Ligation

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Version: 1.0 - Print Version (.PDF)

ATTENTION: This is a low priced protocol. Use it preferably!
The ligation buffer contains ATP and should NOT be freeze/thawed several times. Aliquot it into 20-30 µl aliquots in PCR tubes!

1. **Digest the two vectors** you like to use for 3 hours
   As a rule of the thumb I use 2 µg of plasmid and 1.5-2.0 µl of each enzyme in a 50 µl reaction. If I use SAP (to remove the phosphates) on the verctor, I add 1 µl after 2 hours and another 1 µl after 2.5 hours and inactivate it by heat shock (65 °C for 15 min) after 3 hours.
2. Allways purify all fragments by **gel purification**
   Do NOT make pictures of the gel before you cut out the bands! If you cut out the bands, be fast and expose the DNA to as few UV light as possible!
3. **Measure DNA concentration** by gel or nanodrop
4. **Calculate the amount in ng** needed of each fragment
   We use a vector : instert ratio of 1:3
   For the vector you need 50 fmol:
   ng needed = (length of the plasmid in **bp**) **x 0.033**
   For the insert you need 150 fmol:
   ng needed = (length of the plasmid in **bp**) **x 0.099**
5. **Calculate the amount in µl** needed of each fragment
   µl needed = **ng** needed / (concentration in **ng/µl**)
6. **Prepare the ligation mix**
   We prepare two reactions, one with insert (B) and one without (A):

	A	B
Vector	50 fmol	50 fmol
Insert	---	150 fmol
Buffer	3 µl	3 µl
Ligase	1 µl	1 µl
Water	add to 30 µl	add to 30 µl

7. **Incubate at room temperature for 3 hours**
8. Transform competent cells.
   Use 1 µl for electrocompetent cells and 10 µl for chemical competent cells. Plate on plates using the apropriate antibiotic. Incubate over night.

Useful Formula:

Desired amount in **ng** =
= amount in **fmol x** length of plasmid in **bp** (660 fg / 1 fmol) x (1 ng / 1000000 fg)

Which is equal to:
Desired amount in **ng** = amount in **fmol x** length of plasmid in **bp x 0.00066** x (ng / fmol)

Materials needed:

T4 DNA Ligase (# 716 359) by Roche

Commented Protocol:

1. Digest the two vectors you like to use for 3 hours

As a rule of the thumb I use 2 µg of plasmid and 1.5-2.0 µl of each enzyme in a 50 µl reaction. If I use SAP (to remove the phosphates) on the vector, I add 1 µl after 2 hours and another 1 µl after 2.5 hours and inactivate it by heat shock (65 °C for 15 min) after 3 hours.

Usually one hour is sufficient, but I prefer to have an extended time to allow complete digestion. Use not more than 10% of the digest volume for enzymes, the concentration of glycerol could cause problems.
I only use shrimp alkaline phosphatease (SAP) and not calf intestinal alkaline phosphatase (CIP), because SAP can be heat inactivated and CIP not. Always heat inactivate SAP, even if you purify later on a gel. If you want to clone undigested PCR products, do not use phosphatase because the primers do not have phosphates on their 5' end and ligation will not work.
If you want to clone PCR products with restriction sites introduced on the 5' end of the primers, take care that there are 4-6 extra nucleotides between the restriction site and the end of the primer, otherwise the digestion may not work efficiently.

2. Allways purify all fragments by gel purification

Do NOT make pictures of the gel before you cut out the bands! If you cut out the bands, be fast and expose the DNA to as few UV light as possible!

Load the digest mix on a suitable gel, don't forget the marker, cut out the band you need and make a picture of the gel if you like. Use a kit to purify the DNA from the gel, but don't use too much elution buffer. This step is important to clean of the undigested plasmids.

3. Measure DNA concentration by gel or nanodrop

Usually the concentration is quite low, but a nanodrop can measure it quite well. The better and more time consuming method is to load 10% of the fragment solution on a gel and compare the band densities to the known band densities of the marker.

4. Calculate the amount in ng needed of each fragment

We use a vector : insert ratio of 1:3
For the vector you need 50 fmol:
ng needed = (length of the plasmid in **bp**) **x 0.033**
For the insert you need 150 fmol:
ng needed = (length of the plasmid in **bp**) **x 0.099**

If you use two inserts for a three point ligation, use all three fragments in 100 fmol.
The vector : insert : insert ratio is then 1:1:1.
ng needed = (length of the plasmid in **bp**) **x 0.066**

5. Calculate the amount in µl needed of each fragment

µl needed **= ng** needed / (concentration in **ng/µl**)
This should be easy.

6. Prepare the ligation mix

We prepare two reactions, one with insert (B) and one without (A):

	A	B
Vector	50 fmol	50 fmol
Insert	---	150 fmol
Buffer	3 µl	3 µl
Ligase	1 µl	1 µl
Water	add to 30 µl	add to 30 µl

This allows to check the ligation. You should get 100x more colonies from B than from A. If A has many colonies, the digestion did not work well and using SAP on the vector or to digest longer could help. If you get hardly any colonies, check if the competent cells are still good.

7. Incubate at room temperature for 3 hours

Some people also incubate at 16 °C over night, but I get better results with the 3 hours at room temperature.

8. Transform competent cells.

Use 1 µl for electro competent cells and 10 µl for chemical competent cells. Plate on plates using the apropriate antibiotic. Incubate over night.

The ligation mix is quite salt rich and will make electro competent cells bang if you use too much.

Known Issues:

- If ligation does not work, most of the times the ligation buffer is spoiled (ATP degraded). Buy a new batch!
- Another reason might be the exposure to UV light. Especially with lights which are to strong or with the wrong wavelength, the DNA might be damaged so much that no colonies are obtained after ligation.
- If you don't get any colonies, check your competent cells. Recheck that there are no restriction sites in vector or insert which you overlooked and mess up your strategy.

References and Comments:

This is a collection of all my experiences with ligations in the last eight years. If you follow this protocol, it should work.

How to cite this page in publications:

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