PRODUCT INFORMATION

Rapid DNA Ligation Kit

#__ for __ reactions
Lot: __ Expiry Date: __

Store at -20°C

www.thermoscientific.com/onebio

CERTIFICATE OF ANALYSIS

The Rapid DNA Ligation Kit is functionally tested in cloning experiment:
130 ng of blunt-end 2000 bp insert DNA was ligated with 50 ng of pUC19 DNA digested with SmaI and dephosphorylated with alkaline phosphatase. The yield of white colonies after transformation into competent *E.coli* XL1-Blue cells was >1x10^5 cfu/µg DNA and the transformation efficiency of the cells was 1x10^7 cfu/µg DNA.

Quality authorized by: Jurgita Zilinskiene

COMPONENTS OF THE KIT

Rapid DNA Ligation Kit	#K1422	#K1423
T4 DNA Ligase, 5u/µl *	50 µl	150 µl
5X Rapid Ligation Buffer	1 ml	2x0.75 ml
Water, nuclease-free	1.25 ml	2x1.25 ml

* Weiss unit.

DESCRIPTION

The Rapid DNA Ligation Kit enables sticky-end or blunt-end DNA ligation in only 5 minutes at room temperature (*see* Fig.1).The kit includes T4 DNA Ligase and a specially formulated 5X Rapid Ligation Buffer optimized for fast and efficient DNA ligation. The efficiency of the fast ligation is analogous to that obtained with T4 DNA Ligase in a standard 1 hour ligation. The ligation reaction mixture can be used directly for bacterial transformation using conventional transformation procedures or with the Thermo Scientific TransformAid Bacterial Transformation Kit.

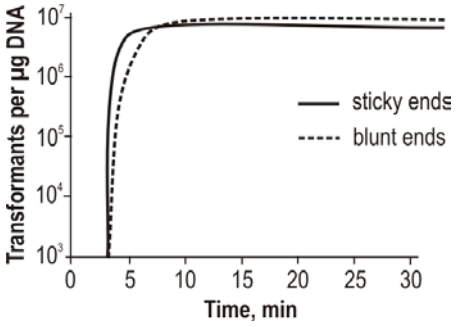

Fig.1. Ligation time course with Rapid DNA Ligation Kit.
Blunt-ends: 2.3 kb blunt-end DNA fragment ligated with pUC19 DNA/SmaI dephosphorylated vector.
Sticky-ends: 2.1 kb sticky-end DNA fragment ligated with pUC19 DNA/PstI dephosphorylated vector.
The efficiency of ligation was estimated by transformation of *E.coli* XL1-Blue cells.

IMPORTANT NOTES

- Thoroughly mix 5X Rapid Ligation Buffer before use.
- Do not exceed the maximum recommended amount of vector DNA of 200 ng in 20 µl reaction volume.
- The optimal insert/vector molar ratio is 3:1. Visit www.thermoscientific.com/reviewer for molar calculations or use following guidelines:

pmol ends = pmol DNA x (number of cuts x 2+2)

Length of DNA fragment (bp)	pmol of ends per 1 µg of DNA
100	30.0
300	10.0
500	6.0
1000	3.0
2000	1.5
3000	1.0

- To minimize recircularization of the cloning vector, dephosphorylate linearized plasmid DNA with Thermo Scientific FastAP Alkaline Phosphatase (#EF0651) or Shrimp Alkaline Phosphatase (#EF0511) prior to ligation. Heat inactivate the phosphatase or remove from the mixture after the dephosphorylation step.
- DNA purity is an important factor for successful ligation. Plasmids should be purified using a method that ensures isolation of high quality DNA (e.g., Thermo Scientific GeneJET Plasmid Miniprep Kit #K0502). Use only high quality agarose and fresh electrophoresis buffers for gel-purification of DNA fragments.
- For efficient cloning of gel-purified DNA fragments, care should be taken to avoid DNA damage with UV light. Always use a long wavelength UV (360 nm) light-box during excision of an agarose gel slice or minimize the UV exposure to several seconds. Keep the gel on a glass or plastic plate during illumination with UV. To avoid DNA exposure to UV altogether, visible dyes can be included into standard agarose gels to visualize migration of DNA bands in ambient light (1, 2).

