Fig. 1

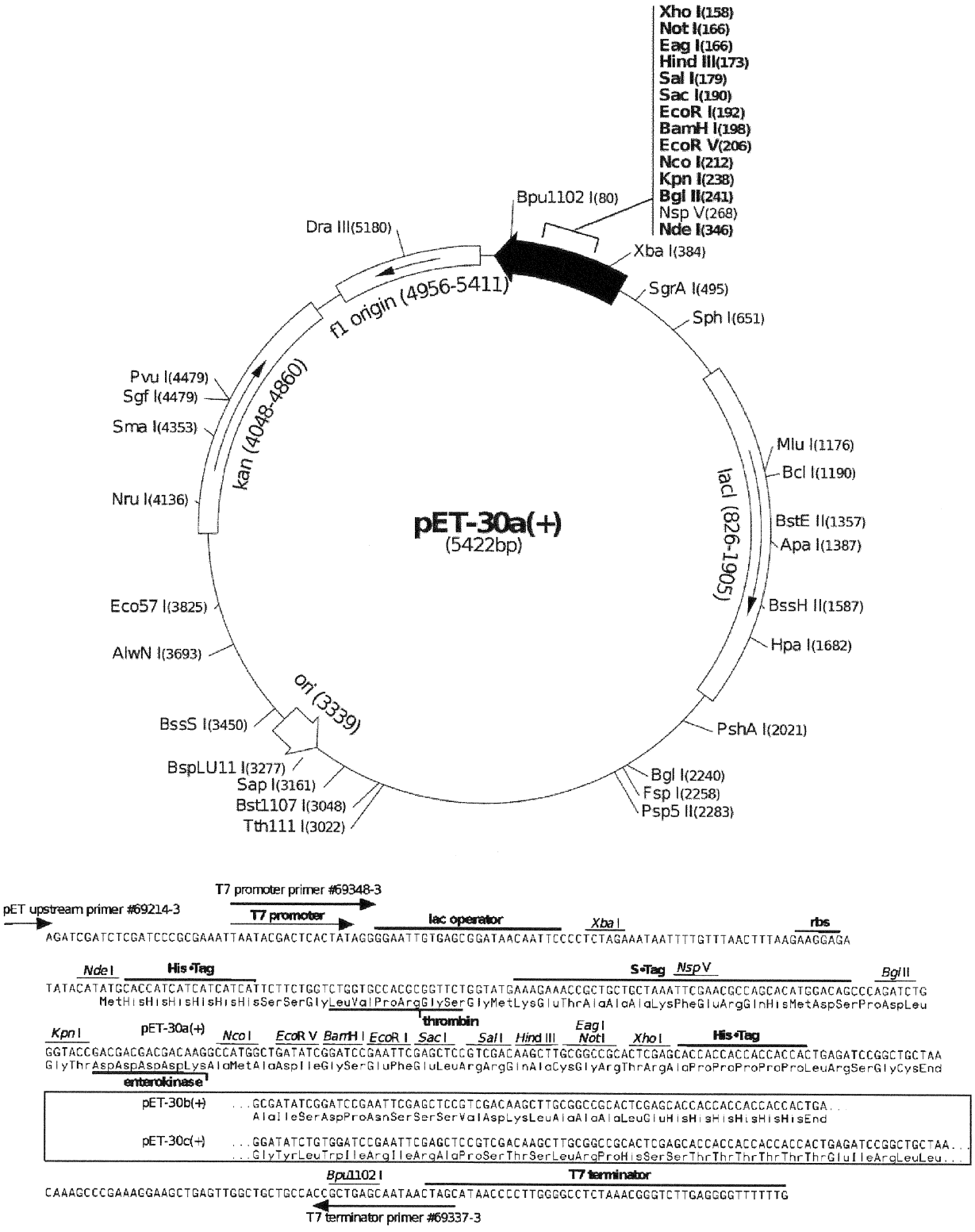

Xho I(158)
Not I(166)
Eag I(166)
Hind III(173)
Sal I(179)
Sac I(190)
EcoR I(192)
BamH I(198)
EcoR V(206)
Nco I(212)
Kpn I(238)
Bgl II(241)
Nsp V(268)
Nde I(346)
Bpu1102 I(80)
Dra III(5180)
Xba I(384)
SgrA I(495)
f1 origin (4956-5411)
Sph I(651)
Pvu I(4479)
Sgf I(4479)
Sma I(4353)
kan (4048-4860)
Mlu I(1176)
Bcl I(1190)
Nru I(4136)
pET-30a(+)
(5422bp)
lacI (826-1905)
BstE II(1357)
Apa I(1387)
Eco57 I(3825)
BssH II(1587)
AlwN I(3693)
Hpa I(1682)
ori (3339)
BssS I(3450)
PshA I(2021)
BspLU11 I(3277)
Sap I(3161)
Bst1107 I(3048)
Tth111 I(3022)
Bgl I(2240)
Fsp I(2258)
Psp5 II(2283)
T7 promoter primer #69348-3
pET upstream primer #69214-3
T7 promoter
lac operator
Xba I
rbs
AGATCGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGA
Nde I
His•Tag
S•Tag
Nsp V
Bgl II
TATACATATGCACCATCATCATCATCATTCTTCTGGTCTGGTGCCACGCGGTTCTGGTATGAAAGAAACCGCTGCTGCTAAATTCGAACGCCAGCACATGGACAGCCCAGATCTG
