NEB® PCR Cloning Kit

(with or without competent cells)

NEB #E1202S, NEB #E1203S

20 reactions

Version 5.0_5/21

Table of Contents

Introduction 2
Cloning Kit Protocol Overview 2
Detailed Protocols 3
Vector Map & Sequence Information 4
Insert Screening Protocols 5
RNA Synthesis of Cloned Transcripts 6
FAQs 7
Ligation Tips 8
What to Expect from Your Cloning Experience 9
Supporting Documents, References 9
Ordering Information 9
Revision History 10

The NEB PCR Cloning Kits Include

A sufficient supply of materials to perform 20 x 10 µl cloning reactions.
Primers are also provided, allowing screening for inserts by colony PCR and/or sequencing.

KIT COMPONENTS	NEB #E1202	NEB #E1203
Cloning Mix 1*	80 µl	80 µl
Cloning Mix 2*	20 µl	20 µl
Linearized pMiniT™ 2.0 Vector	20 µl	20 µl
Amplicon Cloning Control (1 kb)	10 µl	10 µl
Cloning Analysis Forward Primer	35 µl	35 µl
Cloning Analysis Reverse Primer	35 µl	35 µl
NEB 10-beta Competent *E. coli* (Cloning Efficiency)	20 x 50 µl	
pUC19 Control DNA	25 µl	
NEB 10-beta/Stable Outgrowth Medium	25 ml	

**Cloning mixes 1 and 2 provide T4 DNA Ligase, ATP, proprietary ligation enhancers and end polishing components.*

Properties and Storage

NEB PCR Cloning Kit (NEB #E1202):

The kit is shipped on dry ice. Upon arrival, store the competent cells (in the large exterior box) at –80°C, the components in the small interior box at –20°C and the NEB 10-beta/Stable Outgrowth Medium at room temperature or 4°C.

NEB PCR Cloning Kit (without competent cells) (NEB #E1203):

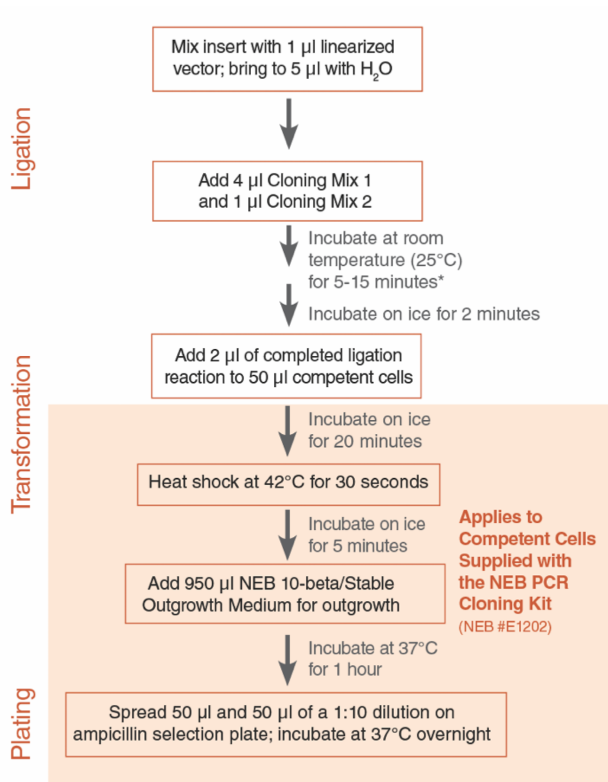
The kit is shipped on wet ice. Upon arrival, store the components at –20°C.

Introduction

This PCR Cloning Kit contains optimized Cloning Mixes featuring a proprietary ligation enhancer, and a linearized vector that uses a novel mechanism for background colony suppression to give a low background. It allows simple and quick cloning of any PCR amplicon, whether the amplification reactions are performed with proofreading DNA polymerases, such as Q5® which produce blunt ends, or nonproofreading DNA polymerases, such as *Taq* or *Taq* mixes (One*Taq*®, LongAmp® *Taq*) which produce single base overhangs. This is possible due to “invisible” end polishing components that are active during the ligation step only if needed. The kit also allows direct cloning from amplification reactions without purification, and works well whether or not the primers used in the PCR possess 5´-phosphate groups.

