

iGEM2017 – Microbiology – BMB – SDU	
Title: Ligation	Date issued: 2013.06.19
SOP number: SOP09	Review date: 2017.09.10
Version number: 03	Written by: ASF
	Modified by: JB, EG

1. **Purpose**
To ligate pieces of DNA
2. **Area of application**
Cloning

3. Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Criteria for approval/rejection
Vortex	Laboratory 1. Floor	•	
Pipettes (p20, p10)	Micro Storage	•	
		•	
		•	
		•	
		•	
		•	

4. Materials and reagents – their shelf life and risk labelling

Name	Components	Supplier / Cat. #	Room (hallway storage)	Safety considerations
Purple pipette tips		Contact lab-manager	Micro storage	
Green pipette tips		Contact lab-manager	Micro storage	
Eppendorf tubes		Contact lab-manager	Micro storage	
Distilled water		Contact lab-manager	Micro storage	
Ligase buffer		Agilent Technologies	Freezer at 1. Floor	
Ligase			Freezer 1. Floor	
DNA piece 1			Refrigerator 1. Floor	
DNA piece 2			Refrigerator 1. Floor	

5. QC – Quality Control

6. List of other SOPs relevant to this SOP

7. Environmental conditions required

8. Procedure

1. Prepare the ligation mixture and mix by pipetting up and down
2. Leave the mixture overnight at 16°C
- 2a. Alternatively, leave the ligation solution at room temperature for 2h.
3. Use ligation solution for transformations

Reagents	Volume
10x T4 DNA ligase buffer	2 µL
T4 DNA ligase (add last!)	0.5 µL
PCR product (cut) of each brick which is to be ligated – or 1 part plasmid and 5 part bricks	10 fmol Plasmid 0, 20 and 50 fmol PCR (or other amounts depending on the available amount)
H ₂ O	to reach a total volume of 20µL

Conversion from (ng/µl) to (fmol/µl):

$$\frac{c_{ng/\mu L} * 1515 \text{ fmol}/\mu L}{n_{bp}} = c_{fmol/\mu L}$$

$c_{ng/\mu l}$ = concentration in ng/µl units.

$c_{fmol/\mu l}$ = concentration in fmol/µl units.

n_{bp} = number of base pairs in the fragment.

9. Waste handling

Chemical name	Concentration	Type of waste (C, Z...)	Remarks
One use Plastic		GMO	Yellow GMO Trash

10. Time consumption

- 3 Hours
- 1 Hour + Ligation overnight

11. Scheme of development

Date / Initials	Version No.	Description of changes
13.06.19 / ASF	01	The SOP has been written
13.06.26 / PRA	01	The SOP has been approved
17.05.01 / JB	02	The SOP has been modified
17.05.01 / EG	02	The SOP has been approved
17.09.10 / EG	03	The SOP has been modified

12. **Appendices**