SCARABGENOMICS LLC
CLEAN GENOME® *E. coli*

Clean Genome® *E. coli*
MDS™ 42*recA*$^-$ *trf*A Electrocompetent Cells
(Cat. No. E-0201-05, E-0201-10, E-0201-20)

FOR RESEARCH USE ONLY

COMPONENTS	**E-0201-05**	**E-0201-10**	**E-0201-20**
MDS™ 42*recA*$^-$ *trfA* Electrocompetent Cells	1 x 0.2 ml	2 x 0.2 ml	4 x 0.2 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

STORAGE CONDITIONS

Store components at –80°C. **Do not store cells in liquid nitrogen.**

BACKGROUND

Using synthetic biology methods, the *Escherichia coli* K-12 genome was reduced by making a series of planned, precise deletions. The multiple-deletion series (MDS™) strains (1), with genome reduction of up to 15%, were designed by identifying non-essential genes and sequences for elimination, including recombinogenic or mobile DNA and cryptic virulence genes, while preserving robust growth and protein production. Genome reduction also led to unanticipated beneficial properties, including high electroporation efficiency and accurate propagation of recombinant genes and plasmids that are unstable in other strains. Subsequent deletions and introduction of useful alleles produce strains suitable for many molecular biology applications.

GENOTYPE

MG1655 multiple-deletion strain (1), *rec*A$^-$ 1819, ara:*trf*A

The *recA* 1819 mutation is a deletion of *recA*$^-$.
The TrfA protein regulates initiation of replication at the *oriV* origin of replication from the RK2 plasmid. A “copy up” *trf*A allele has been cloned into the genome under the control of the *araBAD* promoter. The *trf*A cell lines allow copy number amplification of BAC plasmids containing the oriV origin of replication as described by Wild et al. (2).

ELECTROCOMPETENT CELL 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 > 1 x 10^9 cfu/µg DNA.

BEFORE YOU BEGIN

Please note, Clean Genome® strains do not remain viable for extended periods (greater than 2 weeks) when stored at 4°C. We recommend preparing glycerol stock cultures of clones and storing at -80°C, or keeping plates at room temperature for up to 5 days. **Do not store in liquid nitrogen.**

To ensure that the cells grow on minimal media and to prevent a significant lag when transferring to liquid culture, we streak from glycerol stocks of 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 (RT) for several days (e.g., over the weekend). Colonies picked from these plates are used for cultures. If you are only using rich media (e.g., LB, TB) for all growth, rich medium plates can be used for streaking clones.

Clean Genome strains do not have flagella and tend to aggregate and drop fairly quickly from solution. To obtain OD readings, cells should be mixed just before taking an aliquot for dilution, and dilution samples should be mixed just before taking an OD reading.

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PRE-TRANSFORMATION STEPS

1. Thaw the provided SOC medium and warm it to 37°C to dissolve any visible precipitate.
2. Warm selective antibiotic plates to room temperature or 37°C.
3. If you are transforming a plasmid with antibiotic resistance other than ampicillin, you need to have at least one LB agar plate containing 100 µg/ml ampicillin (or 50 µg/ml carbenicillin) for plating the pUC19 Control DNA transformation.

TRANSFORMATION PROCEDURE

1. Place the required number of **0.1-cm** electrode gap electroporation cuvettes and 1.5-ml microcentrifuge tubes on ice.
2. Add experimental DNA to 1.5-ml microcentrifuge tubes.

   NOTE - Transformation efficiency of ligation products can be increased several fold by diluting the ligation reaction 5-fold with TE or water prior to adding DNA to the cells.
3. For the positive control, add 1 µl of pUC19 Control DNA to a microcentrifuge tube. For the negative control, add 1 µl of Ultrapure H_2O.
4. Thaw Clean Genome MDS™ 42 *recA*$^-$ *trfA* Electrocompetent Cells on ice and use as soon as possible.
5. When the cells are thawed, flick the tube gently 1-3 times to evenly suspend cells. Add 40 µl cells to each pre-chilled microcentrifuge tube containing DNA.
6. Gently pipet the cell/DNA mixture 1-2 times to mix. Discard any unused cells. **Re-freezing cells is not recommended.**
7. Pipet the cell/DNA mixture into a pre-chilled 0.1-cm electroporation cuvette avoiding bubble formation. Gently tap the cuvette several times to remove bubbles and to ensure the cell/DNA mixture is at the bottom of the cuvette.
8. Electroporate samples at 18 kV/cm. Add 960 µl of room-temperature SOC medium to the cuvette of electroporated cells. Mix well by pipetting up and down 2-3 times. Transfer the mixture to a 15-ml culture tube.
9. Incubate at 37°C with shaking at 250–275 rpm for 1 h.
10. For cells transformed with pUC19 Control DNA, dilute the culture 1/50 in SOC medium and plate 100 µl of the dilution onto pre-warmed LB plates containing 100 µg/ml ampicillin.
11. For cells transformed with experimental DNA, plate as required and spread up to 100 µl onto selective plates. **OPTIONAL:** For blue/white screening, spread cells onto LB plates containing the appropriate antibiotic, 80 µg/ml X-Gal, and 0.5 mM IPTG.
12. Incubate plates overnight at 37°C.