The ultimate in flexibility: clone with any amplicon made with any DNA polymerase, with or without 5´ phosphates, purified or not!

Figure 1. Cloning Kit Protocol Overview



Detailed Protocols

Ligation Protocol:

1. Assemble ligation reactions using the chart below as a guide. Mix the first 4 components before adding 5 µl of the cloning mix consisting of 4 µl Cloning Mix 1 and 1 µl Cloning Mix 2, for a total of 10 µl per ligation reaction. This ensures the ligase is not allowed to recircularize the vector backbone before the insert is present. It is recommended that first-time users of this kit perform the positive control ligation reaction.

	LIGATION REACTION	POSITIVE CONTROL
Linearized pMiniT 2.0 Vector (25 ng/µl)	1 µl (25 ng)	1 µl (25 ng)
Insert*	1-4 µl*	–
Amplicon Cloning Control (1 kb) (15 ng/µl)	–	2 µl (30 ng)
H_2O	to 5 µl	2 µl
Cloning Mix 1	4 µl	4 µl
Cloning Mix 2	1 µl	1 µl
Total Volume	10 µl	10 µl

*For purified PCR amplicon products, the amount of insert to be added can be calculated by relative length or molar calculations. For illustrative purposes calculations are shown below; however, the NEBiocalculator web tool (**www.NEBioCalculator.neb.com**) is a quick and convenient way to determine the insert amounts for all cloning reactions. Formulas below use the recommended values of 25 ng of linearized vector (2,588 bp) per reaction and an insert-to-vector ratio of 3:1.

a. Relative length calculations:
   ng insert to be added = (3)(25 ng vector) (bp of insert/2,588 bp of vector)

b. Molar calculations:
   i. Convert the 25 ng vector present in the ligation reaction to pmoles: (25 ng vector)(1,000)/(650 daltons per base pair) (number of base pairs in vector or 2,588) = (25)(1,000)/(650)(2,588) = 0.015 pmoles vector
   ii. Calculate a 3-fold molar amount of insert to add to each ligation: (3)(0.015 pmoles vector) = 0.045 pmoles insert
   iii. Convert the pmoles insert amount to ng insert to be added: ng insert to be added = (0.045 pmoles insert) (base pairs in insert)(650 daltons per base pair)/1000

As examples, these calculations will yield insert levels of 15 ng (500 bp insert), 30 ng (1 kb insert) or 60 ng (2 kb insert).

For unpurified PCR amplicons, analyze 5% of your reaction by agarose gel electrophoresis both to confirm the specificity of the product and to estimate the DNA concentration of the product by comparing the amplicon yield to known amounts of DNA fragments in a marker lane, such as our Quick-Load Purple 2-Log DNA Ladder (NEB #N0550). This quantitation allows estimating the appropriate amount of the PCR volume to achieve a 3:1 ratio of insert:vector backbone. Both too low a level of insert, or such a high level of insert that insert ligates to both ends of the linearized vector, will decrease cloning efficiency. Do not use more than 1 µl of a PCR for cloning reactions to avoid carrying over PCR components that will interfere with cloning.

2. Incubate at room temperature (25°C) for 5–15 minutes. While 5 minutes is recommended, 15 minutes will increase transformation levels for inserts suspected as being difficult to clone.
3. Incubate on ice for 2 minutes.
4. Transform immediately or store at –20°C. For best results, transform into NEB 10-beta Competent *E. coli* (NEB #C3019), which are supplied with NEB #E1202.

