SCARAB GENOMICS

Clean Genome® *E. coli* Chemically competent Cell Protocol

KIT CONTENTS

Catalog Number	C-XXXX-05	C-XXXX-10	C-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

Transformation efficiency is tested using pUC19 control DNA, performed in duplicate. Transformed cells are grown on LB plates containing 50 µg/mL carbenicillin. Transformation efficiency is ≥ $1x10^8$ 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 water bath to 42°C
3. Warm the provided SOC medium to 37°C to dissolve any visible precipitate.
4. Place required number of 17x100-mm culture tubes (14 mL), or 1.5 mL Eppendorf tubes, on ice
5. Thaw competent cells on ice, use as soon as possible to prevent loss of viability
6. 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
7. Add 50 µL cells to each pre-chilled tube
8. 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, add 2-5 µL (2-10 ng DNA) of heat inactivated ligation reaction
9. Gently flick the cell/DNA mixes 2-3 times
10. Incubate tubes on ice for 30 min
11. Heat shock cells for 30 sec in a 42°C water bath without agitation
12. Place tubes on ice for 2 min
13. Add 450 µL pre-warmed SOC medium to each tube
14. Incubate at 37°C with shaking at 250–275 rpm for 1 hour
15. For cells transformed with pUC19 Control DNA, spread 100 µl of the outgrowth culture onto pre-warmed LB agar plates containing 100 µg/mL ampicillin or 50 µg/mL carbenicillin.
16. For cells transformed with experimental DNA, spread up to 150 µL of the outgrowth culture onto selective plates.
17. Store the remaining cultures at 4°C for future plating, if desired. Expect reduced efficiency.
18. Incubate plates overnight at 37°C.

NOTES

- Unused chemically competent cells may be re-frozen in a dry ice/ethanol bath for 5 min before returning to the -80°C freezer. Do not store in liquid nitrogen. Re-frozen cells may have lower transformation efficiency. Clean Genome® *E. coli* strains survive poorly at 4°C.

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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.