PRODUCT INSERT

PUREGENE
GENETIX BRAND

GeneSure™ Bacterial Transformation Kit

Store at -20°C

Cat. No.	**Pack Size**
PGK271	20 Rxns

GeneSure™ Bacterial Transformation Kit	**20 transformations**
C-Medium	35 ml
BT-Solution A	2x1.25 ml
BT-Solution B	2x1.25 ml

STORAGE

The BT-solution A and BT-solution B should be stored at -20°C till the expiry date.The C-medium can be stored at 4°C for up to two months or at -20°C for longer periods.

DESCRIPTION

Puregene's GeneSure™ Bacterial Transformation Kit provides a new method for rapid preparation of chemically competent E.coli cells from overnight bacterial culture or bacterial colonies. The key component of this system is the unique BT-solution, which produces competent cells in a few easy steps. The quick and convenient procedure provides transformation efficiencies in a range of 10^6-10^7 transformants per µg of plasmid DNA. The GeneSure™ Bacterial Transformation Kit can be applied to most E.coli strains commonly used for cloning.

• Use overnight bacterial culture to prepare competent cells in only 50 min (hands on time 20 min). Then the transformation of competent E.coli cells takes only 5 min and the cells are plated immediately. No time is wasted on heat shock or on incubation of transformed cells in SOC medium. This transformation method saves more than one hour compared to transformation of frozen competent cells.

• Use bacterial colonies, if overnight culture is not available. The procedure takes 2.5 hours (hands on time 20 min) nevertheless it allows researchers to transform E.coli strain of choice the same day.

IMPORTANT NOTES

• All procedures are performed on ice. All short centrifugations can be carried out at room temperature in a regular table-top minicentrifuge (10000-12000 x g). Make sure the cells are not left in the centrifuge at ambient temperature for more than 5 min as this will significantly decrease the transformation efficiency.

• Competent cells prepared with GeneSure™ bacterial transformation kit are suitable for direct use only. Freezing down and storage at -70°C is not recommended.

• The GeneSure kit works with ampicillin, tetracycline and chloramphenicol antibiotic resistance systems. Kanamycin systems show reduced transformation efficiencies of 105 cfu/µg DNA, which are still acceptable in routine cloning experiments.

• Do not use more than 5 µl (containing 10-100 ng of vector DNA) of ligation reaction mixture per 50 µl of competent cells.

TRANSFORMATION PROTOCOLS

Please read all IMPORTANT NOTES before starting.

Transformation Protocol from Overnight Bacterial Culture
(for 2 transformations)

• The day before the transformation seed overnight culture by inoculating 2 ml* of C-medium with a single bacterial colony. Make sure to use freshly streaked bacterial colonies (not older than 10 days**). Incubate the culture overnight at 37°C in a shaker.

• The day of transformation pre-warm culture tubes containing the required amount of C-medium (1.5 ml for each 2 transformations) at 37°C for at least 20 min. Pre-warm LB agar plates, supplemented with appropriate antibiotic in a 37°C incubator for at least 20 min before plating.

• Prepare BT-solution: thaw BT-solution A and BT-solution B, mix contents thoroughly. Combine 250 µl of BT-solution A and 250 µl of T-solution B in a separate tube and keep on ice.

Step: Procedure

1: Add 150 µl of the overnight bacterial culture to 1.5 ml of pre-warmed C-medium. Incubate 20 min at 37°C in a shaker.

2: Pellet bacterial cells by 1 min centrifugation, discard the supernatant.

3: Resuspend cells in 300 µl of BT-solution. Incubate on ice for 5 min.

4: Centrifuge for 1 min in a microcentrifuge, discard the supernatant.

5: Resuspend pelleted cells in 120 µl of BT-solution. Incubate 5 min on ice.

6: Add up to 5 µl of ligation mixture (containing 10-100 ng vector DNA) or 1 µl of supercoiled DNA (10-100 pg) into new microcentrifuge tubes. Chill on ice for 2 min.

7: Add 50 µl of the prepared cells to each tube containing DNA, mix and incubate on ice for 5 min.

8: Plate immediately on pre-warmed LB antibiotic agar plates. Incubate overnight at 37°C.

