

Plasmid purification using the Opentrons OT-2

- 1) Prepare a 2 ml deep well plate with square wells for cultivation
 - a. Sterilize the plate (autoclave or UV light)
 - b. Fill each well with 1.5 ml LB, add antibiotic if necessary
- 2) Pick colonies for cultivation

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

- 3) Seal the plate with non-woven sealing foil and incubate overnight (16 h) at 37 °C, 250 rpm and 25 mm shaking diameter.
- 4) Harvest the cultures by centrifuging at 1500 x g for 15 min.
- 5) Load the protocol "plasmid_purification", set up the deck according to instructions in the app and calibrate the robot.

You will need:

- a. The 300 µl Opentrons multichannel pipette (GEN 1)
- b. 11x 300µl tip boxes
- c. 3x round-bottom collection plates
- d. 1x DWP containing pelleted cells
- e. 1x 12-column plate filled with reagents:

Column	Reagent	min. volume for one column [ml] (recommended volume)	min. volume for one plate [ml] (recommended volume)
1	Cell resuspension solution	0.72 (2.0)	8.64 (12.0)
2	Cell lysis solution	0.96 (2.0)	11.52 (14.0)
3	Neutralisation solution	0.96 (2.0)	11.52 (14.0)
4	Magnesil Blue	0.20 (2.0)	2.40 (5.0)
5	Magnesil Red	0.40 (2.0)	4.80 (7.0)
6	80 % (v/v) Ethanol	0.80 (2.0)	9.60 (13.0)
7	80 % (v/v) Ethanol	0.80 (2.0)	9.60 (13.0)
8	80 % (v/v) Ethanol	0.80 (2.0)	9.60 (13.0)
9	Elution Buffer	0.80 (2.0)	9.60 (13.0)
10	-	-	-
11	-	-	-
12	-	-	-

- 6) Start the protocol.
 - a. While the protocol is running, you should watch the trash bin. If it becomes too full, please empty it.
 - b. After approx. 1 h you are required to exchange the collection plates and to fill up the tip boxes. Press resume afterwards.
 - c. After approx. 1:45 h, the robot stops until you confirm that the pellets in the plate on the Magnetic Module are dry. The drying process can take up to 1 h. To accelerate it, you can place the plate under a sterile hood or in a drying oven. Dried pellets are lighter in colour and have a matt finish.
- 7) After completion of the protocol, the purified plasmid DNA is in the plate at position 2. Seal it with adhesive foil and store it at -20 °C until use.