NiCo21(DE3): a BL21(DE3) Derivative Designed for Expression and Purification of His-tagged Recombinant Protein

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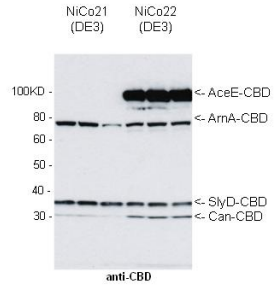
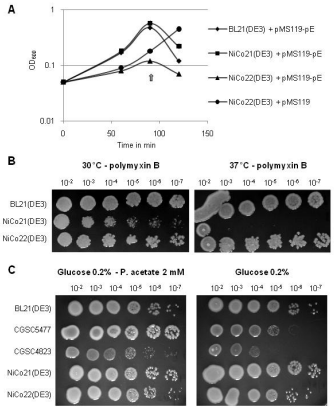
Abstract

Recombinant His-tagged proteins expressed in *Escherichia coli* and purified by immobilized metal affinity chromatography (IMAC) are commonly co-eluted with native *E. coli* proteins, especially if the recombinant protein is expressed at a low level. The *E. coli* contaminants display high affinity to divalent nickel or cobalt ions, mainly due to the presence of clustered histidine residues or biologically relevant metal binding sites. To improve the final purity of expressed His-tagged protein, we engineered *E. coli* BL21(DE3) expression strains in which the most recurring contaminants are either expressed with an alternative tag or mutated to decrease their affinity to divalent cations. The current study presents the design, engineering and characterization of two *E. coli* BL21(DE3) derivatives, NiCo21(DE3) and NiCo22(DE3), which express the endogenous proteins SlyD, Can, ArnA and optionally AceE fused at their C-terminus to a chitin binding domain (CBD), and the protein GlmS with six surface histidines replaced by alanines. We show that each *E. coli* CBD-tagged protein remains active and can be efficiently eliminated from an IMAC elution fraction using a chitin column flow-through step, while the modification of GlmS results in loss of affinity for nickel-containing resin. The "NiCo" strains uniquely complement existing methods for improving the purity of recombinant His-tagged protein.

Major Ni-NTA contaminants SlyD, Can, ArnA and optionally AceE are fused with CBD-tag



Functional analysis of CBD-tagged candidate proteins

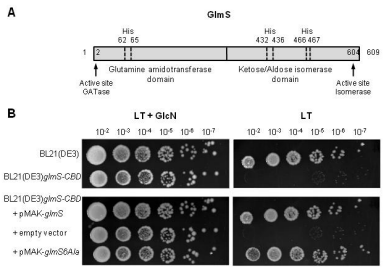


(A) SlyD-CBD function is confirmed by lysis sensitivity of BL21(DE3), NiCo21(DE3) and NiCo22(DE3) upon induction of protein *E* from the bacteriophage φx174.

(B) Polymyxin B resistance is an indicator of ArnA-CBD function. Cells (5 µL of o/n culture diluted 10^{-2} to 10^{-7}) were blotted on LB media with or without 2 µg/mL polymyxin B.

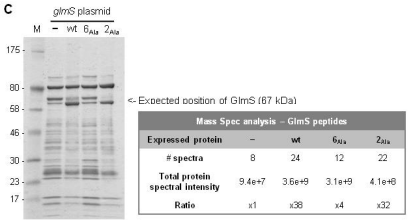
C) Growth on glucose media lacking acetate is an indicator of AceE function. Cells (5 µL of overnight culture diluted 10^{-2} to 10^{-7}) were blotted on minimal media with 0.2 % glucose and supplemented with 2 mM potassium acetate when indicated. CGSC5477 and CGSC4823 are control *aceE* mutant strains.

Mutation of 6 surface exposed histidines eliminates the binding of GlmS to Nickel



(A) Representation of the GlmS protein showing the location of the histidines (His) selected for replacement by alanine.

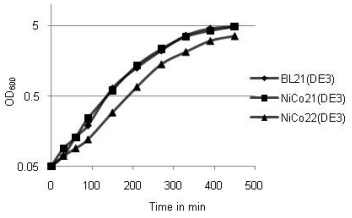
(B) BL21(DE3)*glmS-CBD* strain is non-viable without GlcN or GlcNac. $GlmS_{6His\text{-}Ala}$ protein is able to restore the growth of BL21(DE3)*glmS-CBD* with the same efficiency as wt GlmS.



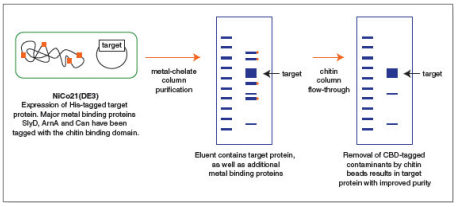
Mass Spec analysis – GlmS peptides				
Expressed protein	–	wt	6_{Ala}	2_{Ala}
# spectra	8	24	12	22
Total protein spectral intensity	9.4e+7	3.6e+9	3.1e+9	4.1e+8
Ratio	x1	x38	x4	x32

(C) SDS-PAGE of elution fractions after loading GlmS or mutant GlmS containing lysates onto a 1 mL His-Trap HP column. Additionally, mass spectrometry analysis of the Ni-NTA elution fractions supports the SDS-PAGE observations. Background level of GlmS (8 spectra) is due to the chromosomal *glmS* gene.

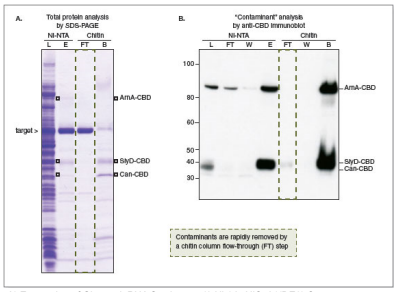
NiCo21(DE3) has the same growth rate as BL21(DE3)



Two-step purification of His-tagged protein that has been expressed in the NiCo21(DE3) strain of *E. coli*



Improved purity of His-tagged proteins with NiCo21(DE3)

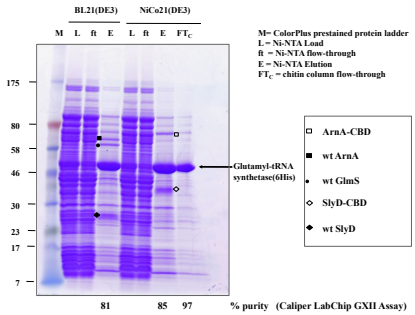


A) Expression of Glutamyl tRNA Synthetase (6-His) in NiCo21(DE3) Competent *E. coli* followed by Ni-NTA purification. Eluent (E) from a Ni-NTA column was passed over a chitin column. The protein of interest elutes in the flow through (FT), while the CBD-tagged metal binding proteins remain bound (B) to the chitin resin (NEB #S6651S). Purifications were performed according to manufacturers' recommended conditions. B) Contaminants ArnA, SlyD and Can are confirmed by Western blot using Anti-CBD Antibody (NEB #E8034S).

Compatible vectors/promoters

T7
T7-*lac*
T5-*lac*
P*tac*
P*trc*
P*lac*
P*lacUV5*
P*araBAD*
P*rhaBAD*

NiCo21(DE3) outperforms BL21(DE3) in final protein purity



Reference:
Robichon et al. Applied and Environmental Microbiology July 2011, pp. 4634-4646, Vol. 77, No. 13

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