

WCSB, Wageningen University	EPP-SOP-WU06
	Version 1.0

EPP - Standard Operating Procedure

(only for selected experiments intended to transfer results from one lab to the other)

Title: Restriction / digestion of DNA

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Instruction

Restriction / digestion of DNA

1. *Introduction / Purpose*

This protocol describes how to restrict or digest DNA with Time Saver Qualified restriction enzymes from New England Biolabs (www.neb.com). If using another brand or type of restriction enzymes, please refer to their specific manuals for using the enzymes.

Keywords: Restriction – digestion - DNA

2. *Equipment and chemicals*

2.1. *Equipment*

- Thermomixer or water bath or PCR machine.

2.2. *Chemicals*

- Time Saver Qualified Restriction enzyme from NEB.

2.3. *Other materials*

- DNA that has to be restricted.

3. *Media and Buffers*

- Restriction buffer (provided with restriction enzyme).

4. *Procedures*

A general rule is that you can restrict 1 µg of DNA with 1 µl of enzyme in 5-15 minutes using Time Saver Qualified Restriction Enzymes produced by New England Biolabs (NEB). Less DNA is of course possible.

A typical reaction includes (always check which buffer to use and at which temperature to incubate):

400-1000 ng DNA

1 µl of each enzyme

2 µl SmartCut buffer

MQ to make a total volume of 20 µl

Incubate for 5-15 minutes at 37°C.

5. Remarks / troubleshooting

- The buffer can be different for different enzymes, check NEB website or package of enzyme.
- Most enzymes are active at 37°C, while some are not. Please always check the temperature for your enzyme!
- For troubleshooting: check NEB website and check expiry date of enzyme.
- Several different enzymes can be used in the same reaction, just make sure that they are all active at the same temperature and in the same buffer.
- If one wants to combine two enzymes that have different temperatures, add them both and first incubate at the lowest temperature.
- Some enzymes can be deactivated at high temperatures.

6. Biosafety

No biosafety issues were associated with this protocol.

7. Acknowledgements



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