MetHisHisHisHisHisHisSerSerGlyLeuValProArgGlySerGlyMetLysGluThrAlaAlaAlaLysPheGluArgGlnHisMetAspSerProAspLeu
thrombin
Kpn I
pET-30a(+)
Nco I
EcoR V
BamH I
EcoR I
Sac I
Sal I
Hind III
Eag I
Not I
Xho I
His•Tag
GGTACCGACGACGACGACAAGGCCATGGCTGATATCGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCACCACTGAGATCCGGCTGCTAA
GlyThrAspAspAspAspLysAlaMetAlaAspIleGlySerGluPheGluLeuArgArgGlnAlaCysGlyArgThrArgAlaProProProProProLeuArgSerGlyCysEnd
enterokinase
pET-30b(+)
...GCGATATCGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCACTGA...
AlaIleSerAspProAsnSerSerSerValAspLysLeuAlaAlaAlaLeuGluHisHisHisHisHisHisEnd
pET-30c(+)
...GGATATCTGTGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCACTGAGATCCGGCTGCTAA...
...GlyTyrLeuTrpIleArgIleArgAlaProSerThrSerLeuArgProHisSerSerThrThrThrThrThrGluIleArgLeuLeu...
Bpu1102 I
T7 terminator
CAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG
T7 terminator primer #69337-3

