GC Cloning & Amplification Kits
with
pGC™ BLUE VECTOR

IMPORTANT!
-80°C and -20°C Storage Required
Immediately Upon Receipt

Lucigen® Corporation
Advanced Products for Molecular Biology

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MA036 v1.1

GC Cloning & Amplification Kits (with pGC™ Blue vector)

Technical Support

Lucigen is dedicated to the success and satisfaction of our customers. Our products are tested to assure they perform as specified when used according to our recommendations. It is imperative that the reagents supplied by the user, especially the DNA targets to be cloned, are of the highest quality. Please follow the manual carefully and contact our technical service representatives if additional information is necessary. We encourage you to contact us with your comments regarding the performance of our products in your applications. Thank you.

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pGC Blue Cloning Kit Designations

Several versions of the pGC Blue Cloning Kit are available. The kits differ in the number of reactions and cells that are included (see table below). Please refer to Appendix B: Application Guide for more information and recommended uses of the kits.

Catalog Numbers of Vector and Cell Combinations

Vector	Reactions	10G ELITE Electrocompetent Cells (SOLOs)	10G SUPREME Electrocompetent Cells (SOLOs)	10G Chemically Competent Cells (SOLOs)
pGC Blue (High Copy Number)	10	40742-1	40741-1	40743-1
	20	40742-2	40741-2	40743-2

Components & Storage Conditions

The pGC Blue Cloning Kits components are shipped in Container 1, which should be stored at **-20°C**. *E. cloni*® 10G Cells are shipped in Container 2, which must be stored at **-80°C**.

Container 1: GC Cloning Kit Components — Store at -20°C

	10 Reactions	20 Reactions
4X pGC Blue Vector Premix Includes Buffer, ATP, and pGC Blue Vector	25 µl	50 µl
CloneSmart® DNA Ligase (2 U/µl)	12 µl	24 µl
T4 Polynucleotide Kinase (10 U/µl) 10X Primer Kinase Buffer (containing ATP)	20 µl 100 µl	20 µl 100 µl
PCR Control Cm^R template plus primers (5 ng/µl template, 25 pmol/µl each primer)	12 µl	12 µl
EconoTaq™ DNA Polymerase (5U/µl) EconoTaq 10X Reaction Buffer 2.5 mM dNTPs	50 µl 1.5 ml 50 µl	50 µl 1.5 ml 50 µl
CloneSmart Sequencing Primers (200 reactions each) Z FOR Primer (3.2 pmol/µl) Z REV Primer (3.2 pmol/µl)	 200 µl 200 µl	 200 µl 200 µl

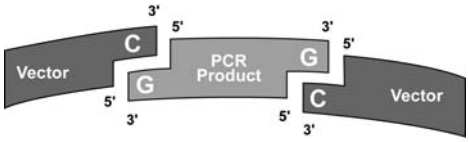
Container 2: *E. cloni* 10G Competent Cells — Store at -80°C

	Catalog #	Reactions
E. cloni 10G ELITE Electrocompetent Cells	60052-1	12 (6 x 50 µl)
or	60052-2	24 (12 x 50 µl)
E. cloni 10G SUPREME Electrocompetent Cells	60080-1	12 (6 x 50 µl)
or	60080-2	24 (12 x 50 µl)
E. cloni 10G Chemically Competent Cells	60107-1	12 (6 x 50 µl)
	60107-2	24 (12 x 50 µl)
Control pUC19 DNA (1 ng/µl) Store at **-20°C** or **-86°C**	----	10 (1 x 10 µl)
Recovery Medium Store at **-20°C** or **-86°C**	----	12 (1 x 12 ml)
	----	24 (1 x 24 ml)
	80030-1	96 (8 x 24 ml)

pGC Blue Cloning Kit Description

The GC Cloning Kits contain everything needed to amplify and efficiently clone PCR products into an unbiased, high-fidelity cloning vector. The Kits are compatible with both NON-proofreading and proofreading PCR polymerases. They can also be used to clone any blunt DNA up to 10 kb.

The GC Cloning Kits are based on a newly discovered attribute of non-proofreading DNA polymerases: these enzymes can add a single 3'-G residue to the ends of DNA molecules. The 3'G tailing occurs during PCR with a NON-proofreading, or it can be performed as a separate G-tailing reaction to the blunt products of proofreading polymerases (or any other type of blunt DNA fragments). The pSMARTGC vectors contain a single 3'-C overhang, which is compatible with the 3'-G overhang generated by the polymerase (see Figures 1 and 2). The unique combination of a G-tailed insert DNA and C-tailed vector is the basis for GC Cloning (patents pending).



GC Cloning technology is analogous to TA cloning® (Mead 1991), in which a non-proofreading polymerase, such as Taq, Tfl, Tth, or Tbr DNA polymerase, adds a single 3'-A residue to the insert DNA. A compatible T-tailed vector is used for the complementary cloning step. However, there are several notable differences between the two technologies: (a) the optimal time for the GC ligation reaction is minutes, compared to the hours required for standard TA cloning; GC Cloning technology rivals TOPO® TA cloning (Invitrogen Corp.) in the time required to complete the reaction; (b) the cloning efficiency and accuracy is higher with GC ends than with TA ends; and (c) the transcription-free pSMARTGC vectors demonstrate much greater stability for cloning large and otherwise unstable PCR products, as described below.

The pGC Blue vector (Figure 2) incorporates Lucigen's CloneSmart® transcription-free cloning technology (U.S. Pat. 6, 709, 861) to reduce bias and maximize cloning efficiency. The pGC Blue vector is supplied pre-digested, with single 3'-C tails and dephosphorylated 5' ends, and is qualified to produce >99% recombinant clones in typical experiments. The very low background of empty vector eliminates the need to screen for recombinants. It also enables PCR cloning and novel library construction methods from nanogram amounts of DNA. In contrast, conventional TA or TOPO TA vectors typically have an empty vector background of 5% or more. In addition, the ampicillin-resistant transformants are often surrounded by non-transformed "satellite" colonies, which complicate colony picking and can contaminate cultures. The growth of satellite colonies is eliminated with the kanamycin-resistant pGC Blue vector. The vector also includes opposed SP6 & T7 promoters for expression studies.

