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Double Digestion of Insert DNA

iGEM Dusseldorf[1]

[1]Heinrich-Heine Universität Düsseldorf

1 Works for me dx.doi.org/10.17504/protocols.io.79ehr3e

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MATERIALS TEXT

Sterile MiliQ water
10x Fast Digest Buffer
PCR-amplified DNA
Fast Digest Enzyme 1
Fast Digest Enzyme 2

Double digest of insert DNA

1m

1 *Combined enzyme volume should not exceed 1/10 of the total reaction volume.*
For **double** digestion with **two** FastDigest restriction enzymes, mix:

Component	Amount
10x FastDigest Buffer	2 µl
DNA	400 ng
FastDigest Enzyme 1	1 µl
FastDigest Enzyme 2	1 µl
Sterile MilliQ Water	Fill up with Sterile Milliq Water to 20 µl

Total Volume can vary. Other components need to be adjusted.

2 Mix gently and spin down

3 Incubate at enzyme suitable conditions 4m

Find out suitable conditions on website of particular science company

4 Inactivate the enzyme at suitable conditions 1m

Find out suitable conditions on website of particular science company

5

Optional: Load on 1% agarose gel and run (duration and voltage vary)