Protocol EMULSIFLEX C5

▶ Suggested lysis buffer (mandatory):

50 mM Tris-HCl pH8.0
50-100 mM NaCl
1 mM EDTA
10 mM $MgCl_2$
DNAse @ 0.1mg/mL
Lysozyme @ 0.1mg/mL
Protease Inhibitors

▶ Incubate the bacterial cell suspension for 30min @ 4°C with gentle shaking.

▶ Wash the Emulsiflex "sample cylinder body" (SCB) with 200 mL dH_2O, to remove Ethanol 20%.

▶ Switch on the pressure knobs (**PK**) up to 10-15 psi. Output pressure should be between 10000-15000 psi for *E. coli* cells (same OD as for the French Press).

▶ Equilibrate the SCB with your lysis buffer (50 mL). Pressure will barely increase with water/buffer.

▶ Transfer your sample into the SCB (7 to 280 mL samples).

▶ 2-3 cycles of lysis at 10000-15000 psi. Let the lysate going through the tubing by adding 10-20 mL of lysis buffer.

▶ Wash with 250 mL dH_2O (pressure ON) and clean up the inside wall with water.

▶ Wash with 50 mL NaOH 0.5 M (pressure OFF).

▶ Wash with 250 mL dH_2O (pressure OFF).

▶ Wash with 100 mL EtOH 20% (pressure OFF). Leave about 25 mL in the system tubing.

▶ Empty the waste…

REMs:

- Please fill up the various cleaning solutions when they are near empty. Many thanks.
- We have an adaptor to mount syringes (20 and 50 mL) on the Emulsiflex. Useful for working with small volume samples.
- Please put DNAse as DNA clumps will probably block the machine for a long time…

Any question: **edurand@imm.cnrs.fr**