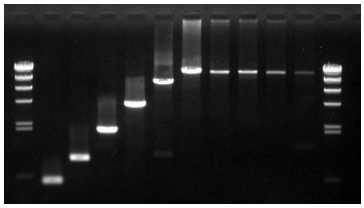
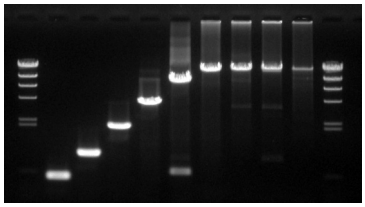
Human genomic DNA
(Template: 100 ng/50 μl reaction)




1. p53 0.5 kb
2. DCLRE 1A 1 kb
3. DCLRE 1A 2 kb
4. DCLRE 1A 4 kb
5. β-globin 8.5 kb
6. β-globin 15 kb
7. β-globin 20 kb
8. β-globin 24 kb
9. β-globin 27 kb
10. β-globin 30 kb

M. λ-*Hin*d III digest

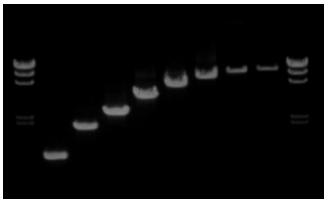
λDNA
(Template: 1 ng/50 μl reaction)




1. 0.5 kb
2. 1 kb
3. 2 kb
4. 4 kb
5. 8 kb
6. 15 kb
7. 20 kb
8. 30 kb
9. 40 kb

M. λ-*Hin*d III digest

cDNA
(Template: equivalent of 250 ng total RNA/50 μl reaction)

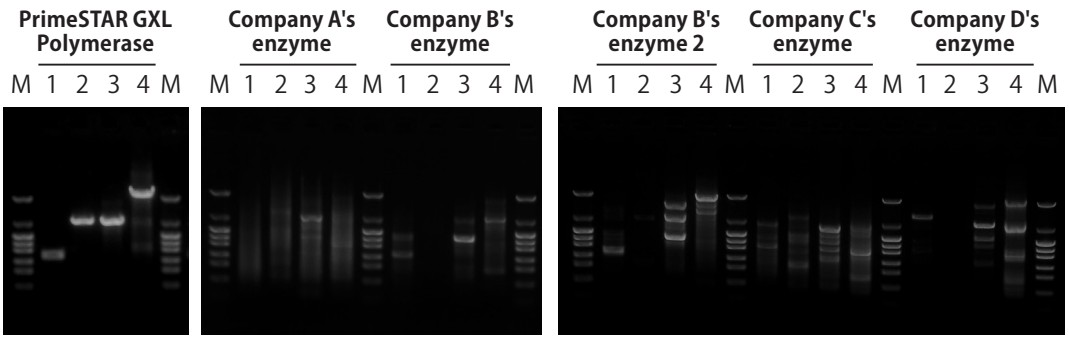



1. Dystrophin 1 kb
2. Dystrophin 2 kb
3. CCND2 2.8 kb
4. TFR 4 kb
5. Dystrophin 6 kb
6. Dystrophin 8 kb
7. Dystrophin 12 kb
8. Dystrophin 13.5 kb

M. λ-*Hin*d III digest

### C. Amplification of GC-rich targets

PrimeSTAR GXL DNA Polymerase allows for highly specific amplification of GC-rich templates that are otherwise challenging. Excellent results are achieved without special buffers or reaction conditions. The performance of PrimeSTAR GXL DNA Polymerase in comparison to other commercially available high-fidelity DNA polymerases and polymerases optimized for GC-rich templates is shown below. Reactions were performed according to the protocols specified by each manufacturer.