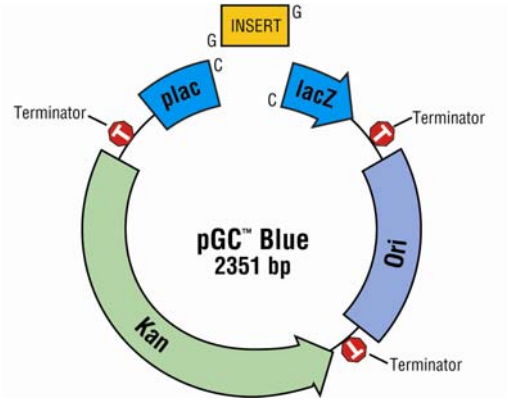
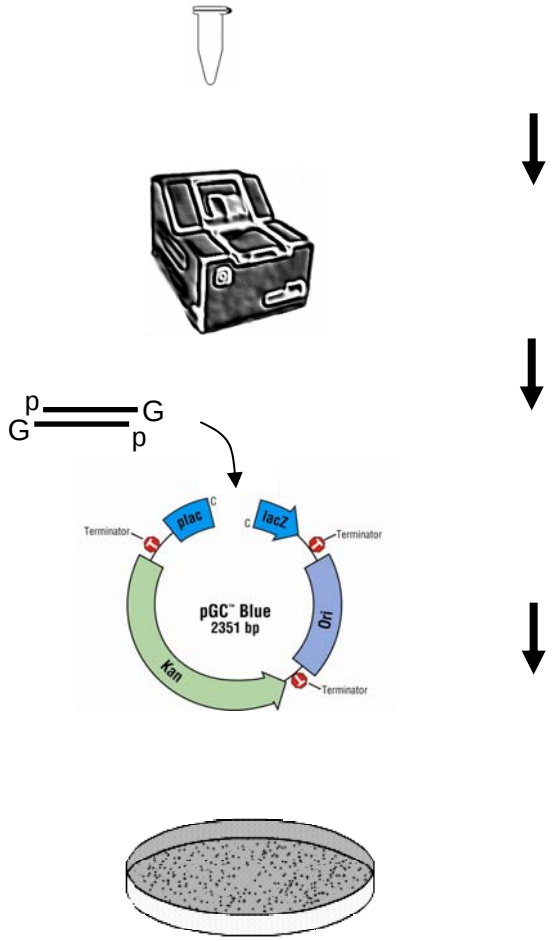


Figure 2. Schematic diagram of the pGC Blue vector. Ori, origin of replication; Kan, Kanamycin resistance gene; plac, lac promter; lacZ, *lac*Zalpha ORF. Approximate positions of sequencing primers and transcriptional terminators (T) are indicated. See Appendix D.

The GC Cloning Kits are convenient to use, containing pre-cut, dephosphorylated pGC Blue cloning vector premixed with buffer and ATP, as well as DNA ligase, Taq DNA polymerase, sequencing primers, and DNA controls. The Kits also contain high-efficiency *E. cloni*® 10G Electrocompetent or Chemically Competent Cells.



PHOSPHORYLATE PRIMERS
with T4 Polynucleotide Kinase
37°C, 10 min

AMPLIFY TARGET
with EconoTaq™ DNA Polymerase or other non-proofreading polymerase

LIGATE TO pGC Blue
with CloneSmart® DNA Ligase
RT, 30 min
70ºC, 15 min.

TRANSFORM
***E. cloni*® 10G Cells**
15 min.

GROW and PLATE
30-60 min.

Figure 1. GC Cloning with EconoTaq DNA Polymerase. The simplest method of GC Cloning uses products from EconoTaq or other NON-proofreading polymerases. PCR primers are phosphorylated in a brief kinase reaction and are then added directly to the PCR reaction. After amplification, PCR fragments are directly ligated to the pGC Blue vector and transformed into *E. cloni* 10G competent cells. Alternate protocols for Proofreading polymerases are described in the Manual.

pGC Blue Cloning Vector

The pGC Blue vector contains a high-copy replication origin and the kanamycin resistance gene. Strong transcription terminators flank the *lac*Z gene to protect the vector from fortuitous transcription from cloned inserts (Figure 2). The vector is supplied pre-cut with single 3'-C overhangs and dephosphorylated 5' ends. The copy number is similar to that of pUC plasmids (~300 copies/cell). Insert DNA that contains 3'-G tails and 5'-phosphate groups is ligated to the GC Cloning vector, transformed into competent cells, and spread on plates containing kanamycin plus XGAL and IPTG. The sequence of the pGC Blue vector is supplied at the back of this manual (Appendix F).

Purification and Size Fractionation of DNA

A PCR reaction that contains one homogeneous DNA band and no primer-dimers can be used directly for cloning with the GC Cloning vectors. However, PCR products often contain numerous spurious bands that must be purified away from the correct product. Agarose gel electrophoresis is highly recommended to size fractionate and isolate the desired DNA fragments.

Sensitivity of DNA to Short Wavelength UV Light

DNA resolved on agarose gels is generally stained with ethidium bromide and visualized by illumination with ultraviolet light. Exposure to short wavelength ultraviolet light (e.g., 254, 302, or 312 nm) can reduce cloning efficiencies by several orders of magnitude (Figure 3). Note that the wavelength of most UV transilluminators, even those designated specifically for DNA visualization, is typically 302 nm or 312 nm, which can cause significant damage to DNA.

Use a long wavelength (e.g., 360 nm) UV lamp and short exposure times when isolating DNA fragments from agarose gels.

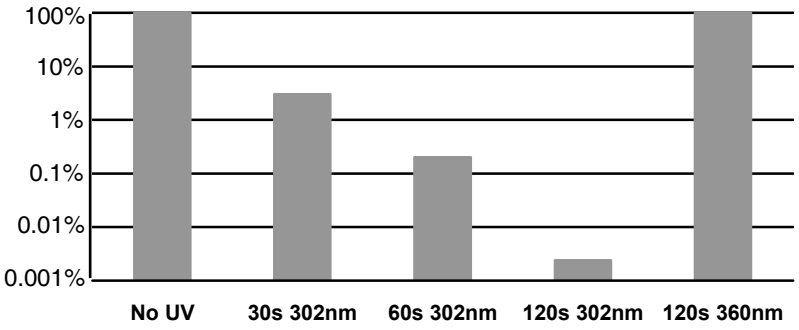


Figure 3. Relative cloning efficiency of pUC19 after exposure to UV light. Intact pUC19 DNA was transformed after no UV exposure ("No UV") or exposure to 302 nm UV light for 30, 60, or 120 seconds ("30s 302nm, 60s 302nm,120s 302nm") or to 360 nm UV light for 120 seconds ("120s 360nm"). Cloning efficiencies were calculated relative to un-irradiated pUC19 DNA.

E. cloni® 10G Competent Cells

For maximum cloning efficiency, Lucigen's *E. cloni* 10G Competent Cells are supplied with all GC Cloning Kits. *E. cloni* 10G Competent Cells are *E. coli* strains optimized for high efficiency transformation. They are ideal for cloning and propagation of BAC, fosmid, or plasmid clones. *E. cloni* 10G Cells give high yield and high quality plasmid DNA due to the *end*A1 and *rec*A1 mutations, and are phage resistant (*ton*A mutation). *E. cloni* 10G strains also contain the inactive *mcr* and *mrr* mutations, allowing methylated genomic DNA that has been isolated directly from mammalian or plant cells to be cloned without deletions or rearrangements.

Lucigen's GC Cloning Kits are available with the following *E. cloni* 10G Competent Cells in convenient SOLO packaging (one transformation per tube):

***E. cloni* 10G SUPREME Electrocompetent Cells** deliver $\geq$ 4 x 10^{10} cfu/μg. SUPREME Cells are ideal for the most demanding applications that require the greatest number of transformants, such as construction of large, high complexity libraries or cloning difficult targets.

***E. cloni* 10G ELITE Electrocompetent Cells** deliver $\geq$ 2 x 10^{10} cfu/μg, providing large numbers of transformants from hard-to-clone fragments or limited DNA at a lower price than SUPREME Cells.

***E. cloni* 10G Chemically Competent Cells** deliver $\geq$ 1 x 10^{8} cfu/μg and offer unbeatable performance and value for routine applications.

