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Double Digestion and Dephosphorylation of Plasmid

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

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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

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