

mi-Flash Link DNA Ligation Kit

Cat. No mi-RL050
[50 Reactions]

This kit is for research purposes only.
Not for use in diagnostic procedures.
For in vitro use only.

don't risk your experiment. trust ... *metabion*

Introduction

The mi-Flash Link DNA Ligation Kit enables the ligation of cohesive end or blunt DNA fragments in only 5 min at room temperature (25°C) with high efficiency.

Applications:

- Cloning into vectors
- Library construction
- TA cloning
- Linker ligation
- Recirculization of linear DNA

Kit Contents

2X mi-Flash Link Ligation Buffer

T4 DNA Ligase

mi-Flash Link DNA Ligation Kit

1ml

(mix thoroughly before use. Avoid repeated freeze/thaw cycles)

100 µl

(supplied in 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA and 50% glycerol),

Required Equipment

Microcentrifuge (13,000 rpm or 12,000 x g)

Vortexer

Microcentrifuge tubes

Distilled water (pH 7-8)

Kit Storage

At -20°C for 6 months.

Precautions

See MSDS on our homepage (www.mymetabion.com).

Quality Control

The mi-Flash Link DNA Ligation Kit is tested for the transformation efficiency using the following protocol:

pUC18 vector is cut with either EcoRV (blunt) or Hind III (cohesive), treated with calf intestinal phosphatase and gel purified. Blunt insert from a EcoRV and cohesive insert from a Hind III digest of lambda DNA are ligated into the respective vectors at an insert:vector ratio of 3:1 using the Flash Link DNA Ligation protocol.

The ligation products are transformed as described.

Each lot exceeds the following standards:

Efficiency (transformants/ μ g)

	Recircularization	Insertion
Cohesive ends	$> 5 \times 10^6$	$> 1 \times 10^6$
Blunt ends	$> 2 \times 10^6$	$> 1 \times 10^6$
Uncut vector	$> 2 \times 10^7$	

mi-Flash Link DNA Ligation Protocol

Note: Before starting, please make sure....

- To mix the 2X mi-Flash Link Ligation Buffer thoroughly before use.

Avoid repeated freeze/thaw cycles!

- Most ligations performed using the mi-Flash Link DNA Ligation Kit reach an end point at 5 min or less at 25°C. An incubation beyond 5 min provides no additional benefit.

The transformation efficiency will begin to decrease after 2 hours and is reduced by up to 75% if the reaction is allowed to go overnight at 25°C!!!

1. Combine 50 ng of vector with a 3-fold molar excess of insert (molar vector : insert ratio 1:3) **.

Adjust the volume to a final volume of 10 µl with distilled water.

** For help have a look at the “Hints and Troubleshooting” section!

2. Add 10 µl of 2X mi-Flash Link DNA Ligation Buffer and mix thoroughly.
3. Add 1 µl of T4 DNA Ligase and mix thoroughly.
4. Spin briefly and incubate at room temperature for 5-30 min.
5. Chill on ice, then transform or store at -20°C.

Note: Do not heat inactivate. Heat inactivation dramatically reduces the transformation efficiency!

Transformation Protocol

The mi-Flash Link DNA Ligation Kit may be transformed by many different methods. The following heat shock transformation protocol is recommended.

1. Thaw competent cells / bacteria on ice.
2. Chill approximately 5 ng (2 µl) of the ligation mix in a 1.5 ml centrifuge tube.
3. Add 50 µl of competent cells to the DNA and mix gently by pipetting up and down.
4. Incubate on ice for 30 min.
5. Heat shock for 2 min at 37°C and chill on ice for 5 min.

6. Add 950 μ l of room temperature media (like LB medium, without antibiotics!) and incubate at 37°C for 1 hour.
7. Spread 100 μ l of the transformed cells onto the appropriate solid medium (containing the appropriate antibiotics).
8. Incubate overnight at 37°C.

Hints and Troubleshooting

** Molar ratio of vector: insert

After the vector and the insert have been prepared for the ligation, estimate the concentration of each by agarose gel electrophoresis along with molecular weight standards of known concentration.

Test various vector:insert ratios in order to find the optimum ratio for the particular vector and insert.

In most cases 1:1 or 1:3 ratios of vector : insert works well.

Here is a formula for the conversion of molar ratios to mass ratios for

$$\frac{\text{Ng of vector} \times \text{kb size of insert}}{\text{kb size of vector}} \times \frac{\text{insert}}{\text{molar ratio of vector}} = \text{ng of insert}$$

Example:

Vector 3kb 100ng
Insert 500bp x ng? (How many ng of insert have to be added to the ligaton?)
The Vector : insert ratio should be 1:3

$$\frac{100\text{ng} \times 0,5\text{kb}}{3 \text{ kb}} \times \frac{3}{1} = 50 \text{ ng of insert}$$

DNA

Purified DNA for the ligation can be dissolved in distilled water, TE buffer.

For optimum ligation, the volume of DNA and insert should be 10 µl before adding the 2x mi-Flash Link Ligation Buffer.

For DNA volumes greater than 10 µl, increase the volume of 2X mi-Flash Link Ligation Buffer, such that it remains 50% of the reaction and correspondingly increase the volume of ligase. The overall concentration of vector and insert should be between 1-10 µg/ml for efficient ligation.

Insert:vector ratios between

2:1 and 6:1 are optimal for single insertions. Ratios below 2:1 result in lower ligation efficiency. Ratios above 6:1 promote multiple insertions. If you are unsure of your DNA concentrations, perform multiple ligations with varying ratios.

Time

Most ligations performed using the mi-Flash Link DNA Ligation Kit reach an end point at 5 min or less at 25°C. An incubation beyond 5 min provides no additional benefit. The transformation efficiency will begin to decrease after 2 hours and is reduced by up to 75% if the reaction is allowed to go overnight at 25°C!!!

Cells

Competent cells can vary by several logs in their competence. The perceived ligation efficiency directly correlates to the competence of the cells used for transformation. Always transform uncut vector as a control for comparisons.

Electroporation

Electroporation can increase the transformation efficiency by several logs. Before using the products of rapid ligation reaction for electroporation, it is necessary to reduce the PEG concentration. We recommend a spin column purification (mi-PCR Purification Kit mi-PP200).

Biology

Some DNA structures, including inverted and tandem repeats, are selected against E.coli. Some recombinant proteins are not well tolerated by E.coli and can result in poor transformation or small colonies.