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Miraprep: Fast Plasmid Prep

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Protocol status: Working

We use this protocol and it's working

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Abstract

An adaptation of the original miraprep protocol published by Pronobis et al. PLoS One (2016).

Introduction

- 1 My adaptation of the miraprep method originally published by **Pronobis et al.** The miraprep method is highly preferred since plasmid prep kits larger than minipreps take too long and require scarier, louder centrifuges.

Protocol

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 - Inoculate an overnight culture in 2xYT and appropriate antibiotic¹
 - The next day, pour confluent culture into 50 mL tubes and centrifuge at 3500rpm 4C for 10-30 min.
 - Decant supernatant
 - Resuspend each tube in 2mL Qiagen Buffer P1
 - To each tube, add 2mL Qiagen Buffer P2. Invert tubes several times and let sit for 3 min.
 - To each tube, 2mL Qiagen Buffer N3. Invert tubes several times and let sit at RT for about 5 minutes or until debris forms a distinct layer on top of the lysate
 - Harvest lysate from bottom layer without touching cell debris and filter through a 0.2-0.4um filter²
 - Add 1mL lysate to as many Qiagen spin columns as needed
 - Spin at max speed in tabletop centrifuge for 1 min.
 - Discard flowthrough, add 1mL Buffer PE to each spin column
 - Spin at max speed in tabletop centrifuge for 1 min.
 - Discard flowthrough, spin again at max speed for 2 minutes.
 - Place each column on an eppendorf tube
 - Add 100uL nuclease-free water to each column and let sit for 2-3 min.³
 - Spin each column at max speed for 1 min to elute plasmid.
 - Collect and pool eluate.

Notes

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 1. 2xYT or another rich medium is preferred over LB for higher plasmid yield
 2. Filtering the lysate precludes the 10 min. spin step used to clear the lysate
 3. I prefer eluting in water over Tris/Buffer EB for better Sanger sequencing and electroporation results