

# Stable transfection of CHO-cells

## transfection

- grow CHO-cells in a 10cm dish till 70% confluent
- dilute 5 ug r144 and 5 ug rB2m in 300 ul serum-free medium
- add 40 ul superfect and mix well
- incubate 5-10'
- wash cells 1x with 10 ml PBS
- add 3 ml medium +serum pipet 2x up/down and put this on the cells
- incubate 2-3 hours
- wash 3x with PBS
- add 10 ml medium + serum

## selection

- grow till 100% confluent
- split in 5x 96 well plates (100ul each)
- grow ON
- start selection with 25 uM MSX, change medium every 3 days
- after 3 weeks select with 100 uM MSX

## screen colonies

- \*start screening of the colonies when you have 2x 24-wells of a colony
- \*Label one well with <sup>35</sup>Smet and grow ~5hours (save other well)
- \*harvest medium and run over Ni column
- \*run different colonies on gel and choose colonies with high expression