CRISPR interference of nucleotide biosynthesis improves production of a single-domain antibody

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Abstract

Growth decoupling can be used to optimize production of biochemicals and proteins in cell factories. Inhibition of excess biomass formation allows for carbon to be utilized efficiently for product formation instead of growth, resulting in increased product yields and titers. Here, we used CRISPR interference (CRISPRi) to increase production of a single domain antibody (sdAb) by inhibiting growth during production. First, we screened 21 sgRNA targets in the purine and pyrimidine biosynthesis pathways, and found that repression of 11 pathway genes led to increased GFP production and decreased growth. The sgRNA targets pyrF, pyrG, and cmk were selected and further used to improve production of two versions of an expression-optimized sdAb. Proteomics analysis of the sdAb-producing pyrF, pyrG, and cmk growth decoupling strains showed significantly decreased RpoS levels and an increase of ribosome-associated proteins, indicating that the growth decoupling strains do not enter stationary phase and maintain their capacity for protein synthesis upon growth inhibition. Finally, sdAb production was scaled up to shake-flask fermentation where the product yield was improved 2.6-fold compared to the control strain with no sgRNA target sequence. An sdAb content of 14.6% was reached in the best-performing pyrG growth decoupling strain.

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Regular growth and production

Growth decoupling
Carbamoyl phosphate

PRPP
purF
purD
purN
purL
purM
purK
purE
purC
purB
purH
purA
guaB

ASP
pyrBi
pyrC
pyrD
pyrE
pyrH
pyrF

GAR
purN
FGAR
purM
AIR
purK
N5-CAIR
purL
FGAM
purE
CAIR
purB
AICAIR
purH
FAICAR

IMP
XMP
GMP
ASC
AMP

guaB
guaA
purA
purB

b

OD
flow cytometry

12h and 24h sampling

overnight cultures

Ptet
dCas9tetR
Ptet
gRNA
Pconst.
Chromosome
ndk

c

12 h - Ratio induced/uninduced cultures

Average cell fluorescence

Cell density

24 h - Ratio induced/uninduced cultures

Average cell fluorescence

Cell density
overnight cultures
a + aTc
- aTc
OD
proteomics
24 h sampling
PT7
Ptet
sgRNA
tetR
pSLQ1236
dCas9
T7
Plac
tetR
psdAb
Chromosome
Plasmids
VHH
CH3
CH2
Chamelid heavy chain antibody
Single-domain antibody

PyrB
PyrI
PyrC
PyrD
PyrE
PyrF
PyrG
PyrH
Ndk
Cmk
pyrF
TIR1
pyrF
TIR2
pyrG
TIR1
pyrG
TIR2
cmk
TIR1
cmk
TIR2
GO process terms
- Ribosomal small subunit assembly
- Ribosomal large subunit assembly
- Response to heat
- rRNA base methylation
- Translation
- Fatty acid biosynthetic process
- PEP-dependent sugar phosphotransferase system
- Cellular response to DNA damage stimulus

GO compartment terms
- Cytosolic small ribosomal subunit
- Cytosolic large ribosomal subunit
- Integral component of plasma membrane
- Outer membrane-bounded periplasmic space
- Periplasmic space
- Plasma membrane
- Cell outer membrane
- Integral component of membrane

| pyrF | pyrF | pyrG | pyrG | cmk | cmk |
|------|------|------|------|-----|-----|
| TIR1 | TIR2 | TIR1 | TIR2 | TIR1 | TIR2 |

Upregulated Downregulated

Figure a: OD, sdAb-TIR1

Figure b: OD, sdAb-TIR2

Content (%), sdAb-TIR1

Content (%), sdAb-TIR2