Transformation Protocol:

The following protocol is designed for NEB 10-beta Competent *E. coli* (NEB #C3019) which are included in the NEB PCR Cloning Kit (NEB #E1202) **only**. Competent cells are also available separately for use with NEB #E1203**. Important – please read the FAQs regarding competent cell requirements before using a different cell strain.**

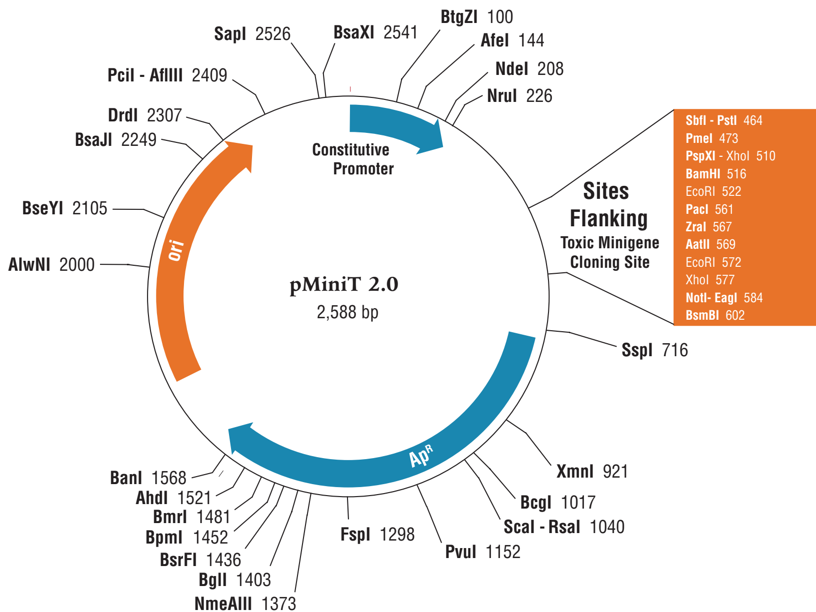
1. Thaw a 50 µl tube of competent cells on ice for 10 minutes.
2. Add 2 µl of ligation reaction; gently mix by flicking the tube 4–5 times.
3. Incubate on ice for 20 minutes.
4. Heat shock at 42°C for 30 seconds.
5. Chill on ice for 5 minutes.
6. Add 950 µl of NEB 10-beta/Stable Outgrowth Medium and place at 37°C for 60 minutes with rotation or shaking at 250 rpm (outgrowth step).

Note: For further general discussion about transformations with NEB 10-beta Competent *E. coli*, please refer to the transformation protocol at www.neb.com/C3019. The cloning efficiency cells provided with the NEB PCR Cloning Kit (NEB #E1202) differ from the high-efficiency strain in their transformation efficiencies only.

Plating Protocol:

1. Mix cells thoroughly by flicking or inversion and spread 50 µl of the 1 ml outgrowth and 50 µl of a 1:10 dilution onto 37°C pre-warmed agar plates containing 100 µg/ml ampicillin. For 15 minute ligations, it is especially important to spread both volumes of outgrowth as the selection for cloned inserts is strongest with plating densities < 400 colonies per plate.
2. Invert plate and incubate overnight at 37°C or for 24 hours at 30°C. Do not use room temperature growth as the slow growth rate will interfere with selection of constructs with inserts.

Vector Maps and Sequence:



Features within Sequence Flanking the Toxic Minigene/Cloning Site

Cloning Analysis Forward Primer →

SbfI/PstI PmeI

5´ ACC TGC CAA CCA AAG CGA GAA CAA AAC ATA ACA TCA AAC GAA TCG ACC GAT TGT TAG GTA ATC GTC ACC TGC AGG AAG GTT
3´ TGG ACG GTT GGT TTC GCT CTT GTT TTG TAT TGT AGT TTG CTT AGC TGG CTA ACA ATC CAT TAG CAG TGG ACG TCC TTC CAA

PmeI | SP6 Promoter → +1 | PspXI/XhoI | BamHI | EcoRI

5´ TAA ACG CAT TTA GGT GAC ACT ATA GAA GTG TGT ATC GCT CGA GGG ATC CGA ATT CAG GAG GTA AAA ACC
3´ ATT TGC GTA AAT CCA CTG TGA TAT CTT CAC ACA TAG CGA GCT CCC TAG GCT TAA GTC CTC CAT TTT TGG