***E. cloni* 10G Genotype:** F$^-$ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80*dlacZ*ΔM15 Δ*lac*X74 *end*A1 *rec*A1 *ara*D139 Δ(*ara*, *leu*)7697 *gal*U *gal*K *rps*L *nup*G λ- *tonA*

- *E. cloni* 10G Competent Cells are provided with supercoiled pUC19 DNA at a concentration of 1 ng/μl as a transformation control. Dilute the pUC plasmid 1:100 in dH_2O for transformation.
- **NOTE:** For optimal results, use the provided Recovery Medium to resuspend the cells after electroporation. Use of TB or other media may result in lower transformation efficiencies.

Materials and Equipment Needed

The GC Cloning Kits supply most of the items needed to efficiently generate recombinant clones. While simple and convenient, successful use of the Kit requires proper planning for each step. Please read the entire manual and prepare the necessary equipment and materials before starting. Following ligation, the following items are required for transformation:

- Electroporation apparatus and 0.1 cm cuvettes (for electrocompetent cells). Successful results are obtained with cuvettes from BTX (Model 610), BioRad (Cat. #165-2089), or Eppendorf (4307-000-569). Users have reported difficulties using *E. cloni* cells with Invitrogen cuvettes (Cat. # 65-0030).

  or

  Water bath at 42°C (for chemically competent cells).
- Thermocycler and gel electrophoresis equipment.
- Wet ice.
- Sterile 17 x 100 mm culture tubes.
- Terrific Broth.
- YT+KanXI agar plates, containing kanamycin, XGAL, and IPTG (see Appendix for recipes).

OVERVIEW OF PROTOCOL

SECTION 1: PREPARATION OF PCR PRODUCTS FOR GC CLONING

PROTOCOL FOR ECONOTAQ (AND OTHER NON-PROOFREADING POLYMERASES)

Steps: 1. Phosphorylate primers
2. Amplify by PCR
3. Purify product

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PROTOCOL FOR PROOFREADING POLYMERASES

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3. Add 3′-G tails
4. Purify product

Steps: 1. Amplify by PCR
2. Add 3′-G tails
3. Phosphorylate product
4. Purify product

SECTION 2: LIGATION AND TRANSFORMATION

Detailed Protocol

The GC Cloning Kit can be used with PCR products from either Non-Proofreading or Proofreading DNA polymerases. However, all DNA fragments used for GC Cloning **MUST** have three features:

1) 5' phosphate groups: PCR products usually do NOT have 5' phosphate groups, regardless of the type of enzyme used for PCR amplification. The required 5' phosphate groups are added by phosphorylating either the PCR primers (Protocol A) or the PCR product (Protocol B). Protocols A and B are provided for both EconoTaq and for Proofreading polymerases.

2) Single 3'G overhangs: EconoTaq™ DNA Polymerase is a NON-proofreading enzyme supplied with the GC Cloning Kit. It adds 3'G tails during the PCR reaction. Therefore, only a subsequent phosphorylation step is needed before ligation to the GC vector. Other NON-proofreaders (e.g., Taq, Tfl, Tbr polymerases) similarly add 3'G tails.

   Proofreading polymerases (e.g., Vent®, Phusion®, Pfu) do NOT add 3'G tails, so both a G-tailing step and a 5'-phosphorylation step are needed. The EconoTaq enzyme is used after the initial PCR to add the 3'G tails.

3) Sufficient purity: In all cases, gel electrophoresis is ***highly recommended*** for purification of the insert DNA, to avoid cloning spurious bands or PCR primers.

Section 1 describes the preparation of 5-phosphorylated, G-tailed fragments. Separate protocols are provided for preparing fragments from NON-proofreading or proofreading polymerases. Section 2 details their ligation into the GC Cloning vector and transformation of *E. cloni* cells.

SECTION 1: PREPARATION OF PCR PRODUCTS FOR GC CLONING

Phosphate groups can be incorporated into the product by either of two methods: Protocol A describes phosphorylation of the primers before starting the PCR; Protocol B describes phosphorylation of the product after the PCR is finished. The following section presents Protocols A and B for EconoTaq and other NON-proofreading polymerases. The subsequent section presents Protocols A and B for Proofreading polymerases

GC Cloning with EconoTaq (and other NON-proofreading polymerases)

Protocol A. Use of Phosphorylated PCR Primers

Step 1: Phosphorylate Primers.

Option A) Synthesize PCR Primers with 5' Phosphates

The easiest way to generate a phosphorylated PCR product is to use PCR primers that have been chemically synthesized with 5 phosphates already attached. Nearly all manufactures of oligonucleotides provide this option, but the user must specifically request addition of 5 phosphates.

Option B) Add 5' phosphates to the primers by T4 PNK

T4 PNK can be used to add 5' phosphates to PCR primers before performing the PCR reaction. T4 PNK and Primer Kinase Buffer (containing ATP) are included in the GC Cloning Kits. Perform the primer kinase reaction as follows:

Primer kinase reaction

2.0 µl Forward primer @ 100 pmol/µl
2.0 µl Reverse primer @ 100 pmol/µl
1.0 µl 10 X Primer Kinase Buffer
1.0 µl T4 PNK (10 U/µl)
4.0 µl H_2O
10.0 µl total
Incubate at 37°C, 10 minutes

After the incubation, add 2-5 µl of this reaction directly to a 50-100 µl PCR mix and amplify by standard PCR (see Step 2).

In rare cases, the Primer Kinase Buffer interferes with the PCR reaction, resulting in reduced yields or smeared bands. In these cases, use chemically phosphorylated primers (Option A, above) or use non-phosphorylated primers followed by kinase treatment of the PCR product (Protocol B, below).

Step 2: Amplify by PCR.

EconoTaq DNA Polymerase, 10X Reaction Buffer, and dNTPs are included with the GC Cloning Kit for amplification. If desired, other NON-proofreading polymerases can be used in this protocol.

Step 3: Purify Insert DNA

DNA products can be cloned directly after the PCR reaction, if desired. However, any spurious PCR products, primers, or primer dimers may also be cloned efficiently. Size selection on an agarose gel therefore is ***highly recommended*** to remove contaminating DNA, aberrant PCR products, PCR primers, and primer-dimers. Purify the DNA bands from the gel by a commercial DNA purification kit.

If the DNA is not gel fractionated, it can be purified using a commercial purification kit or ethanol precipitation.

Proceed to Section 2: Ligation and Transformation.

Protocol B) Addition of 5′ phosphates to the PCR product

PCR products created with non-phosphorylated primers can also be used for GC Cloning. The PCR product MUST be purified away from the PCR buffer before the kinase reaction, because ammonium ions in the PCR buffer strongly inhibit T4 PNK. We recommend use of a purification column to remove the PCR buffer. The product is then treated with T4 PNK, followed by a second purification.

Step 1: Amplify by PCR.

EconoTaq DNA Polymerase, 10X Reaction Buffer, and dNTPs are included with the GC Cloning Kit for amplification. If desired, other NON-proofreading polymerases can be used for PCR in this protocol.

After the PCR is complete, purify the PCR product using a standard DNA purification column and elute in 44 µl of H_2O or the recommended elution buffer. Alternately, precipitate the PCR reaction using sodium acetate plus ethanol. Do not use ammonium acetate for precipitation, as this salt will severely inhibit the PNK reaction. Resuspend the DNA in 44 µl H_20.

Step 2: Phosphorylate PCR Product.

