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Protocol status: Working
 We use this protocol and it's working

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Miracle Prep for Plasmid Isolation

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ABSTRACT

Based on:

<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0160509>

MATERIALS

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- ☒ Sterile deionized H2O **Contributed by users**
 - ☒ LB Broth **Contributed by users**
 - ☒ Buffer P1 **Qiagen Catalog #19051**
 - ☒ Buffer P2 **Qiagen Catalog #19052**
 - ☒ Buffer N3 **Qiagen Catalog #19064**
 - ☒ 96% EtOH **Contributed by users**
 - ☒ Econospin Mini Spin Column 250ct **Epoch Life Science Catalog #1920-250**
 - ☒ Buffer PB **Qiagen Catalog #19066**
 - ☒ Buffer PE **Qiagen Catalog #19065**
- #### STEP MATERIALS
- ☒ Buffer P1 **Qiagen Catalog #19051**
 - ☒ Buffer P2 **Qiagen Catalog #19052**
 - ☒ Buffer N3 **Qiagen Catalog #19064**
 - ☒ Buffer PB **Qiagen Catalog #19066**

Keywords: DNA isolation,
cloning, plasmid prep

Grow bacteria containing plasmid

- 1 Pick colony from plate. Inoculate 50 mL LB broth per vector.
- 2 Allow to grow overnight at 37 °C .

Isolate Plasmid

- 3 Spin culture 00:10:00 4000 x g 4 °C
- 4 Remove supt. Resuspend cells in 2 mL Buffer P1.
Buffer P1 **Qiagen Catalog #19051**
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- 5 2 mL Buffer P2 (invert 3-4 times)

Buffer P2 **Qiagen Catalog #19052**
- 6 Incubate 00:03:00 @ Room temperature

- 7 2 mL Buffer N3 (invert 3-4 times)
- Buffer N3 **Qiagen Catalog #19064**
- 8 Distribute lysate into 4 x 1.5mL tubes
- 9 Centrifuge 00:10:00 @ 13200 x g
- 10 Collect supt, add 1 volume 96% EtOH, mix
- 11 Load on 4 x “QIAprep 2.0 spin columns” or any generic silica membrane mini spin column (Econospin).
- 12 Spin 00:01:00 @ 17900 x g
- 13 Re-load and re-spin until everything is loaded.
- 14 500 µL Buffer PB to each column
- Buffer PB **Qiagen Catalog #19066**

- 15 Spin 00:01:00 @ 17900 x g
- 16 750 uL Buffer PE
- 17 Spin 00:01:00 @ 17900 x g
- 18 Spin again to dry 00:01:00 @ 17900 x g
- 19 Elute with 30-50 uL H₂O
- 20 (Optional) Re-load H₂O on columns and re-spin 00:01:00 @ 17900 x g to increase yield.
Step 20 includes a Step case.
If need higher yield