Fig. 2

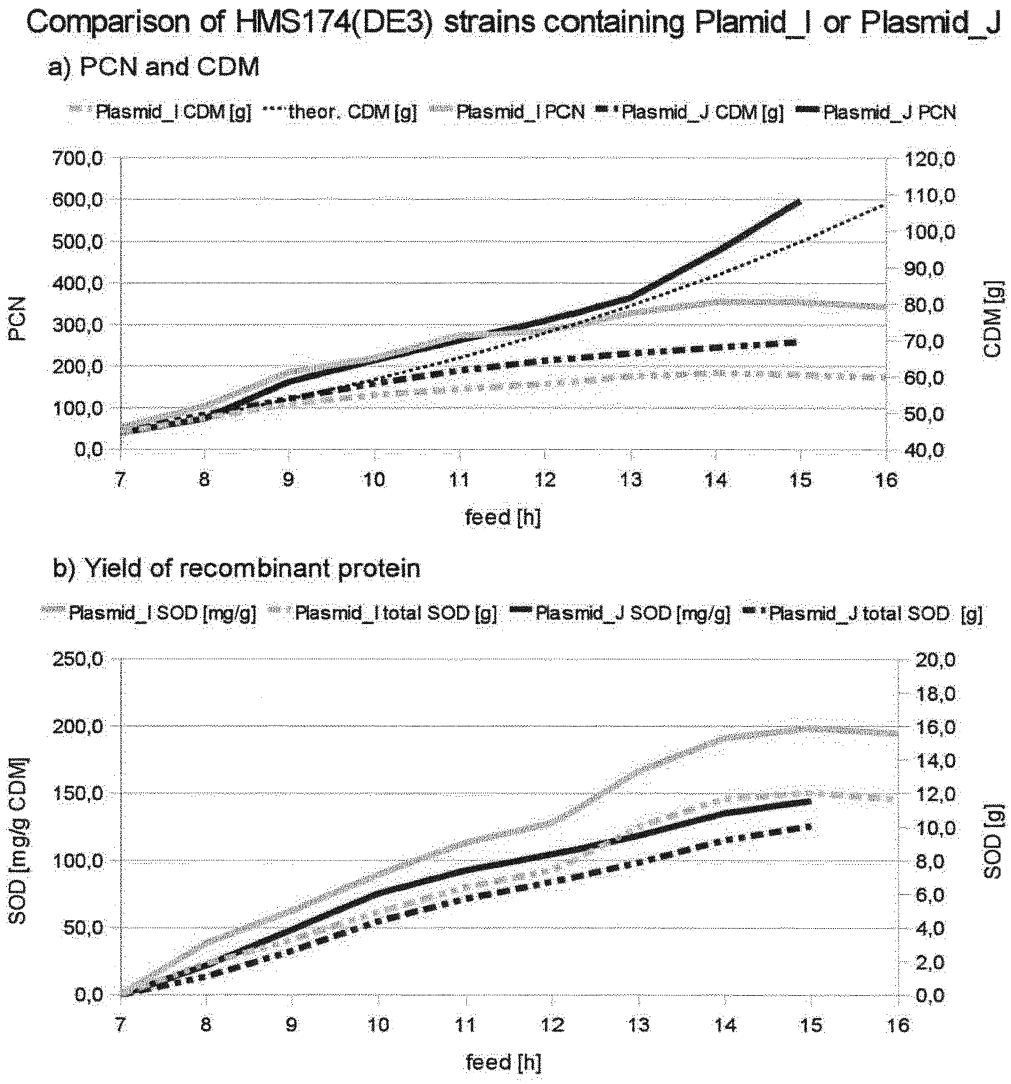

Comparison of HMS174(DE3) strains containing Plamid_I or Plasmid_J
a) PCN and CDM
Plasmid_I CDM [g]
theor. CDM [g]
Plasmid_I PCN
Plasmid_J CDM [g]
Plasmid_J PCN
PCN
CDM [g]
feed [h]
b) Yield of recombinant protein
Plasmid_I SOD [mg/g]
Plasmid_I total SOD [g]
Plasmid_J SOD [mg/g]
Plasmid_J total SOD [g]
SOD [mg/g CDM]
SOD [g]
feed [h]

Fig. 3

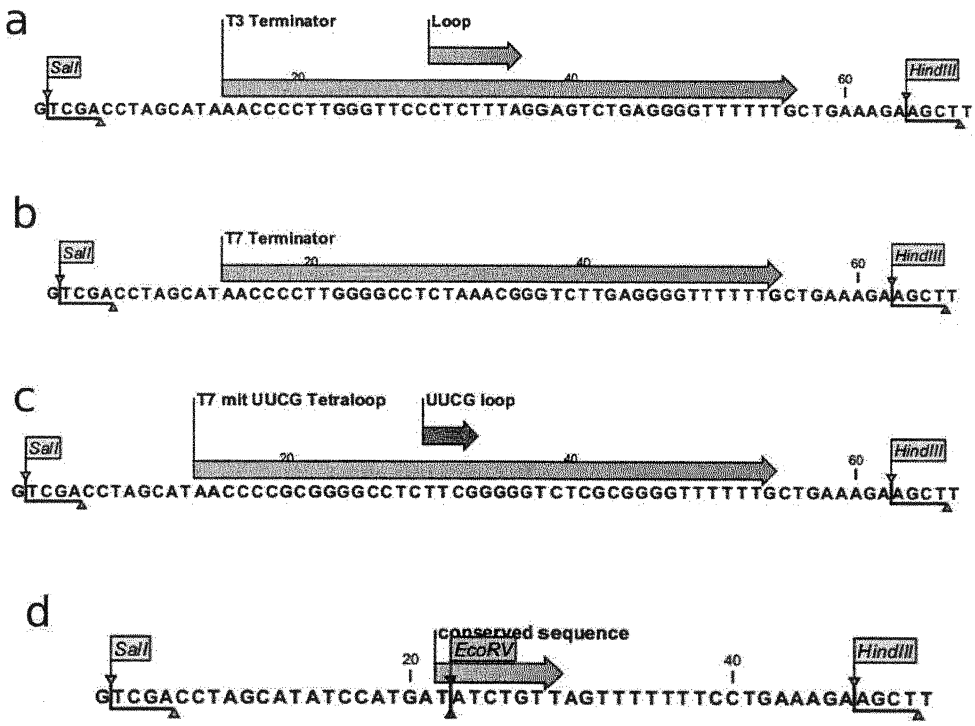

a
T3 Terminator
Loop
SalI
HindIII
GTCGACCTAGCATAAACCCCTTGGGTTCCCTCTTTAGGAGTCTGAGGGGTTTTTTGCTGAAAGAAGCTT
b
T7 Terminator
SalI
HindIII
GTCGACCTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCTGAAAGAAGCTT
c
T7 mit UUCG Tetraloop
UUCG loop
SalI
HindIII
GTCGACCTAGCATAACCCCGCGGGGCCTCTTCGGGGGTCTCGCGGGGTTTTTTGCTGAAAGAAGCTT
d
conserved sequence
SalI
EcoRV
HindIII
GTCGACCTAGCATATCCATGATATCTGTTAGTTTTTTTCCTGAAAGAAGCTT

