Protocol

Cloning Polymerase Chain Reaction (PCR) Products: TA Cloning

Michael R. Green and Joseph Sambrook

The nontemplate-dependent terminal transferase activity inherent in nonproofreading DNA polymerases such as *Taq* provides a highly efficient method to clone PCR products. The enzyme adds a single, unpaired residue—preferentially an adenosyl residue—to each 3' end of a double-stranded amplified product. The unpaired terminal (A) residues can pair with a linear T vector that carries an unpaired 3'-thymidyl residue at each end. The two chief advantages of TA cloning are speed and lack of reliance on restriction enzymes. The major disadvantage is an inability to clone directionally. For this reason, it is important to pick and analyze several transformed clones when a particular orientation of the amplified fragment is required.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at <http://cshprotocols.cshlp.org/site/recipes>.

Reagents

Agarose gels (and other reagents and equipment as required) (see Step 5)

ATP (10 mM)

Omit ATP from the ligation reaction in Step 1 if the ligation buffer contains ATP.

Bacteriophage T4 DNA ligase and buffer

*See Introduction: **Ligation and Ligases** (Green and Sambrook 2019a). Some commercial ligase buffers contain ATP; when using such buffers, addition of ATP is not required.*

SOB <R>, SOC <R>, or LB <R> agar plates containing the appropriate antibiotic

*If blue/white screening is to be used, the plates should also contain X-Gal and IPTG (see Protocol: **Screening Bacterial Colonies Using X-Gal and IPTG: α -Complementation** [Green and Sambrook 2019b]).*

Target DNA (25 ng/mL), amplified by PCR catalyzed by a nonproofreading thermostable DNA polymerase (e.g., *Taq*)

*Typically, only ~30% of the amplified products will carry an unpaired 3'-adenosyl residue. To maximize the efficiency of *Taq*-catalyzed addition of an unpaired A residue use primers carrying either a 5'-G residue or a 5'-A residue (Magnuson et al. 1996). The efficiency of the nontemplated addition reaction can be improved by programming a 10-min incubation at 72°C at the end of the PCR amplification cycles.*

Before cloning, check the size of an aliquot of the PCR product by gel electrophoresis. When the PCR generates more than one or two bands of amplified DNA, purify the target fragment by electrophoresis through low-melting/gelling-temperature agarose. If not purified by gel electrophoresis, PCR-amplified DNA may be prepared for ligation by extracting the PCR with phenol:chloroform and precipitating the amplified DNA with ethanol.

From the Molecular Cloning collection, edited by Michael R. Green and Joseph Sambrook.

© 2021 Cold Spring Harbor Laboratory Press

Cite this protocol as *Cold Spring Harb Protoc*; doi:10.1101/pdb.prot101303

However, many researchers will prefer to use a commercial cleanup kit, such as Wizard SV Gel and PCR Clean-Up System (Promega), PureLink PCR purification kit (Life Technologies), Millipore Ultrafree spin columns, or QIAquick PCR purification kit (QIAGEN). Some researchers recommend using the PCR products within a day or two of synthesis in insurance against loss of the protruding A-residue. Both T vectors and target DNAs tend to lose their unpaired 3'-residues during prolonged storage at 4°C and when frozen and thawed many times.

T vector

For synthesis of T vectors, see Protocol: *Cloning Polymerase Chain Reaction (PCR) Products: Making T Vectors* (Green and Sambrook 2021).

Ready-made T-vectors can be purchased from many commercial suppliers as components of cloning kits (e.g., pCR-Script [SK+] from Stratagene; pCRII in the TA Cloning kit from Life Technologies; pGEM-T from Promega) (Hengen 1995).

