Oct 18, 2019

Double Digestion and Dephosphorylation of Plasmid

iGEM Dusseldorf[1]

[1]Heinrich-Heine Universität Düsseldorf

1 Works for me dx.doi.org/10.17504/protocols.io.79phr5n

iGEM Dusseldorf

1 Mix the following components gently:

Components	ng/µL
Plasmid DNA Tube X	
Component	Volume (µL)
Sterile MilliQ Water	Fill up to 30.0 µl
10x FastDigest Buffer	3.0
Plasmid DNA	Total ng of DNA/Plasmid DNA concentration ng/µl
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

Find out suitable conditions on website of particular science company

3 Inactivate the enzymes at suitable conditions and temperature

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.

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

5 Cut out digested band (~150 mg) and transfer to pre-weighed tube. Check weight.

6 Purify the band

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

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited