Standard BioBrick Assembly

- Modified from silver lab
- This assembly method can be used for BioBricks which are bigger than 150 bp. The BioBrick should be at least 500 bp bigger or smaller than the backbone. The BioBrick, which complies with these conditions, is used as the insert and is assembled into the prefix or suffix of the other used BioBrick, called vector. So you have to differentiate between prefix and suffix insertion.

- Suffix insertion:
  - ◊ Digestion of insert: at least 700 ng DNA / 10 µL volume, 1 µL 10x NEBuffer 2.1, 0.5 µL XbaI, 0.5 µL PstI. Digest for 1 h at 37 °C, afterwards inactivation for 20 minutes at 80 °C. Clean up the insert via gel electrophoresis. When cutting the insert out of the gel, try to avoid staining or exposure to ultraviolet light of the insert.
  - ◊ Digestion of vector about 700 ng DNA / 10 µl volume, 1 µL 10x NEBuffer 2.1, 0.5 µL SpeI, 0.5 µL PstI. Digest for 1 h at 37 °C, afterwards inactivation for 20 minutes at 80 °C. Add 1 µL AP (Antarctic phosphatase) and 1.2 µL 10 x AP reaction buffer, incubate for 1 h at 37 °C. Clean up the vector with a PCR clean-up kit.
  - ◊ Ligation: after digestion and clean-up: 50 - 200 ng of vector, 3 - 10 fold molar access of insert, 20 µL ligation volume, 2 µL T4-Ligase-Buffer, 1 µL T4-Ligase. Incubate for 20 - 30 min at room temperature, afterwards inactivation for 5 minutes at 70 °C. Then: store at -20 °C or transform.

- Prefix insertion:
    - ◊ Digestion of insert: at least 700 ng DNA / 10 µL volume, 1 µL 10x NEBuffer 2.1, 0.5 µL EcoRI, 0.5 µL SpeI. Digest for 1 h at 37 °C, afterwards inactivation for 20 minutes at 80 °C. Clean up the insert via gel electrophoresis. When cutting the insert out of the gel try to avoid staining or exposure to ultraviolet light of the insert.
    - ◊ Digestion of vector about 700 ng DNA / 10 µL volume, 1 µLl 10 x NEBuffer 2.1, 0.5 µL EcoRI, 0.5 µL XbaI. Digest for 1h at 37 °C, afterwards inactivation for 20 minutes at 80 °C. Add 1 µL AP (Antarctic phosphatase) and 1.2 µL 10 x AP reaction buffer, incubate for 1 h at 37 °C. Clean up the vector with a PCR clean-up kit.
    - ◊ Ligation: after digestion and clean-up: 50 - 200 ng of vector, 3 - 10 fold molar access of insert, 20 µL ligation volume, 2 µL T4-Ligase-Buffer, 1 µL T4-Ligase. Incubate for 20 - 30 minutes at room temperature, afterwards inactivation for 5 minutes at 70 °C. Then: store at -20 °C or transform.
- Variations:
    - ◊ A digestion overnight is possible. If you digest overnight use only 0.1 µL restriction enzyme.
    - ◊ It is also possible to use PCR product as insert. Digest after PCR with corresponding restriction enzymes and clean up with a PCR clean-up kit. This could lead to higher yields of insert DNA because a lot of DNA gets lost during the gel electrophoresis clean up.
    - ◊ Sometimes some BioBricks are hard to assemble. Then you have to clean up the vector by gel electrophoresis as well.