

Reconstituted cell-free protein synthesis kit

PURE*frex*[®]

PURE*frex*[®]を用いた

ジスルフィド結合含有タンパク質の合成

ジーンフロンティア株式会社

金森 崇

2017年12月6日 ConBio2017

1. Introduction of PUREfrex[®]

2. Application of PUREfrex[®]

- Optimization of nt sequence at 5'-terminus of ORF*
- Synthesis of proteins containing disulfide bonds*
- Synthesis of antibody-related proteins*



PURE system

Components of the original PURE system

Purified Transcription/Translation Factors

T7 RNA Polymerase

Initiation Factors (IF1, IF2, IF3)

Elongation Factors (EF-Tu, EF-Ts, EF-G)

Release Factors (RF1, RF2, RF3)

Ribosome Recycling Factor

20 Aminoacyl-tRNA synthetases (ARS)

Methionyl-tRNA transformylase (MTF)

E. coli Ribosome

E. coli tRNA mix

Energy Regeneration System
(Enzymes and Substrates)

4 NTPs

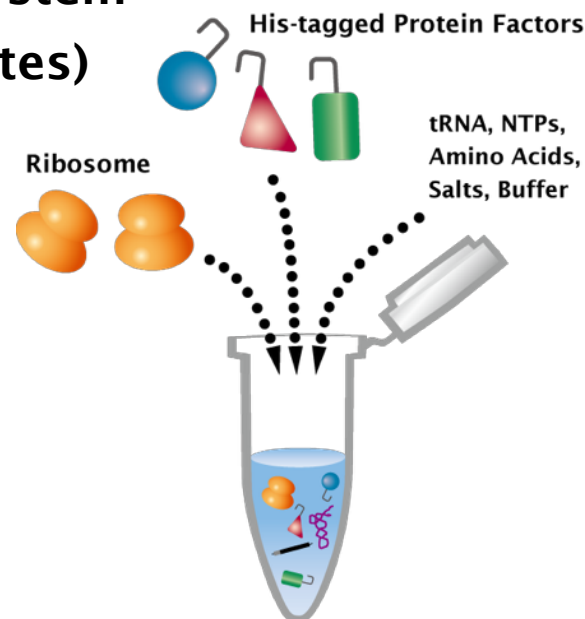
20 Amino Acids

K⁺, Mg²⁺

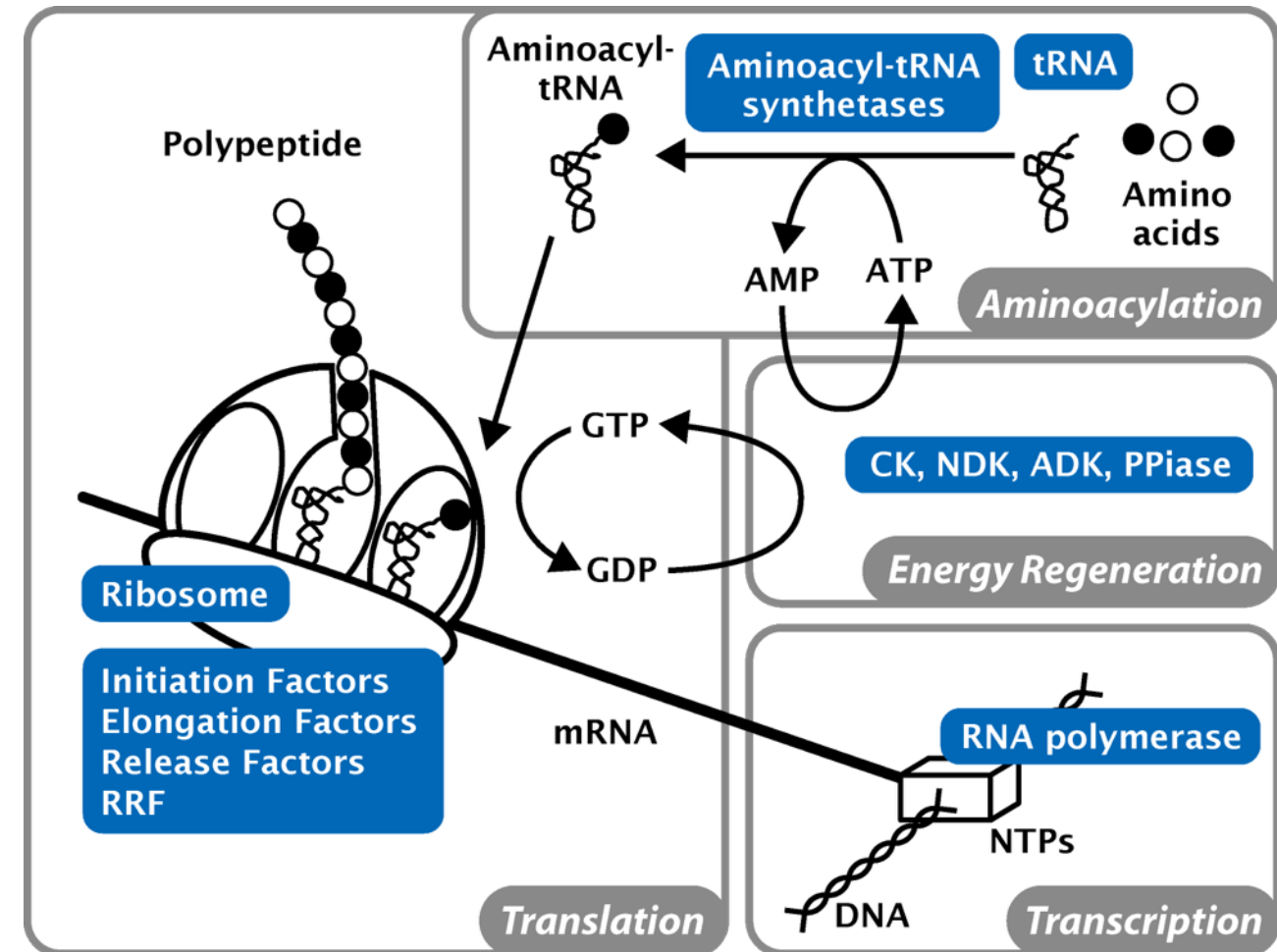
Polyamine

Reductant

Buffer



Outline of protein synthesis in *E. coli*



Shimizu *et al.* (2001) *Nat. Biotechnol.*, vol.19, p.751

Shimizu *et al.* (2005) *Methods*, vol.36, p.299

Development of PURE^{frex}[®]

Summary of the preparation methods of the components

Original PURE system		PURE ^{frex} [®]
Protein		
Tag	His-Tag	None
Number of columns	1	> 3
Wash with detergent	-	+
Ribosome		
Wash with detergent	-	+
tRNA		
Wash with detergent	-	+

Original PURE system: Shimizu *et al.* (2005) *Methods*, vol.36, p.299

Comparison of *E. coli*-based cell-free protein synthesis system

	Extract System	Reconstituted System	
	S30 system	Original PURE system	PUREfrex [®]
Typical Yield	100-1000 µg/mL	10-200 µg/mL	10-200 µg/mL
Contamination			
RNase	very High	Low	very Low
LPS	very High	High	very Low
Template DNA			
Plasmid DNA	OK	OK	OK
PCR product	NG	OK	OK
Customization of Reagent	Difficult	Easy	Easy
Purification of His-tagged product	OK	NG	OK

