**Clean Genome® *E. coli***
**MDS™ 42 Pack, Chemically Competent Cells**
***(Cat. No. C-1000-15)***

***FOR RESEARCH USE ONLY***

| **COMPONENTS** | **C-1000-15** |
|---|---|
| MDS™ 42 Chemically Competent Cells | 1 x 0.25 ml |
| MDS™ 42*recA*⁻ Chemically Competent Cells | 1 x 0.25 ml |
| MDS™ 42*recA*⁻ Blue Chemically Competent Cells | 1 x 0.25 ml |
| pUC19 Control DNA (10 pg/µl) | 1 x 50 µl |
| SOC Medium | 1 x 10 ml |

## STORAGE CONDITIONS

Store it 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.

## GENOTYPES

MG1655 multiple-deletion strain (1)
MG1655 multiple-deletion strain (1), *rec*A1819
MG1655 multiple-deletion strain (1), *rec*A1819, *lacZ* M15⁻

The *recA* 1819 mutation is a complete deletion of *recA*⁻.
The *lacZ* M15 deletion has been created in the genome to allow blue/white screening of inserts in plasmids using the α-complementing fragment of β-galactosidase.

## CHEMICALLY COMPETENT CELL QUALIFICATION

Transformation efficiency is tested using pUC19 control DNA, performed in duplicate. Transformed cells are plated on LB plates containing 50 µg/ml carbenicillin. Transformation efficiency is ≥ $1x10^8$ 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. Equilibrate a water bath to 42°C.
2. Thaw the provided SOC medium and warm it to 37°C to dissolve any visible precipitate.
3. Warm selective antibiotic plates to room temperature or 37°C.
4. If you are transforming a plasmid with antibiotic resistance other than ampicillin, you will need at least one LB agar plate containing 100 µg/ml ampicillin (or 50 µg/ml carbenicillin) for plating the pUC19 Control DNA transformation.

If you are planning to use MDS™ 42*recA*$^{-}$ Blue for blue/white screening, you will need LB agar plates containing the appropriate antibiotic, 80 µg/ml 5-bromo-4-chloro-3-indolyl- β-D-galactopyranoside (X-Gal), and 0.5 mM Isopropyl-β-D-thiogalactopyranoside (IPTG). Alternatively, spread 20 µl 50 mg/ml X-Gal (in dimethylformamide) and 100 µl 0.1 M IPTG onto LB agar plates containing the appropriate antibiotic and incubate for at least 1 h at 37°C, so that the plates absorb the X-Gal and IPTG before plating.

## TRANSFORMATION PROCEDURE

1. Place required number of 17x100mm culture tubes (14 ml), or 1.5 ml Eppendorf tubes, on ice.
2. Thaw MDS™ 42 Chemically Competent Cells on ice.
3. Flick competent cells tube gently 2-3 times to evenly suspend cells. Add 50 µl cells to each pre-chilled tube.
4. Add controls and 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.
   - Gently flick the cells/DNA mix 2-3 times.

***NOTES:***

***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.***

***Unused cells may be re-frozen in dry ice/ethanol bath for 5 min before returning to the -80°C freezer. Expect re-freezing cells to decrease their transformation efficiency.***

5. Incubate tubes on ice for 30 min.
6. Heat shock cells for 30 s in a 42°C water bath without agitation.
7. Place tubes on ice for 2 min.
8. Add 450 µl pre-warmed SOC medium to each tube.
9. Incubate at 37°C with shaking at 250–275 rpm for 1 h.
10. 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.
11. For cells transformed with experimental DNA, spread up to 150 µl of the outgrowth culture 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. Store the remaining cultures at 4°C for future plating, if desired. Expect reduced efficiency.
13. Incubate plates overnight at 37°


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## 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 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

$$\frac{10\ \text{pg DNA}}{500\ \mu\text{l total rxn vol.}} \times 100\ \mu\text{l plated} = 2\ \text{pg DNA plated}$$

$$\frac{200\ \text{colonies}}{2\ \text{pg DNA}} \times \frac{10^6\ \text{pg}}{\mu\text{g}} = 1.0 \times 10^8\ \frac{\text{cfu}}{\mu\text{g}}$$

## TROUBLESHOOTING

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

| Problem | Possible Solution |
|---|---|
| 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 transformation or plate an aliquot of the remaining out growth culture onto new plates.<br>2. Incorrect incubation temperature. Be sure to incubate at 37°C. Verify the temperature setting. Incubate plates at 37° for the remainder of one day. If no/low number of colonies is still observed, repeat transformation or plate an aliquot of the remaining out growth culture onto new plates.<br>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 ready to transform. Repeat transformation. |
| Lawn or confluent cell growth or satellite colonies | 1. Incorrect drug concentration. Verify that LB agar plates contain the appropriate selective antibiotic concentration. Repeat transformation or plate an aliquot of the remaining out growth culture onto new plates.<br>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 transformation or plate an aliquot of the remaining out growth culture onto new plates.<br>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 transformation, or plate an aliquot of remaining out growth culture, and incubate plates for 16-18 h. |
| Colonies from pUC19 control DNA plates are white or light blue when plated on LB agar plates containing X-Gal and IPTG. | 1. Colonies have not grown long enough or are very small due to high plating density. Extend the 37°C incubation, or keep the plates at RT, and allow time for better expression of the β-galactosidase enzyme and color development. The best color development occurs 24 h after plating.<br>2. Colonies are too small due to old/dry plates. Repeat transformation or plate an aliquot of the remaining out growth culture onto new plates.<br>3. Incorrect X-Gal and/or IPTG concentration in the plates. Verify that LB agar plates contain the appropriate X-Gal and IPTG concentration. Repeat transformation or plate some of remaining out growth culture onto new plates. |



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## REFERENCE

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).

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