Promegakit - Assembly of Restriction Enzymes Digestion

Introduction

Digestion by restriction enzymes is used to cut DNA at specific restriction sites. Before use, decide what digestion you want to make and check what restriction enzymes are needed.
In this protocol a general way to do the digestion from Promega's manual will be given. For more info check out the complete manual where additional variants of digestions are to be found (a rapid digestion and PCR-product digestion).

Materials

- Promega Kit
- Restriction enzyme 10X buffer
- Acetylated BSA, 10µg/µL
- Restriction enzyme, 10u/µL
- DNA, 1 µg/µL
- Sterile deionized water

Procedure

Setting up a Restriction enzyme Digestion

1. In a sterile tube, assemble the following components in the order listed below:

2. Mix gently by pipetting, close the tube and centrifuge for a few seconds in a microcentrifuge. Incubate at the enzyme's optimum temperature for 1–4 hours.

Component	Volume
Sterile, deionized water	16.3µl
Restriction Enzyme 10X Buffer	2µl
Acetylated BSA, 10µg/µl	0.2µl
DNA, 1µg/µl	1.0µl
Mix by pipetting, then add:	
Restriction Enzyme, 10u/µl	0.5µl
Final volume	20µl

N.B.Overnight digestions are usually unnecessary and may result in DNA degradation.

References

Schagat, T., 2020. *Rapid DNA Digestion Using Promega Restriction Enzymes*. [online] Se.promega.com. Available at: <https://se.promega.com/resources/pubhub/enotes/rapid-dna-digestion-using-promega-restriction-enzymes/> [Accessed 5 December 2020].