[iGEM 2017] Qiagen Miniprep

Introduction

Miniprep using Qiagen kit/reagents/buffers

Materials

- Spin Columns (either from the Qiagen kit or blue epoch spin columns)
  - Do not use epoch science "min-elute columns"
- Buffer P1 (resuspension buffer)
  - Buffer P1 with RNAse A added (100µg/ml) needs to be stored in the fridge
- Buffer P2
- Buffer N3
- Buffer PE
- Buffer EB
- Temp tubes and final tubes

Procedure

1. In a 1.7ml microcentrifuge tube, pellet 3ml of cells at 6000 rpm, discard supernatant

   Attempt to remove as much supernatant as can reasonably done, (pipetting not necessary). Dabbing tubes on a paper towel can be useful.

2. Re-suspend pellet completely in **250µl** of cold buffer **P1**.

3. Add **250µl** of buffer **P2** and invert tubes gently 6 times.

   CRITICAL Do not vortex or shake tubes violently from this point forward, doing so will shear gDNA

4. Allow lysis to incubate for no longer than 5 minutes (1-2 minutes is more than enough). Then add **350µl** of buffer **N3**. Invert tubes gently at least 10 times.

   To prevent over lysing cells, it is recommended to not do more than 12 tubes at a time.

5. Pellet cell debris at 13,000 rpm for 10 minutes.

6. Aspirate off supernatant (800µl) and apply to spin column. Spin at 13,000 rpm for 1 minute.

   Obtain as much supernatant as is feasible. Do not dislodge pellet, and avoid getting cell debris onto the column.
   The maximum volume of column is 800µl, if you have a greater volume of supernatant, simply perform this step twice

7. Discard flow through. Add **750µl** of buffer **PE** to column. Spin at 13,000 rpm for 1 minute

8. Discard flow through. Spin column at 13000 rpm for 1 minute

9. Transfer column to final tubes. Add **50µl** of 55C buffer **EB** to the spin column (make sure to add to the silica and not the side of the column). Incubate for 1 minute.

Greater elution volumes can be used for greater yield at the cost of lower concentration
Room temperature EB can be used at the cost of slightly reduced yields

10. Spin at 13,000 RPM for 1 minute. Repeat step 9 using the elutent from the final tube.

11. Nanodrop minipreps and add to awaiting confirmation log.