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PRODUCT INFORMATION

Thermo Scientific
CloneJET PCR Cloning Kit
#K1231, #K1232

Note:
revised improved protocol

www.thermoscientific.com/onebio

#___
Lot __
Expiry Date __

CERTIFICATE OF ANALYSIS

All components of the kit were function tested in control experiment as described in the manual.
A 2 µl aliquot of the ligation mixture was used to transform 50 µl of chemically competent XL1-Blue cells of >10^6 cfu/µg DNA transformation efficiency.
Cloning efficiency of the Control PCR Product into the pJET1.2/blunt was >2x10^4 cfu/µg DNA.
>90% of the recombinant plasmids contained the appropriate insert.

Quality authorized by:  Jurgita Zilinskiene

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COMPONENTS OF THE KIT

CloneJET PCR Cloning Kit	20 cloning reactions #K1231	40 cloning reactions #K1232
pJET1.2/blunt Cloning Vector (50 ng/µl)	24 µl	46 µl
2X Reaction Buffer	300 µl	600 µl
T4 DNA Ligase (5 u/µl)	24 µl	46 µl
DNA Blunting Enzyme	24 µl	46 µl
pJET1.2 Forward Sequencing Primer, 10 µM aqueous solution	50 µl	100 µl
pJET1.2 Reverse Sequencing Primer, 10 µM aqueous solution	50 µl	100 µl
Control PCR Product (24 ng/µl) 976 bp, with 3'-dA overhangs	8 µl	12 µl
Water, nuclease-free	1.25 ml	1.25 ml

STORAGE

All components of the Thermo Scientific CloneJET PCR Cloning Kit should be stored at -20°C.

DESCRIPTION

The CloneJET™ PCR Cloning Kit is an advanced positive selection system for the highest efficiency cloning of PCR products generated with *Pfu* DNA polymerase, *Taq* DNA polymerase, Thermo Scientific DreamTaq DNA polymerase or other thermostable DNA polymerases. Additionally, any other DNA fragment, either blunt or sticky-end, can be successfully cloned using the kit.

The kit features the novel positive selection cloning vector pJET1.2/blunt. This vector contains a lethal gene which is disrupted by ligation of a DNA insert into the cloning site. As a result, only cells with recombinant plasmids are able to propagate, eliminating the need for expensive blue/white screening.

The vector contains an expanded multiple cloning site, as well as a T7 promoter for *in vitro* transcription. Sequencing primers are included for convenient sequencing of the cloned insert.

CLONING PRINCIPLE

pJET1.2/blunt is a linearized cloning vector, which accepts inserts from 6 bp to 10 kb. The 5'-ends of the vector cloning site contain phosphoryl groups, therefore, phosphorylation of the PCR primers is not required.

Blunt-end PCR products generated by proofreading DNA polymerases can be directly ligated in just 5 min with the pJET1.2/blunt cloning vector. PCR products with 3'-dA overhangs generated using *Taq* DNA polymerase or other non-proofreading thermostable DNA polymerases are blunted in 5 min with a proprietary thermostable DNA blunting enzyme (included in the kit) prior to ligation. All common laboratory *E.coli* strains can be directly transformed with the ligation product.

Recircularized pJET1.2/blunt vector expresses a lethal restriction enzyme after transformation and is not propagated. As a result, only recombinant clones containing the insert appear on culture plates. Therefore, blue/white screening is not required.

