Protocol: Simple Restriction Digestion with BamHI and NdeI

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Reviewed by: Patrik

This protocol was originally provided by ThermoFisher. We have added additional details, clarifications, and tips for practical use in the lab.

This protocol was used instead of Johan's restriction protocol, as it was quicker and more convenient to use.

Digestion of pET11 with NdeI and BamHI (Thermo Fisher FastDigest enzymes)

Materials

- Plasmid DNA (pET-11a)
- FastDigest NdeI (ThermoFisher)
- FastDigest BamHI (ThermoFisher)
- FastDigest Buffer (10X)
- Sterile Water
- DNA loading dye (Ampliqon 5x DNA Loading Buffer Orange)
- Sterile 1.5 mL microcentrifuge tubes
- Heating blocks (37°C and 80°C)

Instructions

Note!

- The volumes may have to be adjusted depending on the concentration of DNA in your sample.
- Use two different heating blocks - one for 37°C and one for 80°C.
- Always keep enzymes on ice while setting up.
- If you are digesting PCR products or genes as well (see below), you can do it at the same time as the vector, but please make sure to follow the separate protocol for PCR products.
- Evaporation and condensation may occur during the protocol. If this happens, briefly centrifuge the tube to keep the full volume at the bottom of the tube.

Controls: (prepare in parallel with your samples!)

Control Digest	***Enzyme***	***Purpose***	***Details***
Single digest 1 (with NdeI)	NdeI only	Verifies NdeI activity and correct cutting	Instead of BamHI, put MilliQ H2O
Single digest 2 (with BamHI)	BamHI only	Verifies BamHI activity and correct cutting	Instead of NdeI, put MilliQ H2O
No digest (uncut)	None	Confirms plasmid integrity and migration	Not in digestion, just loading on the gel

Digest single digest controls using the same buffer and plasmid amount. Adjust volumes to 20 µL total.

The **No Digest Control** does not go through digestion reactions, just load the same mass of the plasmid onto the gel (combined with the **loading dye**)!

Step 1: Prepare the initial digestion mix

In a sterile 1.5 mL microcentrifuge tube, add the following:

Component	***NdeI Control***	***BamHI***	***Sample***
10X FastDigest Buffer *If you used the green 10X FastDigest Buffer: You do not need to add loading dye!*	2 µL	2 µL	2 µL
FastDigest NdeI	1 µL	0 µL	1 µL
FastDigest BamHI	0 µL	1 µL	1 µL
Plasmid DNA	x µL (calculate based on your DNA concentration to add 0.5 µg total)	x µL (calculate based on your DNA concentration to add 0.5 µg total)	x µL (calculate based on your DNA concentration to add 0.5 µg total)

Sterile MilliQ Water	Up to 20 µL.	Up to 20 µL.	Up to 20 µL.
Total volume	**20 µL**	**20 µL**	**20 µL**

Alternatively, a Master Mix can be made, and aliquoted into multiple 1.5 mL Eppendorf tubes, with **aliquots being 20 µL**. Here is a suggestion for composition of different Master Mix volumes.

Note: controls must be done separately, as Master Mix will contain all reagents for a reaction

Component	***5 Reactions***	***6 Reactions***	***7 Reactions***	***Y Reactions***
10X FastDigest Buffer (not the Green one)	10 µL	12 µL	14 µL	Y*2 µL
FastDigest NdeI	5 µL	6 µL	7 µL	Y µL
FastDigest BamHI	5 µL	6 µL	7 µL	Y µL
Plasmid DNA	2.5 µg (calculate the volume based on your concentration)	3 µg (calculate the volume based on your concentration)	3.5 µg (calculate the volume based on your concentration)	Y*0.5 µg
Sterile MilliQ Water	Up to 100 µL	Up to 120 µL	Up to 140 µL	Y*20 µL
Total volume	**100 µL**	**120 µL**	**140 µL**	**Y*20 µL**

Step 2: Incubate

- Briefly spin down the tube after mixing to collect the liquid at the bottom.
- Incubate at 37°C (heating block or thermocycler) for **15-20 minutes**.

Step 3: Inactivate restriction enzymes

- Put safety caps on the tubes, to make sure they don't explode on high temperature.
- Incubate at 80°C for 5 minutes.
- After heat inactivation, briefly centrifuge the tube to collect any condensation.

Step 4: Prepare for gel electrophoresis

- This step is done to verify if digestion was successful. On the gel, load your **controls** (**all 3 of them**), and one digestion reaction (20 µL) coming from each of the mastermix reactions (to verify that reactions coming from that mastermix are successful)
- If running on a gel, add the appropriate volume of DNA loading dye (4 µL of dye for 20 µL sample).
- If using **5x DNA Loading Buffer Orange**, the sample should be diluted 5 times, this means add approx 4 µL if you have 20µL total volume.
- If you use **Green 10x FastDigestBuffe**r, you do not need to add DNA Loading Dye, as it already contains tracking dyes and glycerol.
- Do not forget to add the **NO DIGEST CONTROL** on the gel, combined with loading dye!

Step 5: NucleoSpin or Gel Extraction

- If the gel electrophoresis showed positive results, proceed onto the NucleSpin PCR Clean-up.
- You can combine more digestion reactions into one Eppendorf tube, and clean them up as one big sample. The limit for optimal recovery is 15 µg of DNA per column (which is much higher than we usually put into restriction digestion).
- After NucleoSpin Clean-up, test the concentration on NanoDrop.

Digestion of PCR products

If digesting the amplified G-Blocks, simply add 1 µL of NdeI and 1 µL of BamHI into the PCR tube, then incubate at 37°C for 45 minutes.

Note: inactivation at 80°C for 5 minutes may be needed, if not directly cleaning up digestion products.