PLASMID COPY NUMBER INDUCTION

The plasmid copy number induction is based on the procedure of Wild *et al.* (2) using the two part *oriV* system. The origin of replication is located on the plasmid while the *trfA* gene encoding the trans-acting initiation protein, is present in the bacterial chromosome under control of the *araBAD* promoter. Two methods for plasmid copy number induction are provided, the original manual method and an autoinduction method.

MANUAL INDUCTION

1. Inoculate isolated colony into sterile LB medium containing the appropriate antibiotic.
2. Grow culture at 37°C with shaking to an OD_{600} of 0.3.
3. Add sterile arabinose to a final concentration of 0.1%.
4. Continue growth at 37°C with shaking for 5 hours.
5. Harvest cells by centrifugation and proceed with plasmid preparation.

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AUTOINDUCTION

1. Inoculate isolated colony into sterile LB medium containing the appropriate antibiotic plus 0.02% glucose, 0.02% glycerol and 0.1% arabinose.
2. Grow culture at 37°C with shaking for 15 to 18 hours.
3. Harvest cells by centrifugation and proceed with plasmid preparation.

CALCULATION OF TRANSFORMATION EFFICIENCY USING pUC19 CONTROL DNA

1. Count cells on each Control plate. Calculate the average colonies/plate for the pUC19 Control DNA plates if more than one replicate was plated (Note: the No DNA Control plate should not have any colonies).
2. Use the formula below to calculate the transformation efficiency in colony forming units (cfu) per µg Control DNA.

$$\frac{\text{Average \# colonies}}{\text{pg DNA plated}} \times \frac{10^6\ \text{pg}}{\mu\text{g}} = \frac{\text{cfu}}{\mu\text{g plasmid DNA}}$$

EXAMPLE OF TRANSFORMATION EFFICIENCY USING pUC19 CONTROL DNA

$$\frac{10\ \text{pg DNA}}{1000\ \mu\text{l total rxn vol.}} \times \frac{1}{50}\ \text{dilution} \times 100\ \mu\text{l plated} = 0.02\ \text{pg DNA plated}$$

$$\frac{200\ \text{colonies}}{0.02\ \text{pg DNA}} \times \frac{10^6\ \text{pg}}{\mu\text{g}} = 1.0 \times 10^{10}\ \frac{\text{cfu}}{\mu\text{g}}$$

TROUBLESHOOTING

The pUC19 Control DNA plasmid provided should be used as a positive control.

Problem	Possible Solution
Arcing or “popping” occurs during electroporation	1. DNA may contain salt. Desalt DNA sample or dilute it 1/5 before electroporation. Repeat electroporations with 1 µl of the desalted or diluted DNA.
No colonies or low number of colonies	1. Incorrect drug selection or drug concentration. Verify that LB agar plates contained the appropriate selective antibiotic. Repeat electroporations and plate on new plates. 2. Plates were too old/dry. Repeat electroporations; plate on new plates. 3. Cells were not handled correctly. Cells must be handled very gently. Do not pipet vigorously or vortex. Always gently pipet or gently flick cells to resuspend. Thaw cells and keep on ice until electroporation. Repeat electroporation.
Lawn or confluent cell growth or satellite colonies	1. Incorrect drug concentration. Verify that LB agar plates contain the appropriate selective antibiotic concentration. Repeat electroporation; use new plates. 2. Antibiotic has degraded. Plates are too old, antibiotic stock(s) have degraded, or antibiotic was added when medium was too hot. Prepare fresh antibiotic stock(s) and fresh plates. Repeat electroporation. 3. Incubated at 37°C too long. Plates should incubate for 16-18 h. Amp^R cells secrete β-lactamase that creates a drug-free zone in the surrounding medium, allowing small amp^S colonies to grow. Carbenicillin (an ampicillin analog) appears to be less vulnerable to degradation. Repeat Electroporation and incubate plates for 16-18 h.

REFERENCES

1. Pósfai, G., Plunkett III, G., Fehér, T., Frisch, D., Keil, G.M., Umenhoffer, K., Kolisnychenko, V., Stahl, B., Sharma, S.S, de Arruda, M., Burland, V., Harcum, S.W., and Blattner, F.R. Emergent Properties of Reduced-Genome *Escherichia coli. Science* **312**, 1044-1046 (2006).

2. Wild, J., Hradecna, Z., and Szybalski, W. Conditionally Amplifiable BACs: Switching from Single-Copy to High-Copy Vectors and Genomic Clones. Genome Research **12**, 1434-1444. (2002).

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