Jan 31, 2023

Ultra-Competent Cells Preparation

DOI
dx.doi.org/10.17504/protocols.io.q26g7yd48gwz/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.q26g7yd48gwz/v1

External link: http://doi:10.1101/pdb.prot101196

Protocol Citation: Jorge Fernández Méndez 2023. Ultra-Competent Cells Preparation . **protocols.io https://dx.doi.org/10.17504/protocols.io.q26g7yd48gwz/v1**

Protocol status: Working
Standard Laboratory Protocol.

Created: January 31, 2023

Last Modified: January 31, 2023

Protocol Integer ID: 76131

Keywords: competent cells preparation, plasmidic dna, cell, dna, adaptation of the inoue protocol, inoue protocol, preparation, adaptation

Abstract

Adaptation of the Inoue protocol for competent cells preparation.

Expected transformation efficiency of 10^8 colonies per µg of plasmidic DNA.

Original Source: doi:10.1101/pdb.prot101196

Materials

- Big 1L E-Flasks, x2
- 50 mL Centrifugue Falcon Tubes
- Centrifugue with 50 mL Tubes adapter
- Incubator

- $MnCl_2 \cdot 4H_2O$
- $CaCl_2 \cdot 2H_2O$
- PIPES Buffer
- DMSO
- KOH
- LB Media (550 mL)

Troubleshooting

Preparation of Transformation Buffer & Reagents

1 Prepare a buffer with the following composition

	Component	Ammount for 0.5 L	Final Concentration
	KCl	9.33 g	250 mM (18.65 g/L)
	CaCl2 · 2 H2O	1.1 g	15 mM (2.2 g/L)
	MnCl2 · 4 H2O	5.44 g	55 mM (10.88 g/L)
	PIPES	10 mL from 0.5 M pH 6.7 Stock	10 mM (3.02 g/L)

Inoue Transformation Buffer Composition

1.1 **Prepare Stock of PIPES 0.5 M**

Measure 7.56 g of PIPES and dissolve it in 40 mL of deionized H2O using a small beaker. Set a magnetic stirrer in the beaker and start stirring. Then, adjust the pH using a pH meter and **10M KOH** solution (Or KOH pellets added one by one to the beaker). Final point is reached at pH 6.7 .

Note

STORAGE of PIPES Stock. The prepared PIPES buffer can be stored for further use. In this case, filter sterilize the solution with a disposable 0.45 µm filter, and freeze it at -20 °C.

1.2 **Prepare the transformation buffer solution**

Place 400 mL of deionized H2O in a big beaker, drop a magnetic stirrer and add in the following order 9.33 g of KCl , 1.1 g of CaCl2 · 2 H2O and 5.44 g of MnCl2 · 4H2O .

Important! Wait until the previous salt has completely dissolved, the add the following. CaCl2 solubilization is highly exothermic, add carefully the pellets. MnCl2 should be added last.

1.3 After dissolving the salts, add 10 mL of PIPES 0.5M pH 6.7 , stir the solution and measure the final pH. It should be close to pH 6.7 . **pH can not be adjusted after MnCl2 addition to avoid precipitation of the metal ions, thus it is extremely important that the PIPES pH is correct.**

1.4 Transfer the solution to a big measuring cylinder and fill with deionized water (Clean freshly treated MiliQ if possible) up to 500 mL final volume.

1.5 Filter Sterilize the transformation buffer using a 0.45 µm filter. It is recommended to aliquot the buffer in 100 mL batches.

Storage. For long term storage freeze the transformation buffer at -20 °C

Direct Utilization. Keep the filter-sterilized buffer at 4 °C

2 Filter sterilize 1-5 mL of pure DMSO . It is recommended to use DMSO of the higher possible purity to ensure optimal competency in the cells.

E. Coli Cultivation

6h

3 Grow the required *E. Coli* strain in an **LB Agar Plate**, streaking the cells to obtain single colonies and incubate at 37 °C Overnight

3.1 The next day, pick a single colony from the plate and inoculate 25 mL of LB . Incubate the cells at 180-240 rpm, 37°C, 06:00:00 . **Recommended doing it early in the morning!**

3.2 After the incubation, prepare 2 big E-Flasks with 200 mL Sterile LB (SOB Media could be used instead). Inoculate one flask with 1 mL of the starter *E. Coli* culture, the second flask receives 10 mL of seed culture instead. Incubate the cells at 180-240 rpm, 18-22°C Overnight .

Note

E. Coli cells grown at low temperatures has been shown to improve it's transformation efficiency, likely due to changes in the membranes composition. However they grow slow and they can take up to 36 hours to grow at the required OD.

It is recommended to start the cultures in the evening of the previous day. Two flasks are used to ensure that at least one of them has the proper OD.

If there are no incubators with temperature control capable of achieving 18 °C (optimal), they can be grown at room temperature in the lab (Normally fluctuating between 20-24 °C).

Previous Preparation of Competent Cells

30m

4 Cool down the centrifugue at 0-4 °C to ensure it is already cold before starting.

4.1 Prepare an ice-bath in a styrofoam box and chill 100 mL of the transformation buffer on it for at least 30 minutes before starting the protocol.

5 Measure the **Optical density at λ = 600 nm (OD_{600}).** When one of the culture reaches 0.55 OD_{600}. Stop the incubation and discard the other culture.

6 Take 200 mL of the *E. Coli* culture at OD_{600} 0.55 and split it into **4, 50 mL Falcon tubes.**

10m

Spin down the cells at:

2500 x g, 0-4°C, 00:10:00 , (Approx 3,900 rpm for standard lab centrifugue).

7 After centrifugation, place immediately the tubes on ice and always keep them there while working.

Discard the supernatant and remove the excess of media by tipping the tubes over paper towels.

Work under a flame or sterile hood when opening the tubes.

Add 16 mL of Transformaion buffer to each falcon tube and gently resuspend the cell pellet by swirling the tube. (Avoid pipetting or vortexing to keep cells integrity).

8 **Spin down the cells at:** 10m

2500 x g, 0-4°C, 00:10:00 , (Approx 3,900 rpm for standard lab centrifugue).

After centrifugation, place the tubes on ice and discard the supernatant.

9 **Add** 4 mL of Transformaion buffer to each falcon tube and gently resuspend the cell pellet. 10m

Then **add** 300 µL of Sterile DMSO to each falcon tube, and mix gently by inverting the tubes 3-4 times.

Incubate the tubes On ice (0 °C) for 00:10:00 .

10 Working as quick as possible, take one of the tubes and dispense 50-200 µL aliquots of the suspensions into chilled, sterile 1.5 eppendorf microfugue tubes.

Immediately after dispensing the aliquots, close the tubes and freeze them on liquid nitrogen.

Note

Freezing on liquid N_2 could be avoided by it enhances the competency of the cells specially during long term storage. Alternatively the aliquots can be kept on ice for some minutes and quickly moved to a -70 °C freezer (or lower temperatures).