Technische Universiteit
Eindhoven
University of Technology

iGEM TU/e 2017
Biomedical Engineering

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Digestion

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Table of contents

Digestion

1 Digestion

Estimated bench time: 30 minutes
Estimated total time: 2 hours
Purpose: Giving sticky ends to the vector and the insert(s) so that they can be ligated.

It is essential to work with gloves at all times to protect the DNA from DNase activity.

1.1 Materials

- 10X Cut-Smart buffer
- Autoclaved H_2O
- Autoclaved PCR tubes
- Bucket with ice
- Insert(s) which is/are to be digested
- Pipettes and tips
- Restriction enzymes
- Thermal cycler
- Vector which is to be digested

1.2 Setup & Protocol

- Construct a PCR mixture in the following way. Use 2 U restriction enzyme per 1 µg DNA. Start with the component with the largest volume and end with the two restriction enzymes. Keep the enzymes on ice.

Component	Quantity/mass/final concentration	Volume (µl)
H_2O	Fill up to 50 µl	
10x Cut-Smart buffer	1 X	5
Plasmid DNA	5 µg	
Restriction enzyme 1	10 U (20 U/µl stock)	0.5
Restriction enzyme 2	10 U (20 U/µl stock)	0.5
Total		50

- Mix well by pipetting up and down.
- Run the following PCR program:

Step	Temp (°C)	Time (min)
Incubation	37	60
Heat inactivation	Variable (65,80,no inactivation possible)[1]	20
Cooling	4	Hold

[1] Typical values for NEB restriction enzymes. It is advisable to use to manufacturers protocol for these experiments before setting the deactivation temperature