After removal of the PCR buffer from the PCR product, perform the following kinase reaction:

Post-PCR Kinase Reaction

44 µl purified PCR product (No PCR buffer present)
5 µl 10 X Primer Kinase Buffer (containing ATP)
1 µl T4 PNK (10 U/µl)
50 µl total

Incubate at 37°C, 10 minutes.

Do not use this material directly in a ligation reaction, as the PNK will phosphorylate the vector, resulting in a high background of non-recombinants. Purification is essential after this step.

Step 3: Purify Insert DNA

Size selection on an agarose gel is ***highly recommended*** to remove contaminating DNA, aberrant PCR products, PCR primers, and primer-dimers. Purify the DNA bands from the gel by a commercial purification kit.

If the DNA is not gel fractionated, it can be purified using a commercial DNA purification kit or ethanol precipitation.

Do NOT attempt to clone the products directly after the phosphorylation reaction, without performing a purification step; doing so will produce a high background of empty vector clones.

Proceed to Section 2: Ligation and Transformation.

GC Cloning with PROOFREADING DNA polymerases

Proofreading DNA polymerases, such as Vent® or Pfu polymerases, result in blunt DNA fragments. A 5' phosphorylation reaction and a 3' G-tailing reaction are both necessary for ligation of these products into the GC Cloning vector.

Preparation of PCR fragments

The most efficient method of GC Cloning with proofreading polymerases is to use phosphorylated primers followed by G-tailing (Protocol A, below). Alternately, the PCR product can be G-tailed first and subsequently phosphorylated (Protocol B). For both methods, EconoTaq™ DNA Polymerase is provided in the GC Cloning Kit to add single 3' G tails to the PCR product.

Proofreaders Protocol A: Phosphorylation followed by G-tailing

Step 1 of this protocol describes two options for incorporating phosphate groups into the PCR primers. Step 2 describes G-tailing of the 5'-phosphorylated PCR products.

Step 1. Phosphorylate PCR Primers

Option A) Synthesize PCR Primers with 5' Phosphates

The easiest way to generate a phosphorylated PCR product to use PCR primers that have been synthesized with 5 phosphates chemically attached. Nearly all manufactures of oligonucleotides provide this option, but the user must specify that 5 phosphates are desired.

Option B) Add 5' phosphates to the primers by T4 PNK

Standard (non-phosphorylated) primers can be treated with T4 PNK to phosphorylate the primers before the PCR reaction. T4 PNK and Primer Kinase Buffer are provided with the GC Cloning Kit. Perform the primer kinase reaction as follows:

Primer kinase reaction

2.0 µl Forward primer @ 100 pmol/µl
2.0 µl Reverse primer @ 100 pmol/µl
1.0 µl 10 X Primer Kinase Buffer (containing ATP)
1.0 µl T4 PNK (10 U/µl)
4.0 µl H_2O
10.0 µl total

Incubate at 37°C, 10 minutes

After the incubation, add 2-5 ul of this reaction directly to a 50-100 µl PCR mix and amplify by standard protocols (see Step 2: PCR Amplification).

In rare cases, the Primer Kinase buffer interferes with the PCR reaction, resulting in reduced yields or smeared bands. In these cases, use chemically phosphorylated primers (Option A) or use non-phosphorylated primers followed by a kinase reaction (Protocol 2, below).

Step 2: PCR Amplification

Amplify the template by standard PCR methods using the phosphorylated PCR primers and a proofreading DNA polymerase (e.g., Vent, Pfu).

After the PCR is complete, proceed directly to Step 3: G-tailing. Do NOT purify the PCR reaction product, as the PCR buffer is required for the G-tailing reaction.

Step 3: G Tailing of Blunt DNA

After the PCR is complete, EconoTaq™ DNA Polymerase adds single 3'G tails to blunt PCR products that contain 5' phosphate groups. The G-tailing reaction is performed in the same tube and buffer as the PCR:

G-Tailing Reaction

50-100 µl completed PCR reaction (AFTER thermal cycling, but NOT purified)
1 µl EconoTaq DNA Polymerase (5 U/µl)
51-101 µl total

Incubate at 72°C, 10 minutes. Do not cycle.

After G-tailing, proceed to Step 4: Purification of Insert DNA.

Step 4: Purification of Insert DNA

DNA products can be cloned directly after the G-tailing reaction, if desired. However, if spurious products or primer dimers are present, they may also be cloned efficiently. Size selection on an agarose gel therefore is ***highly recommended*** to remove contaminating DNA, aberrant PCR products, PCR primers, and primer-dimers. Purify the DNA bands from the gel by a commercial DNA purification kit.

If the DNA is not gel fractionated, it can be purified using a commercial purification kit or ethanol precipitation.

Proceed to Section 2: Ligation and Transformation.

Proofreaders Protocol B: G Tailing followed by phosphorylation

In this protocol, a blunt PCR product is created with a proofreading DNA polymerase using non-phosphorylated primers. Subsequently, the product is G-tailed in Step 2 and phosphorylated with T4 PNK in Step 3.

Step 1: Amplify by PCR

Amplify a template using non-phosphorylated primers and a proofreading polymerase (e.g., Vent, Pfu). After the reaction is complete, proceed directly to Step 2: G-tailing reaction.

Do NOT purify the PCR reaction product, as the G-tailing reaction requires the PCR buffer.

Step 2: G-tailing reaction

EconoTaq™ DNA Polymerase is used to add single 3'G tails to blunt PCR products, as follows:

G-Tailing Reaction

50-100 µl *complete* PCR reaction mix (AFTER thermal cycling, but NOT purified)
1 µl EconoTaq DNA Polymerase (5 U/µl)
51-101 µl total

Incubate at 72°C, 10 minutes. Do not cycle.

Purify the G-tailed PCR reaction to remove the PCR buffer. We recommend use of a DNA purification column, with elution in 44 µl of H_2O or the recommended elution buffer.

Ethanol precipitation with sodium acetate can also be used. Do NOT use ammonium acetate for precipitation, because the ammonium ions strongly inhibit the T4 PNK reaction.

Step 3: Phosphorylation of G-tailed product

The G-tailed product MUST be purified before performing phosphorylation with T4 PNK. The reaction is carried out as follows:

Post-PCR Kinase Reaction
44 µl *purified* PCR product (No PCR buffer present)
5 µl 10 X Primer Kinase Buffer mix
1 µl T4 PNK (10 U/µl)
50 µl total

Incubate at 37°C, 10 minutes.

Proceed to Step 4: Purification of Insert DNA.

Do not use this material directly in a ligation reaction, as the PNK will phosphorylate the vector, resulting in a high background of non-recombinants.

Step 4: Purification of Insert DNA

Size selection on an agarose gel is ***highly recommended*** to remove contaminating DNA, aberrant PCR products, PCR primers, and primer-dimers. Purify the DNA bands from the gel by a commercial purification kit.

If the DNA is not gel fractionated, it can be purified using a commercial purification kit or ethanol precipitation.

Do NOT attempt to clone the products directly after the phosphorylation reaction, without performing a purification step; doing so will produce a high background of empty vector clones.

Proceed to Section 2: Ligation and Transformation.

Control Reaction

The control PCR product must be created by amplification *and* must contain 5' phosphate groups for ligation into the GC Cloning vector! Performing the control reaction is recommended to help evaluate the results obtained with experimental samples. A control template and primers are supplied to produce a diagnostic PCR product of 781 bp that encodes chloramphenicol acetyl transferase (CAT). Successful cloning of the resulting PCR product will produce chloramphenicol resistant colonies. A few white colonies containing small, non-functional CAT inserts may also be observed if the transformants are grown on non-selective plates.

