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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<sup>1</sup>
  - 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<sup>2</sup>
  - 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.<sup>3</sup>
  - 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