Template: Human genomic DNA (100 ng/50 μl reaction)
1. APOE gene 746 bp (GC-content 74%)
2. TGF β1 gene 2,005 bp (GC-content 69%)

Template: *T. thermophilus* HB8 genomic DNA
(10 ng/50 μl reaction)
3. 2,029 bp (GC-content 74%)
4. 4,988 bp (GC-content 74%)

M : pHY Marker

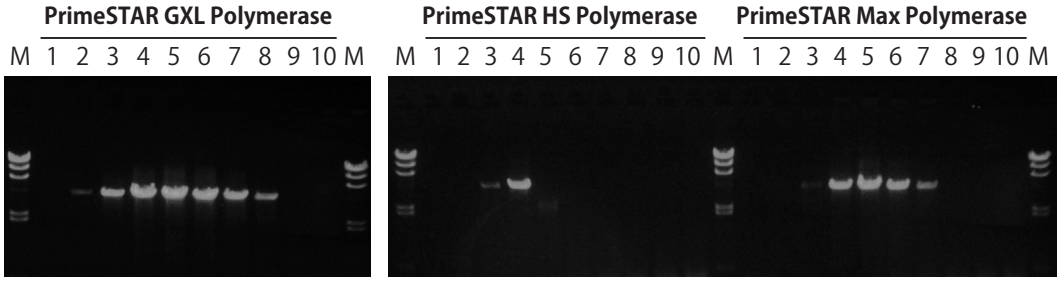
Company B's enzyme 2:
Optimized for GC-rich templates

Company D's enzyme:
Includes buffers optimized for GC-rich templates

**D. Sensitivity and range of template quantity**

Conventional high-fidelity PCR enzymes are relatively easily affected by excess nucleic acid in the reaction mixture, and frequently do not readily amplify cDNA templates. In contrast, PrimeSTAR GXL DNA Polymerase shows excellent activity over a wide range of template quantities, and therefore, is well-suited to amplification of cDNA templates.

(1) Using cDNA templates obtained by reverse transcription of various quantities of total RNA prepared from HL-60 cells, the transferrin receptor (TFR) gene (4 kb) was amplified using each enzyme in the PrimeSTAR series. Sensitivity and the range of template quantity were compared.



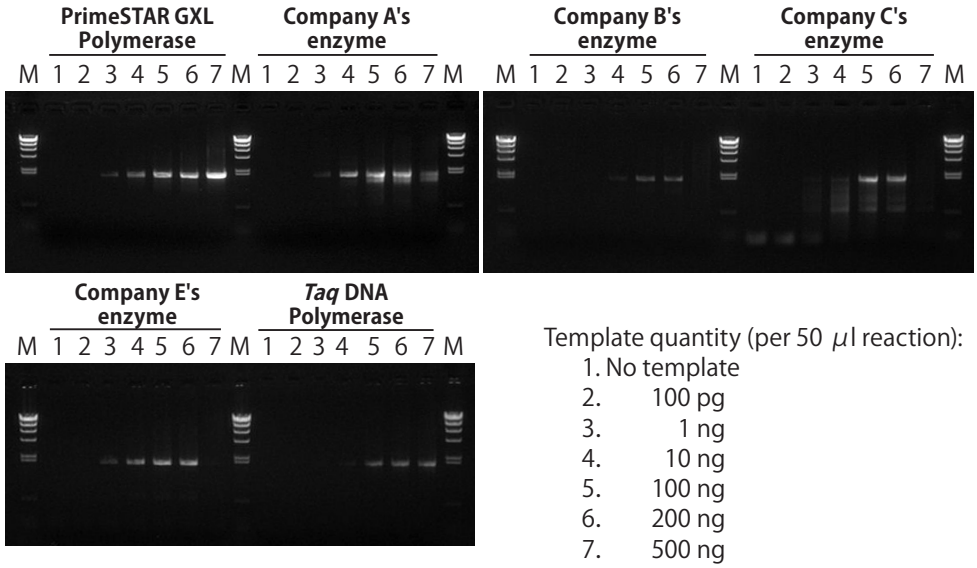

cDNA template quantity (equivalent to total RNA amounts/50 μl reaction):

1. 25 pg
2. 250 pg
3. 2.5 ng
4. 25 ng
5. 250 ng
6. 500 ng
7. 750 ng
8. 1 μg
9. 1.5 μg
10. 2 μg

M. λ-*Hin*d III digest

PrimeSTAR GXL DNA Polymerase demonstrated good amplification over a wide range of template cDNA quantity, as well as excellent sensitivity.

