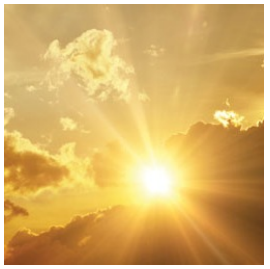
Oct 24, 2019

Miracle Prep for Plasmid Isolation



DOI
dx.doi.org/10.17504/protocols.io.8p2hvqe

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OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.8p2hvqe

Protocol Citation: Blake Flood 2019. Miracle Prep for Plasmid Isolation. **protocols.io** https://dx.doi.org/10.17504/protocols.io.8p2hvqe

Manuscript citation:
Pronobis MI, Deuitch N, Peifer M (2016) The Miraprep: A Protocol that Uses a Miniprep Kit and Provides Maxiprep Yields. PLoS ONE 11(8): e0160509. https://doi.org/10.1371/journal.pone.0160509

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

Created: October 24, 2019

Last Modified: October 24, 2019

Protocol Integer ID: 29146

Keywords: DNA isolation, cloning, plasmid prep

Abstract

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

Materials

MATERIALS

- Sterile deionized H2O
- LB Broth
- Buffer P1 **Qiagen Catalog #**19051
- Buffer P2 **Qiagen Catalog #**19052
- Buffer N3 **Qiagen Catalog #**19064
- 96% EtOH
- 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
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Protocol materials

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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

Isolate Plasmid

4 Remove supt. Resuspend cells in 2 mL Buffer P1.

Buffer P1 **Qiagen Catalog #**19051

.

Isolate Plasmid

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.