Transformation of SHuffle® Competent Cell Strains

SHuffle Competent *E.coli* Cells are engineered to enable the folding of recombinantly expressed proteins *in vivo*. The introduction of the isomerase, DsbC to the SHuffle chromosome enhances the correct formation of disulfide bonds in proteins that require them for biological activity. Biologically important molecules that require disulfide bonds include antibodies, hormones, proteases, toxins and cellular signals.

High Efficiency Transformation Protocol

Perform steps 1–7 in the tube provided.

1. Thaw a tube of SHuffle Competent *E. coli* cells on ice for 10 minutes.
2. Add 1–5 µl containing 1 pg–100 ng of plasmid DNA to the cell mixture. Carefully flick the tube 4–5 times to mix cells and DNA. Do not vortex.
3. Place the mixture on ice for 30 minutes. Do not mix.
4. Heat shock at exactly 42°C for exactly 30 seconds. Do not mix.
5. Place on ice for 5 minutes. Do not mix.
6. Pipette 950 µl of room temperature SOC into the mixture.
7. Place at 30°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
8. Warm selection plates to 30°C.
9. Mix the cells thoroughly by flicking the tube and inverting, then perform several 10-fold serial dilutions in SOC.
10. Spread 50–100 µl of each dilution onto a selection plate and incubate overnight at 30°C. Alternatively, incubate at 25°C for 48 hours.

5 Minute Transformation Protocol

A shortened transformation protocol resulting in approximately 10% efficiency compared to the standard protocol may be suitable for applications where a reduced total number of transformants is acceptable. Follow the High Efficiency Transformation Protocol above with the following changes:

1. Steps 3 and 5 are reduced to 2 minutes.
2. Omit outgrowth (step 7) completely for ampicillin-resistant plasmids or reduce the outgrowth time for other selective media as appropriate.

Protocol for Expression Using SHuffle

1. Transform expression plasmid into SHuffle. Plate on antibiotic selection plates and incubate 24 hours at 30°C.
2. Resuspend a single colony in 10 ml liquid medium with antibiotic.
3. Incubate at 30°C until OD_{600} reaches 0.4–0.8.
4. Add the appropriate inducer, e.g. 40 µl of a 100 mM stock of IPTG. Incubate for 4 hours at 30°C or 16°C overnight.
5. Check for expression either by Coomassie stained protein gel, Western Blot or activity assay. Check expression in both the total cell extract (soluble + insoluble) and the soluble fraction alone.
6. For large scale, innoculate 1 L of liquid medium (with antibiotic) with a freshly grown colony or 10 ml of freshly grown culture. Incubate at 30°C until reaches 0.4–0.8. Add the appropriate inducer, e.g. IPTG to 0.4 mM. Induce 4 hours or 16°C overnight.

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