Technische Universiteit
Eindhoven
University of Technology

iGEM TU/e 2015
Biomedical Engineering

Eindhoven University of Technology
Room: Ceres 0.04
Den Dolech 2, 5612 AZ Eindhoven
The Netherlands
Tel. no. +31 50 247 55 59
2015.igem.org/Team:TU_Eindhoven

Vector Linearization

Where innovation starts

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Vector Linearization

1 Vector Linearization through PCR

Estimated bench time: 45 minutes
Estimated total time: 5-7 hours (depends on the vector)
Purpose: Preparing a linear vector which can be used in the Gibson Assembly reaction.

When linearizing a vector, you are working with DNA. It is essential to work with gloves at all times to protect your vector from DNase activity.

1.1 Materials

- Autoclaved H_2O
- Bucket with ice
- Pair of primers which yield the necessary overlaps for the insert
- PCR tubes
- Pipettes and tips
- Q5 High-Fidelity 2X Master Mix (high-fidelity polymerase to linearize the vector)
- Thermal cycler
- Vector which is to be linearized
- Vortex

1.2 Setup & Protocol

- Thaw the Q5-HF 2X master mix on ice. If the master mix contains a pellet, briefly vortex or flick the tube until the pellet disappears.
- Set up a PCR with the following reaction components for the vector to be amplified. Add the Q5-HF 2X master mix lastly. Quickly transfer the PCR tube to the thermocycler after adding the polymerase:

Component	Stock concentration	In the PCR tube	Volume to be pipetted (ul)
Plasmid	1 ng/ul	1 ng	1
Forward primer	10 uM	0.5 uM	2.5
Reverse primer	10 uM	0.5 uM	2.5
Q5 High-Fidelity 2X Master Mix	2X	1X	25
H_2O			19
Total			50

- Run the following thermal cycling program:

Step	Temp (°C)	Time (sec.)	Cycles
Initial denaturation	98	120 (2 min.)	1
Denaturation Annealing Extension	98 X[1] 72	15 20 30/kb	35
Final extension	72	120 (2 min.)	1
Hold	4		

2 (Optional) DpnI digestion

Estimated bench time: 5 minutes + 1 minute per sample
Estimated total time: 1.5 hours
Purpose: Digestion of the template vector from the PCR product mixture. This will remove the number of background colonies which do not carry the desired insert after Gibson Assembly.

2.1 Materials

- 10X CutSmart buffer from New England Biolabs
- Bucket with ice
- DpnI restriction enzyme
- PCR Product
- Thermal cycler

2.2 Setup & Protocol

- Thaw the 10X CutSmart buffer at room temperature and thaw the DpnI restriction enzyme on ice. Setup the following reaction:

Component	Stock concentration	In the PCR tube	Volume in ul
PCR Product			50
10X CutSmart buffer	10X	1X	5.7
DpnI	20U/ul	20U	1
Total	2X	1X	56.7

- Digest the vector for 1 hour at 37°C. Heat inactivate DpnI for 20 minutes at 80°C

3 (Optional) PCR Purification

Estimated bench time: 45 minutes
Estimated total time: 45 minutes

[1] The annealing temperature can be calculated for the set of primers using New England Biolabs Tm calculator. An annealing temperature of 3°C lower than the lowest melting temperature was used to increase yields.

Purpose: If the PCR product is <90% pure, large volumes of unpurified PCR product could significantly inhibit the Gibson Assembly [1]. PCR purification may be performed to increase the efficiency.

For more information, see our general PCR purification protocol.

4 NanoDrop

Estimated bench time: 5 minutes start-up and 2 minutes per sample
Estimated total time: 5 minutes start-up and 2 minutes per sample
Purpose: Measuring the concentration of the PCR product which is necessary to set up the Gibson Assembly reaction.

For more information, see our general NanoDrop protocol.

5 (Optional) Gel Electrophoresis

Estimated bench time: 40 minutes
Estimated total time: 1.5 hours
Purpose: Agarose gel electrophoresis may be used to verify the purity of your PCR product. If the product is pure, a single bond will show up during the gel electrophoresis.

For more information, see our general Gel Electrophoresis protocol.

6 References & Acknowledgements

This protocol was based on information from New England Biolabs NEBuilder HiFi DNA Assembly Cloning Kit manual as well as on Integrated DNA Technologies' gBlocks Gene Fragments Cloning Protocols.

[1] New England Biolabs, "NEBuilder HiFi DNA Assembly Master Mix / NEBuilder HiFi DNA Assembly Cloning Kit." [Online]. Available: https://www.neb.com/~/media/Catalog/All-Products/709D232D72C045D2B2B1089A89DC879F/Datacards or Manuals/manualE2621.pdf.