(2) Using various quantities of human genomic DNA as a template, the amplification efficiency of PrimeSTAR GXL DNA Polymerase was compared to the efficiencies of other commercially available high-fidelity PCR enzymes and *Taq* DNA Polymerase. Reactions were performed according to the protocols specified by each manufacturer.




Template: Human genomic DNA
Target: DCLRE 1A gene (2 kb)

Template quantity (per 50 μl reaction):
1. No template
2. 100 pg
3. 1 ng
4. 10 ng
5. 100 ng
6. 200 ng
7. 500 ng
M. λ-*Hin*d III digest

PrimeSTAR GXL DNA Polymerase demonstrated superior sensitivity and amplification efficiency in comparison to other commercially available high-fidelity PCR enzymes and *Taq* DNA Polymerase. High activity was observed for PrimeSTAR GXL DNA Polymerase even in the presence of excess template DNA that suppressed the activity of high-fidelity PCR enzymes from other companies.

## IX. Related Products

PrimeSTAR® Max DNA Polymerase (Cat. #R045A/B)
PrimeSTAR® HS DNA Polymerase (Cat. #R010A/B)
PrimeSTAR® HS (Premix) (Cat. #R040A)
TaKaRa PCR Thermal Cycler Dice™ Gradient (Cat. #TP600)
Mighty Cloning Reagent Set (Blunt End) (Cat. #6027)
NucleoSpin Gel and PCR Clean-Up (Cat. #740609.50/.250)*
Agarose L03「TAKARA」(Cat. #5003)
PrimeGel™ Agarose PCR-Sieve (Cat. #5810A)

＊ Not available in all geographic locations. Check for availability in your area.

PrimeSTAR is a registered trademark of Takara Bio Inc.
Thermal Cycler Dice and PrimeGel are trademarks of Takara Bio Inc.

# NEB Stable Heat Shock Transformation

**Date:** 2023-09-07
**Tags:** Cloning | Protocol | Molecular biology
**Category:** Protocols
**Created by:** Ben Rasmus Lenkewitz

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Note:

There are different kind of competent cells and transformation protocols. Usually all of them work, but here are some general guidelines:

- Work on ice!
- Avoid shaking the cells (especially when plasmid was added).

Here, a high efficiency protocol by NEB is presented:

1. Thaw a tube of NEB Stable Competent *E. coli* cells on ice for 10 minutes.
2. Add 1 -2 µl containing 100 pg – 100 ng of plasmid DNA to the cell mixture. Carefully flick the tube 4-5 times to mix cells and DNA. Do not vortex.
3. Place the mixture on ice for 30 minutes. Do not mix.
4. Heat shock at exactly 42°C for exactly 30 seconds. Do not mix. Place on ice for 5 minutes. Do not mix.
5. Pipette 950 µl of room temperature NEB 10-beta/Stable Outgrowth Medium into the mixture and place at 30°C for 60 minutes. Shake the tube horizontally at 250 rpm or rotate.
6. Warm selection plates to 30°C.
7. Mix the cells thoroughly by flicking the tube and inverting. Then spread 50-100 µl of cells or diluted cells onto a selection plate. Incubate plates 24 hrs at 30°C or overnight at 37°C.

