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Miraprep: Fast Plasmid Prep

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

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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 antibiotic[1]
- 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 filter[2]
- 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

3 1. 2xYT is 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

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