Transformation of Bacterial Cultures Using Hexamine Cobalt Chloride

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Abstract

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Guidelines

Materials:

1) SOB medium

- 1.0% Bacto-tryptone
- 0.5% Bacto-yeast extract
- 10.0 mM NaCl
- 2.5 mM KCl
- 10.0 mM $MgCl_2$
- 10.0 mM $MgSO_4$
- Prepare the $MgCl_2$ and $MgSO_4$ as 1 M stock solutions and autoclave separately.
- Add the $MgCl_2$ and $MgSO_4$ after sterilization of the remainder of the components.

2) SOC medium

- SOB media, supplemented with 20mM glucose

3) TFB buffer

- 10 mM K-MES, pH 6.2, 100 mM KCL, 45 mM $MnCl_2$:$4H_2O$, 10 mM $CaCl_2$:$2H_2O$, 3 mM $HaCoCl_3$
- Weigh out the components. Add the MES to d-H_2O and adjust the pH with KOH.
- Add the remaining components (in order), waiting until one component is in solution before adding the next. Adjust the volume to the final volume. Filter sterlize. Store frozen at -20^{o}C in 15 mL aliquots.

4) DTT solution

- 2.25 M DTT, 40 mM KOAc, pH 6.0. Filter sterilize, Store frozen at -20°C.

5) DMSO (Dimethylsulfoxide)

Reference

D. Hanahan. (1983). Studies on Transformation of *Escherichia coli* with plasmids. Journal of Molecular Biology **166**: 557-580.

Protocol

Step 1.
Prepare the SOB medium.

PROTOCOL

. SOB medium

CONTACT: Irina Agarkova
Step 1.1.

Prepare the $MgCl_2$ and $MgSO_4$ as 1 M stock solutions and autoclave separately.

Step 1.2.
Add the $MgCl_2$ and $MgSO_4$ after sterilization of the remainder of the components.

Step 2.
Prepare the SOC medium by using the SOB media supplemented with 20mM glucose.

Step 3.
Prepare the TFB buffer.

PROTOCOL

. TFB buffer

CONTACT: Irina Agarkova
Step 3.1.
Weigh out the components.

Step 3.2.
Add the MES to d-H_2O and adjust the pH with KOH.

Step 3.3.
Add the remaining components (in order), waiting until one component is in solution before adding the next.

Step 3.4.
Adjust the volume to the final volume.

Step 3.5.
Filter sterilize.

Step 3.6.
Store frozen at -20°C in 15 ml aliquots.

Step 4.
Prepare the DTT solution.

PROTOCOL

. DTT solution

CONTACT: Irina Agarkova

Step 4.1.
Add components together and adjust pH as necessary.

Step 4.2.
Filter sterilize.

Step 4.3.

Store frozen at -20°C.

Step 5.
Grow 5 mL of the host cells overnight in SOB media at 37°C.

DURATION
18:00:00

Step 6.
Inoculate 40 mL of SOB medium with 0.8 mL of the overnight culture.

Step 7.
Grow to an A_{550} of 0.45-0.55 at 37°C (approximately 3-4 hours).

DURATION
04:00:00

Step 8.
Centrifuge the cells in the Sorvall SS34 rotor at 5,000 rpm, 5 min, 4°C.

DURATION
00:05:00

Step 9.
Discard the supernatant.

Step 10.
Resuspend the pellet with 12.5 mL of the TFB solution.

Step 11.
Hold the remaining 2.5 mL of TFB for use later.

Step 12.
Chill the cells on ice for 15 min.

DURATION
00:15:00

Step 13.
Centrifuge the cells in the Sorvall SS34 rotor at 5,000 rpm, 5 min, 4°C.

DURATION
00:05:00

Step 14.
Discard the supernatant.

Step 15.
Resuspend the pellet with 2.4 mL of TFB solution.

Step 16.
Add DMSO to 3.5% (84µL), mix and chill on ice for 5 min.

DURATION
00:05:00

Step 17.
Add DTT solution to 75 mM (84 µL), mix and chill on ice for 10 min.

DURATION
00:10:00

Step 18.
Add an equal volume of DMSO as before (84 µL), mix and chill on ice for 5 min. The cells are now "competent".

DURATION
00:05:00

Step 19.
Pipet 21 µL competent cells per prechilled microfuge tube.

NOTES
Irina Agarkova 14 Apr 2016
One tube will be spread on one plate.

Step 20.
Add the DNA (in as small a volume as possible, 1-2 µL/tube), mix and chill on ice for 30 min.

DURATION
00:30:00

Step 21.
Heat pulse the tubes at 42°C for 3 min.

DURATION
00:05:00

Step 22.
Then chill on ice for 2 min.

DURATION
00:02:00

Step 23.
Add 80 µL of SOC medium per tube and incubate the tubes at 37°C for 60 min.

DURATION
01:00:00

Step 24.
Spread 100 µL onto each plate.

Step 25.
Incubate the plates at 37°C overnight.

DURATION
18:00:00