CloneCatcher™ DH5G*

Gold Electrocompetent *E. coli*

Genlantis
A division of Gene Therapy Systems, Inc.

Cat. #	Contents	Quantity
C810111	CloneCatcher™ DH5G Gold Electrocompetent *E. coli.* (8 x 10^{10} - 1.2 x 10^{11} cfu/µg)	10 x 20.0 µl
	Plating Medium	2 x 6.0 ml
	pUC19 Positive Control Plasmid	20.0 µl (10 pg/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.

Related Products	Catalog #
CloneCatcher™ DH5S Silver Electrocompetent *E. coli* 3-8 x 10^{10} cfu/µg.	C810310 (10 x 20.0 µl)
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)

Introduction: The CloneCatcher™ DH5G Gold Electrocompetent *E. coli* strain is a variant of the widely-known DH5α. When using pUC19 DNA as a control plasmid, the CloneCatcher cells provide the highest electroporation efficiencies available commercially between 8 x 10^{10} and 1.2 x 10^{11} cfu/µg. These efficiencies represent a 3 to 5 fold improvement over other available electrocompetent cells, strains, or preparations when using pUC19 control plasmid DNA. Most importantly, the CloneCatcher DH5G Gold cells will result in the highest possible efficiencies, and as much as 10 fold improvements over other available strains on the market today when electroporating ligation reactions that use conventional T4 DNA ligase. Scientists in the field of Metagenomics, mutagenesis, or those seeking to create more complex libraries and increase the chances of finding difficult clones, will now find the best possible success with the CloneCatcher DH5G Gold Electrocompetent cells. Alternatively, the increased efficiency of the CloneCatcher cells will allow users to dramatically decrease the amount of needed topoisomerase-loaded cloning vectors, resulting in a substantial cost savings in the long run.

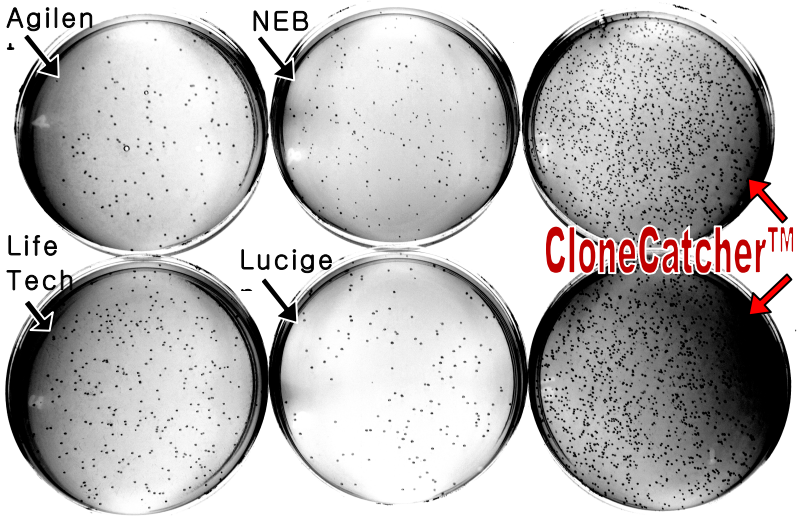
CloneCatcher™ DH5G GoldGenotype
F- endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)U169, hsdR17(rK- mK+), λ–

† *The CloneCatcher DH5G Gold strain contains uncharacterized mutations obtained through a proprietary process. These mutations provide maximum efficiency.*

METHODS AND PROCEDURES

A. General Notes and Positive Control Reaction

The CloneCatcher DH5G Gold Electrocompetent *E. coli* have minimum transformation efficiencies between 8 x 10^{10} and 1.2 x 10^{11}. 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 1 x 10^{11} efficiency will yield 1,000 colonies.



CloneCatcher DH5G Gold Electrocompetent *E. coli* versus major competitors.
One µl of a T4 Ligase-generated library electroporated into CloneCatcher DH5M cells and competitor cells following each manufacturer's instructions.

* *U.S. Patents Pending*

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B. 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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