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Preparing Chemically Competent E coli for Transformation

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Competent Cell Preparation

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We use this protocol and it's working

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Abstract

This protocol prepares chemically competent E coli for plasmid transformation. The competency is high enough for routine cloning but working with libraries may require higher competency. The protocol has worked with every E. coli type I've tried it with. Frozen cells are stable for years at -80, and can be refrozen with minimal effects to their competency. Recommended to get a friend or two to help with aliquoting the bacteria at the end, both so that they stay cool (important) and so that you stay cool (important).

Adapted from Promega Cloning Manual Notes and Inoue/Hanahan protocol, DNA Cloning 1985. With help from Amy Lee, Brandeis (http://www.bio.brandeis.edu/leelab/).

Attachments

PDF

bakktw.pdf

42KB

Guidelines

Additional Protocol Notes

- Protocol works well to prepare cells with 1 plasmid already inside (Cam^{+}tRNA RIL plasmid for example)
- Add selection to original plate and growth media, but do not add selection to TFB1/TFB2 buffers
- Can prepare plates with odd antibiotic by first plating antibiotic onto a LB agar plate
- Pre-mix enough antibiotic for 15 ml of LB into 100 µl of LB and evenly spread mixture onto the plate and allow to dry at RT for ~30 min before streaking cells.

Materials

TFB1 (Prepare 300 ml)

30 mM KOAc (98.15 $^{g}/_{mol}$) 0.883 g

10 mM $CaCl_2 \cdot 2H_2O$ (147.01 $^{g}/_{mol}$) 0.441 g

50 mM $MnCl_2 \cdot 4H_2O$ (197.91 $^{g}/_{mol}$) 2.969 g

100 mM RbCl (120.92 $^{g}/_{mol}$) 3.628 g

15% Glycerol 45 ml (100% glycerol stock)

dH_2O 220 ml, qs to 290 ml

*pH to 5.8 with 1.75 M Acetic Acid 10–20 drops (**SLOWLY**)

- Dilute glacial acid $^{1}/_{10}$ for use
- pH of starting solution is ~7.1

*qs to 300 mL with dH_2O and 0.2 µm-filter sterilize

TFB2 (Prepare 300 ml)

100 mM MOPS (209.26 $^{g}/_{mol}$) 6.278 g

75 mM $CaCl_2 \cdot 2H_2O$ (147.01 $^{g}/_{mol}$) 3.308 g

10 mM RbCl (120.92 $^{g}/_{mol}$) 0.363 g

15% Glycerol 45 ml (100% glycerol stock)

dH_2O 220 ml, qs to 290 ml

*pH to 6.5 with 2 M KOH 2–4 ml (**SLOWLY**)

- 2 M KOH: 1.122 g (56.11 $^{g}/_{mol}$) in 10 mL dH_2O
- pH of starting solution is ~4.2

*qs to 300 mL with dH_2O and 0.2 µm-filter sterilize

1 M $MgSO_4$ (Prepare 45 ml): Add 11.09 g $MgSO_4 \cdot 7H_2O$ (246.47 $^{g}/_{mol}$) to 40 ml, qs to 45 ml and 0.2 µm-filter sterilize

Safety warnings

See SDS (Safety Data Sheet) for hazards and safety warnings.

Before start

For Competent Cell Preparation

- Be prepared to work in 4°C room.
- Pre-chill buffers TFB1 and TFB2 on ice.
- Pre-chill p1000 tips at 4°C.
- Label and pre-chill 1.5 mL microfuge tubes on ice and in 4°C room.

Starter Culture Growth

1 Streak cell-stock on an LB agar plate, and grow o/n at 37 °C.

2 Inoculate a single colony from the plate into a 5 mL LB liquid culture and grow o/n at 37 °C with shaking.

3 Typically prepare **TFB1/TFB2** solutions now, and store at 4 °C o/n before preparing cells in the next day.

Competent Cell Preparation

4 Supplement 250 mL of LB in a 1 L flask with [M] 20 millimolar (mM) $MgSO_4$.

4.1 Inoculate with 2.5 mL of overnight 5 mL culture.

4.2 Add 5 mL of [M] 1 Molarity (M) $MgSO_4$ stock.

5 Grow cells by shaking at 37 °C until OD_{600} = 0.4 –0.6, typically takes 02:00:00 – 03:30:00 for most cell lines.

	Cell Line	Inoculum	Estimated Growth Time
	Top10	2.5 ml	2 h 45 min

	Whelan DH5α	2.5 ml	2 h 30 min
	RIL	2.5 ml	4 h
	Stbl 3	3.5 ml	2 h 45 min

6 Pre-chill buffers TFB1 and TFB2 **on ice**. Pre-chill p1000 tips at 4 °C.

7 Gently pellet culture by centrifugation at 4500 x g for 00:05:00, 4 °C.

Note

4,500 x g = ~5,500 RPM in SLA-1500, SLA-3000 or F10BCI-FibreLite “F500”

8 Gently re-suspend the cell pellet in 100 mL ice-cold TFB1 (0.4 original volume).

Note

Essential to obtain optimal competency: complete the following steps **on ice** and while working at **4°C.**

8.1 Pour off media, and place cell pellet on ice. Then gently re-suspend pellet while working on ice in 4 °C room.

Note

It is normal for the pellet to be somewhat difficult to re-suspend in TFB1 (should easily re-suspend in TFB2).

9 Incubate the re-suspended cells on ice for 00:05:00 in 4 °C room.

10 Gently pellet culture by repeating centrifugation at 4500 x g for 00:05:00, 4 °C.

11 Gently re-suspend cells in 10 mL of ice-cold TFB2 (1/25th original volume).

12 Incubate the re-suspended cells on ice for 00:20:00 in 4 °C room.

13 Label and **pre-chill** 1.5 mL microfuge tubes on ice and in 4 °C room.

14 Aliquot cells as 220 µL stocks in 1.5 mL microfuge tubes on ice.

15 Flash-freeze aliquoted tubes by submerging in liquid-nitrogen, and store at -80 °C.

Note

Typically use 25–50 µl of cell stock for each transformation.
Tubes can be freeze-thawed multiple times with minimal competency loss.

Testing Competency of Cell Stocks (optional, recommended if high competency is required)

16 Measure competency by transforming 0.1 ng of plasmid DNA (within 10 µL total dH2O volume).

Note

Optionally, also plate 50 µL of 1/10 dilution of transformation (in LB) mixture for easier colony counting.

16.1 Transform 10 µL of a 0.01 ng/µL stock of pGEM9z into 50 µL of competent cells and plate 50 µL on Amp^{+}

LB plate.

17 Count number of colonies and calculate cell competency

Expected result

EX. Counted 832 colonies from 50 µl plate.

a. 0.1 ng of DNA in 0.560 ml = 0.17857 ng DNA / mL.
b. 0.17857 ng DNA / ml x 0.05 ml plated = 0.00892857 ng DNA.
c. 832 cfu / 0.00892857 ng = 9.318×10^4 cfu/ng → 9.32×10^7 cfu/µg.

Note

Optional: also plate cells on Amp^+ plates to verify no background resistance.