Diagnostic Restriction Digestions

Introduction

In order to ensure that our cloning was successful we will do Diagnostic Restriction Digestions with NEB's Restriction Digest Protocol. Since, we are only using the protocol to do diagnostic digestions we will scale down the volume of our enzymes.

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

Materials

- Materials
  - Plasmids
  - EcoRI HF
  - PstI
  - 10X NEBuffer 2.1
  - Nuclease free Water
- Equipment
  - Incubator
  - Pipettes
  - Tips
  - Eppendorfs

Procedure

Preparation

1. Set up the following reaction (enzymes should be added last) :

Table1

	A	B
1	Reagents	Volume
2	Nuclease free water	to 25 µl
3	Buffer 2:1 (10X)	2.5 µl
4	DNA	250 ng
5	EcoRI-HF	0.25 µl
6	PstI	0.25 µl
7	Total	25 µl

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

3. Use the protocol Electrophoresis to visualize the results of the digestions