Preparation of cells for Blue Native electrophoresis

(1) trypsinize cells, wash once with PBS

(2) resuspend cells in cold PBS (0.5 ml PBS per 1 plate 10cm, 1 ml PBS - 15 cm)

(3) measure the protein concentration (use Bradford method, sonicate cells)

(4) add more PBS, spin the cells

(5) resuspend cells in PBS to final concentration of 5 mg/ml (according the protein concentration measured above) - put them in eppendorf tube

(6) add equal volume of digitonin (4mg/ml) and incubate on ice for 5 min (final concentration of cells is 2.5mg/ml, final concentration of digitonin is 2 mg/ml, digitonin/protein ratio is 0.8)

(7) add PBS to final volume app. 1.5 ml

(8) centrifuge 10 000g, 10 min

(9) resuspend sediment (membranes) in MB2 buffer (half volume of PBS in step 5)

(10) add lauryl malthoside (LM, dodecyl malthoside 10%) to final concentration of 1 % (1/10 volume of MB2), incubate on ice 15 min

(11) centrifuge 20 000g 20min

(12) put supernatant in a new tube and measure the protein concentration

(13) add SBG - 1/2 volume of lauryl malthoside

(14) freeze -20C and run the gel