For convenience, the control primers and template are supplied in a single tube. The Primer Kinase reaction will also phosphorylate the Control PCR template, which does not affect the results.

1. Primer Kinase reaction:

4.0 µl PCR control CAT template plus primers (5 ng/µl template, 25 pmol/µl each primer)
1.0 µl 10X Primer Kinase buffer
4.0 µl H_2O
1.0 µl T4 Polynucleotide kinase (10 U/µl)
10.0 µl total

Incubate at 37°C for 10 minutes. Use 5.0 µl of the reaction directly for PCR amplification (below).

2. PCR

5.0 µl Primer Kinase reaction (PCR control CAT template plus primers)
5.0 µl 10X EconoTaq™ Reaction Buffer
2.5 µl dNTP mix (2.5 mM each)
37.0 µl H_2O
0.5 µl EconoTaq DNA Polymerase (5 unit/µl)
50.0 µl total

3. Amplify using the following cycling parameters:

STEP	TIME	TEMPERATURE	CYCLES
Denaturation	15 seconds	94ºC	25X
Annealing	15 seconds	60ºC	
Extension	60 seconds	72ºC	
Final Extension	10 minutes	72ºC	1X

4. Analyze 5 µl of the reaction by agarose gel electrophoresis. A distinct band at 781 bp should be visible. Use this product in the GC Cloning reaction described below.

Size Fractionation and Purification of Control PCR Fragments

Size selection on an agarose gel is highly recommended to remove aberrant PCR products, PCR primers, and primer-dimers. Purify the 781-bp DNA band from the gel by a commercial purification kit.

If the DNA is not gel fractionated, it can be purified using a commercial purification kit or ethanol precipitation.

DNA product can be cloned directly after the PCR reaction, if desired. However, if spurious products and primer dimers are present, they may also be cloned efficiently, resulting in numerous white colonies.

SECTION B: Ligation to the pGC Blue Cloning Vector

In the ligation reaction, the G-tailed, phosphorylated insert is ligated with pre-processed pGC Blue vector. Successful cloning can be achieved routinely with as little as 10 ng of insert, but using low amounts of insert will result in significantly fewer transformants. The ligation is performed as follows:

1. Briefly centrifuge the GC Cloning Vector Premix before use. Mix by gently pipeting up and down several times.

2. Combine the following components in a 1.5 ml tube, adding the ligase last:

x µl	Insert DNA (10-400 ng, with 3′G tails and 5' phosphates)
2.5 µl	4X pGC Blue Vector Premix
1.0 µl	CloneSmart® DNA Ligase (2 U/µl)
y µl	H_2O
10.0 µl	total reaction volume

3. Mix by gently pipeting the reaction mixture up and down. Incubate at room temperature (21-25°C) for 30 minutes. To obtain the maximum number of clones, ligation time can be extended to 2 hours.

Optional control reactions include the following:

Positive Control Insert DNA	To determine the ligation and transformation efficiency with a known insert, use 100 ng (~1.0 µl) of the PCR amplified control CAT template.
Vector Background	To determine the background of empty vector, omit Insert DNA in the above reaction.

4. Heat denature the ligation reaction at 70°C for 15 minutes.

Preparation for Transformation

1. Heat denature the ligation reaction at 70°C for 15 minutes if you have not done so already.
2. Cool to room temperature for 15 seconds followed by 0-4°C for 15 seconds to condense water vapor inside the tube.
3. Spin 1 minute at 12,000 rpm to collect condensation and pellet precipitated material.
4. The sample is ready for transformation; precipitating the DNA is not necessary.

To ensure optimal cloning results, we strongly recommend the use of Lucigen's *E. cloni*® 10G ELITE or SUPREME Electrocompetent Cells. These cells yield $\geq 2 \times 10^{10}$ or $\geq 4 \times 10^{10}$ cfu/µg of pUC19, respectively, to maximize the number of transformants. For less demanding applications, *E. cloni* 10G Chemically Competent Cells may be used.

Most laboratory strains of competent *E. coli* can be effectively transformed with GC Cloning ligation reactions. The number of clones will be proportionate to the competency of the cells.

The following protocols are provided for transformation of *E. cloni* 10G Competent Cells.

Heat Shock Transformation of *E. cloni* Chemically Competent Cells

E. cloni 10G Chemically Competent Cells are provided in 40 µl aliquots (SOLOs), sufficient for one transformation reaction. Transformation is performed by heat shock at 42ºC, followed by incubation on ice. To ensure successful transformation results, the following precautions must be taken:

***ESSENTIAL:* After ligation, the reaction must be heat killed at 70°C for 15 minutes!**

- Heat-killed ligation reactions can be used directly, without purification of the ligation products.
- All microcentrifuge tubes must be thoroughly pre-chilled on ice before use.
- The cells must be completely thawed **on ice** before use.

Transformation Protocol for Chemically Competent cells

1. Remove *E. cloni* cells from the -80°C freezer and thaw completely on wet ice (10-15 minutes).
2. Add 2-4 µl of the heat-denatured GC Cloning ligation reaction to the 40 µl of cells on ice. **Failure to heat-inactivate the ligation reaction will prevent transformation**. Stir briefly with pipet tip; **do not** pipet up and down to mix, which can introduce air bubbles and warm the cells.
3. Incubate cells/ligation mixture on ice for 30 minutes.
4. Heat shock cells by placing them in a 42°C water bath for 45 seconds.
5. Return the cells to ice for 2 minutes.
6. Add 260 µl of room temperature Recovery Medium to the cells in the culture tube.
7. Place the tubes in a shaking incubator at 250 rpm for 1 hour at 37°C.
8. Plate 50-250 µl of transformed cells on YT agar plates containing 30 µg/ml kanamycin plus XGAL and IPTG. Incubate the plates overnight at 37°C.
9. Transformed clones can be further grown in TB or any other rich culture medium

EXPECTED RESULTS USING *E. cloni* 10G CHEMICALLY COMPETENT CELLS

Plating chemically transformed cells and expected results.

Reaction Plate	µl/Plate	CFU/Plate	Efficiency
Experimental Insert (100 ng per ligation)	50 & 250	variable	NA
CAT PCR Amplified Insert (Positive Control)	50	> 200	> 99% inserts
No-Insert Control (Vector Background)	250	< 2	<1% background
Supercoiled pUC19 Transformation Control Plasmid (10 pg, Amp^R)	2	> 200	> 1 x 10^8 cfu/µg plasmid

The results presented above are expected when cloning 100 ng of intact, PCR amplified *CAT* DNA, with G-tailed ends and 5' phosphate groups, into Lucigen's pGC Blue Cloning vector. When transforming *E. cloni* 10G Chemically Competent Cells (transformation efficiency $\geq$1 x 10^8 cfu/ug pUC19 DNA) the number of recombinant clones is typically 100-fold greater than the background of self-ligated vector. The background of empty GC Cloning vector is constant (< 2 colonies per 250 µl of cells plated), unless contaminants are introduced. However, use of too little insert DNA, or insert DNA that is improperly phosphorylated or G-tailed, can yield significantly fewer recombinant clones. Cloning AT-rich DNA and other recalcitrant sequences may also lead to fewer colonies. With relatively few recombinant clones, the number of empty vector colonies becomes noticeable. For example, if the Experimental Insert reaction produces 50 colonies from 250 µl of cells, then the 2 colonies obtained from 250 µl of the No-Insert Control ligation will represent a background of 4%.

