Transformation of DNA in competent *E. coil*

Reagents:

SOC medium (1L)

(a) 20g tryptone, 5g yeast extract, 0.5g NaCl in 950ml dH_2O.

(b) 250mM KCl: 1.86 KCl in 100ml dH_2O.

Add 10ml of solution (b) to solution (a). Adjust the pH to 7.0 with 5N NaOH and bring the volume to 980ml with dH_2O

(c) 1M $MgCl_2$: 20.33g of $MgCl_2$ in 100ml dH_2O.

Autoclave solutions (ab) and (c) separately.

(d) 2M Glucose: 36g Glucose in 100ml dH_2O. Filter sterilize it.

Finally cool the autoclaved solutions to 55°C. Add 10ml of solution (c) and solution (d) to first solution (ab). Final volume is 1L.

Store at room temperature or at 4°C.

LB/antibiotic plate

5g tryptone, 5g NaCl, 2.5g yeast extract and 7.5g agar in 480ml dH_2O, Adjust pH to 7.0 by NaOH and complete the volume to 500ml. Autoclave and allow the solution to cool up to 55-50C before adding the antibiotics.

Kanamycin:

Prepare ***25mg/ml*** water stock and the final concentration should be **50µg/ml**. Take 1ml (1000µl) of the stock and add it to 500ml LB.

Ampecillin:

Prepare ***50mg/ml*** water stock solution and store in the freezer. The final concentration should be **100µg/ml**. Thus, take 1ml (100µl) of the stock in 500ml LB.

Chloramphenicol:

Dissolve ***34mg/ml*** ethanol stock and store in the freezer. The working solution is **34µg/ml**. Thus, take 500µl (i.e. 0.5ml) of the stock in 500ml LB medium to get 34µg/ml.

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Transformation in Competent *E. coli*

* **Run under aseptic conditions in steps of this procedure**.

* Treat the competent cells very gently. These cells are so weak that the heat from your fingers or vigorous mixing will destroy them (Avoid touching the bottom of the competent cell containing tube).

(1) Bring an ice bucket.

(2) Place one eppendorf tube containing competent cells **DH5α** (stored at -121°C on the ice. **Do not touch** the tube from the bottom as cells will lose their activity (These cells are so weak, thus treat them very gently).

(3) Pipet **50µl** of competent cells in an autoclaved eppendort tube. Add **1-2µl** of vector or ligate product and **mix gently** (**Do not vortex**; weak cells will be damaged if they are mixed vigorously).

(4) Incubate them on ice for 30 minutes.

(5) Heat shock the cells by placing them in water bath at **42°C** for 30-45 seconds (don't exceed this duration).

(6) Ice them rapidly for 2minutes

(7) Add **250µl of SOC** medium (This medium will let them relax and recover after heat shock treatment).

(8) Incubate at 37°C for 1 hour in shaking incubator at 225rpm.

(9) After 1 hour incubation, take **50µl** and **150µl** of cell suspension and inoculate onto separate plates of LB + antibiotic (in our case, LB/Kanamycin since the plasmid pET30a carries Kanamycin resistance gene). Spread the inoculum evenly.

(10) Wait for 10-15 minutes till the spread inoculum is absorbed by the agar.

(11) Invert the plate and incubate it in a 37°C incubator for at least 8-12 hours or O/N.

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Transformation in Expression Competent *E. coli*

* **Run under aseptic conditions in steps of this procedure**.

* Treat the competent cells very gently. These cells are so weak that the heat from your fingers or vigorous mixing will destroy them (Avoid touching the bottom of the competent cell containing tube).

(1) Bring an ice bucket.

(2) Place one eppendorf tube containing expression competent host cells **Rosetta (DE3)** (stored at -80°C) in the ice bucket. Try not to touch the tube from the bottom or cells will lose their activity (These cells are so weak, thus treat them very gently).

(3) Pipet **50µl** of competent cells in an autoclaved eppendort tube. Add **1-2µl** of vector or ligate product and **mix gently** (Do not vortex; weak cells will be damaged if they are mixed vigorously).

(4) Incubate them on ice for 30 minutes.

(5) Heat shock the cells by placing them in water bath at **42°C** for 30-45 seconds (don't exceed this duration).

(6) Ice them rapidly for 2minutes

(7) Add **250µl of SOC** medium (This medium will let them relax and recover after heat shock treatment).

(8) Incubate at 37°C for 1 hour in shaking incubator at 225rpm.

(9) After 1 hour incubation, take **50µl** and **150µl** of cell suspension and inoculate onto separate plates of LB + antibiotic (in our case, LB/Kanamycin/ Chloramphenicol, since the plasmid pET30a carries Kanamycin resistance gene while Rosetta (DE3) expression host cells bear Chloramphenicol resistance gene). Spread the inoculum evenly.

(10) Wait for 10-15 minutes till the spread inoculum is absorbed by the agar.

(11) Invert the plate and incubate it in 37°C incubator for at least 8-12 hours or O/N.

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