OCT 18, 2019

Double Digestion and Dephosphorylation of Plasmid

Igem Dusseldorf¹

¹Heinrich-Heine Universität Düsseldorf

Igem Dusseldorf

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.79phr5n

Protocol Citation: Igem Dusseldorf 2019. Double Digestion and Dephosphorylation of Plasmid.

protocols.io

<https://dx.doi.org/10.17504/protocols.io.79phr5n>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: Oct 15, 2019

Last Modified: Oct 18, 2019

PROTOCOL integer ID:

28687

1

Mix the following components gently:

Component	ng/ μ L
Plasmid DNA Tube X	
Component	Volume (μ L)
Sterile MilliQ Water	Fill up to 30.0 μ l
10x FastDigest Buffer	3.0
Plasmid DNA	Total ng of DNA/Plasmid DNA concentration ng/ μ l
FastDigest Enzyme 1	1.0
FastDigest Enzyme 2	1.0
FastAP Alkaline Phosphatase	1.0
Total Volume	30.0
Total ng of DNA	1000.0

2 Incubate at enzyme suitable conditions 00:30:00 - 01:00:00 and temperature

Note

Find out suitable conditions on website of particular science company

- 3 Inactivate the enzymes at suitable conditions and temperature

Note

Find out suitable conditions on website of particular science company

- 4 Run gel at 80-150 V until the dye line is approximately 75-80% of the way down the gel. A typical run time is about 1-1.5 hours, depending on the gel concentration and voltage.
- 5 Cut out digested band (~150 mg) and transfer to pre-weighed tube. Check weight.
- 6 Purify the band