- Transformation efficiency: 0.6-1 x 10^9 cfu/µg pUC19 DNA
- Enhanced BL21 derivative ideal for P_{lac}, P_{tac}, P_{trc} expression vectors
- Deficient in proteases Lon and OmpT
- Resistant to phage T1 *(fhuA2)*
- Does not restrict methylated DNA ($McrA^-$, $McrBC^-$, $EcoBr^-m^-$, Mrr^-)
- Free of animal products

Description:

Chemically competent *E. coli* cells suitable for high efficiency transformation and protein expression. Recommended host strain for pMAL Protein Fusion and Purification System.

Reagents Supplied:

C2523H:

20 x 0.05 ml/tube of chemically competent NEB Express Competent *E. coli* cells (**Store at -80°C**)

20 ml of SOC outgrowth medium (**Store at room temperature**)

0.025 ml of 50 pg/µl pUC19 Control DNA (**Store at -20°C**)

C2523I:

6 x 0.2 ml/tube of chemically competent NEB Express Competent *E. coli* cells (**Store at -80°C**)

25 ml of SOC outgrowth medium (**Store at room temperature**)

0.025 ml of 50 pg/µl pUC19 Control DNA (**Store at -20°C**)

Genotype: *fhuA2 [lon] ompT gal sulA11 R(mcr-73::miniTn10--*Tet^S*)2 [dcm] R(zgb-210::Tn10--*Tet^S*) endA1 Δ(mcrC-mrr)114::IS10*

Transformation Protocol Variables:

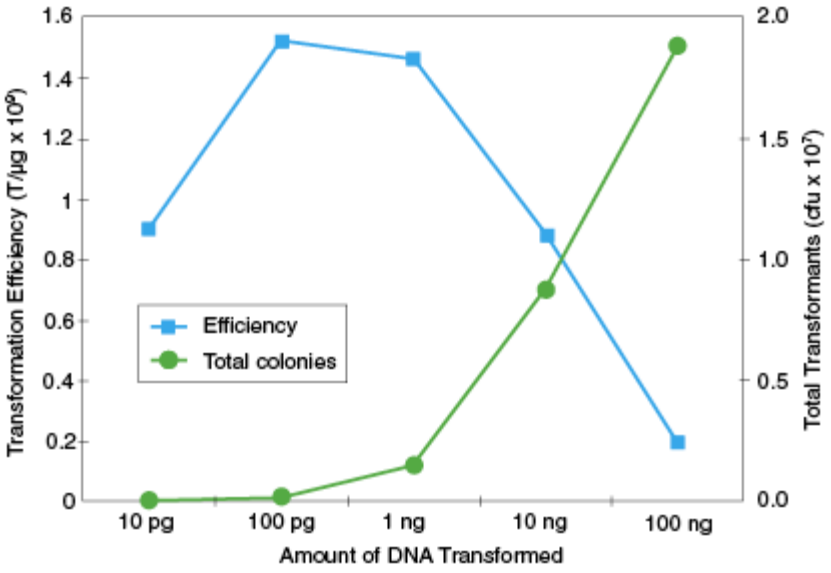
Thawing: Cells are best thawed on ice and DNA added as soon as the last bit of ice in the tube disappears. Cells can also be thawed by hand, but warming above 0°C will decrease the transformation efficiency.

Incubation of DNA with Cells on Ice: For maximum transformation efficiency, cells and DNA should be incubated together on ice for 30 minutes. Expect a 2-fold loss in transformation efficiency for every 10 minutes this step is shortened.

Heat Shock: Both the temperature and the timing of the heat shock step are important and specific to the transformation volume and vessel. Using the transformation tube provided, 20 seconds at 42°C is optimal.

Outgrowth: Outgrowth at 37°C for 1 hour is best for cell recovery and for expression of antibiotic resistance. Expect a 2-fold loss in transformation efficiency for every 15 minutes this step is shortened. SOC gives 2-fold higher transformation efficiency than LB medium; and incubation with shaking or rotating the tube gives 2-fold higher transformation efficiency than incubation without shaking.

Plating: Selection plates can be used warm or cold, wet or dry without significantly affecting the transformation efficiency. However, warm, dry plates are easier to spread and allow for the most rapid colony formation.



DNA Effects on Transformation Efficiency and Colony Output: The optimal amount of DNA to use in a transformation reaction is lower than commonly recognized. Using clean, supercoiled pUC19, the efficiency of transformation is highest in the 100 pg-1 ng range. However, the total colonies which can be obtained from a single transformation reaction increase up to about 100 ng.

