Johan’s Protocol: Restriction Digestion with BamHI and NdeI

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

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)
- FastAP Thermosensitive Alkaline Phosphatase (ThermoFisher)
- Sterile Water
- DNA loading dye (typically 6X)
- Sterile 1.5 mL microcentrifuge tubes
- Heating blocks (37°C and 80/75°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 80/75°C. They heat up fast but it takes a while for them to cool down if temperatures are very different.
- 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***
Single digest 1 (with NdeI)	NdeI only	Verifies NdeI activity and correct cutting
Single digest 2 (with BamHI)	BamHI only	Verifies BamHI activity and correct cutting
No digest (uncut)	None	Confirms plasmid integrity and migration

Digest all controls using the same buffer and plasmid amount (0.25 µg DNA). Adjust volumes to 21 µL total.

For the **No Digest Control**, do not add it into the 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	***Volume***
Sterile Water	Adjust based on volume of Plasmid DNA. The total volume should be 19 µL.
10X FastDigest Buffer *If you used the green 10X FastDigest Buffer: You do not need to add loading dye!*	2 µL
Plasmid DNA	x µL (calculate based on your DNA concentration to add 0.25 µg total)
FastDigest NdeI	1 µL
Total volume	**19 µL** *Final reaction volume after all additions (BamHI, and FastAP) will be ~21 µL.*

Step 2: Incubate

- Incubate at 37°C (heating block or thermocycler) for 45 minutes.
- Briefly spin down the tube after mixing to collect the liquid at the bottom.

Step 3: Add second enzyme

- Add 1 µL FastDigest BamHI directly to the reaction.
- Gently mix (pipette up and down or flick the tube).

Step 4: Second incubation

- Incubate at 37°C for an additional 10 minutes.

Step 5: Inactivate restriction enzymes

- Incubate at 80°C for 5 minutes.
- After heat inactivation, briefly centrifuge the tube to collect any condensation.

Step 6: Dephosphorylation (Dephosphorylation of the vector reduces self-ligation during ligation steps)

- Add 1 µL FastAP directly to the reaction.
- Mix gently.
- Incubate at 37°C for 10 minutes.

Step 7: Inactivate FastAP

- Incubate at 75°C for 5 minutes.

Step 8 (Optional): Prepare for gel electrophoresis

- If running on a gel, add the appropriate volume of DNA loading dye. It should often be diluted 6 times (please look if it says 6X on the tube), this means add approx 4µL if you have 20µL total volume.
- If you used **green 10X FastDigest Buffer**, you do **not** need to add additional DNA loading dye, it already contains tracking dyes and glycerol.

- Do not forget to add the **NO DIGEST CONTROL** on the gel!

Digestion of PCR products

Use the same basic protocol as above. Digest 0.2 µg DNA, adjust H_2O accordingly. DO NOT dephosphorylate with FastAP (skip steps 6 and 7).