** 2 ml of overnight culture is sufficient for 26 transformations. The culture can be kept at 4°C for one week and used for preparation of competent cells.*

*** E.coli DH5a® shoud only be <24 hours fresh.*

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Transformation Protocol from Bacterial Colonies (for 2 transformations)

• Seed a LB plate with a single bacterial colony using the streak plate method and incubate the plate overnight at 37°C. Use freshly streaked bacterial colonies (not older than 10 days*), for preparation of competent cells.
• Before the transformation pre-warm culture tubes containing the required amount of C-medium (1.5 ml for each 2 transformations) at 37°C for at least 20 min. Pre-warm LB antibiotic agar plates in a 37°C incubator for at least 20 min before plating.
• Prepare T-solution: thaw BT-solution A and BT-solution B, mix contents thoroughly. Combine 250 µl of BT-solution A and 250 µl of BT-solution B in a separate tube and keep on ice.

Step : Procedure

1: Transfer a portion of freshly streaked bacterial culture (4 x 4 mm size) to 1.5 ml of pre-warmed C-medium using an inoculating loop. Suspend the cells by gently mixing and incubate the tubes at 37°C for 2 hours in a shaker.
2: Pellet bacterial cells by 1 min centrifugation, discard the supernatant.
3: Resuspend cells in 300 µl of BT-solution. Incubate on ice for 5 min.
4: Centrifuge for 1 min in a microcentrifuge, discard the supernatant.
5: Resuspend pelleted cells in 120 µl of BT-solution. Incubate 5 min on ice.
6: Add up to 5 µl of ligation mixture (containing 10-100 ng vector DNA) or 1 µl of supercoiled DNA (10-100 pg) into new microcentrifuge tubes. Chill on ice for 2 min.
7: Add 50 µl of the prepared cells to each tube containing DNA, mix and incubate on ice for 5 min.
8: Plate immediately on pre-warmed LB antibiotic agar plates. Incubate overnight at 37°C.

Scaling up

To prepare larger volumes of competent cells for transformation the procedure can be scaled up by increasing volumes of all components accordingly. In steps 2 and 4 the cells are centrifuged in large centrifuge tubes at 5000-10000 x g for 5 min at 4°C.

Plaque Formation

For plaque formation follow 1-5 steps of one of transformation protocols on p.4 or p.5. In step 6 use 10 ng of double-stranded phage DNA (e.g. M13mp19) to transform cells. In step 8, mix the transformed cells (from step 7) with 4 ml of top agar (0.7%, 55°C). Mix gently and pour the soft agar/cell mixture onto an agar plate. The number of plaques obtained depends on the bacterial strain used (e.g.CJ236: ~60 plaques, JM109: ~350 plaques).

CONTROL TRANSFORMATION

Control transformation with 100 pg of supercoiled DNA (e.g. pUC18 DNA,) should yield approximately 100 - 2000 colonies depending on the bacterial strain and antibiotic resistance system used.

Sample calculation:

If 500 colonies are counted on a control plate, the transformation efficiency is calculated as follows:

$$\frac{500 \text{ cfu on control plate}}{0.1 \text{ ng of control DNA plated}} \times 1000 \text{ ng}/\mu\text{g} = 5\times10^6 \text{ cfu}/\mu\text{g DNA}$$

LIGATION PROTOCOLS

DNA Insert Ligation into Vector DNA
1. Prepare the following reaction mixture:

Component	Sticky-end ligation	Blunt-end ligation
Linear vector DNA	20-100 ng	
Insert DNA	1:1 up to 5:1 molar ratio over vector	
10X T4 DNA Ligase buffer	2 µl	
50% PEG 4000 solution	–	2 µl
T4 DNA Ligase	0.2 µl (1 u)	1 µl (5 u)
Water, nuclease-free	to 20 µl	
Total volume	20 µl	20 µl

2. Sticky-ends: incubate for 10 min at 22°C. Blunt-ends: incubate for 1 hour at 22°C.
3. Use up to 5 µl of the mixture for transformation of 50 µl of prepared competent cells.

