05_02 Digest a Vector

DIENSTAG, 1.6.2021

Goal-Setting

- Vector linearisation

Terms / abbreviations

- CIP = Calf Intestinal Phosphatase
- DNA-template = Purified DNA-template
- FastAP = Thermosensitive Alkaline Phosphatase
- NEB = New England Biolabs
- Thermo = ThermoFisher Scientific

Risk areas

- None

Required materials and / or information

- Chemicals:
  - DNA-Template: circular vector
  - Enzymes, NEB or Thermo Fisher Scientific
  - Enzyme-buffer, CutSmart or FastDigestBuffer
  - Performance Chart for NEB Restriction Enzymes
  - Performance Chart Thermo Scientific FastDigest Restriction Enzymes
- Materials:
  - 1.5 mL tubes, Eppendorf
  - Pipettes, Eppendorf
  - Timer

Templates, devices, software

- Micorcentrifuge with 1,5 mL rotor, Bio-Rad
- Thermomixer comfort with 1,5 mL exchangeable block, Eppendorf

Preliminary work

- 04_01 Spectralphotometer NanoDrop

Operation

1. If it is not possible to digest with both enzymes in the same buffer, digest with one enzyme each (time/buffer according to the manufacturer's instructions) and purify in between. Only digest both enzymes together if both have > 75% activity in the same buffer. If the enzymes require the same buffer but different incubation temperatures, then initially only prepare the restriction mixture with one enzyme (with the lower temperature) and incubate. The first enzyme is then heat-inactivated (according to the manufacturer's instructions). Then dialyze and add the second enzyme and incubate at the appropriate temperature.
2. Amount of enzyme: 1 µL/µg DNA (for each enzyme)
3. Pipette digest, mix well and centrifuge. Incubate the solution at ideal temperature or at ideal temperatures. Duration: see manufacturer information.

4. After digestion: Add 1 µL of CIP or Fast AP/10 µg DNA, centrifuge, mix well and centrifuge again. Incubate the solution at 37 °C. Duration: 30 min (NEB) or 10 min (Thermo). Incubate immediately at 75 °C for 5 min. Then prepare the sample for gel electrophoresis.

Troubleshooting

- None

Follow-up work

- 01_02 Sample Preparation for Gel Electrophoresis