Original PURE system: Shimizu *et al.* (2005) *Methods*, vol.36, p.299



Comparison of *E. coli*-based cell-free protein synthesis system

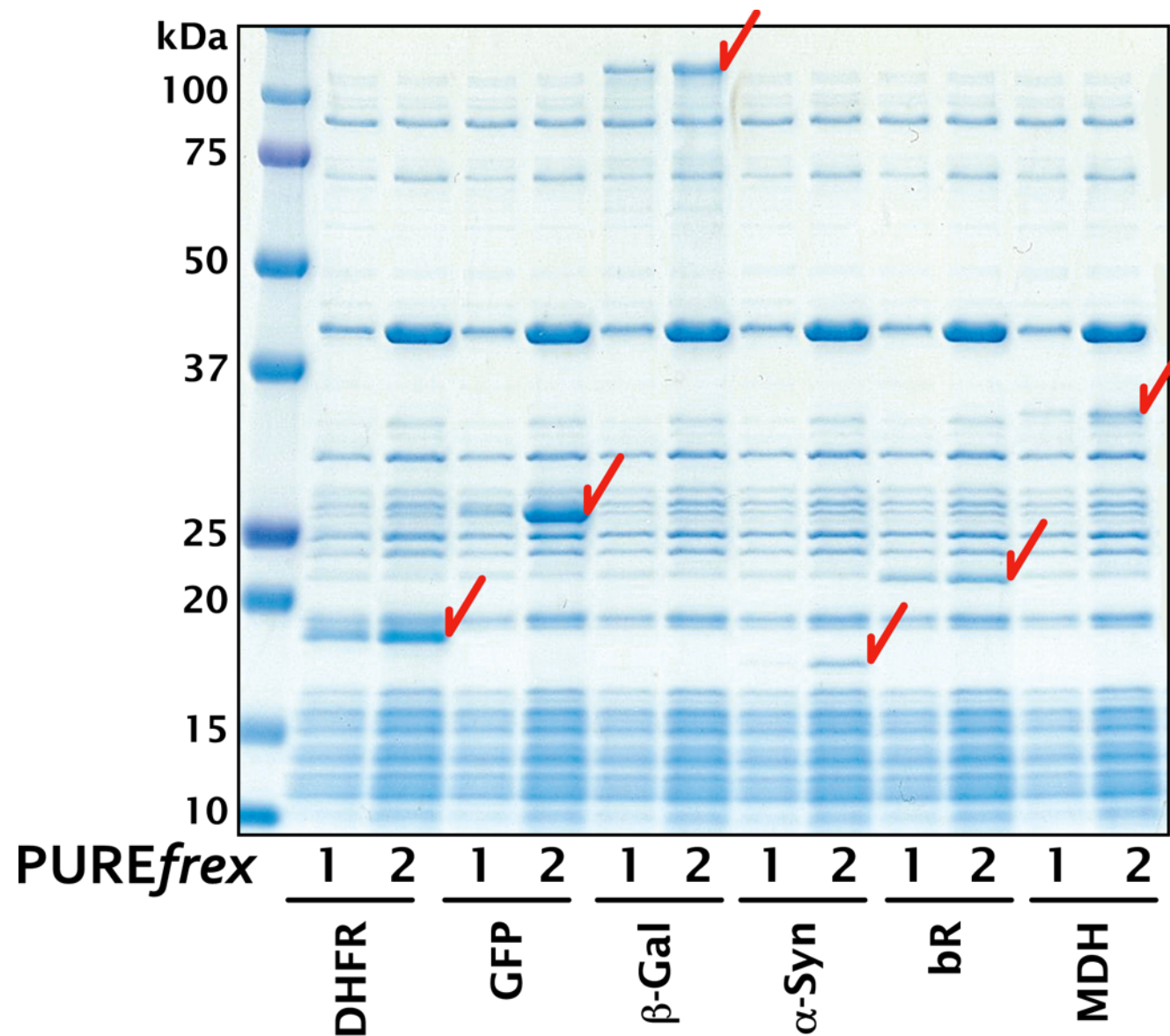
	Extract System	Reconstituted System	