ATG AT | INSERT | C TGA TAA TAA
TAC TA | | G ACT ATT ATT

Met Ile (interrupted) * * *

Two Amino Acid Toxic Minigene with Cloning Site Shown

(As diagrammed, minigene inactivated if insert cloned into site)

PacI | ZraI/AatII | EcoRI/XhoI | NotI/EagI | BsmBI

5´ TTA ATT AAG ACG TCA GAA TTC TCG AGG CGG CCG CAT GTG CGT CTC CCT ATA GTG AGT CGT ATT AAT TTC GCG GGC
3´ AAT TAA TTC TGC AGT CTT AAG AGC TCC GCC GGC GTA CAC GCA GAG GGA TAT CAC TCA GCA TAA TTA AAG CGC CCG

+1 ← T7 Promoter

5´ GGA ACC CCT ATT TGT TTA TTT TTC TAA ATA CAT TCA AAT ATG TAT CCG CTC ATG AGA CAA TAA CCC TGA 3´
3´ CCT TGG GGA TAA ACA AAT AAA AAG ATT TAT GTA AGT TTA TAC ATA GGC GAG TAC TCT GTT ATT GGG ACT 5´

← Cloning Analysis Reverse Primer

Top map shown above displays the construct formed if no insert is present. Unique restriction sites are shown in bold. Additional restriction sites that can be used for subcloning are also shown. Expanded box below shows location of sequencing primers, restriction sites for subcloning or linearization for in vitro transcription, RNA Polymerase promoter sequences and placement of insertion site within the toxic minigene.

DNA Sequence Information:

Cloning Analysis Forward Primer: `5´ ACCTGCCAACCAAAGCGAGAAC 3´`

Cloning Analysis Reverse Primer: `5´ TCAGGGTTATTGTCTCATGAGCG 3´`

Amplicon Cloning Control: Purified 1,003 bp ("1 kb") amplicon produced by using the L30350F and L71-1R primers as described in Wang, Y. et al. (1).

Sequence of 1 kb (1,003 bp) Amplicon Cloning Control:

```
                                                           c ctgctctgcc
gcttcacgca gtgcctgaga gttaatttcg ctcacttcga acctctctgt ttactgataa
gttccagatc ctcctggcaa cttgcacaag tccgacaacc ctgaacgacc aggcgtcttc
gttcatctat cggatcgcca cactcacaac aatgagtggc agatatagcc tggtggttca
ggcggcgcat ttttattgct gtgttgcgct gtaattcttc tatttctgat gctgaatcaa
tgatgtctgc catctttcat taatccctga actgttggtt aatacgcttg agggtgaatg
cgaataataa aaaaggagcc tgtagctccc tgatgatttt gcttttcatg ttcatcgttc
cttaaagacg ccgtttaaca tgccgattgc caggcttaaa tgagtcggtg tgaatcccat
cagcgttacc gtttcgcggt gcttcttcag tacgctacgg caaatgtcat cgacgttttt
atccggaaac tgctgtctgg ctttttttga tttcagaatt agcctgacgg gcaatgctgc
gaagggcgtt ttcctgctga ggtgtcattg aacaagtccc atgtcggcaa gcataagcac
acagaatatg aagcccgctg ccagaaaaat gcattccgtg gttgtcatac ctggtttctc
tcatctgctt ctgctttcgc caccatcatt tccagctttt gtgaaaggga tgcggctaac
gtatgaaatt cttcgtctgt ttctactggt attggcacaa acctgattcc aatttgagca
aggctatgtg ccatctcgat actcgttctt aactcaacag aagatgcttt gtgcatacag
cccctcgttt attatttatc tcctcagcca gccgctgtgc tttcagtgga tttcggataa
cagaaaggcc gggaaatacc cagcctcgct ttgtaacgga gtagacgaaa gtgattgcgc
ctacccggat attatcgtga ggatgcgtca tc
```

Insert Screening Protocols

Screening for inserts can be performed by colony PCR, restriction enzyme digestion or PCR of miniprep plasmid DNA, or by sequencing of the miniprep plasmid DNA.