Fig. 4

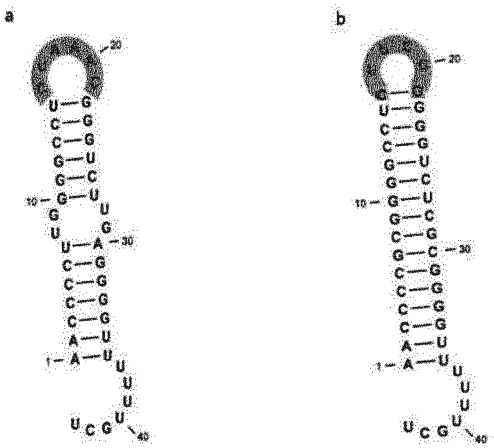

a
b

Fig. 5

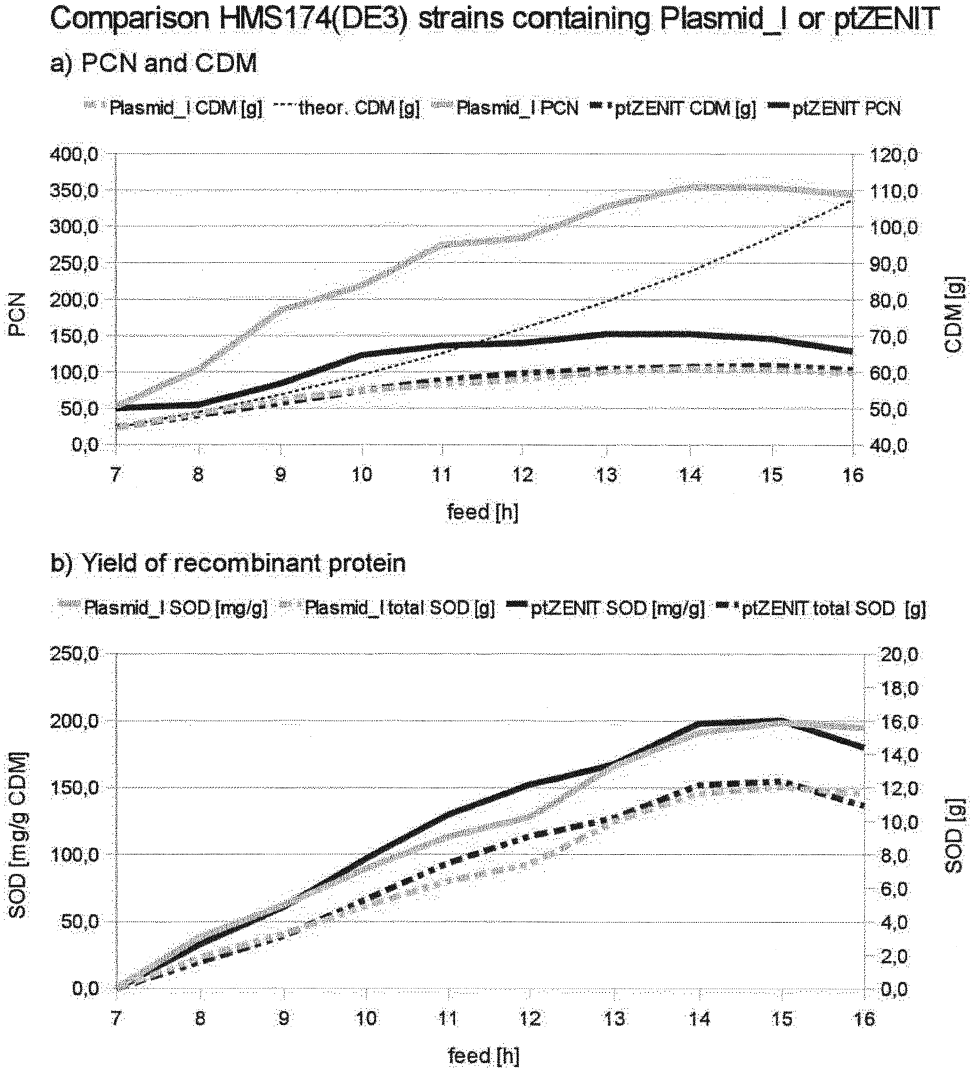

Comparison HMS174(DE3) strains containing Plasmid_I or ptZENIT
a) PCN and CDM
Plasmid_I CDM [g]
theor. CDM [g]
Plasmid_I PCN
ptZENIT CDM [g]
ptZENIT PCN
PCN
