CloneCatcher™ DH5S*

Silver Electrocompetent *E. coli*

Genlantis
A division of Gene Therapy Systems, Inc.

Cat. #	Contents	Quantity
C810310	CloneCatcher™ DH5S Silver Electrocompetent *E. coli.* (3 - 8 x 10^{10} cfu/µg)	10 x 20.0 µl
	Plating Medium	2 x 6.0 ml
	pUC19 Positive Control Plasmid	20.0 µl (10 pg/µl)

Related Products	Catalog #
CloneCatcher™ DH5G Gold Electrocompetent *E. coli* 8 x 10^{10}-1.2 x 10^{11} cfu/µg.	C810111 (10 x 20.0 µl)
SoluLyse™ Bacterial Protein Extraction Reagent (Phosphate Buffer)	L100125 (125 ml); L100500 (500 ml)
SoluLyse™ Bacterial Protein Extraction Reagent (Tris Buffer)	L200125 (125 ml); L200500 (500 ml)
EZ-Spread ™ Beads, Single-Use Tubes	C400050 (50 tubes)
EZ-Spread ™ Beads, Dispenser Bottle	C400100 (1 bottle)
SoluBL21 Chemically Competent *E. coli*	C700200 (10 x 50 µl)
SoluBL21 Electrocompetent *E. coli*	C700210 (10 x 20 µl)
TurboCells® Competent *E. coli*	C300020 (20 x 50 µl)
TurboCells® BL21(DE3) Competent *E. coli*	C302020 (20 x 50 µl)
SmartCells™ Competent *E. coli*	C101020 (20 x 50 µl)

Shipping	Shipped on dry ice.
Storage	Store the CloneCatcher kit at -70°C. The Plating Medium may be stored at 4 °C. Stable for 6 months.

Introduction: The CloneCatcher™ DH5S Silver Electrocompetent *E. coli* strain is a variant of the standard DH5α bacterium. When using pUC19 DNA as a control plasmid, the CloneCatcher cells provide highly competitive electroporation efficiencies, between 3 and 8 x 10^{10} cfu/µg, while providing cost savings over competitors. In comparisons with other strains, the CloneCatcher cells perform same or better than competitors when electroporating with a pUC19 plasmid, and substantially better than any other competitor strain when electroporating T4-ligated DNA. The CloneCatcher Silver Electrocompetent *E. coli* are ideal for scientists seeking better performing electrocompetent cells while achieving substantial cost, time, and material savings.

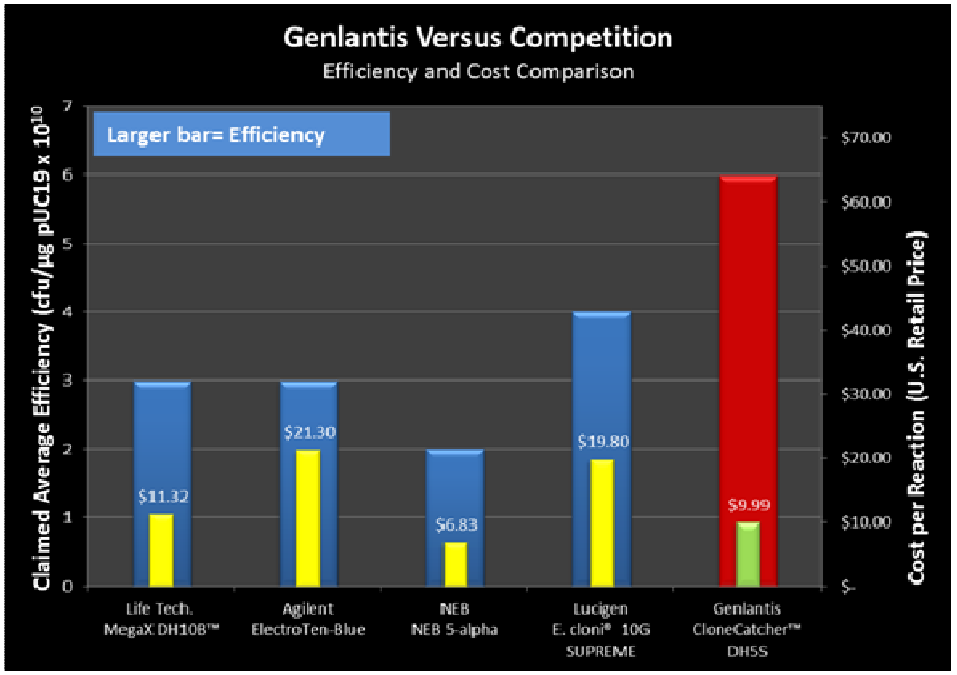
CloneCatcher™ DH5S Genotype
F- endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ80d*lac*ZΔM15 Δ(*lacZYA-argF*)U169, hsdR17(rK- mK+), λ–