IMPORTANT NOTES

- Thoroughly mix every vial before use.
- The CloneJET PCR Cloning Kit is compatible with all PCR buffers supplied by Thermo Scientific.
- Gel-analyze the PCR product for specificity and yield before cloning.
- Specific PCR products of <1 kb appearing as one discrete band on the gel can be used for ligation directly from PCR reaction mixture without any purification.
- Do not use more than 1 µl of unpurified PCR product in the blunting or ligation reaction. Excess polymerase (*Taq, Pfu* or other) or salts from the PCR reaction mixture may result in background colonies and may reduce the efficiency of the cloning procedure.
- Gel purification of the PCR product (e.g. with Thermo Scientific GeneJET Gel Extraction Kit, #K0691) is recommended to increase the number of recombinants containing full length inserts in following cases:
  - PCR product is longer than 1 kb;
  - PCR product is contaminated with non-specific PCR products;
  - PCR product is contaminated with primer-dimers;
  - PCR template contains β-lactamase (ampicillin resistance) gene, which may result in background colonies on LB-ampicillin agar plates.
- For efficient cloning of gel-purified DNA fragments, it is important to avoid DNA damage by ethidium bromide and UV light. Use a long wavelength UV (360 nm) light-box when excising DNA from the agarose gel. When using a short-wavelength (254-312 nm) light-box, limit DNA exposure to UV to a few seconds. Keep the gel on a glass plate or on a plastic plate during UV illumination. Alternatively, use dyes, like crystal violet, to visualize DNA in ambient light (1, 2).
- The kit performs well over a wide range of insert/vector molar ratios (0.5:1 to 15:1). However the optimal insert/vector ratio is 3:1. Vector pJET1.2/blunt is supplied at a concentration of 0.05 pmol DNA ends/µl. To calculate optimal amount of the PCR product for ligation (0.15 pmol of DNA ends respectively), refer to Table 1 on page 4 or use dedicated software (e.g., www.thermoscientific.com/reviewer) for calculations.

CONSIDERATIONS FOR CLONING LONG PCR PRODUCTS

Short DNA fragments (<1 kb) are cloned with a much higher efficiency compared to long ones. Therefore, long PCR products must be purified to remove any smaller fragments from the solution.

- Optimize PCR conditions to increase specificity and yield of full length PCR product.
- Gel-purify PCR products (e.g., with GeneJET™ Gel Extraction Kit, #K0691) to minimize presence of primer dimmers or non-specific short PCR products in the ligation reaction, even if these are not visible on the gel.
- Protect long PCR products from mechanical sharing and damage by nucleases:
  - Store the PCR product at -20°C if it is not used immediately.
  - Use clean labware, razor blade and electrophoresis tank. Prepare fresh electrophoresis running buffer for gel purification procedure.
- Avoid DNA damage by UV light (*see* Important Notes on page 3).
- Before ligation, verify the quantity and quality of the purified PCR product on a gel. The optimal insert/vector ratio for the ligation reaction is 3:1. Determine the required amount of PCR product for the reaction (0.15 pmol of DNA ends) referring to Table 1 or use dedicated software (like www.thermoscientific.com/reviewer) for calculations.

Table 1. Recommended amount of PCR product for the ligation reaction.

Length of PCR product (bp)	Optimal PCR product quantity for ligation reaction, (0.15 pmol ends)
100	5 ng
300	15 ng
500	25 ng
1000	50 ng
2000	100 ng
3000	150 ng
4000	200 ng
5000	250 ng

- Prolong the ligation time to 30 min for inserts of >3 kb. Ligation times longer than 30 min are not recommended and may decrease cloning efficiency.
- Use competent *E.coli* cells of high transformation efficiency (*see* p 5. Transformation)
- Success in cloning of long PCR products may also depend on the DNA sequence of the insert. PCR products may contain toxic sequences not tolerated by *E.coli*, therefore multicopy vectors like pJET1.2 may not be suitable cloning these PCR products.

Blunt-End Cloning Protocol

- For cloning blunt-end PCR products generated by proofreading DNA polymerases, such as *Pfu* DNA polymerase. (If the DNA end structure of the PCR products is not specified by the supplier of the DNA polymerase, follow the Sticky-End Cloning Protocol on page 6).
- For cloning of blunt-end DNA fragments generated by restriction enzyme digestion. Gel-purify the DNA fragment prior to ligation and use in a 3:1 molar ratio with pJET1.2/blunt (*see* Table 1).

1. Set up the ligation reaction on ice:

Component	Volume
2X Reaction Buffer	10 µl
Non-purified PCR product *or* purified PCR product/other blunt-end DNA fragment	1 µl 0.15 pmol ends
pJET1.2/blunt Cloning Vector (50 ng/µl)	1 µl (0.05 pmol ends)
Water, nuclease-free	up to 19 µl
T4 DNA Ligase	1 µl
Total volume	20 µl

Vortex briefly and centrifuge for 3-5 s.

2. Incubate the ligation mixture at room temperature (22°C) for 5 min.
Note. For PCR products >3 kb, ligation can be prolonged to 30 min. Ligation times longer than 30 min are not recommended and may decrease cloning efficiency.