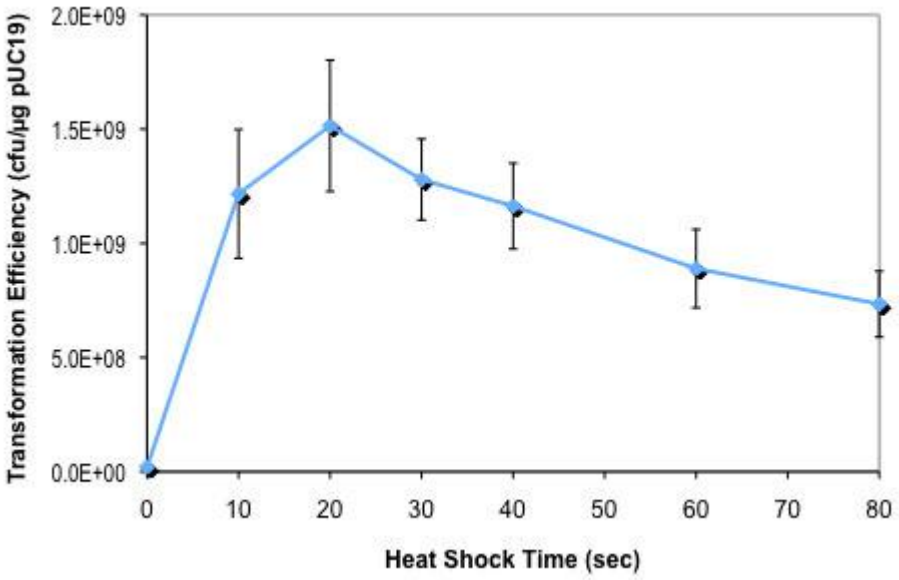
DNA Contaminants to Avoid

Contaminant	Removal Method
Detergents	Ethanol precipitate
Phenol	Extract with chloroform and ethanol precipitate
Ethanol or Isopropanol	Dry pellet before resuspending
PEG*	Column purify or phenol/chloroform extract and ethanol precipitate
DNA binding proteins* (e.g. Ligase)	Column purify or phenol/chloroform extract and ethanol precipitate

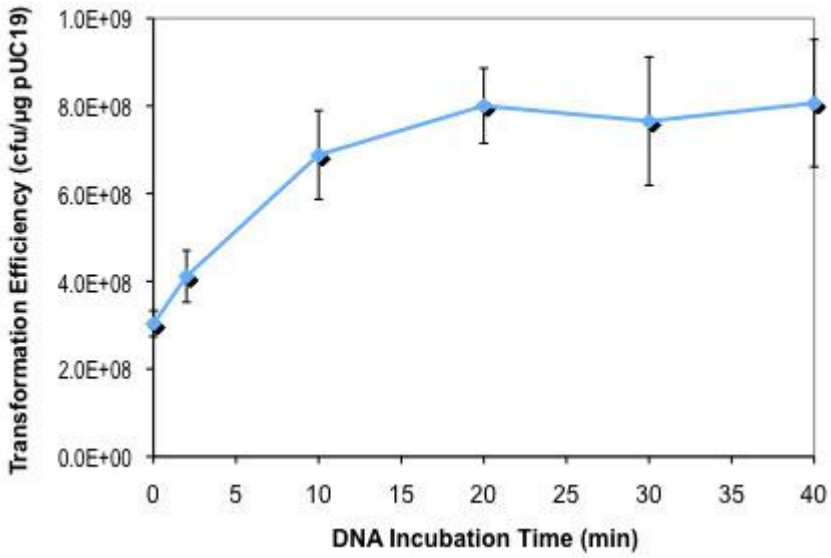
* Ideally, DNA for transformation should be purified and resuspended in water or TE. However, up to 10 µl of DNA directly from a ligation mix can be used with only a two-fold loss of transformation efficiency. Where it is necessary to maximize the number of transformants (e.g. a library), a purification step, either a spin column or phenol/chloroform extraction and ethanol precipitation should be added.

Antibiotics for Plasmid Selection

Antibiotic	Working Concentration
Ampicillin	100 µg/ml
Carbenicillin	100 µg/ml
Chloramphenicol	33 µg/ml
Kanamycin	30 µg/ml
Streptomycin	25 µg/ml
Tetracycline	15 µg/ml



Effect of heat shock time on NEB Express competent *E. coli* transformation efficiency: 50 µl of competent cells were transformed with 100 pg of pUC19 control DNA following the provided High Efficiency Transformation Protocol except heat shock time varied from 0 to 80 seconds.



Effect of DNA incubation time on NEB Express competent *E. coli* transformation efficiency: 50 µl of competent cells were transformed with 100 pg of pUC19 control DNA following the provided High Efficiency Transformation Protocol except DNA incubation time varied from 0 to 40 minutes.