† *The CloneCatcher DH5S strain contains uncharacterized mutations obtained through a proprietary process.*

METHODS AND PROCEDURES

General Notes and Positive Control Reaction

The CloneCatcher DH5S electrocompetent cells have transformation efficiencies between 3 and 8 x 10^{10} cfu/µg of pUC19. To perform a positive control reaction we recommend using 1 picogram (pg) of the pUC19 Positive Control Plasmid. To do this, dilute 5 µl of the provided pUC19 plasmid in 45 µl of sterile water (1:10 dilution), and use 1 µl of this dilution. Plate 10 µl of the electroporation mix on an LB agar plate with 100 µg/ml carbenicillin; a 3 x 10^{10} efficiency will yield 300 colonies.



* *U.S. Patents Pending*

Genlantis
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A. Electroporation Protocol

1. Place 0.1 cm cuvette on ice for at least 5 minutes.

2. Thaw CloneCatcher cells on ice.

3. For purified plasmid DNA, proceed to Step 6 below.

4. For **a topoisomerase cloning system,** follow the manufacturer's recommendations for electroporation buffer, and then continue to Step 6 below.

5. For **T4 ligations**, we recommend using a spin column based PCR purification kit. Below is a sample protocol from a QIAquick® PCR Purification Kit* (Qiagen, Catalog Numbers 28104 or 28106). If using a kit from another vendor, please use the specific kit protocol.
   a. After ligation reaction is done, add water to bring final volume up to 20 µl.
   b. Add 100 µl PBI Buffer to diluted ligation reaction.
   c. Transfer the PBI/ligation reaction to a QIAquick spin column.
   d. Wash with 0.75 ml PE Buffer, twice.
   e. Perform one extra spin without adding any PE Buffer to ensure the column is dry enough before elution.
   f. Elute with 20 µl of water, and proceed to Step 6.

6. Add 1 µl of DNA directly to 20 µl of cells from either Steps 3, 4, or 5 above.
   a. Incubate on ice for 5 minutes.
   b. Transfer cells + DNA into prechilled cuvette. Keep on ice.
   c. Wipe cuvette free of ice and moisture and place in electroporator chamber.
   d. Set electroporation parameters as follows:

      2.5 Kilovolts (KV)
      100 Ohms

   e. **Pulse the cells 3 times**.

      NOTE: to perform 3 pulses, keep cuvette in chamber after the first pulse, wait until electroporator is ready again (light and/or audio notification, according to manufacturer), and pulse cells again; repeat one more time for third pulse.

   f. Recover the electroporated cells by adding 980 µl of Plating Medium to cuvette. Pipet up and down 6-8 times to completely suspend cells and transfer to 2059 culture tube.

   g. Recover for 90 minutes in a 37ºC shaker at 200 rpm. **NOTE**: for experiments requiring room temperature recovery, extend the incubation time to 120 minutes.

      IMPORTANT: The CloneCatcher cells grow more slowly than parental DH5α cells, therefore longer recovery times (as specified above) are essential.

   h. Plate the contents of Step 6.f. on an LB Agar plate containing an antibiotic that is appropriate for the electroporated plasmid DNA.

   i. Incubate plates overnight in a 37ºC air incubator.

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