3. Use the ligation mixture directly for transformation (*see* page 7 for Transformation).
Note. Keep the ligation mixture at -20°C if transformation is postponed. Thaw on ice and mix carefully before transformation.

Sticky-End Cloning Protocol

- For cloning PCR products with 3'-dA overhangs generated by *Taq* DNA polymerase, DreamTaq™ DNA polymerase or enzyme mixtures containing *Taq* DNA polymerase.
- For cloning PCR products when DNA end structure of the generated PCR products is not specified by the supplier of the DNA polymerase.
- For cloning DNA fragments with 5'- or 3'-overhangs generated by restriction enzyme digestion. Gel-purify the DNA fragment prior to ligation and use in a 3:1 molar ratio with pJET1.2/blunt (*see* Table 1).

Note. The DNA Blunting Enzyme is a proprietary thermostable DNA polymerase with proofreading activity. It will remove 3'- overhangs and fill-in 5'-overhangs. Nucleotides for the blunting reaction are included in the reaction buffer.

1. Set up the blunting reaction on ice:

Component	Volume
2X Reaction Buffer	10 µl
Non-purified PCR product *or* purified PCR product/other sticky-end DNA fragment	1 µl 0.15 pmol ends
Water, nuclease-free	to 17 µl
DNA Blunting Enzyme	1 µl
Total volume	18 µl

Vortex briefly and centrifuge for 3-5 s.

2. Incubate the mixture at 70°C for 5 min. Chill on ice.

3. Set up the ligation reaction on ice. Add the following to the blunting reaction mixture:

Component	Volume
pJET1.2/blunt Cloning Vector (50 ng/µl)	1 µl (0.05 pmol ends)
T4 DNA Ligase	1 µl
Total volume	20 µl

Vortex briefly and centrifuge for 3-5 s to collect drops.

4. Incubate the ligation mixture at room temperature (22°C) for 5 min.

   Note. For PCR products >3 kb, ligation can be prolonged to 30 min. Ligation times longer than 30 min are not recommended and may decrease cloning efficiency.

5. Use the ligation mixture directly for transformation (*see* page 7 for Transformation).

   Note. Keep the ligation mixture at -20°C if transformation is postponed. Thaw on ice and mix carefully before transformation.

Transformation

- The CloneJET PCR Cloning Kit is compatible with all common *E.coli* laboratory strains. Transformation of competent *E.coli* cells with the ligation mixture can be performed using different transformation methods (Table 2 and 3).
- The number of transformants on the plates directly depends on the transformation efficiency of the competent cells.
- For successful cloning, competent *E.coli* cells should have an efficiency of at least $1x10^6$ cfu/ µg supercoiled plasmid DNA. To check the efficiency, prepare a control transformation with 0.1 ng of a supercoiled vector DNA, e.g., pUC19 DNA, #SD0061 (Table 2).

Table 2. Evaluation of transformation efficiency of competent cells (control transformation).

Transformation method	Number of transformants per µg of supercoiled plasmid DNA	Amount of pUC19 DNA for control transformation (to yield ~ 1000 colonies per plate)	Volume of competent cells
Thermo Scientific TransformAid Bacterial Transformation Kit (#K2710)*	~$1x10^7$	0.1ng	50 µl
Calcium chloride transformation	~$1x10^6$	1ng	50 µl
Electro-transformation	~$1x10^9$	0.01 ng	40 µl

* XL1-Blue, ER2267, ER1727 *E. coli* strains are the best strains for transformation with TransformAid™ Bacterial Transformation Kit. DH10B, DH5α and TOP10 strains are not efficient with TransformAid but are recommended for calcium chloride transformation or electroporation.

- For transformation of the ligation mixture, refer to Table 3.

Table 3. Recommendations for transformation of ligation mixture.

Tranformation method	Treatment of the ligation mixture before transformation	Volume of the ligation mixture for transformation	Volume of competent cells for transformation
TransformAid Bacterial Transformation Kit (#K2710)	Not necessary	≤2.5 µl	50 µl
Calcium Chloride Transformation	Not necessary	≤5 µl	50 µl
Electro-transformation	Spin column (GeneJET PCR Purification Kit, #K0701) or chloroform extraction (*see* protocol on p. 11)	1 µl of purified ligation mixture	40 µl

Analysis of recombinant clones

Analyze 4-6 colonies for the presence and orientation of the DNA insert using one of the following methods:

Colony PCR

Use the following protocol for colony screening by PCR if the cloned PCR fragment is shorter than 3kb. For longer inserts, perform restriction analysis.

