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## Ouble Digestion and Dephosphorylation of Plasmid

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

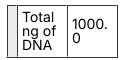
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Mix the following components gently:

Comp onent s	ng/μL
Plasm id DNA Tube X	
Comp onent	Volu me (µL)
Steril e MilliQ Water	Fill up to 30.0 μl
10x FastD igest Buffe r	3.0
Plasm id DNA	Total ng of DNA/ Plasm id DNA conc entrat ion ng/µl
FastD igest Enzy me 1	1.0
FastD igest Enzy me 2	1.0
FastA P Alkali ne Phos phata se	1.0
Total Volu me	30.0



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