Clean Genome® *E. coli* MDS™ 42 Glycerol Stock *(Cat. No. G-0742)*

FOR RESEARCH USE ONLY

COMPONENTS

Clean Genome® *E. coli* MDS™ 42 Glycerol Stock	0.4 ml

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.

STORAGE CONDITIONS

Store at –80°C.

GENOTYPE

MG1655 multiple-deletion strain (1)

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.

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.

Scarab Genomics – 1202 Ann Street – Madison, WI 53713 – Tel: 888 513-7075 – Fax: 608 257-2043 – www.scarabgenomics.com

REVIVAL PROCEDURE

1. Scrape the frozen glycerol stock with a sterile inoculating loop. To ensure that the cells grow on minimal media and to prevent a significant lag when transferring to liquid culture, we recommend streaking from the glycerol stock onto a fresh MOPS minimal (2,3) or M9 (3) minimal medium agar plate containing 0.2% glucose. Incubate plate at 37°C for 24–36 h.

NOTE - If you will only be using rich media (e.g., LB, TB) for all growth, rich medium plates can be used for streaking.

2. Inoculate an isolated colony into M9 or MOPS minimal medium containing a carbon source (e.g., 0.2% glucose) or any other medium of choice. Incubate culture at 37°C for 15–18 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. Neidhardt, F.C., Bloch, P.L., and Smith, D. F. Culture Medium for Enterobacteria. *J Bacteriol*. **119**, 736-47 (1974).
3. Commercially available from Teknova, Inc. (http://www.teknova.com).

LIMITED PRODUCT WARRANTY

Recipient acknowledges that the Material is experimental and is supplied to Recipient WITHOUT ANY WARRANTIES, EXPRESS OR IMPLIED, INCLUDING ANY WARRANTY OF TITLE, MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE OR ANY WARRANTIES REGARDING INFRINGEMENT OF THIRD PARTY RIGHTS. Recipient agrees to rely solely upon its own opinion of the Material with regard to their safety and suitability for any purpose.

Recipient agrees to waive all claims against Scarab except as may be limited by state law governing Recipient. Recipient agrees to defend and indemnify Scarab, its employees and agents from all claims asserted by any third party and any damages and recoveries arising from the use, storage, or handling of the Material caused by or allowed by Recipient. Scarab makes no representation that the use of the Material will not infringe any patent or proprietary rights of any third parties.

USAGE RESTRICTIONS (LICENSING)

Scarab is providing you with this Material subject to the non-transferable right to use the subject amount of the Material for your research at your academic institution. The Recipient agrees not to sell or otherwise transfer this Material, or anything derived or produced from the Material to a third party. NO RIGHTS ARE PROVIDED TO USE THE MATERIAL OR ANYTHING DERIVED OR PRODUCED FROM THE MATERIAL FOR COMMERCIAL PURPOSES. If the Recipient makes any changes to the chromosome of the Material that results in an invention in breach of this limited license, then Scarab will have a worldwide, exclusive, royalty-free license to such invention whether patentable or not.

If the Recipient is not willing to accept the terms of this limited license, Scarab is willing to accept return of this product with a full refund, minus shipping and handling costs. For information on obtaining a license to this Material for purposes other than research, please contact Scarab's Licensing Department.

Scarab Genomics – 1202 Ann Street – Madison, WI 53713 – Tel: 888 513-7075 – Fax: 608 257-2043 – www.scarabgenomics.com