Many DNA polymerases are suitable for colony PCR. For inserts < 1 kb, NEB recommends OneTaq DNA Polymerase (NEB #M0480) or One*Taq* Hot Start DNA Polymerase (NEB #M0481). *Taq* DNA Polymerase (NEB #M0267) can also be used. For inserts > 1 kb NEB recommends One*Taq* formulations, LongAmp *Taq* DNA Polymerase (NEB #M0323) or LongAmp Hot Start *Taq* DNA Polymerase (NEB #M0534); the LongAmp *Taq* formulations are especially useful for larger inserts. These products are also available in easy-to-use master mix formats. Following your choice of DNA polymerase, use the provided sequence of the cloning analysis forward and reverse primers and the Tm calculator found on the NEB website (TmCalculator.neb.com) to determine the annealing temperature for your PCR reactions.

Screening Protocol 1: Colony PCR using One*Taq* 2X Master Mix with Standard Buffer (NEB #M0482) or LongAmp *Taq* 2X Master Mix (NEB #M0287)

1. Prepare a PCR mix of sufficient volume to allow 50 µl per screened colony:

	AMOUNT	FINAL CONCENTRATION
One*Taq* or LongAmp *Taq* 2X Master Mix	25.0 µl	1X
Cloning Analysis Forward Primer (100 µM)	0.15 µl	300 nM
Cloning Analysis Reverse Primer (100 µM)	0.15 µl	300 nM
H_2O	24.7 µl	
Total	to 50 µl	

2. Use a sterile toothpick or pipette tip to pick an individual colony, and dip into each amplification reaction. To create a stock of each individual colony, either dip the same toothpick or pipette tip into 3 ml growth media containing ampicillin, or use a separate agarose-ampicillin plate to prepare a streak or patch of the colony material.

2a. Transfer the reactions to a thermocycler and perform PCR with the following conditions:

STEP	TEMP	TIME
Initial Denaturation	94°C	2 minutes
30 Cycles	94°C	15 seconds
	53-57°C*	15 seconds
	68°C	60 seconds/kb
Final Extension	68°C	5 minutes
Hold	4-10°C	∞

*For One*Taq*, use 53°C; for LongAmp, use 57°C

3. Load 5 µl of each completed PCR onto an agarose gel alongside an appropriate DNA ladder [e.g., Quick-Load Purple 2-Log DNA Ladder (NEB #N0550)]. For reference, the amplicon length in the absence of an insertion would be 309 bp in length. The amplicon length with the positive control insert would be 1,312 bp.

Screening Protocol 2: Sequence Analysis

The Cloning Analysis Forward and Reverse Primers can also be used for sequencing inserts. This can be performed with purified plasmids from overnight cultures from each colony, or with amplicons from the above colony screening PCRs. The primers anneal 155 bp upsteam and 154 bp downsteam (measured from the 5´ end of each primer to cloning insertion site), ensuring complete reads of the insert.

RNA Synthesis of Cloned Insert Transcripts:

The NEB PCR Cloning Kit vector, pMiniT 2.0, has *in vitro* transcription capabilities. NEB has a variety of T7 RNA polymerase-based high yield transcription kits (NEB #E2040, #E2050) and mRNA synthesis kits (NEB #E2065, #E2060). We suggest using the "Recommended HiScribe™ RNA Synthesis Kits by Application" selection chart in our Tools & Resources section.* These kits include optimized protocols to synthesize high yield RNA for a variety of applications. For RNA synthesis using the stand-alone enzymes T7 (NEB #M0251) or SP6 (NEB #M0207) RNA Polymerases, the protocol below can be used; companion products are listed on page 9.

*Chart can also be located by use of the search window on the main landing page using this title.