*Note: For best clone stability, incubate plates and liquid cultures at 30°C and prepare plasmid DNA from fresh transformants (from plates not greater than 3 days old). Store unstable clones as plasmid DNA, rather than cell-based glycerol stocks.*

# High Efficiency Transformation for NEB® Stable Competent *E. coli* (C3040H)

1. Thaw a tube of NEB Stable Competent *E. coli* cells on ice for 10 minutes.
2. Add 1 -2 µl containing 100 pg – 100 ng of plasmid DNA to the cell mixture. Carefully flick the tube 4-5 times to mix cells and DNA. Do not vortex.
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## Links to this resource

**Product Categories:** Cloning Competent Cell Strains Products

**Applications:** Transformation

**Related Products:** NEB® Stable Competent *E. coli* (High Efficiency)

## Attached files

High-Efficiency-Transformation-Protocol-C3040H-NEB.jpg
sha256: b7da4a55166558a3cb415e60ea04181d78e2b2e2d45dff85a928ae2c1bcc67de

High-Efficiency-Transformation-Protocol-C3040H-NEB.pdf
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Unique eLabID: 20230907-6bdc22349ab53e6296778c45a63eeaf05f77f91b
Link: https://elabftw-lehre.hhu.de/database.php?mode=view&id=93

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*Note: For best clone stability, incubate plates and liquid cultures at 30°C and prepare plasmid DNA from fresh transformants (from plates not greater than 3 days old). Store unstable clones as plasmid DNA, rather than cell-based glycerol stocks.*

## Links to this resource

**Product Categories:** Cloning Competent Cell Strains Products

**Applications:** Transformation

**Related Products:** NEB® Stable Competent *E. coli* (High Efficiency)

# LiAc transformation protocol

**Date:** 2023-08-16
**Tags:** Yeast Protocol Microbiology
**Category:** Protocols
**Created by:** Julia Smoluk

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Inoculate 5 ml preculture (YPD)
Incubate at 28°C overnight
Inoculate 25 ml main culture (YPD) with an OD600 of 0.15
Incubate at 28°C for 3-5 h
25 ml are enough for ≤ 5 transformations
Defrost PEG & carrier DNA
Preheat heating block (99°C)
Thaw PEG and carrier DNA
After 2-3 cell divisions (3-5 h):
harvest cells at 1600 × g for 5 min
Wash cells with 25 ml sterile H2O

Resuspend cells in 1 mL 100 mM lithium acetate
Boil carrier DNA at 99°C for 10 min and chill it on ice
Transfer cell suspension to a 1.5 ml eppi

13,000 × g, 1 min → discard supernatant
Resuspend cells in 500 µl 100 mM lithium acetate

For each transformation, transfer 100 µl cell suspension to a new 1.5 ml eppi
13,000 × g, 1 min → remove supernatant

Add in the following order:
240 µl PEG 3350 (50%)
36 µl lithium acetate (1 M)
20 µl boiled carrier DNA (Salmon testes DNA, 5 mg/ml)

DNA:
e.g. two PCRs or
2 µl self-replicating plasmid or
20 µl plasmid digest containing ~ 2-5 µg plasmid
Add 360 µl with H2O if necessary

Vortex vigorously until the cell pellet is completely resuspended
Incubate at 30°C for 30 min (heating block without shaking)

Incubate at 42°C for 35 min (heating block without shaking)
13,000 × g, 1 min → discard supernatant

Only for selection with antibiotics:
Resuspend cells in 1 ml YPD

Incubate at 30°C for > 1 h at 200 rpm (lying eppi in the shaker)
13,000 × g, 1 min → discard supernatant
Resuspend cells in 400 µl sterile H2O
Streak cells on 2 selection plates (1x 100 µl, 1x 300 µl) using a 1 ml glass pipette
Incubate plates at 28°C for ~ 3-5 days

Hegemann JH, Heick SB, Pöhlmann J, Langen MM, Fleig U. Targeted gene deletion in Saccharomyces cerevisiae and Schizosaccharomyces pombe. Methods Mol Biol. 2014;1163:45-73. doi: 10.1007/978-1-4939-0799-1_5. PMID: 24841299.

## Attached file

hegemann2014.pdf
sha256: 82bf70899b42af17df07e1cf1c5e9294bf860d62e0e6ba0d0e6a02ff4aa20bc5

Unique eLabID: 20230816-d940a3ad61045341ed501e497a70984a8bc8e564
Link: https://elabftw-lehre.hhu.de/database.php?mode=view&id=84