



Plasmid Extraction from High-Density Cell Cultures Using the CosMCPrep Plasmid Prep Kit

Introduction

Please refer to the current CosMCPrep protocol for product information (part numbers A37064, A29174)

This supplemental protocol is intended for researchers performing plasmid extractions from high-cell density cultures, including Gigaprep-scale preparations. Gigaprep-scale prep is achieved by aliquoting 250 mL of culture into a 24-well block for processing, then combining the eluted aliquots to achieve a Gigaprep equivalent (≥ 10 mg) of plasmid DNA as the final sample.

Note: This high-throughput 24-well layout can be used to generate 6 Maxi-preps by combining eluates from 4 wells, or 24 Midi-preps when each well is loaded with 5-10 mL of a given culture.

Purpose

The purification of plasmids is a fundamental technique in Synthetic Biology and Molecular Biology assays. The standard CosMCPrep protocol is optimized for mini- or midi-scale preparations for both high- and low-copy plasmids. This supplemental protocol provides modifications to the standard IFU, enabling the use of CosMCPrep reagents for efficient plasmid extraction from high-density cultures.

Materials Used

Materials and Reagents

Material/Reagent	Part Number/Supplier
RE1 Resuspension and Elution Solution	C42188, C42166 (Part of A37064, A29174) / Beckman Coulter Life Sciences
L2 Lysis Solution	C42189, C42167 (Part of A37064, A29174) / Beckman Coulter Life Sciences
N3 Neutralization Solution	C42190, C42168 (Part of A37064, A29174) / Beckman Coulter Life Sciences
Pur4 Plasmid Binding Solution	C42192, C42170 (Part of A37064, A29174) / Beckman Coulter Life Sciences
RNase A	B85620 / Beckman Coulter Life Sciences

Additional Material/Reagents Required But Not Supplied

Item	Part Number/Supplier
Magnum FLX®24HV, Universal 24-Well Magnet Plate	A000640/Alpaqua
10 mL 24-Well U-bottom Blocks	19583/Qiagen or equivalent
Microcentrifuge tubes 2mL	0030120094/Eppendorf
24-well Cell Clarification and Sterile Filtration Plate-Depth Filter Plus 0.65/0.2 µm Supor EKV Filter Plates	97026/AcroPrep or equivalent
Reagent Grade Water	AM9932/Ambion, or equivalent
Endotoxin-free Tris-EDTA, BioUltra, Molecular Biology, pH 8.0	41105319/Sigma or equivalent
100% Isopropanol	AB-07015/American Bioanalytical or equivalent
80% Ethanol (EtOH)	AB-00138/American Bioanalytical, or equivalent
Plasmid+® Media	446300 /Thompson
Centrifuge	Allegra V15R/Beckman Coulter Life Sciences

Manual Preps

Item	Part Number/Supplier
Mixmate Plate and Tube Mixer	41103800/Eppendorf® or equivalent
Eppendorf Research® Plus Move It®	3125000192/Eppendorf® or equivalent
50 mL Sterile Storage Reservoir	41121522/Corning or equivalent