RNA Synthesis Protocol

1. Linearize the purified plasmid containing your cloned insert downstream of the insertion site utilizing any of the restriction enzyme sites flanking the insertion site after confirming your insert does not contain any internal sites for your chosen restriction enzyme. We recommend restriction digests leaving blunt or 5´ overhang ends.
2. Purify and quantitate your DNA by use of Nanodrop, Qubit or UV absorption approaches.
3. Assemble the RNA synthesis reaction at room temperature in the following order:

COMPONENTS	AMOUNT	FINAL CONCENTRATION
Nuclease-free Water	X µl	
RNAPol Reaction Buffer (10X)	2 µl	1X
Ribonucleotide Solution Mix (25 mM each)	3.2 µl	4 mM each
Template DNA	X µl	0.2-1 µg
$MgCl_2$ (100 mM)	2.8 µl	Additional 14 mM*
RNase Inhibitor, Murine or Human Placenta (40 units/µl)	0.5 µl	1 unit/µl final
Fresh DTT (100 mM) (optional)	1 µl	5 mM final
T7 RNA Polymerase (50 units/µl) or SP6 RNA Polymerase (20 units/µl)	2 µl	100 units (T7) or 40 units (SP6)
Total Volume	20 µl	

*1X reaction buffer contributes 6 mM for a final concentration of 20 mM.

4. Incubate at 37°C for 1 hour. For shorter (< 300 nt) transcripts incubate at 37°C for 2-4 hours.

Note: Higher yields of RNA using this standard protocol may be obtained by adding higher levels of RNA polymerase and increasing the amount of template in the reaction.

Frequently Asked Questions (FAQs)

For a complete list of FAQs, please visit the product page at www.neb.com

Q1. How does the NEB PCR Cloning Kit work?

A1: Open reading frames coding for 5 amino acid residues or less are difficult for *E. coli* to process (2,3). Such small genes, or minigenes, lead to premature release of the very small peptide from the ribosome, with the last amino acid residue still covalently attached to its tRNA, such that the tRNA is not readily available for protein synthesis. If the pMiniT 2.0 vector recircularizes without an insert, it will cause lethal inhibition of protein synthesis and no colony will result. If the pMiniT 2.0 vector carries an insert, a colony will grow.

Q2. How can the cloning vector work with both blunt-ended amplicons and single-base overhang-containing amplicons?

A2: The mixes contain end-polishing components that will convert single-base overhangs, such as those present in amplicons made by *Taq* DNA polymerase or *Taq*-based mixes, to blunt ends. The blunt-end form then can be ligated into the pMiniT 2.0 vector.

Q3. Do these polishing components present in the mix affect my cloning efficiency if my insert already has blunt ends?

A3: No. The concentrations of the end-polishing components are carefully optimized so that any single base overhang will be removed to form a blunt end; already blunt-ended PCR products are not affected.

Q4. Do my inserts have to possess 5´ phosphates?

A4: No, this cloning strategy works well with inserts whether or not they possess 5´ phosphates. In fact, using non-phosphorylated primers for PCR eliminates the possibility of multiple inserts being cloned into the pMiniT 2.0 vector.

Q5. Can the cloning kit be used for inserts that are not necessarily PCR amplicons?

A5: Yes, the kit works equally well with blunt-ended and single-base overhang restriction fragments or synthetic DNA.

Q6. Can the cloning kit be used with inserts containing 5´ or 3´ overhangs greater than the single-base overhang achieved by PCR with *Taq* DNA Polymerase?

A6: Yes, but with lower efficiencies. The polishing components are optimized for removal of a single base overhang. As an extreme test example, cloning a DNA fragment possessing a 4-base, 5´ overhang requiring a fill in and a 5-base, 3´ overhang requiring a chew back resulted in 10-fold fewer transformants than blunt end or single-base overhang DNA fragments.

Q7. Can I use the NEB PCR Cloning Kit featuring pMiniT 2.0 for Golden Gate Assembly?

A7: pMiniT 2.0 has no BsaI sites so it can be used to clone Golden Gate Modules that have BsaI sites.

Q8. Does the PCR product need to be purified?

A8. No, although higher transformation efficiencies are achieved with purified inserts. If using unpurified PCR amplicons, use 1 µl or less of the PCR as insert material to achieve a 3:1 molar ratio of insert:vector. It is especially important for high yield PCR situations to avoid adding a vast excess of insert that would result in the insert ligating to both ends of the vector backbone.