1. Prepare enough PCR master mix for the number of colonies analyzed plus one extra. For each 20 µl reaction, mix the following reagents:

Component	Using *Taq* DNA Polymerase	Using 2X PCR Master Mix
10X *Taq* buffer	2.0 µl	–
dNTP mix, 2 mM each	2.0 µl	–
25 mM $MgCl_2$	1.2 µl	–
pJET1.2 Forward Sequencing Primer, 10 µM	0.4 µl	0.4 µl
pJET1.2 Reverse Sequencing Primer, 10 µM	0.4 µl	0.4 µl
Water, nuclease-free	13.9 µl	9.2 µl
Taq DNA Polymerase 5 u/µl, #EP0401 *or* DreamTaq Green DNA Polymerase, #EP0711	0.1 µl	–
PCR Master Mix (2X), #K0171 *or* DreamTaq Green PCR Master Mix (2X), #K1081	–	10 µl
Total volume	20 µl	20 µl

2. Mix well. Aliquot 20 µl of the mix into the PCR tubes on ice.
3. Pick an individual colony and resuspend in 20 µl of the PCR master mix.
4. Perform PCR: 95°C, 3 min; 94°C, 30 s, 60°C, 30 s, 72°C 1 min/kb; 25 cycles.
5. Analyze on an agarose gel for the presence of the PCR product.

Note
Due to considerable amount of recircularised vector plated on the surface of plate, colony PCR may give some false-negative results. Prior to clone analysis propagate short strikes of individual colonies on ampicillin plates. Then use small amount of each for colony PCR.

Restriction analysis

Isolate plasmid DNA from an overnight bacterial culture. To speed up the process and to assure the quality of purified plasmid DNA, use the GeneJET Plasmid Miniprep Kit (#K0503). To digest DNA from recombinant clones in just 5 minutes, use Thermo Scientific FastDigest restriction enzymes.

Sequencing

Use the pJET1.2 Forward Sequencing Primer or pJET1.2 Reverse Sequencing Primer supplied with the kit to sequence the cloned insert. *See* page 11 for primer sequences.

CONTROL CLONING EXPERIMENT

The control reaction should be used to verify the efficiency of the blunting and ligation steps. The 976 bp control PCR product (nucleotide sequence is available at www.thermoscientific.com/onebio) has been generated with *Taq* DNA polymerase, which adds extra nucleotides to the 3'-end. Therefore, the **Sticky-End Protocol** must be followed.

1. Set up the blunting reaction **on ice**:

Component	Volume
2X Reaction Buffer	10 µl
Control PCR Product (24 ng/µl)	2 µl
Water, nuclease-free	5 µl
DNA Blunting Enzyme	1 µl
Total volume	18 µl

   Vortex briefly and centrifuge for 3-5 s to collect drops.

2. Incubate the mixture at 70°C for 5 min. Chill on ice.

3. Set up the ligation reaction **on ice**. Add the following to the blunting reaction mixture:

Component	Volume
pJET1.2/blunt Cloning Vector (50 ng/µl)	1 µl
T4 DNA Ligase	1 µl
Total volume	20 µl

   Vortex briefly and centrifuge for 3-5 s to collect drops.

4. Incubate the ligation mixture at room temperature (22°C) for 5 min.

5. Use the ligation mixture directly for transformation (*see* page 7 for Transformation). Keep the ligation mixture at -20°C if transformation is postponed. Thaw on ice and mix carefully before transformation.
   Analyze colonies by colony PCR (*see* page 8). At least 9 of 10 analyzed colonies should contain recombinant plasmid with the 976 bp insert.
   The number of transformants depends on the transformation efficiency of the *E. coli* cells. Verify the transformation efficiency by transforming supercoiled plasmid, e.g., pUC19 DNA (#SD0061) in parallel. Refer to page 7 Table 2 for correct control transformations.

