Digestion of pSB1C3 Vector

Introduction

In order to digest our Vector we will use the Digestion Protocol for BioBrick Assembly Kit (E0546) from NEB. The restriction enzymes that will be used are EcoRI HF and PstI.

For more information check the following link: [https://international.neb.com/protocols/0001/01/01/digestion-protocol-e0546]

Materials

Materials

- pSB1C3 Backbone
- EcoRI HF
- PstI
- 10X NEBuffer 2.1
- Nuclease free Water

Equipment

- Incubator
- Pipettes
- Tips
- Eppendorfs

Procedure

Preparation

1. Digest the Vector with EcoRI-HF® and PstI: The Plasmid DNA should either be prepared with PCR or contain a toxic gene (e.g. ccdB, sacB) in the cloning site to avoid the need for gel purification .Set up the following reaction (enzymes should be added last) :

Table1

	A	B
1	Reagents	Volume
2	Nuclease free water	to 50 µl
3	Buffer 2:1 (10X)	5 µl
4	DNA	500 ng
5	EcoRI-HF	1 µl
6	PstI	1 µl
7	Total	50 µl

2. Incubate all restriction digest reactions at 37°C for 10 minutes and then heat inactivate at 80°C for 20 minutes.