Note
• To increase the overall number of transformants, prolong the reaction time to 1 hour.
• T4 DNA Ligase inactivated by heating at 65°C for 10 min or at 70°C for 5 min.

Self-circularization of Linear DNA

1. Prepare the following reaction mixture:

Linear DNA	10-50 ng
10X T4 DNA Ligase buffer	5 µl
T4 DNA Ligase	1 µl (5 u)
Water, nuclease-free	to 50 µl
Total volume	**50 µl**

2. Mix thoroughly, spin briefly and incubate for 10 min at 22°C.
3. Use up to 5 µl of the mixture for transformation of 50 µl of prepared competent cells.

Note
• To increase the overall number of transformants, prolong the reaction time to 1 hour.
• T4 DNA Ligase inactivated by heating at 65°C for 10 min or at 70°C for 5 min.

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RECIPES

Ampicillin Stock Solution (50 mg/ml)
Dissolve 2.5 g of ampicillin sodium salt in 50 ml of deionized water. Filter sterilize and store in aliquots at -20°C.

X-Gal Stock Solution (20 mg/ml)
Dissolve 200 mg of X-Gal (5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside) in 10 ml dimethylformamide (DMF) or dimethylsulfoxide (DMSO). Store at -20°C in a dark. Alternatively, use X-Gal Solution, ready-to-use. Spread 40 µl on plate surface.

IPTG Stock Solution (100 mM)
Dissolve 1.2 g of IPTG (isopropyl-b-D-thiogalactopyranoside) in 50 ml of deionized water. Filter-sterilize, aliquote and store at -20°C. Alternatively, use IPTG Solution, ready-to-use. Spread 40 µl on plate surface.

LB antibiotic Plates
Prepare LB agar medium (1 liter), weigh out:

Bacto Tryptone	10 g,
Bacto Yeast extract	5 g
NaCl	5 g.

Dissolve in 800 ml of water, adjust pH to 7.0 with NaOH and adjust the volume with water to 1000 ml. Add 15 g of Agar and autoclave.
Before pouring LB ampicillin agar plates, allow the medium to cool to 55°C. Then, add 1 ml of ampicillin stock solution (50 mg/ml) to a final concentration of 50 µg/ml*. Mix gently and pour plates. Allow the LB ampicillin agar medium to solidify.

For blue/white selection, spread 40 µl of each X-Gal stock solution (20 mg/ml) and IPTG stock solution (100 mM) on the surface of the plate. Alternatively, for batch use, add 1 µl of each stock per 1 ml of LB agar (cooled to 55°C).

* When using other antibiotics, the following final concentrations are recommended: kanamycin, 30 µg/ml; tetracycline, 12 µg/ml; chloramphenicol, 20 µg/ml.

TROUBLE SHOOTING

Problem
Transformation efficiency is too low (evaluated in control transformation with supercoiled DNA)

Cause and Solution

Old bacterial cultures were used to prepare competent cells.
Seed overnight culture from a freshly streaked bacterial culture plate. Refresh bacterial strains weekly. For seeding of overnight E.coli DH5a culture, use only <24 hours fresh culture plates.

Problem
Low number or no transformants

Cause and Solution

Volume of ligation reaction mixture too large.
Do not use more than 5 µl of ligation reaction mixture per 50 µl of competent cells.

DNA amount is too high.
Do not use more than 100 ng of plasmid DNA for transformation of 50 µl of competent cells.

Inefficient ligation.
Simple sticky end ligation reactions should yield 50-200 colonies. If the efficiency of competent cells was acceptable, but transformation of ligation mixture yielded no or only few transformants, repeat cloning experiment. Use high quality DNA, enzymes and follow the recommended ligation protocol

Problem
Background colonies without plasmid

Cause and Solution

Insufficient amount of antibiotic in agar medium.
Use recommended amount of appropriate antibiotic in LB agar plates. Antibiotics are heat-sensitive therefore allow the LB medium to cool to 55°C before addition of the antibiotic to it.

Satellite colonies.
Some fast growing strains (e.g. C600) lead to formation of smaller satellite colonies around transformants after >16 hours of incubation. Use shorter incubation times and do not involve such small colonies into clone analysis.