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Cloning with restriction enzymes protocol

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

Abstract

How to quickly clone your gene fragment of interest into a vector, using restriction enzymes.

Materials

- Centifuge 5804R (#5805000010, Eppendorf) or 5810R (#5811000015, Eppendorf)
- Heatblock (#SBH130D3 or #SBH200D3, Stuart)
- 1.5ml tubes (Eppendorf, #0030 120.086)
- Serologicla Pipettes (Greiner, cellstar)
- Milli-Q water system (Merck, Advantage A10)

- E.coli DH5***α*** (Thermo Fisher, EC0112)
- XhoI (Biolabs, #R01468)
- NotI (Biolabs, #R3189L)
- T4 ligase (Biolabs, #M0202T)
- agarose (Roth, #22675)
- Gel extraction kit (QIAEX®II-QIAGEN, #20021)
- DNA clean up kit (Zymo DNA Clean and Concentrate, #D4006)
- ice
- Tryptone (XXX)
- NaCl (XXX)
- Yeast (XXX)
- Ampicillin (1XX)
- Agar (XXX)
- Mini-Prep kit for DNA isolation (QIAprep®spin, 27106)

Generating Gene Fragment and cutting vector

1

Restriction Enzymes depending on your choice, here for example XhoI and NotI (both Biolabs, R01468 and R3189L , respectively), digesting gene fragments for 3h 37°C, or following the enzyme manufacturer's instruction

+1µl XhoI
+1µl NotI
+2µl cut smart buffer
+ Xµl gene fragment to be cut
Up to 20µl with ddH2O

Note: If not sure which enzymes to use, check your sequence, for example with SnapGene

DNA clean up

2 Either run the digested fragment on 2% agarose gel (Roth, 22675), cut out bands and use a gel extraction kit (QIAEX®II-QIAGEN, 20021),
or, use directly DNA clean up kit by for example Zymo DNA Clean and Concentrate (D4006) and follow manufacturer's instruction

Ligation

3 Ligation with T4 ligase (Biolabs) at 16°C o/n
+ 2µl T4 ligase buffer
+ 1µl T4 ligase
+ Xµl cut gene fragment
+ Xµl cut vector
Up to 20µl with ddH2O

Note: to calculate the ratio needed between gene fragment and vector, we recommend using the NEB® Calculator, as the amount required will vary depending on the length of gene fragment and vector

Transformation

4 Transformation into competent E.coli DH5***α*** (Thermo Fisher, EC0112)
+ 5µl ligation sample
+ 40µl E.coli DH5***α***

- incubate for 30min on ice
- heat shock at 42°C for 1min
- 5min on ice
- 1h in LB media 1ml at 37°C
- centrifuge and discard ~900µl of supernatent
- resuspend in leftover media and plate ~50-100µl on LB-plates containing antibiotic (selected resistance of plasmid)
- incubate at 37°C o/n

Amplification

5 pick single colonies from plate and transfer to 5ml LB-medium containing antibiotic of choice
incubate at 37°C on shaker o/n

Note:
LB-medium composition: MilliQ H2O (filled to 1l, and adjusted pH 7.4), Tryptone (10g), NaCl (10g) and Yeast (5g); autoclaved and added for example Ampicillin (100mg/ml stock, add 1ml to 1l LB-medium) when broth is cooled to ~50°C;
Same composition for LB-plates, with addition of agar (15g for 1l) before autoclaving

DNA extraction

6 use Mini-Prep kit for DNA isolation following the manufacturers instructions (QIAprep®spin, 27106)

Sequence validation

7 Sequence plasmid with appropriate primers and check sequencing results