Transformation protocol

1. warm up heat block to 42 °C
2. competent cells from -80 °C freezer
3. XL1-blue cells for all cloning + DNA-related; BL21 cells for protein expression (aliquots of competent cells in 2 ml tubes; ca. 50 µl)
4. keep competent cells 15 min on ice
5. add DNA (depending on concentration) into cell-suspension; 0.5 µl for retransformation; 10 µl for transformation
6. incubation on ice ca. 30 min
7. heat shock for 45 sec
8. put tubes back on ice for 5 min
9. add 850 µl pre-warmed (37 °C) LB-medium (antibiotic-free)
10. incubate cells 1 h at 37 °C and 900 rpm (depending on shaker)
11. centrifuge cells for 2 min at 2500 rcf
12. decant supernatant; ca. 50 µl remain in tube
13. resuspend cell pellet
14. plate 20 µl supernatant on LB-plates with adequate antibiotics
15. over-night incubation at 37 °C in incubator
16. pick colonies after 18 – 24 h

Info to:

XL1-blue cells (E.coli):

The XL1-Blue strain allows blue-white color screening for recombinant plasmids and is an excellent host strain for routine cloning applications using plasmid or lambda vectors. XL-1 cells are tetracycline resistant. XL1-Blue cells are endonuclease (endA) deficient, which greatly improves the quality of Miniprep DNA, and are recombination (recA) deficient, improving insert stability. The hsdR mutation prevents the cleavage of cloned DNA by the EcoK endonuclease system.

BL21 cells (E.coli):

BL21(DE3)pLysS Competent Cells allow high-efficiency protein expression of any gene that is under the control of a T7 promoter and has a ribosome binding site. BL21(DE3)pLysS is lysogenic for λ-DE3, which contains the T7 bacteriophage gene I, encoding T7 RNA polymerase under the control of the lac UV5 promoter. BL21(DE3)pLysS also contains a plasmid, pLysS, which carries the gene encoding T7 lysozyme. T7 lysozyme lowers the background expression level of target genes under the control of the T7 promoter but does not interfere with the level of expression achieved following induction by IPTG.