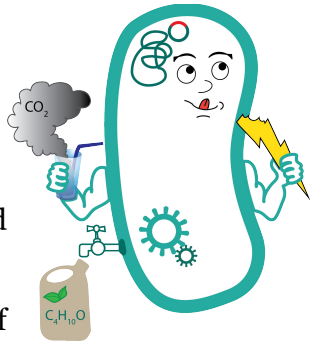
RuBisCO activity assay

- Cultivation and cell lysis
  - Grow overnight culture of your cells containing BBa_K1465213 or the construct T7_Hneap RuBisCo in LB medium containing specific antibiotic. Grow at 37 °C
  - Dilute overnight culture 1:50 in LB medium containing antibiotic and grow cells at 37 °C
  - When the culture reaches an OD_{600} of 0.6-0.8, shift the temperature to 20 °C
  - Induce protein expression using the T7 promotor by adding rhamnose to a final concentration of 0.1 %. When using the ptac promotor, induction concentration is 0,5 mM.
  - Grow the culture at 20 °C overnight
  - Harvest cells by centrifugation at 4 °C
  - Discard supernatant
  - Add 5 ml 20 mM sodium phosphate, 500 mM NaCl buffer to each gram of cell pellet
  - Add 0.2 mg/ml lysozyme, 20 µg/ml DNAse, 1 mM $MgCl_2$, 1 mM PMSF
  - Stir for 30 min at +4 °C
  - Centrifuge 30 min at 4 °C
  - Transfer supernatant (cell extract) to a new tube
- RuBisCo activity assay
  - For the assay, make a reaction mixture containing:
    - 750 µL bicine buffer (200 mM bicine solution, containing 0.4 mM EDTA and 1 mM DTT, pH 8.2)
    - 10 µL 2 M $MgCl_2$
    - 450 µL H_2O
    - 40 µL 250 mM $NaHCO_3$
    - 200 µL 12.5 mM ribulose-1,5-bisphosphate solution
    - 20 µL cell extract
    - Add $NaHCO_3$, ribulose-1,5-bisphosphate solution and cell extract last
  - Incubate the reaction at 37 °C with continous purge of carbon dioxide.



- Take samples in 5 min intervalls Freeze them immediatly at -20 °C
- Before measuring the sample in the HPLC, centrifuge the samples through a Amicon Ultra filter device with a 10 kDa cut-off. Further informations are given in this protocol
- Analyze samples in HPLC following the protocol for HPLC