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Double Digestion of Insert DNA

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Protocol status: Working
We use this protocol and it's working

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Materials

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

Double digest of insert DNA

1 *Combined enzyme volume should not exceed 1/10 of the total reaction volume.* 1m
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

Note

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

2 Mix gently and spin down

3 Incubate at enzyme suitable conditions 4m

Note

Find out suitable conditions on website of particular science company

4 Inactivate the enzyme at suitable conditions 1m

Note

Find out suitable conditions on website of particular science company

5

Note

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