LIGATION PROTOCOLS

Ligation of insert DNA into plasmid vector DNA

Thoroughly mix the 5X Rapid Ligation buffer prior to use.

1. Add the following to a microcentrifuge tube:

Linearized vector DNA	10-100 ng
Insert DNA (at 3:1 molar excess over vector)	variable
5X Rapid Ligation Buffer	4 µl
T4 DNA Ligase, 5 u/µl	1 µl
Water, nuclease-free	to 20 µl
Total volume	20 µl

2. Vortex and spin briefly to collect drops.
3. Incubate the mixture at 22°C for 5 min.
4. Use 2-5 µl of the ligation mixture for transformation.

The reaction mixture can be stored at 0-4°C until used for transformation. Prior to electroporation, chloroform extract the ligation mixture and use 1 µl for the electroporation reaction.

Recircularization of linear DNA

Thoroughly mix the 5X Rapid Ligation buffer prior to use.

1. Add the following to a microcentrifuge tube:

Linearized vector DNA	10-50 ng
5X Rapid Ligation Buffer	10 µl
T4 DNA Ligase, 5 u/µl	1 µl
Water, nuclease-free	to 50 µl
Total volume	50 µl

2. Vortex and spin briefly to collect drops.
3. Incubate the mixture at 22°C for 5 min.
4. Use 2-5 µl of the ligation mixture for transformation.

The reaction mixture can be stored at 0-4°C until used for transformation. Prior to electroporation chloroform extract the ligation mixture and use 1 µl for the electroporation reaction.

(continued on reverse page)

TRANSFORMATION

The number of transformants on the plates or the perceived cloning efficiency, is dependant on the transformation efficiency of competent cells used. For successful cloning, competent *E.coli* cells should have an efficiency of at least 1 x 10^6 transformants per µg of supercoiled plasmid DNA. Control transformation with 0.1 ng of a supercoiled vector DNA (e.g., pUC19 DNA, #SD0061) is always recommended.
Transformation of competent *E.coli* cells with the ligation mixture can be performed using a number of different methods. Electroporation gives the highest transformation efficiencies (10^9 cfu/µg DNA) and is generally recommended for cloning of large inserts (>3 kb).
When using commercially competent cells, follow the recommendations from the supplier. For fast cloning we recommend using competent cells prepared with the TransformAid™ Bacterial Transformation Kit (#K2710).

Transformation tips

Use up to 2.5 µl of the reaction mixture to transform 50 µl of competent *E.coli* cells prepared with the TransformAid Bacterial Transformation Kit (#K2710).
To transform chemically competent *E.coli* cells, use up to 5 µl of the ligation mixture per 50 µl of competent cells.

Analysis of ligation products by agarose gel electrophoresis

Ligation efficiency can be assesed by agarose gel electrophoresis of ligation reaction products. To load the sample, use SDS-supplemented loading dye (e.g., 6X DNA Loading Dye & SDS Solution, #R1151) to eliminate band shifts caused by T4 DNA ligase binding to the DNA (*see* Fig.2).

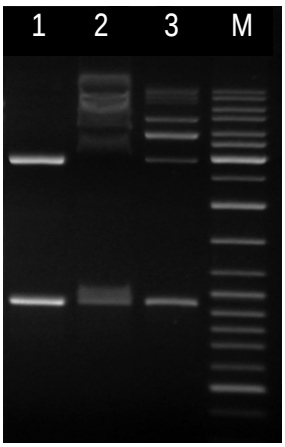

Fig.2. Analysis of ligation products on agarose gel.
Gel analysis of a ligation reaction using 400 ng of vector and insert in total. In general ligation reactions contain less DNA, therefore bands visualized on the gel may appear at lower intensity.