Electroporation of *E. cloni* 10G Electrocompetent Cells

E. cloni 10G SUPREME and ELITE Electrocompetent Cells are provided in 25-µl aliquots (SOLOs), sufficient for one transformation each.

Transformation is carried out in a 0.1 cm gap cuvette. Optimal settings for electroporation are listed in the table below. Typical time constants are 3.5 to 4.5 msec.

Optimal Setting		Alternate Settings (~ 20-50% lower efficiencies)
1.0 mm cuvette 10 µF 600 Ohms 1800 Volts		1.0 mm cuvette 25 µF 200 Ohms 1400 – 2000 Volts

Suggested Electroporation Systems:

Bio-Rad Micro Pulser #165-2100; Bio-Rad *E. coli* Pulser #165-2102; Bio-Rad Gene Pulser II #165-2105; BTX ECM630 Electroporation System.

Optional transformation control reactions include electroporation with 10 pg of supercoiled pUC19 DNA (1 µl of a 1:100 dilution of the provided stock solution of pUC19).

To ensure successful transformation results, the following precautions must be taken:

***ESSENTIAL:* After ligation, the reaction must be heat killed at 70°C for 15 minutes!**

- Microcentrifuge tubes and electroporation cuvettes must be thoroughly pre-chilled on ice before use. Successful results are obtained with cuvettes from Eppendorf (Model 4307-000-569), BTX (Model 610), or BioRad (Cat. #165-2089). Users have reported difficulties using *E. cloni* cells with Invitrogen cuvettes (Cat. # 65-0030).
- The cells must be completely thawed **on ice** before use.

Transformation Protocol for Electrocompetent cells

1. Have Recovery Medium and 17 mm x 100 mm sterile culture tubes readily available at room temperature (one tube for each transformation reaction). Transformation efficiency may decrease with the use SOC or other media.
2. Place electroporation cuvettes (0.1 cm gap) on ice.
3. Remove *E. cloni* cells from the -80°C freezer and place on wet ice until they thaw **completely** (10-15 minutes).
4. When cells are thawed, mix them by tapping gently.
5. Add 1 µl of the heat-denatured GC Cloning ligation reaction to the 25 µl of cells on ice. **Failure to heat-inactivate the ligation reaction will prevent transformation.** Stir briefly with pipet tip; **do not** pipet up and down to mix, which can introduce air bubbles and warm the cells. Use of more than 2 µl of ligation mix may cause electrical arcing during electroporation.
6. Carefully pipet 25 µl of the cell/DNA mixture into a chilled electroporation cuvette without introducing bubbles. Quickly flick the cuvette downward with your wrist to deposit the cells across the bottom of the well. Electroporate according to the conditions recommended above.
7. Within 10 seconds of the pulse, add 975 µl of Recovery Medium to the cuvette and pipet up and down three times to resuspend the cells. Transfer the cells and Recovery Medium to a culture tube.
8. Place the tube in a shaking incubator at 250 rpm for 1 hour at 37°C.

9. Spread up to 100 µl of transformed cells on YT agar plates containing 30 µg/ml kanamycin.

10. Incubate the plates overnight at 37°C.

11. Transformed clones can be further grown in TB or in any other rich culture medium.

EXPECTED RESULTS USING *E. cloni* 10G ELECTROCOMPETENT CELLS

Plating electrocompetent transformed cells and expected results.

Reaction Plate	µl/Plate	CFU/Plate	Efficiency
Experimental Insert (100 ng per ligation)	5 & 50	variable	NA
CAT PCR Amplified Insert (Positive Control)	5	> 400	> 99% inserts
No-Insert Control (Vector Background)	100	< 25	<1% background
Supercoiled pUC19 Transformation Control Plasmid (10 pg, Amp^R)	2	> 200	$\geq 2 \times 10^{10}$ cfu/µg plasmid

The results presented above are expected when cloning 100 ng of intact, PCR amplified *lac*Z DNA, with G-tailed ends and 5' phosphate groups, into Lucigen's pGC Blue Cloning vectors. When transforming *E. cloni* 10G ELITE Electrocompetent Cells (transformation efficiency $\geq 2 \times 10^{10}$ cfu/µg pUC19 DNA) the number of recombinant clones is typically 100-fold greater than the background of self-ligated vector (>400 colonies per 5µl plated). The background number of empty GC Cloning vectors is constant (< 25 colonies per 100 µl of cells plated), unless contaminants are introduced.
Use of too little insert DNA, or insert DNA that is improperly 5-phosphorylated or G-tailed, can yield significantly fewer recombinant clones. Cloning AT-rich DNA and other recalcitrant sequences may also lead to fewer colonies. With relatively few recombinant clones, the number of empty vector colonies becomes noticeable. For example, if the Experimental Insert ligation reaction produces only 5 colonies from 5 µl of cells plated, then the 25 colonies obtained from 100 µl of the No-Insert Control ligation will represent a background of 2.5%.

Use of *E. cloni* SUPREME Electrocompetent cells (transformation efficiency $\geq 4 \times 10^{10}$ cfu/µg pUC19 DNA) will result in proportionately more colonies. Use of competent cells with a transformation efficiency of less than 2×10^{10} cfu/µg will result in proportionately fewer colonies. Most chemically competent cells will yield ~1% of the number of colonies shown above.

Getting More Recombinants

Increasing the ligation reaction time to 2 hours can increase the yield of recombinants by 4-5 fold. Ligation times beyond 2 hours will not improve the results further. Use of more efficiency competent cells will also increase recombinant yields. Use of more PCR amplicon in the ligation reaction can dramatically improve the number of recombinants.

Certain PCR products can prove recalcitrant to cloning due to a large size, the presence of toxic gene products or secondary structures, extremely biased base composition, or other unknown reasons. For these very challenging templates, we strongly recommend the use of the BigEasy™ v2.0 Linear Cloning Kit. The lack of supercoiling in the pJAZZ™-OC linear vector contained in the BigEasy v2.0 Cloning Kit alleviates many problems caused by secondary structure of the insert.

Colony Screening

The pGC Blue vector uses the standard blue/white colony screen based on *lac*Zalpha complementation. Transformants are grown on plates containing kanamycin, XGAL, and IPTG. Recombinant colonies will be white, and non-recombinant colonies will be blue. However, some insert DNAs (e.g., small open reading frames) produce blue colonies in this screen. Therefore, if the proportion of blue colonies is unexpectedly high, it may be useful to analyze blue colonies for the presence of inserts.

Inserts that are large or have unusual base composition may produce very few colonies. Lucigen's cloning kits containing transcription-free vectors are recommended for such inserts (see Appendix B and Lucigen's website: www.lucigen.com).

DNA Isolation and Sequencing

Grow transformants in TB medium plus 30 μg/ml kanamycin. Use standard methods to isolate plasmid DNA suitable for sequencing. The pGC Blue plasmid contains the high copy number pUC origin of replication, yielding 20-80 μg of plasmid DNA per ml of culture. The *E. cloni*® 10G Competent Cells are *recA* and *endA* deficient and will provide high quality plasmid DNA. GC Cloning Kits are provided with the sequencing primers Z FOR and Z REV. The sequence of the primers and their orientation relative to the GC Cloning plasmid are shown in Appendix D.