	S30 system	Original PURE system	PUREfrex [®] 2.0
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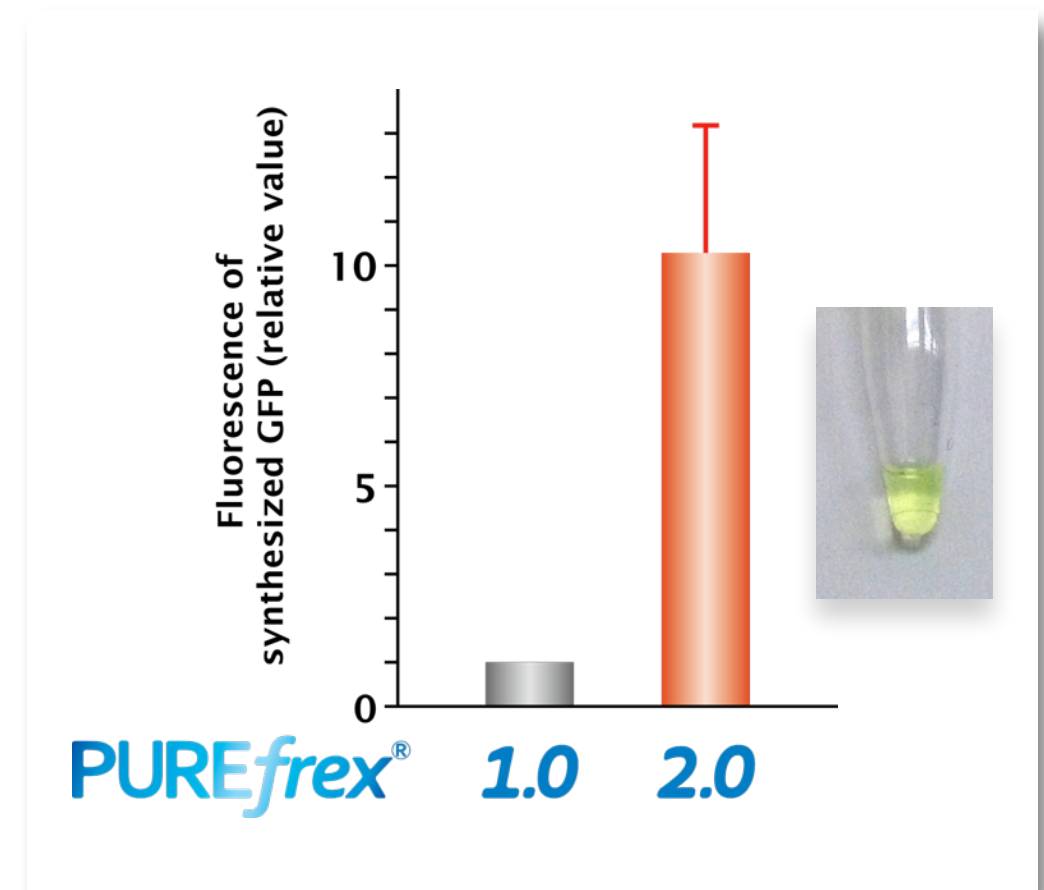
Protein synthesis using PUREfrex[®] 1.0 and 2.0

