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## Double Digestion of Insert DNA

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Works for me

[dx.doi.org/10.17504/protocols.io.79ehr3e](https://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

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

1m

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)*



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