Automated Preps

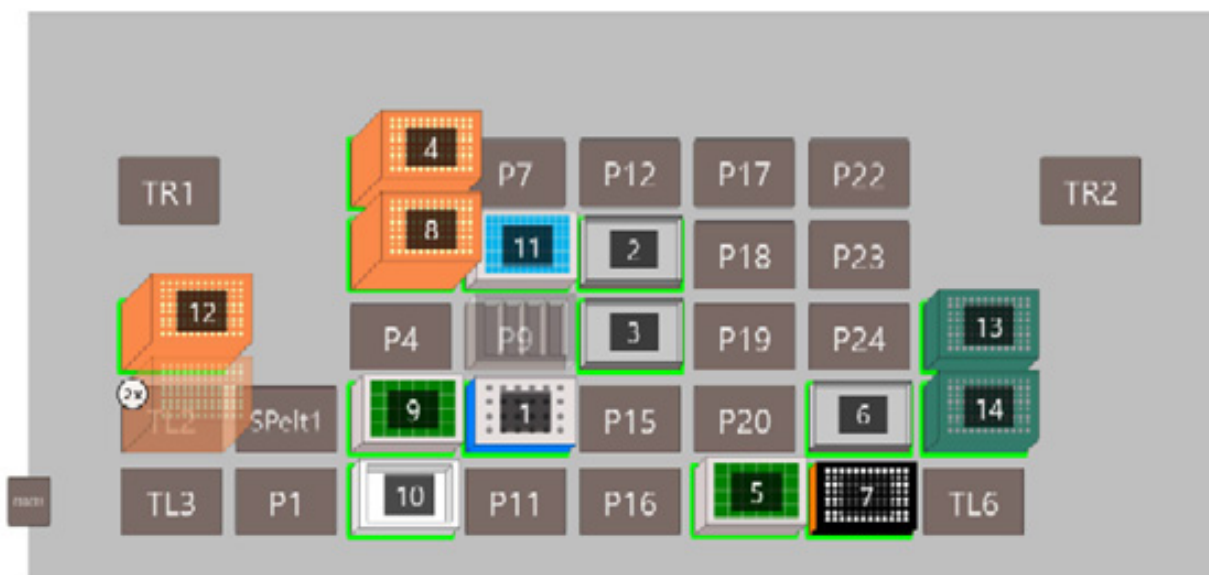
Item	Part Number/Supplier
Thermoshake Classic	7100146/Inheco or equivalent

Users must supply standard microbiological equipment and consumables for growing bacteria, such as a shaking incubator or equivalent in brackets, baffled flasks, selective antibiotics, selective agar plates, and inoculating loops.

Deck Setup on Biomek i7 Dual MC (1200µl/300µl) Workstation for Automated Run With Consumables

Final Deck Setup

Below is the final deck layout. Ensure that all the highlighted labware are in the specified positions.



#	Name	Type	Position	Stack Depth
1	Magnet	Alpaqua_Magnum_24well	P10	1
2	Isopropanol	BCUpsideDownTipBoxLid	P13	1
3	Ethanol	BCUpsideDownTipBoxLid	P14	1
4	Reformatting_Tips2	BC1025F	P2	1
5	Clear_Lysate	Qiagen_24Well_UBottom	P21	1
6	Elution_Buffer	BCUpsideDownTipBoxLid	P25	1
7	Elution	FluidX96_500uL	P26	1
8	Bind_Tips	BC1025F	P3	1
9	Samples	Qiagen_24Well_UBottom	P5	1
10	Waste	Opentron_195ml_Reservoir	P6	1
11	Reagents	Greiner96RoundDeepSquare	P8	1
12	Empty_Box	BC1025F	TL1	1
13	Empty_Box_Elution	BC230	TL4	1
14	Elution_Tips	BC230	TL5	1

1. Select a single colony from a freshly streaked selective plate and use it to inoculate a culture of 1–5 mL of LB medium that contains the appropriate selective antibiotic. Then, incubate this culture overnight at 37°C with shaking at 225 rpm.
2. The following day, inoculate 1:1000 of the overnight culture into 250 mL of Plasmid+® Media that contains the appropriate selective antibiotic, and grow this culture for 16–18 hours at 37°C with shaking at 225 rpm.

Please note that growth beyond 18 hours is not recommended, as cells may begin to lyse, potentially reducing plasmid DNA yields.

3. Lysis
 - a. Dispense 10 mL of the cultured cells into each well of a 24-well block. Seal the block with adhesive plate seals, foil sheet, or silicone sealing mat.
 - b. Centrifuge at 3000 x g for 10 minutes at 4°C.
 - c. Remove the sealing foil or mat and discard the supernatant without disrupting the pellet. Note: Record the weight of the wet pellet.
 - d. Store the pellet at 4°C while reconstituting and dispensing reagents, or freeze it at -20°C for short-term storage or -80°C for long-term storage.

Reconstitute RE1+ RNase A as described in the IFU. Check the L2 for precipitation. Precipitation can occur at lower temperatures; if observed, prewarm the solution to 37°C to avoid lysis issues. Defrost cell pellet if starting from frozen samples.