Transformation-competent *E. coli* cells (and other reagents and equipment for transforming *E. coli* as required, described in Protocol: *The Hanahan Method for Preparation and Transformation of Competent Escherichia coli: High-Efficiency Transformation* [Green and Sambrook 2018] and Protocol: *The Inoue Method for Preparation and Transformation of Competent Escherichia coli: "Ultracompetent" Cells* [Green and Sambrook 2020a])

Transformation-competent *E. coli* cells can be bought commercially or prepared as described in Protocol: *The Hanahan Method for Preparation and Transformation of Competent Escherichia coli: High-Efficiency Transformation* (Green and Sambrook 2018), Protocol: *The Inoue Method for Preparation and Transformation of Competent Escherichia coli: "Ultracompetent" Cells* (Green and Sambrook 2020a), Protocol: *Easy Transformation of Escherichia coli: Nanoparticle-Mediated Transformation* (Green and Sambrook 2019c), and Protocol: *Transformation of Escherichia coli by Electroporation* (Green and Sambrook 2020b).

Equipment

Incubator (37°C)
Water bath (preset to 14°C)

METHOD

For more information on this method, see Clark (1988); Holton and Graham (1991); Marchuk et al. (1991); Trower and Elgar (1994); Zhou and Gomez-Sanchez (2000).

1. In a microcentrifuge tube, set up a ligation reaction.

Amplified target DNA (25 ng/μL)	1 μL
T-tailed plasmid (75 ng/μL)	1 μL
Ligation buffer (10×)	1 μL
Bacteriophage T4 DNA ligase	3 U
H ₂ O	to 10 μL

The optimal molar ratio of vector:amplified DNA in the ligation mixture is 3:1. The amounts of the two DNAs suggested for the ligation reaction (above) assume that the vector and amplified DNA are of equal size. This will rarely be the case. When the size of the target DNA is known, the relative amounts of the two types of DNA included in the ligation reaction should be altered to achieve a molar ratio of 3:1 (vector:amplified DNA).

If necessary, add ATP to a final concentration of 1 mM. Set up a control reaction that contains all the reagents listed above except the amplified target DNA.

2. Incubate the ligation mixtures for 4 h at 14°C.
3. Dilute 5 μL of each of the two ligation mixtures with 10 μL of H₂O and transform a suitable strain of competent *E. coli* to antibiotic resistance as described in Protocol: *The Hanahan Method for Preparation and Transformation of Competent Escherichia coli: High-Efficiency Transformation* (Green and Sambrook 2018), Protocol: *The Inoue Method for Preparation and Transformation of Competent Escherichia coli: "Ultracompetent" Cells* (Green and Sambrook 2020a), Protocol: *Easy Transformation of Escherichia coli: Nanoparticle-Mediated Transformation* (Green and Sambrook 2019c), and Protocol: *Transformation of Escherichia coli by Electroporation* (Green and Sambrook 2020b). Plate the transformed cultures on media containing the

appropriate antibiotic and, depending on the genotype of the host and vector, IPTG and X-Gal (see Protocol: **Screening Bacterial Colonies Using X-Gal and IPTG: α -Complementation** [Green and Sambrook 2019b]).

4. Calculate the number of colonies obtained from each of the ligation mixtures. If the plasmid is equipped for blue/white screening, pick a number of white colonies obtained by transformation with the ligation reaction containing the target DNA. In different experiments, the ratio of blue: white colonies can vary between 1:5 and 2:1.
5. Confirm the presence of the amplified fragment by isolating the recombinant plasmid DNAs and digesting them with the appropriate restriction enzymes. Fractionate the restricted DNA by electrophoresis through an agarose gel using appropriate DNA size markers.

RELATED INFORMATION

TA cloning is universal in the sense that any T vector can be used to clone any A-tailed segment of DNA. PCR may be the method most commonly used to generate A-tailed DNA molecules, but it is not the only way. DNA fragments with blunt ends or 5'-protruding termini can be equipped with 3'-adenosyl tails in a reaction containing all four dNTPs that is catalyzed by *Taq* polymerase. The enzyme first polishes the termini and then adds the supernumerary 3'-A residues. An extra step is required to attach unpaired 3'-adenosyl residues to DNA with protruding 3' tails. The termini are removed in a reaction catalyzed by T4 DNA polymerase and containing all four dNTPs. The blunt-ended molecules can then be equipped with 3'-A tails, as described above.

