CloneCatcher DH5G™

Electrocompetent E. *coli*

Genlantis
A division of Gene Therapy Systems, Inc.

Cat. #	Contents	Quantity
C810111	CloneCatcher™ DH5G Gold Electrocompetent *E. coli.* (≥ 1 x 10^{11} cfu/µg)	10 x 20.0 µl
	SOC Medium	2 x 6.0 ml
	pUC19 Positive Control Plasmid	20.0 µl (10 pg/µl)
C810310	CloneCatcher™ DH5G Silver Electrocompetent *E. coli.* (≥ 3 x 10^{10} cfu/µg)	10 x 20.0 µl
	SOC Medium	2 x 6.0 ml
	pUC19 Positive Control Plasmid	20.0 µl (10 pg/µl)

Related Products	Catalog #
Zeus Electroporation Cuvettes, 0.1 cm	C901050 (50/bag)
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 SOC Medium may be stored at 4 °C. Stable for 6 months.

Introduction: The CloneCatcher™ DH5G 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. Genlantis offer two versions of the CloneCatcher cells: the CloneCatcher DH5G Silver, with efficiencies at 3 x 10^{10} cfu/µg or higher, and the CloneCatcher DH5G Gold, with efficiencies at 10^{11} cfu/µg or higher. The CloneCatcher Silver cells are suitable for users looking for competitive efficiencies at a better cost, while the CloneCatcher Gold cells offer the highest efficiencies available on the market today and represent a 3 to 5 fold improvement over other available electrocompetent cells, strains, or preparations. The CloneCatcher Gold cells are suitable for users seeking to create more complex libraries and increase the chances of success in relatively difficult cloning experiments. In addition, the increased efficiency of the CloneCatcher Gold Electrocompetent *E. coli* cells from Genlantis will allow users to dramatically decrease the amount of needed ligation mixes, including topoisomerase-loaded cloning vectors, resulting in a substantial cost savings in the long run.

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

† *The CloneCatcher DH5G strain contains uncharacterized mutations obtained through a proprietary process. These mutations allow the strain to have a high survival rate post electroporation and provide maximum efficiency.*

METHODS AND PROCEDURES

A. General Notes and Positive Control Reaction

The CloneCatcher DH5G electrocompetent cells have minimum transformation efficiencies of either 3 x 10^{10} (for CloneCatcher Silver) or 10^{11} (for CloneCatcher Gold). To perform a positive control reaction, or check the cells' efficiency, we recommend using 1 picogram (pg) of the pUC19 Positive Control Plasmid. To do this, dilute 1 µl of the provided pUC19 plasmid in 9 µl of sterile water (1:10 dilution), and use 1 µl of this dilution. Plate 5 µl of the electroporation mix on an LB agar plate with 100 µg/ml carbenicillin; a 1 x 10^{11} efficiency will yield 500 colonies.

Telephone: (858) 457-1919 • (888) 428-0558 (U.S. Toll-free) • Fax: 858-623-9494 • www.genlantis.com

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 and pulse the cells as follows:

For Purified Plasmid DNA (from Step 3)	**For Ligated DNA** (from cloning or ligation reactions in Steps 4 or 5)
1.8 Kilovolts (KV)	2.5 Kilovolts (KV)
400 Ohms (Ω)	100 Ohms (Ω)
1 pulse	**2 pulses***

*To perform 2 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 one more time.

   e. Recover the electroporated cells as follows:
      i. If plating an aliquot of the reaction, add 980 µl of SOC media to cuvette. Pipet up and down 6-8 times to completely suspend cells and transfer to 2059 culture tube.
      ii. If plating entire reaction, add 350 µl of SOC and suspend cells as above.
   f. Recover for 90 minutes in a 37ºC shaker at 200 rpm.
      IMPORTANT: **Do not recover for less than 90 minutes.**
   g. Plate the contents of either Step e.i. or e.ii. on an LB Agar plate containing the antibiotic that is appropriate for the electroporated plasmid DNA.
   h. Incubate plates overnight in a 37ºC air incubator.

**QIAquick is Registered Trademark of Qiagen, Inc.*

Telephone: (858) 457-1919 • (888) 428-0558 (U.S. Toll-free) • Fax: 858-623-9494 • www.genlantis.com