CDM [g]
feed [h]
b) Yield of recombinant protein
Plasmid_I SOD [mg/g]
Plasmid_I total SOD [g]
ptZENIT SOD [mg/g]
ptZENIT total SOD [g]
SOD [mg/g CDM]
SOD [g]
feed [h]

Fig. 6

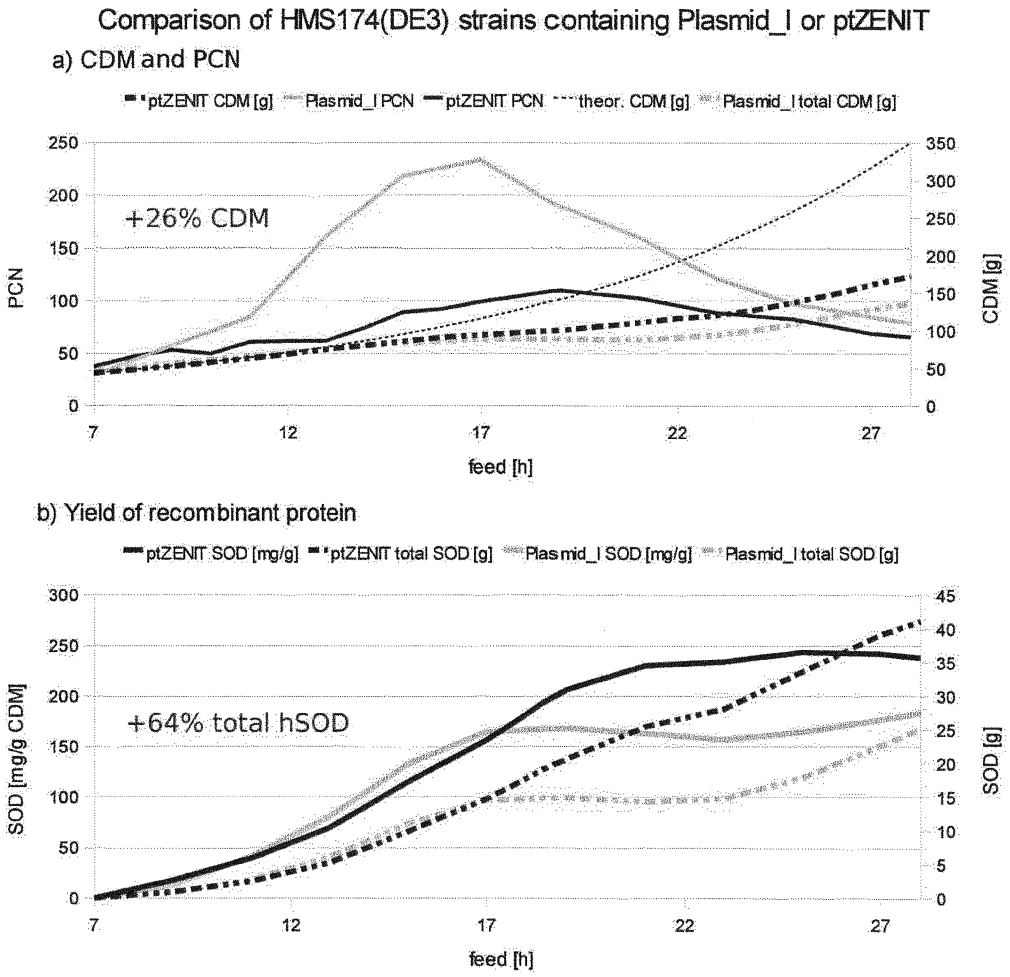
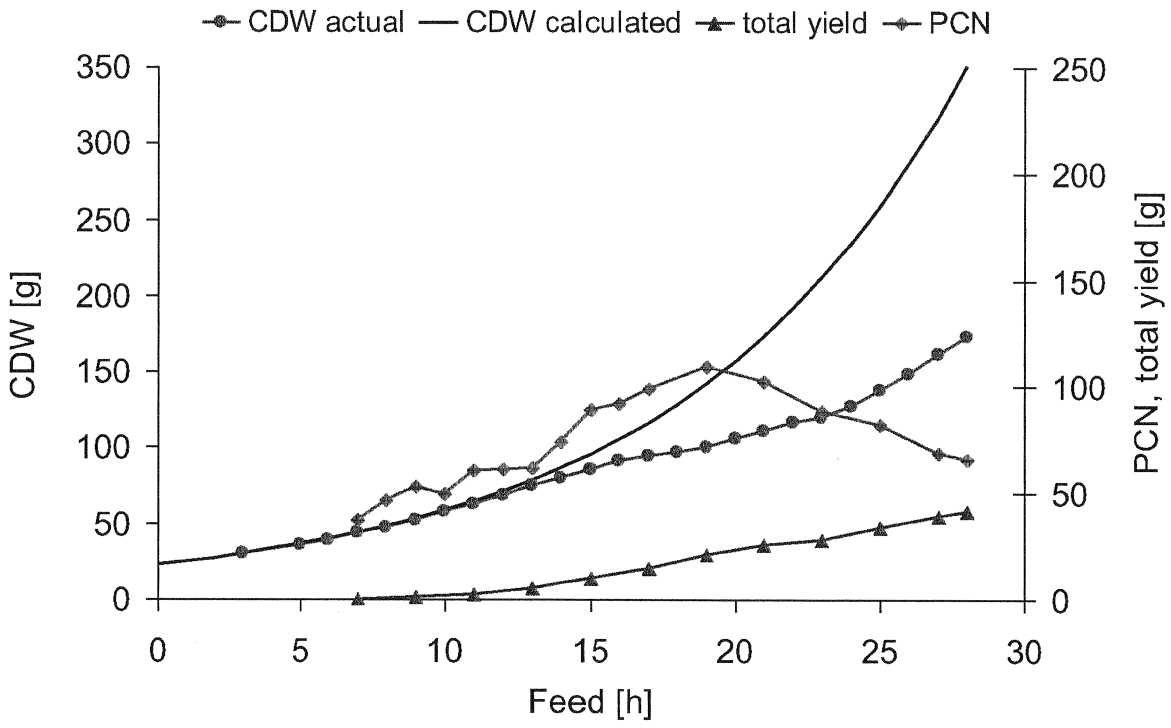