RECIPES

LB (Luria-Bertani) Liquid Medium

Reagent	Amount to add
H ₂ O	950 mL
Tryptone	10 g
NaCl	10 g
Yeast extract	5 g

Combine the reagents and shake until the solutes have dissolved. Adjust the pH to 7.0 with 5 N NaOH (~0.2 mL). Adjust the final volume of the solution to 1 L with H₂O. Sterilize by autoclaving for 20 min at 15 psi (1.05 kg/cm²) on liquid cycle.

For solid medium, see the recipe entitled "Media containing agar or agarose."

Media Containing Agar or Agarose

Prepare liquid media according to the recipe given. Just before autoclaving, add one of the following:

Bacto agar (for plates)	15 g/L
Bacto agar (for top agar)	7 g/L
Agarose (for plates)	15 g/L
Agarose (for top agarose)	7 g/L

Sterilize by autoclaving for 20 min at 15 psi (1.05 kg/cm²) on liquid cycle. When the medium is removed from the autoclave, swirl it gently to distribute the melted agar or agarose evenly throughout the solution. *Be careful!* The fluid may be superheated and

may boil over when swirled. Before adding thermolabile substances (e.g., antibiotics), allow the medium to cool to 50°C–60°C, and mix the medium by swirling to avoid producing air bubbles.

Before pouring the plates, set up a color code (e.g., two red stripes for LB-ampicillin plates; one black stripe for LB plates, etc.), and mark the edges of the plates with the appropriate colored markers. Pour plates directly from the flask; allow ~30–35 mL of medium per 90-mm plate. To remove bubbles from the medium in the plate, flame the surface of the medium with a Bunsen burner before the agar or agarose hardens. When the medium has hardened completely, invert the plates and store them at 4°C until needed.

The plates should be removed from storage 1–2 h before they are used. If the plates are fresh, they will “sweat” when incubated at 37°C. When this condensation drops on the agar/agarose surface, it allows bacterial colonies or bacteriophage plaques to spread and increases the chances of cross-contamination. This problem can be avoided by wiping off the condensation from the lids of the plates and then incubating the plates for several hours at 37°C in an inverted position before they are used. Alternatively, remove the liquid by shaking the lid with a single, quick motion. To minimize the possibility of contamination, hold the open plate in an inverted position while removing the liquid from the lid.

SOB Medium

Per liter: To 950 mL of deionized H₂O, add:

Tryptone	20 g
Yeast extract	5 g
NaCl	0.5 g

Shake until the solutes have dissolved. Add 10 mL of a 250 mM solution of KCl. (This solution is made by dissolving 1.86 g of KCl in 100 mL of deionized H₂O.) Adjust the pH of the medium to 7.0 with 5 N NaOH (~0.2 mL). Adjust the volume of the solution to 1 L with deionized H₂O. Sterilize by autoclaving for 20 min at 15 psi (1.05 kg/cm²) on liquid cycle. Just before use, add 5 mL of a sterile solution of 2 M MgCl₂. (This solution is made by dissolving 19 g of MgCl₂ in 90 mL of deionized H₂O. Adjust the volume of the solution to 100 mL with deionized H₂O and sterilize by autoclaving for 20 min at 15 psi [1.05 kg/cm²] on liquid cycle.)

For solid medium, please see the recipe entitled “Media containing agar or agarose.”

<R>

SOC Medium

Per liter: To 950 mL of deionized H₂O, add:

Tryptone	20 g
Yeast extract	5 g
NaCl	0.5 g

SOC medium is identical to SOB medium, except that it contains 20 mM glucose. To prepare SOB medium, combine the above ingredients and shake until the solutes have dissolved. Add 10 mL of a 250 mM solution of KCl. (This solution is made by dissolving 1.86 g of KCl in 100 mL of deionized H₂O.) Adjust the pH of the medium to 7.0 with 5 N NaOH (~0.2 mL). Adjust the volume of the solution to 1 L with deionized H₂O. Sterilize by autoclaving for 20 min at 15 psi (1.05 kg/cm²) on liquid cycle. Just before use, add 5 mL of a sterile solution of 2 M MgCl₂. (This solution is made by dissolving 19 g of MgCl₂

M.R. Green and J. Sambrook

in 90 mL of deionized H₂O. Adjust the volume of the solution to 100 mL with deionized H₂O and sterilize by autoclaving for 20 min at 15 psi [1.05 kg/cm²] on liquid cycle.)

