SCARAB GENOMICS

Clean Genome® *E. coli* Electrocompetent Cell Protocol

KIT CONTENTS

Catalog Number	E-XXXX-05	E-XXXX-10	E-XXXX-20
Chemically Competent Cells	1 x 0.25 mL	2 x 0.25 mL	4 x 0.25 mL
pUC19 Control DNA (10 pg/µl)	1 x 50 µL	1 x 50 µL	1 x 50 µL
SOC Medium	1 x 10 mL	1 x 10 mL	2 x 10 mL

QUALITY CONTROL

Transformation efficiency is tested using pUC19 Control DNA, in duplicate. Transformed cells are plated onto LB plates containing 50 µg/mL carbenicillin. Transformation efficiency is > 5 x 10^9 cfu/µg DNA for the core Clean Genome® *E. coli* strain, but may be lower for strains carrying mutations that reduce recombination and or mutation frequencies.

TRANSFORMATION PROCEDURE

1. Prepare selective antibiotic plates and warm to room temperature or 37°C
2. Equilibrate a shaker to 37°C
3. Warm the provided SOC medium to 37°C to dissolve any visible precipitate.
4. Place the required number of 0.1-cm electrode gap electroporation cuvettes and 1.5-mL microcentrifuge tubes on ice.
5. Thaw competent cells on ice, use as soon as possible to prevent loss of viability
6. Add control and experimental DNA samples to culture tubes:
    - For no DNA Control, add 1 µL Ultrapure water
    - For pUC19 Control DNA, add 1 µL DNA
    - For ligation products, desalt ligations for 20 minutes by adding the ligation mixture to a VSWP membrane (Millipore VSWP02500) that has been carefully placed on the surface of ultrapure water, then add 2-5 µL (2-10 ng DNA) of heat inactivated ligation reaction
7. Gently flick competent cells tube 2-3 times to suspend cells evenly
    - Clean Genome® *E. coli* strains lack flagella and settle towards the bottom of the suspension more quickly than do wild-type strains
8. Add 40 µL cells to each pre-chilled microcentrifuge tube
9. Gently pipette the cell/DNA mixes 2-3 times then add to the chilled cuvettes, taking care to avoid air bubbles
    - If bubbles are present, gently tap the cuvettes to dislodge the bubbles; make sure cell mixture is at the bottom of the cuvette
    - Re-freezing cells is not recommended due to losses in viability and transformability, so discard any unused cells.
10. Electroporate samples at 1.8 to 2 kV/cm
11. Add 960 µL of room temperature SOC medium to the cuvette of electroporated cells. Mix well by pipetting up and down 2–3 times
12. Transfer the mixture to a 15-mL culture tube and incubate at 37°C with shaking at 250–275 rpm for 1 hour
13. For cells transformed with pUC19 Control DNA, dilute the culture 50-fold in SOC medium then plate 100 µl of the diluted culture onto pre-warmed LB agar plates containing 100 µg/mL ampicillin or 50 µg/mL

carbenicillin.

14. For cells transformed with experimental DNA, spread a 100 µL of each of a series of dilutions of the outgrowth culture onto selective plates.
15. Store the remaining cultures at 4°C for future plating, if desired. Expect reduced efficiency.
16. Incubate plates overnight at 37°C.

Ensuring IS Element-Free Plasmids

Unlike other strains of *E. coli*, Clean Genome® *E. coli* contains zero IS elements. IS elements are undesirable because they can transpose into any part of the genome, including extrachromosomal elements such as plasmids. Such transpositions may mutate the gene you want to express, resulting in mutant product, or into its regulatory elements, resulting in diminished expression. Commercial plasmid products typically contain IS elements. Therefore, Scarab strongly recommends that prior to transforming cells, you test your vector DNA for IS elements using the Insertion Element Detection Kit, Catalog number S-1109-10.

Where IS element contamination is found or suspected, treat plasmid vector DNA preparations with a deoxyribonuclease that does not degrade circular double-stranded DNA to remove any chromosomal DNA that might contain IS elements. Then use just 10 pg plasmid DNA for the transformation, thereby reducing the number of plasmids transformed per cell. Such transformations should yield no more than 1000 transformed cells. Plate at sufficiently low density so that well-isolated bacterial transformants can be cloned. Test each of several clones for IS elements to confirm IS-free status.

Tips for Optimizing Production of Recombinant Proteins and Nucleic Acids

- Use Minimal Medium for growth of Clean Genome® *E. coli* with glucose as the carbon source. This will not only provide superior production, but will also aid product purification
- To prevent a lag when transferring bacteria to liquid culture for production, you may streak clones onto minimal plates with 0.2% glucose and grow at 37°C for 24 h, at 30°C for 48 h, or at room temperature over the weekend

Troubleshooting

If no colonies are obtained from transformations:

The selective drug may be inactive or at too low a concentration. Verify that selective plates contain the appropriate selective antibiotic at the correct concentration.

The post-transformation incubation temperature may be suboptimal. Be sure to incubate at 37°C.

Cells were damaged. Clean Genome® *E. coli* must be handled very gently—do not pipet vigorously or vortex-mix. Rather, gently pipet or gently flick cells to resuspend.

If a lawn of confluent cells is obtained or satellite colonies grow on selective plates

The selective drug concentration may be too low or the drug is inactive. Verify that selective plates contain the appropriate selective antibiotic at the correct concentration.

Selective plates my have been incubated at 37°C for too long. Plates should incubate for 16-18 h. Drug-resistant cells may secrete enzymes that degrade the drug around individual colonies, allowing non-resistant satellite colonies of cells to grow. Note that carbenicillin appears to be less susceptible to this problem than is ampicillin.

Trademarks and Patents

Clean Genome® *E. coli* is a registered trademark of Scarab Genomics, LLC. Clean Genome® *E. coli* strains (the "Material") are covered by U.S. Patent Nos. 6,989,265, 8,039,243, 9,340,791, 9,902,965 and corresponding continuations, divisionals, and foreign patents.