- e. Add 800 µL of RE1 + RNase A. Mix thoroughly by pipetting up and down 10–20 times until the cell pellet is completely resuspended and the mixture appears homogeneous with no cell clumps. Alternatively, shake for 4 minutes at 600 to 1,200 rpm on a shaker. Note that shaking speeds will vary depending on the orbit of the shaker.
- f. Add 800 µL of L2 to each sample.
- g. Mix gently by pipetting up and down 2 times, then shake gently at 300 rpm for 4 minutes. Do not exceed a 5-minute incubation in this step.
- h. Add 800 µL of N3 to each sample.
- i. Mix by shaking at 600 rpm for 2 minutes at room temperature, alternating between clockwise and counterclockwise motion.
- j. Pour the sample into a filtration plate, seal the plate, and centrifuge at 3500 x g for 5 minutes to collect the clear lysate in a new 24-well block.

New users may find it helpful to centrifuge the block with the crude lysate at 3500 x g for 5 minutes before transferring the semi-clarified supernatant to the filtration plate and proceeding with the filtration step described above.

4. Bind

- a. In a clean reservoir or tube, add 2 mL of Pur4 and 33 mL of 100% isopropanol. Mix thoroughly by pipetting up and down 4 times or vortex for 30 seconds.
- b. Add 1.4 mL of the Pur4/isopropanol mix to each well of the 24-well block containing the clarified lysate. Mix by pipetting up and down 4 times. Incubate for 10 minutes with shaking at 800 rpm.
- c. Place the block on a magnet and allow the beads to separate for 2-10 minutes, or until the supernatant is clear.

5. Wash

- a. Remove the supernatant while the block remains on the magnet.
- b. Dispense 500 μ L of 80% ethanol into each well of the block to wash the magnetic beads.
- c. Incubate samples for 30 seconds on the shaker at 300 rpm.
- d. Return the block to the magnet and allow the beads to separate for 2 minutes.
- e. Remove and discard the ethanol wash while the block remains on the magnet.
- f. Repeat steps b-e twice for a total of three washes.
- g. Dry the beads at 37°C for 5 minutes.

Avoid over-drying the beads. If the beads begin to form a crackle pattern, reduce the drying time to 1 or 2 minutes.

6. Elute

- a. Add 300 μ L of Endotoxin-free TE to each well of the block and incubate at room temperature or 45°C with shaking at 600 rpm for 20 minutes.
- b. Place the tube/block back on the magnet for 2-5 minutes, then transfer the eluted samples to a clean, new collection tube/plate.

Table 1. Comparison of Time for Manual or Automated Plasmid Preparation.

		4 Samples		24 Samples		96 Samples (Mini-prep Only)	
	Step	Manual	Auto	Manual	Auto	Manual	Auto
Aliquot Reagents	Reformatting	0	1.5	0	2.5	0	0
	Pipetting	7	4.5	7	4.5	7	4.5
Sample Resuspension	Reformatting	0	1.5	0	2.5	0	0
	Pipetting	1	1	1	1	1	1
	Incubation	5	5	5	5	5	5
Sample Lysis	Reformatting	0	1.5	0	2.5	0	0
	Pipetting	1	2	1	2	1	2
	Incubation	5	5	5	5	5	5
Sample Neutralizing	Reformatting	0	1.5	0	2.5	0	0
	Pipetting	1	1	1	1	1	1
	Incubation	2	2	2	2	2	2
Sample Filtration	Centrifugation	15	15	15	15	15	15
Sample Binding	Reformatting	0	1.5	0	3.5	0	0
	Pipetting	2	6	2	6	2	6
	Incubation	10	10	10	10	10	10
	Magnet/Waste	2	12.5	2	12.5	2	12.5
	Relocating Items	0	1	0	1	0	1
Sample Washing	Reformatting	0	3.5	0	7	0	0
	Pipetting	3	7	3	7	3	7
	Incubation	9	6	9	6	9	6
	Magnet/Waste	2	9	2	9	2	9
	Drying	5	5	5	5	5	5
Sample Elution	Reformatting	0	3	0	5	0	0
	Pipetting	4	3.5	4	3.5	4	3.5
	Incubation	20	20	20	20	20	20
	Magnet	2	5	2	5	2	5
Total Hands-On Time		57	15	57	15	57	15
Automation Run Time		NA	119.5	NA	131	NA	105.5
Total Process Time*		102	133.5	102	145	102	119.5

*Includes incubation and centrifuge times



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