A) SDS-PAGE



DHFR: Dihydrofolate reductase
GFP: Green Fluorescent Protein
β-Gal: β-Galactosidase
α-Syn: α-Synuclein
bR: Bacteriorhodopsin
MDH: Malate Dehydrogenase

B) Fluorescence of synthesized GFP



1. Introduction of PUREfrex[®]

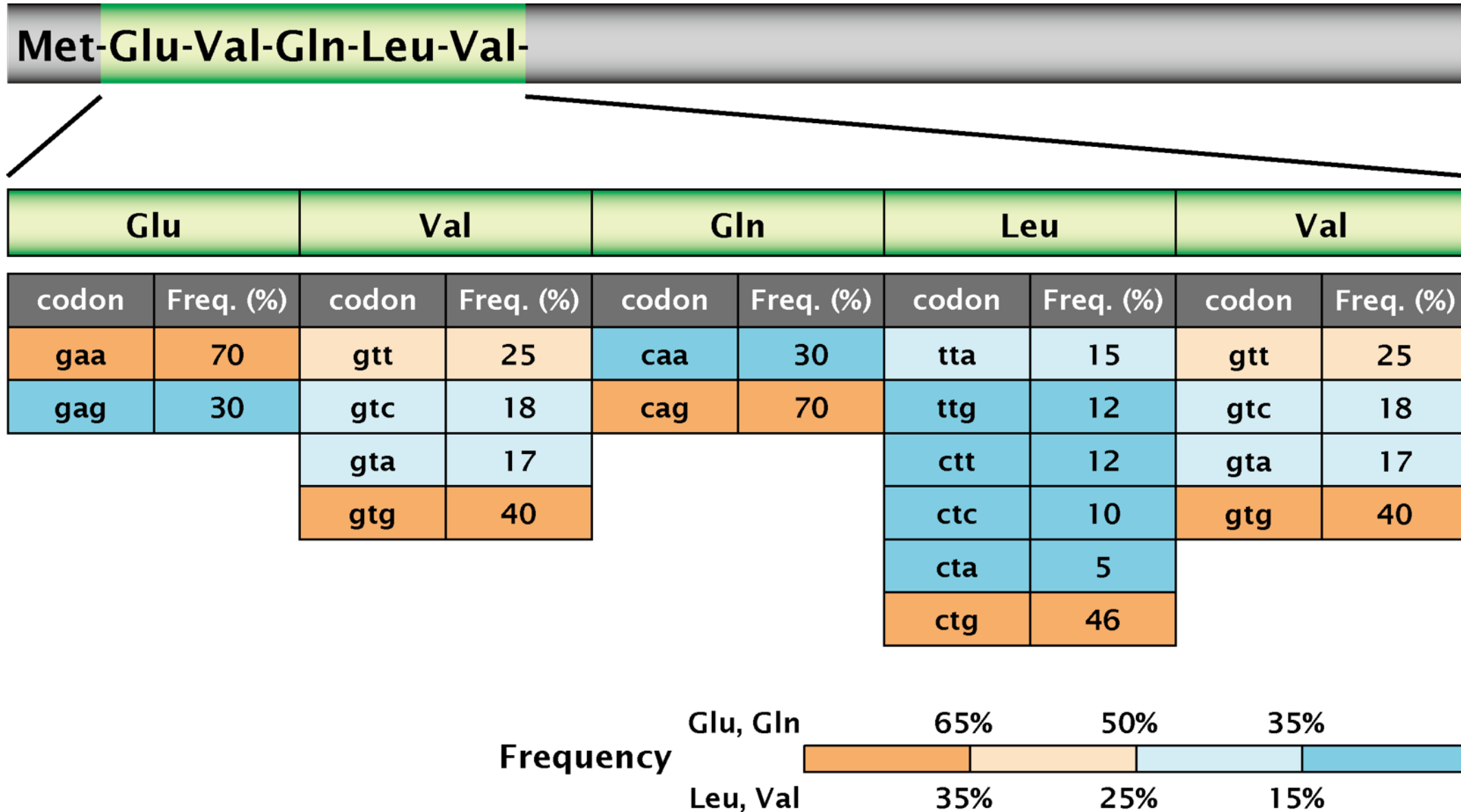
2. Application of PUREfrex[®]

- ***Optimization of nt sequence at 5'-terminus of ORF***
- *Synthesis of proteins containing disulfide bonds*
- *Synthesis of antibody-related proteins*



Effect of 5'-terminal nucleotide sequence on the yield

Trastuzumab Heavy chain (VH+CH1)