MAP AND FEATURES OF pJET1.2/blunt CLONING VECTOR

The pJET1.2/blunt cloning vector has been linearized with Eco32I (EcoRV) (GenBank/EMBL Accession number EF694056). The blunt ends of the vector contain 5'-phosphoryl groups. The nucleotide sequence of pJET1.2/blunt is available at www.thermoscientific.com/onebio

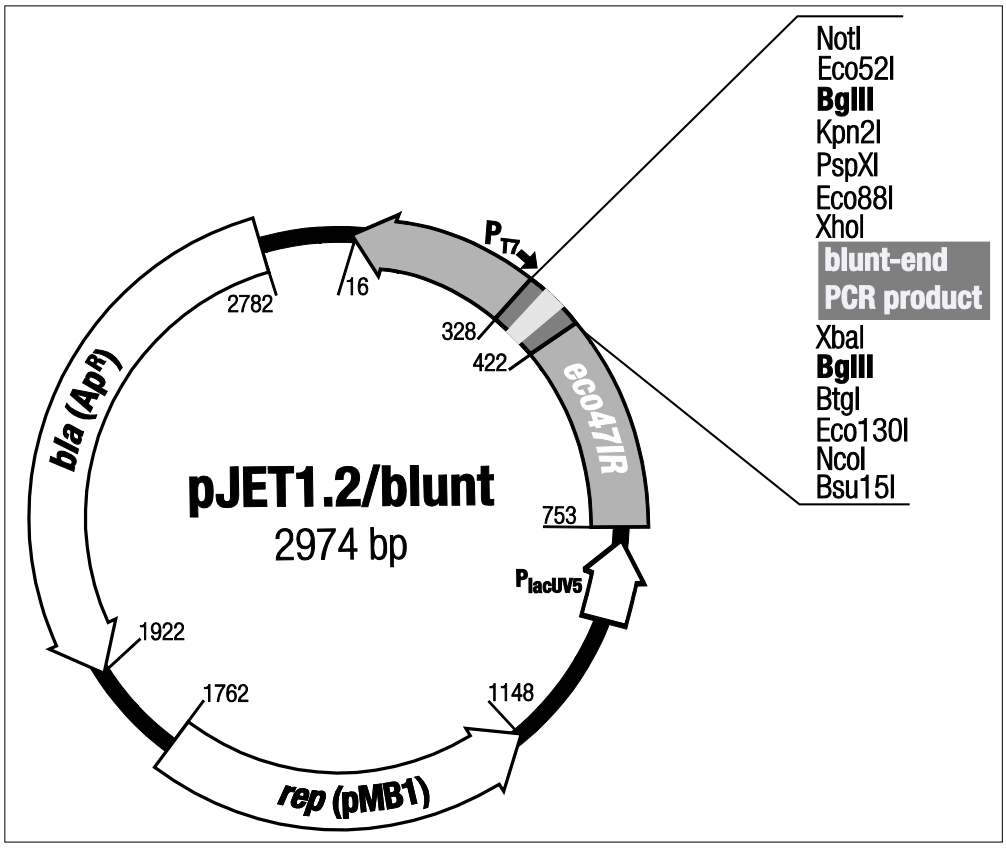

Fig. 1. pJET1.2/blunt Vector Map.

