Cloning – Classic LR-Reaction

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Version: 1.0 - Print Version (.PDF)

ATTENTION: This is expensive. One reaction as described here is 5 Euro!

If possible use Gateway LR-Reaction II because the enzyme is more stable and available in smaller amounts.

1. Make sure you have one ENTR and one DEST clone for "classic" Gateway
2. Measure the DNA concentration of both constructs
3. Calculate the volume in µl needed of ENTR plasmid (25 ng)
   µl needed = **25 ng** needed / (concentration in **ng/µl**)
4. Calculate the volume in µl needed of DEST plasmid (50 ng)
   µl needed = **50 ng** needed / (concentration in **ng/µl**)
5. Prepare in a new eppi the Gateway reaction

ENTR™-vector (25 ng)	1µl
DEST™-vector (50 ng)	1µl
add water to a total volume of **3 µl**	1µl

6. Remove the 5 x LR-Clonase Reaction Buffer from -80°C
7. Pipett 1 µl of this buffer solution to the Gateway reaction
8. Remove the LR-Clonase Enzyme mix from -80°C and vortex 2 x 2 sec
   This is expensive stuff don't leave it to rot in the ice-bucket!
9. Add 1 µl of the Enzyme mix to the Gateway reaction and mix well
10. Store the enzyme mix and buffer immediately at -80°C !!!
11. Incubate at room temperature for 1 hour
12. Add 0,5 µl of Proteinase K solution and incubate for 10 min at 37°C
13. Transform bacteria
    For electro competent cells use 2 µl, for chemical competent all.
14. Plate bacteria with proper antibiotic selection

Materials needed:

Gateway® Enzyme: LR-Clonase ™ (# 11791-019) by Invitrogen

Commented Protocol:

1. Make sure you have one ENTR and one DEST clone for "classic" Gateway

You need one ENTR™ clone with the "classical" combination attL1 and attL2 and the DEST™ vector MUST have attR1 and attR2 sites, or it will not work.

2. Measure the DNA concentration of both constructs

The amount of ENTR™ is not so important as in a multiple Gateway® reactions, because it is more efficient. If you want to optimize you can calculate equimolar amounts of both plasmids as described in the multiple Gateway® protocol. Here we use double the amount of DEST™-vector, because most of the ones we use are round and about double the size of the ENTR™ clones.

3. Calculate the volume in µl needed of ENTR plasmid (25 ng)

µl needed = **25 ng** needed / (concentration in **ng/µl**)

Dilute in such way that 1 µl contains 25 ng - this should be easy.

4. Calculate the volume in µl needed of DEST plasmid (50 ng)

µl needed = **50 ng** needed / (concentration in **ng/µl**)

Dilute in such way that 1 µl contains 50 ng - this should be easy.
The DEST™-vector should be tested for low background colonies (due to a mutated ccdB-gene) when transferred in DH5alpha-bacteria.

5. Prepare in a new eppi the Gateway reaction

ENTR™-vector (25 ng)	1µl
DEST™-vector (50 ng)	1µl
add water to a total volume of **3 µl**	1µl

6. Remove the 5 x LR-Clonase Reaction Buffer from -80°C

7. Pipett 1 µl of this buffer solution to the Gateway reaction

8. Remove the LR-Clonase Enzyme mix from -80°C and vortex 2 x 2 sec

This is expensive stuff don't leave it to rot in the ice-bucket!

9. Add 1 µl of the Enzyme mix to the Gateway reaction and mix well

It is most efficiently mixed by pipetting up and down, do not vortex.

10. Store the enzyme mix and buffer immediately at -80°C !!!
This is expensive stuff don't leave it to rot in the ice-bucket!

11. Incubate at room temperature for 1 hour

12. Add 0,5 µl of Proteinase K solution and incubate for 10 min at 37°C
This step will enhance the reaction ca. 2fold.

13. Transform bacteria
For electro competent cells use 2 µl, for chemical competent all.

Electroporation requires low concentrations of salts, that's why we can not use too much.

14. Plate bacteria with proper antibiotic selection
Plate 1/10 on one plate and the rest on another plate. Sometimes you get so may clones that it could be difficult to pick one.
All our Binary-vectors are spectinomycin resistant. If you are not sure, check first.

Known Issues:

- The reaction is very efficient. You can obtain about 2000 colonies of which about 95 % are correct.
- The obtained plasmids are big. To check for correct clones digest with Sty I and in parallel with Eco RI and Hind III. Compare the pattern of bands with the predicted band size to find the correct clones.

References and Comments:
I developed this protocol myself, because the supplied instructions were not clear enough and complex. I did it as described before many times and never had any problems.
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