Comparison of HMS174(DE3) strains containing Plasmid_I or ptZENIT
a) CDM and PCN
*ptZENIT CDM [g]
Plasmid_I PCN
ptZENIT PCN
theor. CDM [g]
Plasmid_I total CDM [g]
+26% CDM
PCN
CDM [g]
feed [h]
b) Yield of recombinant protein
ptZENIT SOD [mg/g]
*ptZENIT total SOD [g]
Plasmid_I SOD [mg/g]
Plasmid_I total SOD [g]
+64% total hSOD
SOD [mg/g CDM]
SOD [g]
feed [h]

Fig. 7

A: pTZENIT



B: pET30a

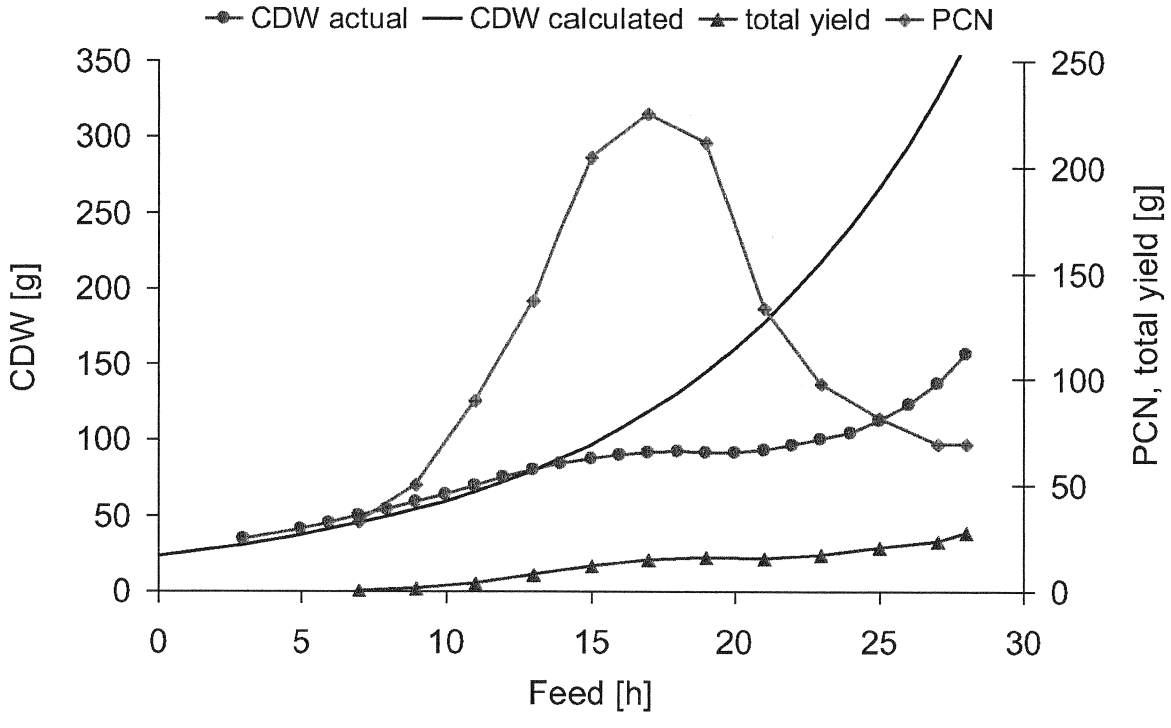
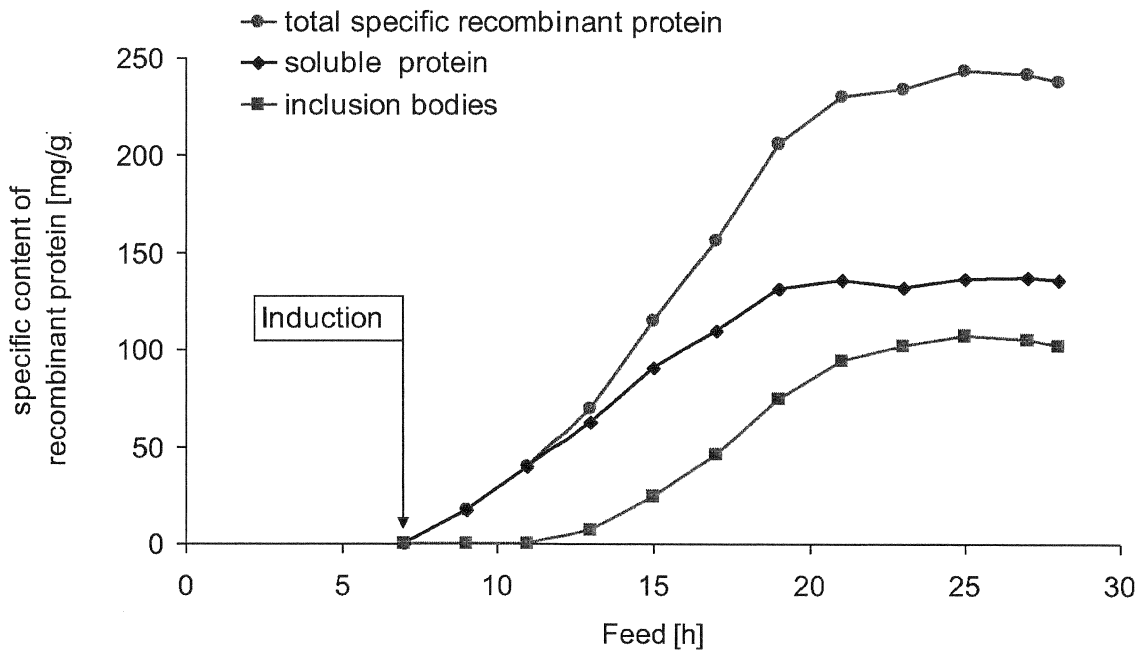


Fig. 8:

