

iGEM TU/e 2017
Biomedical Engineering

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Ligation

Table of contents

Ligation	1	Ligation	3
	1.1	Materials	3
	1.2	Setup & Protocol	3

1 Ligation

Estimated bench time: 20 minutes

Estimated total time: 3 hours

Purpose: Ligating the digested vector and the digested insert(s) so that a circular plasmid is created

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

1.1 Materials

- 10x T4 ligase buffer
- Autoclaved H₂O
- Autoclaved PCR tubes
- Bucket with ice
- Digested insert(s)
- Digested vector
- Pipettes and tips
- T4 ligase
- Thermal cycler

1.2 Setup & Protocol

- Construct a PCR mixture in the following way. Start with the component with the largest volume and end with T4 ligase. Keep the T4 ligase on ice.

Component	Quantity/mass/final concentration	Volume (µl)
Digested vector	100 ng	
Digested insert	(vector:insert molar ratio 1:5) ¹	
10x T4 ligase buffer	1X	5
T4 ligase		1
Autoclaved H ₂ O	Fill up to 50 µl	
Total		50

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

Step	Temp (°C)	Time (min)
Ligation	25	120 (2 hours)
Heat inactivation	65	10
Cooling	4	Hold

¹ NEBcalculator can be useful to plan these experiments