BECKMAN COULTER
Life Sciences

Mini prep of plasmids using CosMCprep

Introduction

Please reference the current CosMCprep protocol for product information.
(Part number: A37064, A29174)

Researchers should use this protocol when prepping plasmids from less than 10 samples, or when a plate centrifuge is unavailable.

Purpose

The purification of plasmids is essential for most cloning technologies. The current CosMCprep manual is geared towards high throughput labs, but the basic protocol works well with lower through puts as well. This protocol provides a method that takes under 30 minutes to prep a plasmid.

Materials Used

Material	Part Number	Supplier
Tube Magnet (1.5, 1.7, and 2 mL)	A29182	Beckman
Microcentrifuge tubes 1.5 mL	357448	Beckman
100 % Isopropanol (Molecular Grade)	AB07015-01000	AmericanBio
TE pH 8.0 (Molecular Grade)	AM9849	ThermoFisher Scientific
100% Ethanol (Molecular Grade)	AB00138	AmericanBio
2xYT	AB15063-01000	AmericanBio

Protocol

1. **Grow cells up in a YT (or other rich media) for 17-22 hours**
2. **Lysis**
    a. **Mix** the culture by briefly vortexing
    b. Add **800 µL** of **fresh culture** to a 1.7 mL micro-centrifuge tube
    c. Pellet cells by spinning for **2 minutes** at **10,000 x g**
    d. Remove and discard the supernatant without disrupting the pellet
    e. Add **100 µL** of **RE1** to the tube
    f. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    g. Add **100 µL** of **L2** to the tube
    h. **Mix** by pipetting up and down **gently** 10 times
    i. **Incubate** for **5 minutes** at **room temperature**
    j. Add **100 µL** of **N3** to the tube
    k. **Mix** by inverting the tube **4-6 times**
    l. Pellet the cellular debris by spinning for **5 minutes** at **max speed (~16,000 x g)**
    m. Transfer **110 µL** of the **supernatant** to a new 1.7 mL micro-centrifuge tube

3. **Bind**
    a. Add **10 µL** of **Pur4** and **80 µL** of 100 % **Isopropanol**
        i. See CosMCprep protocol if you plan on transferring more supernatant
    b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    c. Place the tube on a **magnet** for **5 minutes** (or until supernatant is clear)
    d. Remove and discard the supernatant without disrupting the beads
4. **Wash (Leave samples on magnet for the following steps)**
    a. Add **200 µL** of **70% ethanol** to the tube
    b. Incubate the plate on magnet for **30 seconds** at **room temperature**
    c. Remove and discard the supernatant without disrupting the beads
    d. Repeat steps 4.a-4.c for a total of **3 washes**
    e. **Incubate** the plate on magnet for **1-10 minutes** or until all ethanol has evaporated
5. **Elute**
    a. Add **40 µL** of **RE1** or **TE pH 8** to tube
    b. **Incubate** the tube for **5 minutes** at **37°C**
    c. Place the plate on a **magnet** for **3 minutes** (or until supernatant is clear)
    d. Remove and **Save** the supernatant without disrupting the beads

Example Data:

Plasmid DNA was isolated using this supplementary protocol for the CosMCprep kit from two *E. coli* strains and from *P. aurginosa.* The resulting DNA concentration and purity was assessed using the NanoDrop (Thermo Fisher Scientific)

Plasmid Type	Concentration (ng/ul)	Yield (µg)
High copy number small (<10kb) plasmid	359.2	14.4
High copy number small (<10kb) plasmid isolated from Pseudomonas	120.0	4.8
Naturally occurring large (>100kb) plasmid	90.3	3.6

Table: The average concentration and yield of plasmid DNA from three replicates as quantified using the NanoDrop (Thermo Fisher Scientific).

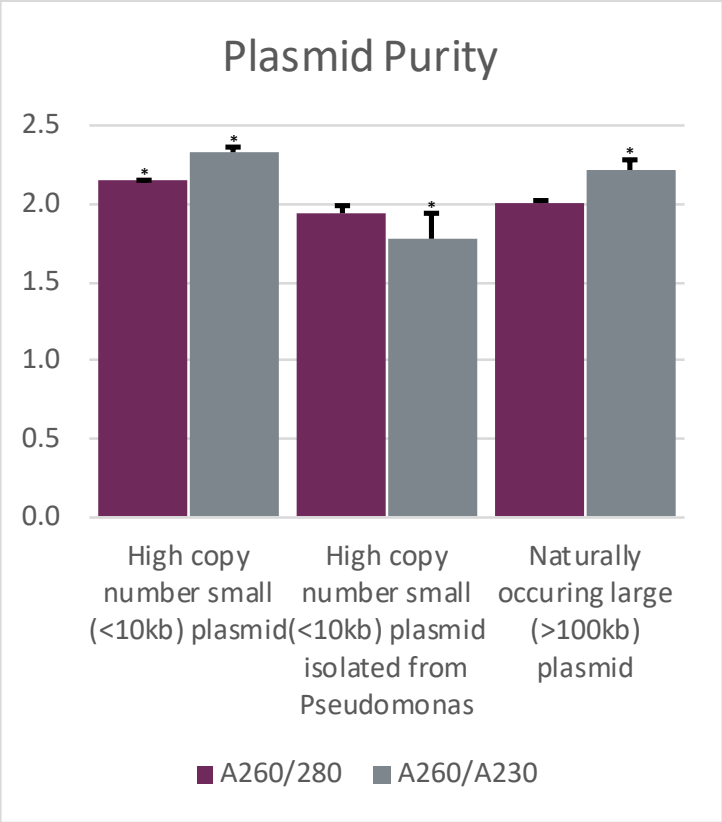

Figure 1. The plasmids purity was assessed using the NanoDrop (Thermo Fisher Scientific). Both the A260/280 and A260/230 ratios for both of the plasmids are within satisfactory ratios for downstream applications. Error bars represent the standard deviation of three replicates.

*Acceptable ratios for A260/280 are 1.8 and 2.0 and for A260/230 are 2.0-2.2, but due to varying nucleotide ratios and DNA concentrations the ratios can be higher. (ThermoScientific T042-Technical Bulletin)

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