

iGEM2014 – Microbiology – BMB – SDU	
Title: Fast digest SOP number: SOP0017_v01 Version number: 01	Date issued: 2013.06.28 Review date: 2014.07.01 Written by: Hwj

1. Purpose

To digest DNA-pieces with fast digest restriction enzymes

2. Area of application

Cloning

3. Apparatus and equipment

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	
FastDigest enzyme		Agilent Technologies	Freezer at 1. Floor	
Fast digest green buffer		Agilent Technologies	Freezer at 1. Floor	

5. **QC – Quality Control**

6. **List of other SOPs relevant to this SOP**

7. **Environmental conditions required**

8. **Procedure**

1. Combine the components at room temperature in the order indicated in the table below. see appendix for specific tables for *ecoRI*, *pstI*, *XbaI* and *BamHI*

#		Plasmid DNA	PCR product	Genomic DNA
1	Sterile water	x μ l	x μ l	x μ l
2	10x FastDigest or 10x FastDigest Green Buffer	2 μ l	3 μ l	5 μ l
3	DNA	depends on konc. \sim 1 μ g	depends on konc. (up to \sim 0.2 μ g)	depends on konc. (5 μ g)
4	FastDigest enzyme	1 μ l	1 μ l	5 μ l
5	FastAP (only when digesting backbone)	1 μ l		
	Total volume	20 μ l	30 μ l	50 μ l

2. Mix gently and spin down
3. Incubate at 37°C in a heat block. see appendix for reaction times.
4. The enzyme can sometimes be inactivated by heating, this is optional.
5. If the 10x FastDigest Green buffer was used, the mixture can be added directly to the gel without loading buffer.
6. Run the gel and purify the appropriate band following SOP0014

9. Waste handling

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

10. Time consumption

- Total-time 1½ hours. Including time for running a gel.
- Hands-on-time 30 min.

11. Scheme of development

Date / Initials	Version No.	Description of changes
13.06.18 / Hwj	01	The SOP has been written
14.07.01 / DWP	01	The SOP has been approved

12. Appendices

Table 12.1 shows the reaction conditions for the 4 iGEM restriction enzymes.

	EcoRI	PstI	XbaI	SpeI
Plasmid DNA digestion time	5min	5min	5min	5min
PCRproduct digestion time	20min	5min	5min	5min
Genomic DNA digestion time	5min	5min	10min	5min
Inactivation of enzymes	5min at 80°C	phenol/chloroform extraction	20min at 65°C	5min at 80°C

The enzyme volume may not rise above 1/10 of the total volume

Table 12.2 shows digestion with 2 fast digest enzymes.

		Plasmid DNA	PCR product	Genomic DNA
1	Sterile water	x µl	x µl	x µl
2	10x FastDigest or 10x FastDigestGreen Buffer	2 µl	3 µl	5 µl
3	DNA	depends on konc. ~1 µg	depends on konc. (up to ~0.2 µg)	depends on konc. (5 µg)
4	FastDigest enzyme 1	1 µl	1 µl	5 µl
5	Fast digest enzyme 2	1 µl	1 µl	5 µl
	Total volume	20 µl	30 µl	50 µl