References

1. Mead DA, Pey NK, Herrnstadt C, Marcil RA, Smith LM. A universal method for the direct cloning of PCR amplified nucleic acid.Biotechnology (N Y). 1991 Jul;9(7):657-63.

2. Sambrook, J. and Russell, DW. Molecular Cloning: A Laboratory Manual (Third Edition). 2001. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Appendix A: Media Recipes

YT+KanXI Agar Medium for Plating of Transformants. Per liter: 8 g Bacto-tryptone, 5 g yeast extract, 5 g NaCl, 15 g agar, plus antibiotic. Mix all components except antibiotic; autoclave and cool to 55°C. Prepare YT+KanXI agar medium by adding kanamycin to a final concentration of 30 mg/l (equal to 30 μg/ml). Add XGAL to a final concentration of 50 μg/ml and add IPTG to 1mM final concentration. Pour into petri plates.

TB Culture Medium. Per liter: 11.8 g Bacto-tryptone, 23.6 g yeast extract, 9.4 g dipotassium hydrogen phosphate (K_2HPO_4; anhydrous), 2.2 g potassium dihydrogen phosphate (KH_2PO_4; anhydrous), 0.4% glycerol. Mix all components except glycerol; autoclave and cool to 55°C. Add 8 ml filter-sterilized 50% glycerol per liter prior to using.

Growing Transformed Cultures. Colonies obtained from a pSMART®GC transformation can be further grown in TB or LB culture medium, containing 30 μg/ml kanamycin. Transformed cultures can be stored by adding sterile glycerol to 20% (final concentration) and freezing at –70°C. Unused portions of the ligation reactions may be stored indefinitely at –20 °C.

Appendix B: CloneSmart® Application Guide

GC Cloning Kits accommodate any cloning situation. For routine applications, we recommend using the pSMARTGC HCKan vector or pGC Blue vector. For cloning toxic genes or particularly difficult DNA sequences, we recommend using the pSMARTGC LCKan vector or the pJAZZ™GC linear vector (coming soon).

Use of the *E. cloni®* 10G strain is essential for cloning inserts that may be methylated, such as genomic DNA isolated directly from plant or mammalian cells, as this strain contains the inactive *mcr* and *mrr* alleles [*mcr*A Δ(*mrr-hsd*RMS-*mcr*BC)]. The 10G SUPREME preparation of these cells is recommended for cloning difficult or very small quantities of insert DNA.

Vector		Insert DNA Source			Intended Use
Vector Name	Copy #	PCR (NON-proofreader)	PCR (Proofreaders)	AT-Rich, "Difficult"	Digestion, Subcloning, Sequencing
pSMARTGC LK	Low	++	+	++	+
pSMARTGC HK	High	++	+	+	++
pGC Blue	High	++	+	-	++
pSMART LCKan	Low	+	++	++	+
pSMART HCKan	High	+	++	+	++
pJAZZ-OC	Low-Mid	+	+	+++	+

Appendix C: Abbreviated Protocol (with EconoTaq™ PCR)

(Please see Manual for detailed instructions.)

1. Phosphorylate the primers.

Primer kinase reaction

2.0 µl Forward primer @ 100 pmol/µl
2.0 µl Reverse primer @ 100 pmol/µl
1.0 µl 10 X Primer Kinase Buffer
1.0 µl T4 PNK (10 U/µl)
4.0 µl H_2O

10.0 µl total

Incubate at 37°C, 10 minutes

After the incubation, add 2-5 ul of this reaction directly to a 50-100 µl PCR mix and amplify according to standard protocols

2. PCR amplify DNA using the provided EconoTaq DNA Polymerase (or other non-proofreading enzyme, such as Taq, Tfl, Tth, or Tbr DNA polymerase)**.**

3. Purify DNA by affinity matrix or gel electrophoresis. Do NOT use short wave UV light.

4. Ligation to pGC Blue Cloning Vector. Mix the following in a 1.5-ml tube. Add ligase last.

x µl Insert DNA (10-400 ng, 5'-phosphorylated, G tailed)
y µl H_2O
2.5 µl 4X pGC Blue Vector Premix
1.0 µl CloneSmart® DNA Ligase (2 U/µl)

10.0 µl total reaction volume

Incubate 30 minutes at room temperature (incubate 2 hours for maximum number of clones). Heat denature the ligation reaction 15 minutes at 70°C.

5. Transformation. *Important*: Use only Electrocompetent cells for Electroporation and Chemically Competent cells for Heat Shock Transformation!

Thaw *E. cloni®* Competent Cells on wet ice. Pipet cells into a pre-chilled tube on ice. Add 1-2 µl of heat-treated ligation reaction to an aliquot of chilled cells on ice.

Electroporation	Heat Shock Transformation
A) Pipet 25 µl of the cell/DNA mixture to a chilled electroporation cuvette. B) Electroporate. Immediately add 975 µl of room temperature Recovery Medium. C) Place in culture tube.	A) Incubate 30 minutes on ice. B) Incubate 45 seconds at 42 °C; then 2 minutes on ice. Add 260 µl of room temperature Recovery Medium to the culture tube.

Shake at 250 rpm for 1 hour at 37°C. Spread up to 100 µl on YT+KanXI agar plate. Incubate at 37°C.

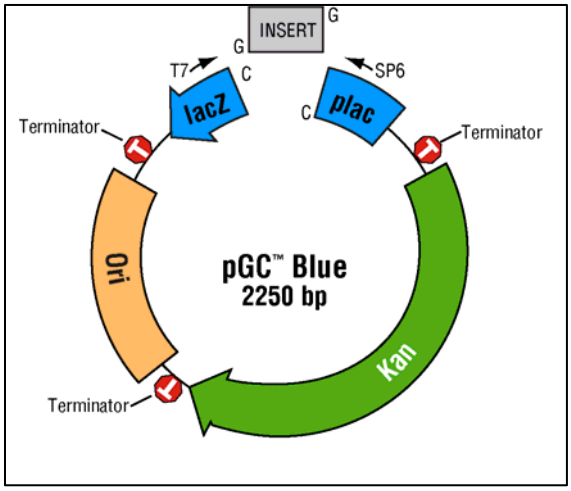
6. Colony Growth. Pick white colonies and grow in TB+Kan.

Appendix D: Vector Map, Cloning Sites, and Sequencing Primers

The pGC Blue vector is supplied predigested, with dephosphorylated 5' ends and a single 3'-C overhang. Transcriptional terminators border the *lac*Z gene to prevent transcription from the insert into the vector. Another terminator at the 3' end of the kanamycin resistance gene prevents this transcript from reading into the insert DNA.

