipette 1.5 mL of overnight culture into a microcentrifuge tube. Centrifuge at >8000rpm for 3 minutes and discard the supernatant. Repeat until there is no culture left (Doing this in several times (ex 3x 1.5 ml) yields better results)
2) Resuspend pelleted bacterial cells in 250 µL buffer P1
3) Add 250µL buffer P2 and mix thourougky by inverting the tube 4-6 times until the solution becomes clear. Do not let the lysis reaction proceed for more than 5 minutes
4) Add 350µL Buffer N3 and mix immediatly by inversting the tube 4-6 times.
5) Centrifuge for 10 minutes at 13000 rpm.
6) Apply 800µL of the supernatant to the spin column by pipetting
7) Centriguge for 30-60 seconds and discard flow-through
8) Wash the spin colom by adding 750µL Buffer PE. Centriguge for 30-60 sec and discard flow-through

9) Centrigufe for 60 sec to remove residual wash buffer

10) Place QIAprep 2.0 spin column in a clean 1.5 mL microcentrifuge tube.

11) To elute DNA, add 50 µL Buffer EB or water to the center of the QIAprep 2.0 spin column, let
stand for 1 minute, and centrifuge for 1 minute

12) Check the purity and concentration of the sample using the Nanodrop and record the
values

13) Store the samples in a -20^{o}C freezer