Restriction digest

Aim of the Experiment

This experiment can be used to digest plasmid DNA from various sources, such as Gibson-assembly, ligations, pure plasmids or g-Blocks.

Materials

- target DNA (to be digested)
- Restriction enzymes (NEB, Germany)
- 10x NEBuffer ™(buffer for restriction digestion, NEB, Germany, note: depending on selected enzymes, other buffers might be recommended)
- Nuclease-free H_2O (nf-water) (Carl Roth, Germany)

Table 1: Reaction Mixture

Concentration	Chemicals
1 μg	DNA
1x	NEBuffer
10U / μg DNA	restriction enzyme
to 50 μl	nf H_2O

Procedure

1. Prepare reaction mixture according to table 1. Multiple types of restriction enzymes can be used in one mix. As a rule of thumb, 5 μlof 10x buffer and 1 μlof each enzyme are added.
2. Mix components by resuspending with pipette but do not vortex.
3. Incubate reaction mix for 1 h at 37 °C
4. Heat inactivate the restriction enzymes for 20 min at either 65 or 80 °C depending on respective enzyme. Alternatively to heat inactivation, a perform PCR clean-up can be used to remove active enzymes.

Possible follow up protocols

The following protocols are the next steps of a possible cloning cycle after a restriction digest:

1. Dephosphorylation
2. Ligation
3. PCR clean-up
4. Agarose gel electrophoresis