

**iGEM TU/e 2017**  
Biomedical Engineering

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## Vector Linearization

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# 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<sub>2</sub>O
- 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 <sub>2</sub> O			19
<b>Total</b>			<b>50</b>

- Run the following thermal cycling program:  
Ingestelde settings thermocycler 2s (naam = IGPCR) 15-6-17:
- 1) 98°C – 0:30
  - 2) 98°C – 0:10
  - 3) 56°C – 0:30
  - 4) 72°C – 2:45
  - 5) Go back to 2, 2x
  - 6) 98°C – 0:10
  - 7) 61°C – 0:30
  - 8) 72°C – 2:45
  - 9) Go back to 6, 25x
  - 10) 72°C – 2:00
  - 11) 4°C - Hold

Step	Temp (°C)	Time (sec.)	Cycles
Initial denaturation	98	120 (2 min.)	1
Denaturation	98	15	35
Annealing	X <sup>1</sup>	20	
Extension	72	30/kb	
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

<sup>1</sup> 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.

<b>Total</b>	2X	1X	56.7
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- 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

**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 band 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](https://www.neb.com/~media/Catalog/All-Products/709D232D72C045D2B2B1089A89DC879F/Datacards%20or%20Manuals/manualE2621.pdf).