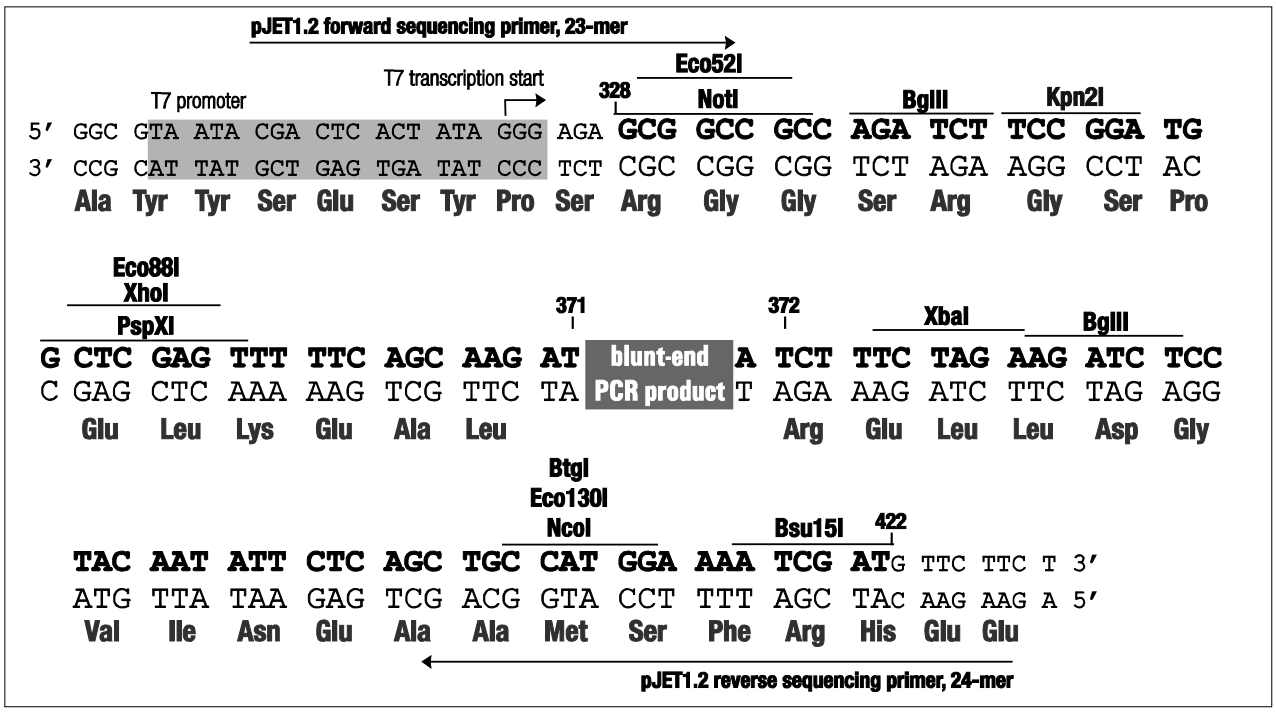

Fig. 2. DNA Sequence of MCS region.

Genetic elements of the pJET1.2/blunt cloning vector

Element	Function	Position (bp)
rep (pMB1)	Replicon (rep) from the pMBI plasmid responsible for the replication of pJET1.2	1762-1148
Replication start	Initiation of replication	1162±1
bla (Ap^R)	β-lactamase gene conferring resistance to ampicillin. Used for selection and maintenance of recombinant *E.coli* cells	2782-1922
eco47IR	Lethal gene *eco47IR* enables positive selection of recombinant plasmid	753-16
P_{lacUV5}	Modified P_{lac} promoter for expression of the *eco47IR* gene at a level sufficient to allow for positive selection	892-769
T7 promoter	T7 RNA polymerase promoter for *in vitro* transcription of the cloned insert	305-324
Multiple cloning site (MCS)	Mapping, screening and excision of the cloned insert	422-328
Insertion site	Blunt DNA ends for ligation with insert	371-372
Primer binding sites:		
pJET1.2 forward sequencing primer	Sequencing of insert, colony PCR	310-332
pJET1.2 reverse sequencing primer	Sequencing of insert, colony PCR	428-405

Primer sequences

pJET1.2 forward sequencing primer, 23-mer	5'-CGACTCACTATAGGGAGAGCGGC-3'
pJET1.2 reverse sequencing primer, 24-mer	5'-AAGAACATCGATTTTCCATGGCAG-3'

Fermentas restriction enzymes that do not cut pJET1.2/blunt

For complete list of enzymes *see* www.thermoscientific.com/reviewer.

AarI, Acc65I, AjiI, AjuI, AlfI, ApaI, BamHI, BclI, BcuI, BoxI, BplI, Bpu1102I, BseJI, BshTI, Bsp119I, Bsp120I, Bsp1407I, Bsp68I, BspTI, Bst1107I, BstXI, Cfr42I, Cfr9I, CpoI, Ecl136II, Eco105I, Eco147I, Eco24I, Eco32I, Eco47III, Eco72I, Eco81I, Eco91I, EcoO109I, EcoRI, EheI, FspAI, HincII, KpnI, KspAI, MlsI, MluI, Mph1103I, NdeI, NheI, OliI, PaeI, PasI, PauI, PdiI, Pfl23II, PfoI, Ppu21I, Psp5II, PsyI, SacI, SalI, SdaI, SfiI, SgsI, SmaI, TstI, Van91I, XagI, XmaJI, XmiI.