After the SOB medium has been autoclaved, allow it to cool to 60°C or less. Add 20 mL of a sterile 1 M solution of glucose. (This solution is made by dissolving 18 g of glucose in 90 mL of deionized H₂O. After the sugar has dissolved, adjust the volume of the solution to 100 mL with deionized H₂O and sterilize by passing it through a 0.22-μm filter.)

REFERENCES

- Clark JM. 1988. Novel non-templated nucleotide addition reactions catalyzed by procaryotic and eucaryotic DNA polymerases. *Nucleic Acids Res* **16**: 9677–9686.
- Green MR, Sambrook J. 2018. The Hanahan method for preparation and transformation of competent *Escherichia coli*: High-efficiency transformation. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot101188.
- Green MR, Sambrook J. 2019a. Ligation and ligases. *Cold Spring Harb Protoc* doi: 10.1101/pdb.top101352.
- Green MR, Sambrook J. 2019b. Screening bacterial colonies using X-Gal and IPTG: α-Complementation. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot101329.
- Green MR, Sambrook J. 2019c. Easy transformation of *Escherichia coli*: Nanoparticle-mediated transformation. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot101204.
- Green MR, Sambrook J. 2020a. The Inoue method for preparation and transformation of competent *Escherichia coli*: “Ultracompetent” cells. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot101196.
- Green MR, Sambrook J. 2020b. Transformation of *Escherichia coli* by electroporation. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot101220.
- Green MR, Sambrook J. 2021. Cloning polymerase chain reaction (PCR) products: Making T vectors. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot101295.
- Hengen PN. 1995. Methods and reagents. Cloning PCR products using T-vectors. *Trends Biochem Sci* **20**: 85–86.
- Holton TA, Graham MW. 1991. A simple and efficient method for direct cloning of PCR products using ddT-tailed vectors. *Nucleic Acids Res* **19**: 1156.
- Magnuson VL, Ally DS, Nylund SJ, Karanjawala ZE, Rayman JB, Knap JJ, Lowe AL, Ghosh S, Collins FS. 1996. Substrate nucleotide-determined non-templated addition of adenine by *Taq* DNA polymerase: Implications for PCR-based genotyping and cloning. *Biotechniques* **21**: 700–709.
- Marchuk D, Drumm M, Saulino A, Collins FS. 1991. Construction of T-vectors, a rapid and general system for direct cloning of unmodified PCR products. *Nucleic Acids Res* **19**: 1154.
- Trower MK, Elgar GS. 1994. PCR cloning using T-vectors. *Methods Mol Biol* **31**: 19–33.
- Zhou MY, Gomez-Sanchez CE. 2000. Universal TA cloning. *Curr Issues Mol Biol* **2**: 1–7.

Cold Spring Harbor Protocols

Cloning Polymerase Chain Reaction (PCR) Products: TA Cloning

Michael R. Green and Joseph Sambrook

Cold Spring Harb Protoc; doi: 10.1101/pdb.prot101303

Email Alerting Service

Receive free email alerts when new articles cite this article - [click here](#).

Subject Categories

Browse articles on similar topics from *Cold Spring Harbor Protocols*.

[Cloning of PCR Products](#) (20 articles)
[Molecular Biology, general](#) (1293 articles)
[Plasmids](#) (154 articles)
[Polymerase Chain Reaction \(PCR\)](#) (139 articles)
[Polymerase Chain Reaction \(PCR\), general](#) (184 articles)
[Vectors](#) (141 articles)

To subscribe to *Cold Spring Harbor Protocols* go to:
<http://cshprotocols.cshlp.org/subscriptions>