A: pTZENIT



B: pET30a

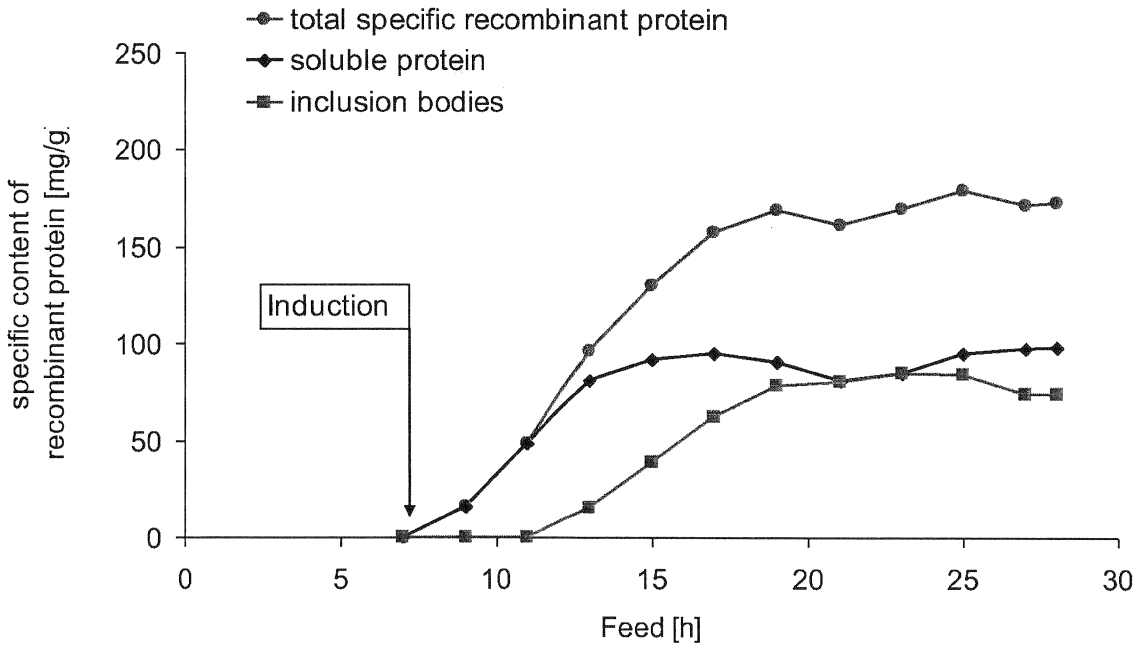
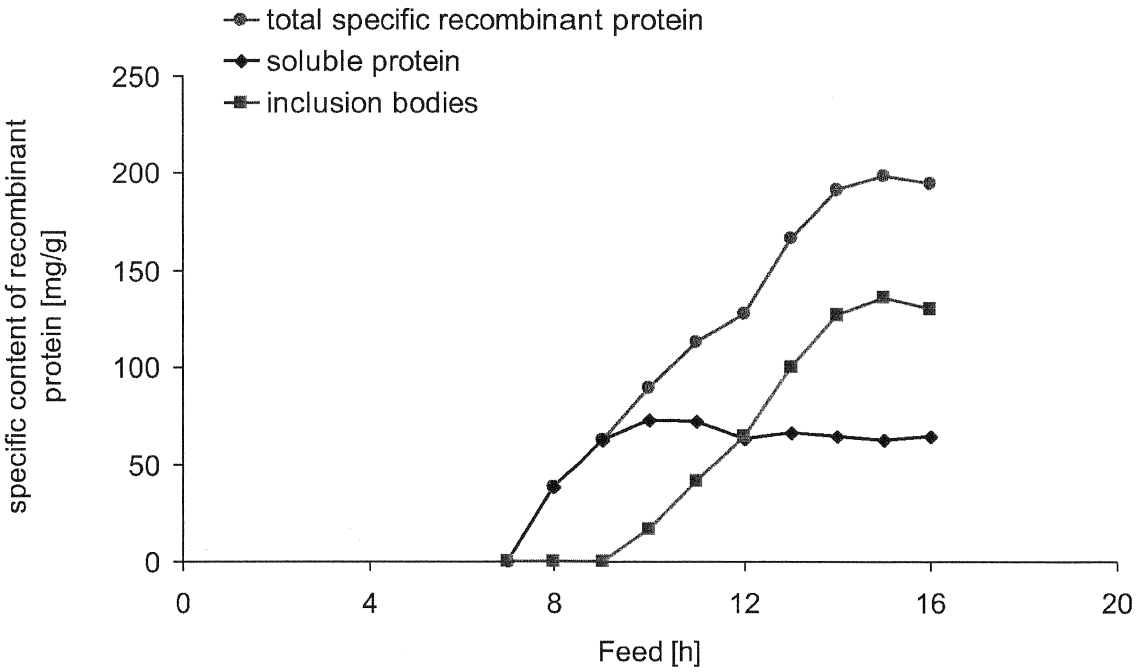


Fig. 9

A: pTZENIT



B: pET30a

