Gibson Assembly

- Modified from Gibson *et al.* (2009)
- This assembly method is an isothermal, single-reaction method for assembling multiple overlapping DNA molecules. By coordinating the activity of a 5‘ exonuclease, a DNA polymerase and a DNA ligase two adjacent DNA fragments with complementary terminal sequence overlaps can be joined into a covalently sealed molecule, without the use of any restriction endonuclease.
- Preparation of DNA molecules for *in vitro* recombination.
- Generate the complementary sequence overlaps by PCR using the Phusion DNA polymerase. If necessary add 5 M Betaine to the reaction mix by reducing the amount of H_2O to decrease the number of false PCR products.
- Identify the PCR products of interest by gel electrophoresis with known DNA standards.
- Extract the PCR products from the gel by cutting out the DNA fragments and clean them up by using a commercial gel clean-up kit.
- *in vitro* recombination:
- Assembly mixture:
  - ◊ 320 µL 5x isothermal reaction buffer
  - ◊ 0.64 µL of 10 U mL-1 T5 exonuclease (for DNA molecules overlapping by greater than 150 bp add 3.2 µL of 10 U mL–1 T5 exonuclease)
  - ◊ 20 µL of 2 U mL-1 Phusion DNA polymerase
  - ◊ 160 µL of 40 U mL-1 taq DNA ligase
  - ◊ add ddH_2O water up to a final volume of 1.2 mL
- Aliquot 15 µL of the reagent-enzyme mix and store it at –20 ˚C.
- Thaw 15 µL assembly mixture aliquot and keep it on ice until use.

- Add 5 µl of the purified DNA molecules in equimolar amounts (between 10 and 100 ng of each DNA fragment).

- Incubate the resulting mixture at 50 ˚C for 15 to 60 min, with 60 min being optimal.

- Transformation (via heat shock or via electroporation) without cleaning up the assembly product.

From: iGEM Bielefeld-CeBiTec