E. coli–based Nanobody Production in Bioreactors and Benchmarking of Strain-Plasmid Combinations

DoE-guided bioreactor process optimization

2 L STBR
HCDC fed-batch

screened parameter
OD_{600nm}: 50 - 150
T: 20 - 30 °C
IPTG: 80 - 650 µM

Operating window
late induction OD_{600nm}: 125 - 150
with low IPTG: 100 µM
Production T: 21-22.5 °C

E. coli strain screening

Cytoplasmic vs. periplasmic route
Screening in shake-flasks

Cytoplasmic

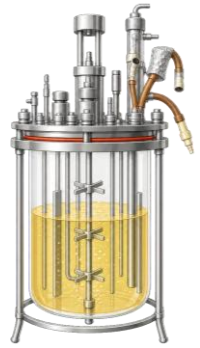
BL21(DE3)
BLR(DE3)
SHuffle T7 Express
Shuffle Express lac
Rosetta 2(DE3)

Periplasmic
ompA / pelB

BL21 (DE3)
SHuffle T7 Express
NEB Express
XL1-Blue MRF

Best host-plasmid combinations
→ Benchmarked under identical
2 L fed-batch conditions

Fed-batch strain benchmarking

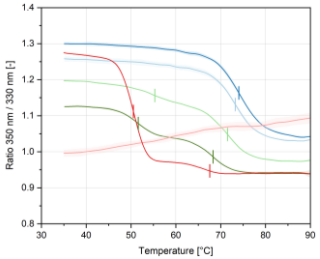


OD_{600nm} 150
T: 22.5 °C
IPTG: 0.1 mM
20 h production

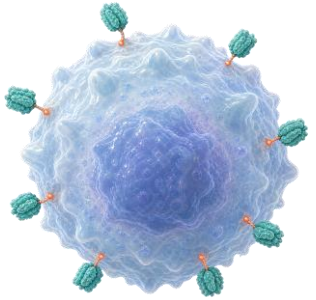
Cytoplasmic	Periplasmic
SHuffle T7	NEB Express
2.19 g L^{-1}	2.43 g L^{-1}

volumetric productivity
0.110 g L^{-1} h^{-1} 0.122 g L^{-1} h^{-1}

Quality control



nanoDSF thermal unfolding
periplasmic: Single transition
up to 74.05 °C



CD45+ Jurkat cells
>99 % positive
NB-dependent binding in
flow cytometry

Optimized process conditions and best strain-plasmid combination enable > 2.5 g L^{-1} functional anti-CD45 nanobody production.