Fermentas restriction enzymes that cut pJET1.2/blunt once

For complete list of enzymes *see* www.thermoscientific.com/reviewer.

Enzyme	Location
AasI	1204
AdeI	120
AloI	284
BfiI	2040
BglI	2109
BpiI	58
Bpu10I	717
Bsu15I	417
BveI	219
CaiI	1513
Cfr10I	2075
Csp6I	2474
Eam1105I	1990

Enzyme	Location
Eco130I	408
Eco31I	2062
Eco52I	329
Eco88I	352
Esp3I	739
FaqI	103
GsuI	2080
HindIII	624
Kpn2I	343
LguI	979
MssI	761
MunI	892
Mva1269I	722

Enzyme	Location
NcoI	408
NotI	328
NsbI	2215
PdmI	2590
PstI	5
PvuI	2362
RsaI	2474
ScaI	2473
SmuI	951
TatI	2473
XbaI	377
XhoI	352

RECIPES & SUPPLEMENTARY PROTOCOLS

Chloroform extraction of the ligation mixture prior electroporation

1. Add an equal volume (20 µl) of the chloroform to the ligation mixture. Mix well.
2. Centrifuge at 10,000 rpm for 3 min at room temperature.
3. Carefully transfer the upper aqueous phase to a fresh tube.
4. Use 1 µl of the purified mixture for transformation of 40 µl of electrocompetent cells.

Keep the purified ligation mixture at -20°C if transformation is postponed. Thaw on ice and mix carefully before transformation.

Ampicillin stock solution (50 mg/ml)

1. Dissolve 2.5 g ampicillin sodium salt in 50 ml of deionized water.
2. Filter sterilize and store in aliquots at 4°C.

LB-ampicillin plates

1. Prepare LB-agar Medium (1 liter), weigh out:
   Bacto® Tryptone 10 g,
   Bacto Yeast extract 5 g,
   NaCl 5 g.
2. Dissolve in 800 ml of water, adjust pH to 7.0 with NaOH and add water to 1000 ml.
3. Add 15 g of agar and autoclave.
4. Allow the medium to cool to 55°C.
5. Add 2 ml of ampicillin stock solution (50 mg/ml) to a final concentration of 100 µg/ml.
6. Mix gently and pour plates.

For fast and easy preparation of LB medium and LB agar plates supplemented with ampicillin, use pre-mixed and pre-sterilized microwaveable Thermo Scientific FastMedia LB Liquid Amp (#M0011) and FastMedia™ LB Agar Amp (#M0021).

TROUBLESHOOTING

Problem	Cause and Solution
Few or no transformants	**Low transformation efficiency of competent *E. coli* cells.** Verify transformation efficiency with supercoiled plasmid DNA (e.g., pUC19). Refer to page 7 Table 2 for control transformations. **Ligation mixture was not purified prior to electroporation.** Always column-purify the ligation mixture using e.g. GeneJET PCR Purification Kit, #K0701 or chloroform extract it prior to electroporation (*see* protocol on p. 12). Electroporation is inhibited by the presence of proteins and salts in the mixture. This can cause not only low transformation efficiency, but also arcing of the sample during electroporation **Incorrect protocol was used.** If *Taq* DNA polymerase or any enzyme mix containing *Taq* DNA polymerase was used for PCR, always follow the Sticky-End Protocol to blunt the PCR product prior to ligation. **T4 DNA Ligase was inhibited by salts present in the PCR buffer.** Do not use more than 1 µl of the PCR mixture in the ligation reaction to avoid inhibition of T4 DNA ligase by salts. **PCR product was damaged by UV light during excision from the agarose gel.** Use a long wavelength UV (360 nm) light-box when excising DNA from the agarose gel. When using a short-wavelength (254-312 nm) light-box, limit DNA exposure to UV to a few seconds. Keep the gel on a glass plate or on a plastic plate during UV illumination. Alternatively, use dyes, like crystal violet, to visualize DNA in ambient light (1, 2). **Cloned sequence is not tolerated by *E.coli*.** Check the target sequence for strong *E.coli* promoters or other potentially toxic elements, as well as inverted repeats. In cases where the product of a cloned gene is toxic to the host, use promoters with a very low expression background, choose a low copy plasmid as cloning vehicle or change the host. **Incorrect insert/vector ratio used for ligation** The kit performs well over a wide range of insert/vector molar ratios (0.5:1 to 15:1). The optimal insert/vector ratio is 3:1. To calculate optimal amount of the PCR product for ligation, refer to a Table 1 on p. 4 or use dedicated software (like www.thermoscientific.com/reviewer) for calculations.