The sequences of the Z FOR and Z REV primers are as follows:

Z FOR: 5'–CGCCAGGGTTTTCCCAGTCACGAC–3'
Z REV: 5'–AGCGGATAACAATTTCACACAGGA–3'



Z Rev Primer — *lac*Zα start — SP6

```
AGCGGATAACAATTTCACACAGGAAACAGCT ATG ACC ATG ATT ACG CCA AGC TAT TTA GGT
TCGCCTATTGTTAAAGTGTGTCCTTTGTCGA TAC TGG TAC TAA TGC GGT TCG ATA AAT CCA
```

Promoter — HindIII KpnI SacI BamHI

```
GAC ACT ATA GAA TAC TCA AGC TAT GCA TCA AGC TTG GTA CCG AGC TCG GAT CCA
CTG TGA TAT CTT ATG AGT TCG ATA CGT AGT TCG AAC CAT GGC TCG AGC CTA GGT

  SpeI          BstXI          EcoRI                              EcoRI
CTA GTA ACG GCC GCC AGT GTG CTG GAA TTC GAC CTC [PCR Product] GGG GTC GAA
GAT CAT TGC CGG CGG TCA CAC GAC CTT AAG CTG GAG                CCC CAG CTT

         EcoRV  BstXI         NotI      XhoI           XbaI    ApaI
TTC TGC AGA TAT CCA TCA CAC TGG CGG CCG CTC GAG CAT GCA TCT AGA GGG CCC
AAG ACG TCT ATA GGT AGT GTG ACC GCC GGC GAG CTC GTA CGT AGA TCT CCC GGG

AAT TCG CCC TAT AGT GAG TCG TAT TAC AAT TCA CTG GCC GTC GTT TTA CAA
TTA AGC GGG ATA TCA CTC AGC ATA ATG TTA AGT GAC CGG CAG CAA AAT GTT
```

T7 Promoter

```
CGT CGT GAC TGG GAA AAC CCT GGC GTT
GCA GCA CTG ACC CTT TTG GGA CCG CAA
```

Z For Primer

Appendix E: Troubleshooting Guide

Problem	Probable Cause	Solution
Very few or no transformants	PCR amplicon is not phosphorylated.	The GC Cloning vectors are dephosphorylated, requiring insert DNA to have 5' phosphates.
	Contaminating enzymes in ligation reaction.	Heat-denature enzymes used to prepare DNA 15 minutes at 70°C. Purify DNA by extraction or adsorption to matrix.
	No DNA, degraded DNA, or insufficient amount of DNA.	Check insert DNA by gel electrophoresis. Determine concentration of insert and add the correct amount. Use the supplied control insert to test the system.
	Ligation reaction failed.	Check the insert DNA for self-ligation by gel electrophoresis. Repeat G-tailing reaction if necessary. Be sure insert DNA is phosphorylated. Use the supplied control insert to test ligation reaction.
	Inadequate heat denaturation of ligation reaction.	Be certain to heat denature for 15 min at 70°C. Skipping this step may lower the number of transformants by 2-3 orders of magnitude.
	Loss of DNA during precipitation.	DO NOT precipitate DNA after ligation reaction. It is not necessary with this protocol and these cells.
	Incorrect recovery media.	Use Recovery Medium following transformation.
	Improper electroporation conditions.	Use Eppendorf, BTX, or BioRad electroporation cuvettes with a gap of 0.1 cm. Pre-chill cuvettes on ice. Add the 1 µl of DNA to 25 µl of pre-aliquotted cells on wet ice; DO NOT add the cells to the DNA.
	Addition of XGAL/DMSO to competent cells.	DO NOT add additional compounds to competent cells, as they are fragile.
	Incorrect amounts of antibiotic in agar plates. Wrong antibiotic used.	Add the correct amount of kanamycin to molten agar at 55°C before pouring plates (see Appendix A). DO NOT spread antibiotic onto the surface of agar plates.
High background of transformants that do not contain detectable inserts.	Small inserts from primer dimer amplification are preferentially cloned.	Gel purify PCR amplicons away from primer-dimers.
	Incorrect amount of antibiotic in agar plates.	DO NOT spread antibiotic onto the surface of agar plates. Add the correct amount of kanamycin to molten agar at 55°C before pouring plates (see Appendix A).
	Unstable DNA Inserts	Use pSMARTGC LCKan or pJAZZ™GC linear vector for maximum clone stability.

Appendix F: Sequence of pGC Blue vector

Note: During processing, the pGC Blue vector is linearized by removal of the underlined region.

ATTTAAATGGTCAGTGCTAGCCGTAAAGGCATATGAATGTGAGTTAGCTCACTCATTAGG
CACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGAT
AACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTATTTAGGTGACACT
ATAGAATACTCAAGCTATGCATCAAGCTTGGTACCGAGCTCGGATCCACTAGTAACGGCC
GCCAGTGTGCTGGAATTCGAC**CTCGTGTCGTGTGTTAACGCACGACTCG**GGGTCGAATTC
TGCAGATATCCATCACACTGGCGGCCGCTCGAGCATGCATCTAGAGGGCCCAATTCGCCC
TATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAAC
CCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAAT
AGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGG
ACGCGCCCTGTAGCGGCGCATTAACGTAGCCCTTATCGCATATGTCAGACTCCAGCGTAA
CTGGACTGCAATCAACTCACTGGCTCACCTTCACGGGTGGGCCTTTCTTCGGTAGAAAAT
CAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAA
ACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAG
GTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTA
GGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTA
CCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAG
TTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTG
GAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACG
CTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAG
CGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGC
CACCTCTGACTTGAGCATCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAA
AACGCCAGCAACGCAGAAAGGCCCACCCGAAGGTGAGCCAGGTGATTACATTTAGATCTT
TATCAGAAGAACTCGTCAAGAAGGCGATAGAAGGCGATGCGCTGCGAATCGGGAGCGGCG
ATACCGTAAAGCACGAGGAAGCGGTCAGCCCATTCGCCGCCAAGCTCTTCAGCAATATCA
CGGGTAGCCAACGCTATGTCCTGATAGCGGTCCGCCACACCCAGCCGGCCACAGTCGATG
AATCCAGAAAAGCGGCCATTTTCCACCATGATATTCGGCAAGCAGGCATCGCCATGGGTC
ACGACGAGATCCTCGCCGTCGGGCATGCGCGCCTTGAGCCTGGCGAACAGTTCGGCTGGC
GCGAGCCCCTGATGCTCTTCGTCCAGATCATCCTGATCGACAAGACCGGCTTCCATCCGA
GTACGTGCTCGCTCGATGCGATGTTTCGCTTGGTGGTCGAATGGGCAGGTAGCCGGATCA
AGCGTATGCAGCCGCCGCATTGCATCAGCCATGATGGATACTTTCTCGGCAGGAGCAAGG
TGGGATGACAGGAGATCCTGCCCCGGCACTTCGCCCAATAGCAGCCAGTCCCTTCCCGCT
TCAGTGACAACGTCGAGCACAGCTGCGCAAGGAACGCCCGTCGTGGCCAGCCACGATAGC
CGCGCTGCCTCGTCCTGCAGTTCATTCAGGGCACCGGACAGGTCGGTCTTGACAAAAAGA
ACCGGGCGCCCCTGCGCTGACAGCCGGAACACGGCGGCATCAGAGCAGCCGATTGTCTGT
TGTGCCCAGTCATAGCCGAATAGCCTCTCCACCCAAGCGGCCGGAGAACCTGCGTGCAAT
CCATCTTGTTCAATCATTTTAGCTTCCTTAGCTCCTGAAAATCTCGATAACTCAAAAAAT
ACGCCCGGTAGTGATCTTATTTCATTATGGTGAAAGTTGGAACCTCTTACGTGCCGATCA
AGTCAAAAGCCTCCGGTCGGAGGCTTTTGACTTTCTGCTATGGAGGTCAGGTATG