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Miracle Prep for Plasmid Isolation

Blake Flood[1]

[1]University of Chicago

1 Works for me dx.doi.org/10.17504/protocols.io.8p2hvqe

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ABSTRACT

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

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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

MATERIALS

NAME	CATALOG #	VENDOR
Sterile deionized H2O		
LB Broth		
Buffer P1	19051	Qiagen
Buffer P2	19052	Qiagen
Buffer N3	19064	Qiagen
96% EtOH		
Econospin Mini Spin Column 250ct	1920-250	Epoch Life Science
Buffer PB	19066	Qiagen
Buffer PE	19065	Qiagen

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Buffer P1	19051	Qiagen
Buffer P2	19052	Qiagen
Buffer N3	19064	Qiagen
Buffer PB	19066	Qiagen

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.

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5 **2 ml** Buffer P2 (invert 3-4 times)

Buffer P1
by Qiagen
Catalog #: 19051

Buffer P2
by Qiagen
Catalog #: 19052

6 Incubate **00:03:00** @ **Room temperature**

7 **2 ml** Buffer N3 (invert 3-4 times)

Buffer N3
by 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
by 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 case

If need higher yield

step case

If need higher yield