Problem	Cause and Solution
Background colonies without plasmid	Insufficient amount of antibiotic in agar medium. Use 100 µg/ml of ampicillin in LB-ampicillin agar plates. Allow the LB medium to cool to 55°C before addition of the ampicillin.
Background colonies that contain plasmids with incorrect inserts	PCR products are contaminated with a template which encodes ampicillin resistance. Gel-purify the PCR product if the PCR template encodes a β-lactamase to avoid background colonies on LB-ampicillin agar. Non-specific PCR products or primer dimers were cloned into pJET1.2/blunt. Gel-analyze the PCR product prior to ligation with the pJET1.2/blunt. If non-specific PCR products or primer-dimers were generated during the PCR reaction, gel-purify the target PCR product. Otherwise, optimize the PCR conditions to increase specificity. Short DNA fragments (<1kb) are cloned with a much higher efficiency compared to long ones. Therefore, long PCR products must be purified to remove any smaller fragments from the solution. To guaranty DNA integrity, preserve long PCR product from both mechanical sharing and damage by nucleases: • Store PCR product at -20°C if it is not used immediately. • Use clean labware and razor blade, prepare fresh electrophoresis running buffer for gel purification procedure.
Background colonies that contain plasmids without inserts	Nuclease contamination. Use only components provided with the kit. Nuclease contamination (e.g., from low quality water) can impair the integrity of the lethal gene, thus disabling positive selection with pJET1.2/blunt. Assemble ligation and blunting reactions on ice to minimize the risk of nucleases damaging vector ends. Do not perform ligation for longer than 30 minutes. Use the ligation mixture directly for transformation. Keep the ligation mixture at -20°C if transformation is postponed. Thaw ligation mixture on ice before transformation. Vector ends damaged by thermophilic polymerases Do not use more than 1 µl of unpurified PCR product for the ligation or blunting reactions to minimize amount of thermophilic polymerases (*Taq, Pfu* or other) that can damage the integrity of DNA ends and result in false positive colonies. False-negatives in colony PCR. Due to considerable amount of recircularized vector plated on the surface of plate, colony PCR may give some false-negative results. Prior to clone analysis propagate short strikes of individual colonies on ampicillin plates. Then use small amount of each for colony PCR.

Problem	Cause and Solution
Sequence errors in the cloned insert	PCR product was damaged by UV light during excision from agarose gel. Use a long wavelength UV (360 nm) light-box when excising DNA from the agarose gel. When a short-wavelength (254-312 nm) light-box is used, limit DNA exposure to UV to a few seconds. Keep the gel on a glass or on plastic plate during UV illumination. Alternatively, use dyes visible in ambient light to visualize DNA in standard agarose gels (1, 2). Low fidelity DNA polymerase was used in PCR. If PCR product will be used for cloning it is always recommended to use high fidelity DNA polymerase with proofreading activity, such as *Pfu* DNA Polymerase (#EP0671) or High Fidelity PCR Enzyme Mix (#K0191). Follow recommendations for high fidelity PCR (adjust concentration of magnesium chloride and other parameters). Errors in PCR primers. If the cloned PCR product contains sequence errors or is missing 5' bases and the same error persists in more than one clone, re-order the PCR primers from a reliable supplier and repeat the procedure starting from the PCR step.

References

1. Rand, K.N., Crystal Violet can be used to Visualize DNA Bands during Gel Electrophoresis and to Improve Cloning Efficiency, Elsevier Trends Journals Technical Tips, Online, T40022, 1996.
2. Adkins, S., Burmeister, M., Visualization of DNA in agarose gels and educational demonstrations, Anal Biochem., 240 (1), 17-23, 1996.

NOTICES

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