M – Thermo Scientific GeneRuler DNA Ladder Mix (#SM0331)
1 – mixture of DNA insert and vector (400 ng DNA in total) in 5X Rapid Ligation buffer. Sample loaded with 6X DNA Loading Dye (#R0611)
2 – mixture of DNA insert and vector (400 ng DNA in total) after the ligation. Sample loaded with 6X DNA Loading Dye (#R0611)
3 – mixture of DNA insert and vector (400 ng DNA in total) after the ligation. Sample loaded with 6X DNA Loading Dye & SDS Solution (#R1141).

Interpretation of results

- Appearance of higher molecular weight bands and decreased intensity of the vector and insert bands indicate a successful ligation.
- Unchanged band pattern after ligation indicates an unsuccessful ligation.

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.

TROUBLESHOOTING

Problem	Possible Cause and Solution
Low yield or no transformants	**Low transformation efficiency of competent *E.coli* cells.** Check transformation efficiency with 0.1 ng of a supercoiled vector DNA (e.g., pUC19, #SD0061). Competent cells should yield at least 1 x 10^6 transformants per µg of supercoiled DNA. **Ligase was not removed prior to electroporation.** Make sure that the ligation reaction mixture was extracted with chloroform or spin column prior to electroporation. **Volume of ligation mixture used for transformation was in excess of recomendations.** Do not use more than 5 µl of ligation mixture per 50 µl of competent cells. **Sample DNA contains contaminants.** Ensure DNA is free of contaminants (e.g., excess salts, EDTA, proteins, phenol, etc.) that may inhibit ligation. Gel purity and/or phenol/chloroform extract the vector and insert prior to ligation. **DNA 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 several seconds. Keep the gel on a glass plate or on a plastic plate during illumination with UV. Alternatively, use dyes visible in ambient light to visualize DNA in standard agarose gels (1, 2). **Both vector and insert lack phosphates.** When using dephosphorylated vectors, make sure the 5' end of the insert is phosphorylated. PCR products generally lack phosphate groups and need to be phosphorylated with T4 Polynucleotide Kinase (#EK0031) prior to ligation. **Incompatible vector and insert ends.** Recheck the cloning strategy. **Suboptimal vector:insert ratio.** Use 1:3 vector: insert molar ratio. **Inefficient digestion of PCR product.** When introducing restriction enzyme sites into primers for subsequent digestion and cloning, refer to the table "Cleavage efficiency close to the termini of PCR fragments" (www.thermoscientific.com/onebio) to determine the number of extra bases required for efficient cleavage. Purification of PCR products prior to digestion is highly recommended to remove the active thermophilic DNA polymerase from the PCR mixture. DNA polymerases may alter the ends of the cleaved DNA and reduce the ligation yield. **Cloned sequence is toxic to the *E.coli* strain used for transformation.** If possible, check the target sequence for strong *E.coli* promoters or other potentially toxic elements, as well as inverted repeats. In case the product of a cloned gene is detrimental to the host, use promoters with very low expression background.
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 to it. **Satellite colonies.** Some strains (e.g., C600) degrade ampicillin faster, which leads to formation of smaller satellite colonies around transformants after >16 hours of incubation. Use shorter incubation times and do not include satellite colonies into clone analysis.
Background colonies that contain plasmids without insert	**Vector recircularization.** Dephosphorylate vector DNA with FastAP™ Thermosensitive Alkaline Phosphatase (#EF0651) or Shrimp Alkaline Phosphatase (#EF0511) prior to ligation. Make sure phosphatase is heat inactivated or removed after the dephosphorylation step. The DNA insert must possess 5'-phosphate groups.
Increased number of sequence errors in the cloned insert	**DNA was damaged by UV light during the 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 several seconds. Keep the gel either 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).