Q9. Can I use a different competent *E. coli* strain than the recommended NEB 10-beta strain?

A9. While this cloning system works well with a variety of cell strains, it is important to use a cell strain with robust growth to maintain selection pressure towards plasmid constructs containing your insert. In addition to the provided NEB 10-beta Competent *E. coli* (Cloning Efficiency) in NEB PCR Cloning Kit (NEB #E1202), cell strains that work well with this cloning kit include NEB Turbo (NEB #C2984) and NEB Express (NEB #C2523) Competent *E. coli*. The 5-alpha strains are not recommended, as their slightly slower growth rate does not support strong background suppression.

Q10. How can I maximize the number of transformants?

A10. Use the longer suggested time periods for the protocol steps. If desired, plate additional 50 µl aliquots of the 1 ml outgrowth onto additional plates, but do not plate more than 50 µl per plate to maintain a low background.

Q11. Can I scale down the reactions to use less vector?

A11. The vector used per reaction can be decreased from 25 ng to 10 ng. Decrease the amount of insert proportionally to maintain the optimal 3:1 insert:vector molar ratio, and also the amount of added Cloning Mix 1 and Cloning Mix 2.

Q12. Are there limits regarding the size of inserts that can be cloned?

A12. NEB 10-beta supports large plasmid stability, and the small size of pMiniT 2.0 allows large insert cloning. Inserts of 4–5 kb have been easily cloned. Note that larger inserts may require lower insert-to-vector ratios.

Q13. Are the NEB 10-beta Competent *E. coli* (Cloning Efficiency) provided with NEB PCR Cloning Kit (NEB #E1202) the same cells as NEB 10-beta Competent *E. coli* (High Efficiency)?

A13. The cells are the same, but differ 10-fold in their transformation efficiencies. Our testing indicates the supplied cloning efficiency level supports excellent results for PCR amplicon cloning.

Q14. How can I determine if my NEB 10-beta cells are competent?

A14. The pUC19 control DNA included in the kit is provided in case the user would like to confirm the competency of the provided NEB 10-beta Competent *E. coli* cells. While this is not routinely required, the transformation efficiency can be independently confirmed by transforming 100 pg (2 µl of the 50 pg/µl stock) into 50 µl competent cells and following the normal transformation and plating protocols. The resulting transformation efficiency should be > 1 x 10^8 cfu/µg pUC19 DNA.

Q15. Can Cloning Mix 1 and Cloning Mix 2 be mixed together before adding them to the ligation reaction?

A15. Cloning Mix 1 and 2 can be mixed before setting up the reaction. For long term storage Cloning Mix 1 and Cloning Mix 2 should be stored in their separate vials. Storing mixed Cloning Mix 1 and 2 together will result in reduced enzyme activity.

Q16. Where are the +1 transcription positions for the SP6 and T7 promoters for *in vitro* transcription and translation located?

A16. The +1 position for the SP6 promoter is located 50 base pairs upstream from the cloning site for transcription of inserts cloned in a CW orientation, while the +1 position for the T7 promoter is located 62 base pairs downstream from the cloning site for transcription of inserts cloned in a CCW orientation.

Q17. What is the difference between the original pMiniT and the pMiniT 2.0 linearized vector backbone now provided in the kit?

A17. The 2.0 version features three improvements:

a. Addition of T7 and SP6 RNA polymerase promoter sequences flanking the cloning site for *in vitro* transcription of cloned inserts.

b. Addition of seven new restriction sites including four 8 base recognition restriction enzyme sites flanking the cloning site to allow easier linearization downstream of the cloned insert for transcription studies, and to increase options for subcloning strategies.

c. Elimination of the BsaI site present in the ApR gene through site-directed mutagenesis to allow easier downstream use of cloned inserts/modules for Golden Gate Assembly.

Both versions of the linearized vector backbone have identical functionality in the cloning kit.

