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Vector Digestion and Purification V.3

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We use this protocol and it's working

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Abstract

Protocol for plasmid digestion and purification

Materials

Reagents:

- Restriction Enzymes (New England Biolabs)
- 10x Cutsmart Buffer (New England Biolabs)
- Agarose
- EtBr
- NEB Monarch DNA Gel Extraction kit (NEB #T1020)

Digestion:

1h 10m

1 1. Double digest Vector with New England Biolabs Restriction Enzymes: 80uL reaction 1h 10m

	A	B
	Component	Volume (uL)
	DNA plasmid	X ul for 4 ug of plasmid
	10x Cutsmart Buffer	8 uL
	Enzyme 1	4 uL
	Enzyme 2	4 uL
	NucleaseFree H2O	64-X uL

2. Incubate for 00:30:00 at 37 °C .

3. Add 16 µL of 6x loading Dye to reaction and vortex briefly to mix.

4. Make 1% agarose gel.

a) Mix 1 g of Agar with 100 mL of TAE Buffer.

b) Microwave to boil agarose and let cool until you can touch bottle, but gel is not solid.

c) Add 2 µL of EtBr to agarose and pour into DNA gel mold with 10 well comb.

d) Let gel solidify.

5. Load 100 µL of reaction into well of gel

6. Run gel for 00:40:00 at 120V.

7. Visualize band with UV light and cut out section of gel with band with new razor blade and place in 1.5 mL tube.

Notes:

- Not all two enzymes can be used together in a double digestion. Think about it ahead and you can test your two enzymes here: https://nebcloner.neb.com/#!/redigest.
- Enzymes are different, but most can be digested in CutSmart buffer at 37°C within 30 minutes. This is a general protocol. Please check the best digestion conditions for your enzymes in this web：https://nebcloner.neb.com/#!/redigest. It will provide the temperature, buffer, and incubation time specific to the two enzymes you are using.

Purification bands from gel with Monarch DNA Gel Extraction kit:

2 1. Weigh the gel slice.

2. Add 4 volumes of Monarch Gel Dissolving Buffer to the tube with the gel slice (e.g., 400 µl buffer per 100 mg agarose). If the gel slice is >150 mg, consider reducing the amount of Gel Dissolving Buffer to 3 or 3.5 volumes to minimize the guanidine salt present in the workflow.
3. Incubate the sample between 37–55°C (typically 50°C), inverting periodically until the gel slice is completely dissolved (generally 5–10 minutes).
4. Insert the column into collection tube and load sample onto the column. Spin for 1 minute, then discard flow-through.
5. Re-insert column into collection tube. Add 200 µl DNA Wash Buffer and spin for 1 minute. Discarding flow-through is optional.
6. Repeat wash (Step 5).
7. Re-spin the column and tubes.
8. Transfer column to a clean 1.5 ml microfuge tube. Use care to ensure that the tip of the column has not come into contact with the flow-through. If in doubt, re-spin for 1 minute before placing into clean microfuge tube.
9. Add ≥ 6 µl of DNA Elution Buffer to the center of the matrix. Wait for 1 minute, and spin for 1 minute to elute DNA.

Notes:

- *Step2: If the volume of the dissolved sample exceeds 800 µl, the loading of the sample onto the column should be performed in multiple rounds to not exceed the volume constraints of the spin column.*
- *Step3: For DNA fragments > 8 kb, an additional 1.5 volumes of water should be added after the slice is dissolved to mitigate the tighter binding of larger pieces of DNA (e.g., 100 mg gel slice: 400 µl Gel Dissolving Buffer: 150 µl water). Failure to dissolve all the agarose will decrease the recovery yield due to incomplete extraction of the DNA and potential clogging of the column by particles of agarose.*

Protocol references

Monarch DNA Gel Extraction Kit Protocol
https://www.neb.com/en-us/protocols/2015/11/23/monarch-dna-gel-extraction-kit-protocol-t1020?srsltid=AfmBOopHTsWwc4YwVyiO2Plq7zqd6dZL-3cMFxWlUQeZdJGdAVZ68Ltz