| iGEM2013 – Microbiology – BMB – SDU | |
|---|---|
| **Title:** Fast digest<br><br>**SOP number:** SOP0017_v01<br>**Version number:** 01 | **Date issued:** 2013.06.28<br><br>**Review date:** 2013.06.<br><br>**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, Xbal and BamHI

| | | **Plasmid DNA** | **PCR product** | **Genomic DNA** |
|---|---|---|---|---|
| 1 | **Sterile water** | 15µl | 16 µl | 30 µl |
| 2 | **10x FastDigest or 10x FastDigest Green Buffer** | 2 µl | 3 µl | 5 µl |
| 3 | **DNA** | 2 µl (up to 1 µg) | 10 µl (up to ~0.2 µg) | 10 µl (5 µg) |
| 4 | **FastDigest enzyme** | 1 µl | 1 µl | 5 µl |
| | Total volume | 20 µl | 30 µl | 50 µl |

2. Mix gently and spin down
3. Incubate at 37ºC 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 |
|---|---|---|---|
| | | | |

## 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 |
| 13. | 01 | The SOP has been approved |
| | | |
| | | |
| | | |

## 12. Appendices

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

| | EcoRI | PstI | XbaI | BamHI |
|---|---|---|---|---|
| Plasmid DNA digestion time | 5min | 5min | 5min | 5min |
| PCRproduct digestion time | 20min | 30min | 5min | 5min |
| Genomic DNA digestion time | 5min | 5min | 10min | 5min |
| Inactivation of enzymes | 5min at 80ºC | phenol/chloroform extraction | 20min at 65ºC | 5min at 80ºC |

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** | 14µl | 15 µl | 29 µl |
| 2 | **10x FastDigest or 10x FastDigestGreen Buffer** | 2 µl | 3 µl | 5 µl |
| 3 | **DNA** | 2 µl (up to 1 µg) | 10 µl (up to ~0.2 µg) | 10 µl (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 |