Ligation Tips

- It is recommended that first-time users of the kit prepare the positive control reaction (using the provided 1 kb amplicon as insert) as described in the protocol.
- Experience has shown that a 3:1 insert:vector molar ratio is best, but ratios from 1:1 to 10:1 can also be utilized. If your insert has 5´phosphates that can lead to multiple insertions, screen for this by colony PCR or sequencing.
- While larger ligation reaction volumes and vector amounts can be used, the 25 ng linearized vector in a total reaction volume of 10 µl works well in most situations.
- If your PCR yields nonspecific products in addition to your amplicon of interest, it is preferable to gel purify your amplicon before using the material for ligations. Use minimal UV exposure and a 360 nm long wave UV source to protect your DNA from damage.
- If you wish to store your ligations to allow transformations at a later time, make sure your freezer is cold enough (-20°C) to freeze the ligations. Or, you may quick freeze with a dry ice/alcohol bath before transferring the samples to -20°C. If you find your freezer-stored ligations have remained in liquid form, this may have allowed further low-level ligation of the vector backbone to occur. In this circumstance, plate less outgrowth (< 50 µl).

What to Expect from Your Cloning Experience

A 5 minute room temperature ligation with the 1 kb *Taq* DNA polymerase-produced positive control amplicon (with single base overhangs) will yield more than 100 colonies when 50 µl of the 1 ml outgrowth is plated. The same level would be achieved if the 1 kb amplicon had been produced with a proofreading DNA polymerase such as Q5 High-Fidelity DNA Polymerase. Smaller inserts will yield higher levels of transformants (e.g., a 500 bp insert increases transformant levels 6 fold), while larger inserts will yield lower levels.

When screened for inserts, > 95% of colonies will have an insert present.

Supporting Documents

The material safety datasheet is available from the link on our website at **www.neb.com/products/E1202** and **www.neb.com/products/E1203**

References

1. Wang, Y. et al. (2004) *Nucleic Acids Research*, 32, 1197–1207.
2. Heurgue-Hamard, V. et al. (2000) *The EMBO Journal*, 19, 2701–2709.
3. Tenson, T. et al. (1999) *Journal of Bacteriology*, 181, 1617–1622.

Ordering Information

NEB #	PRODUCT	SIZE
E1202S	NEB PCR Cloning Kit	20 reactions
E1203S	NEB PCR Cloning Kit (without competent cells)	20 reactions

COMPANION PRODUCTS

NEB #	PRODUCT	SIZE
N0550S	Quick-Load® Purple 1 kb Plus DNA Ladder (0.1-10.0 kb)	125-250 gel lanes
M0287S/L	LongAmp *Taq* 2X Master Mix	100/500 reactions
M0482S/L	One*Taq* 2X Master Mix with Standard Buffer	100/500 reactions
N0447S/L	Deoxynucleotide (dNTP) Solution Mix	8 /40 µmol of each
M0323S/L	LongAmp *Taq* DNA Polymerase	100/500 units
M0480S/L/X	One*Taq* DNA Polymerase	200/1,000/5,000 units
C3019I/H	NEB 10-beta Competent *E. coli* (High Efficiency)	6 x 0.2 ml/20 x 0.05 ml
E2060S	HiScribe T7 ARCA mRNA Kit (with tailing)	20 reactions
E2065S	HiScribe T7 ARCA mRNA Kit	20 reactions
E2050S	HiScribe T7 Quick High Yield RNA Synthesis Kit	50 reactions
E2040S	HiScribe T7 High Yield RNA Synthesis Kit	50 reactions
M0207S/L	SP6 RNA Polymerase	2,000/10,000 units
M0251S/L	T7 RNA Polymerase	5,000/2,500 units
N0466S/L	Ribonucleotide Solution Mix	10/50 µmol
M0314S/L	RNase Inhibitor, Murine	3,000/15,000 units
M0307S/L	RNase Inhibitor, Human Placenta	2,000/10,000 units

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	7/15
1.1		1/16
2.0		9/16
3.0		4/17
4.0	New Format Applied	4/20
5.0	Update to vector map	5/21

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be INSPIRED
drive DISCOVERY
stay GENUINE

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