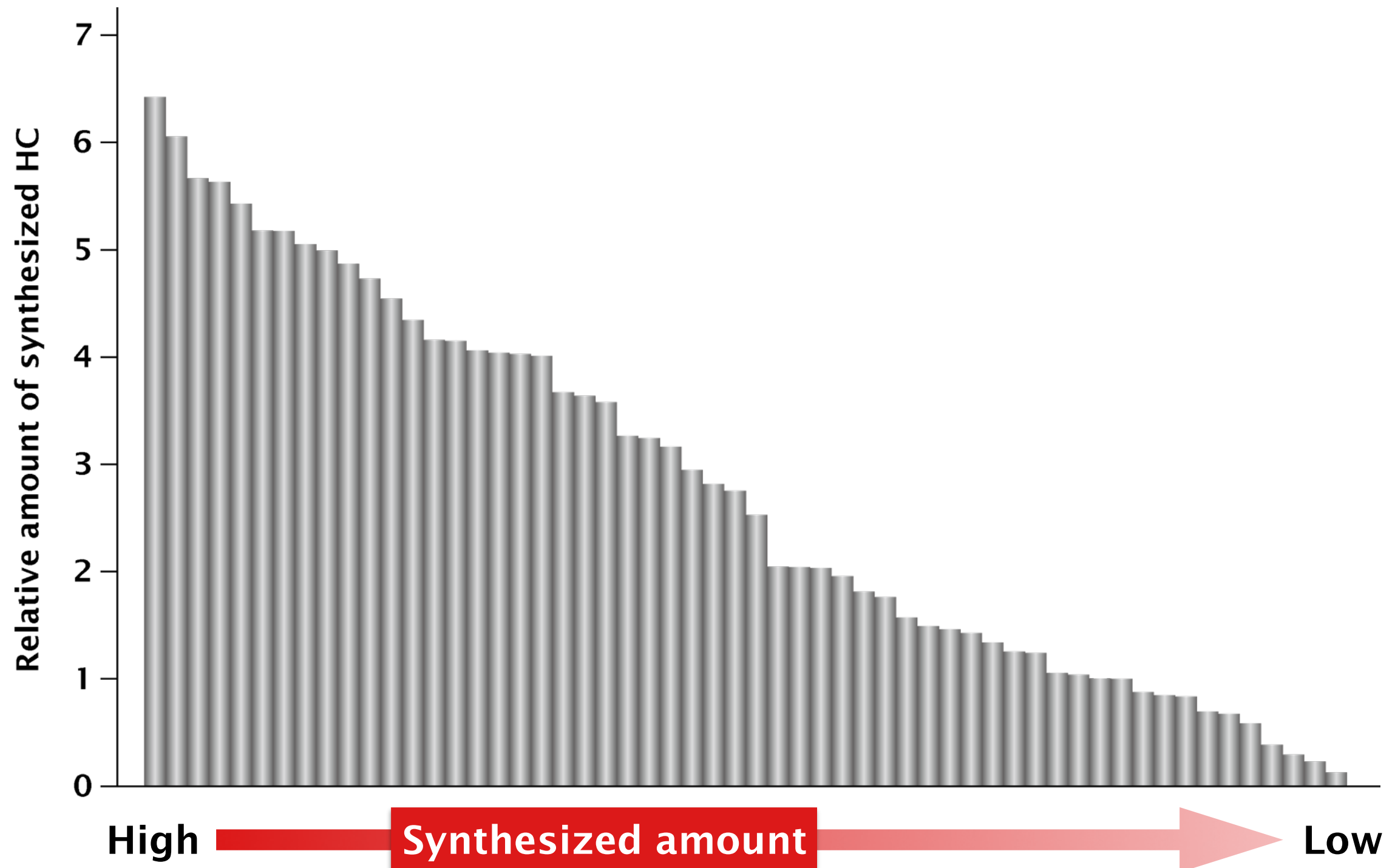


*Frequency is calculated from Codon Usage Database in Kazusa DNA Res. Inst. (*E. coli* K-12 strain)

Effect of 5'-terminal nucleotide sequence on the yield

Trastuzumab Heavy chain (VH+CH1)

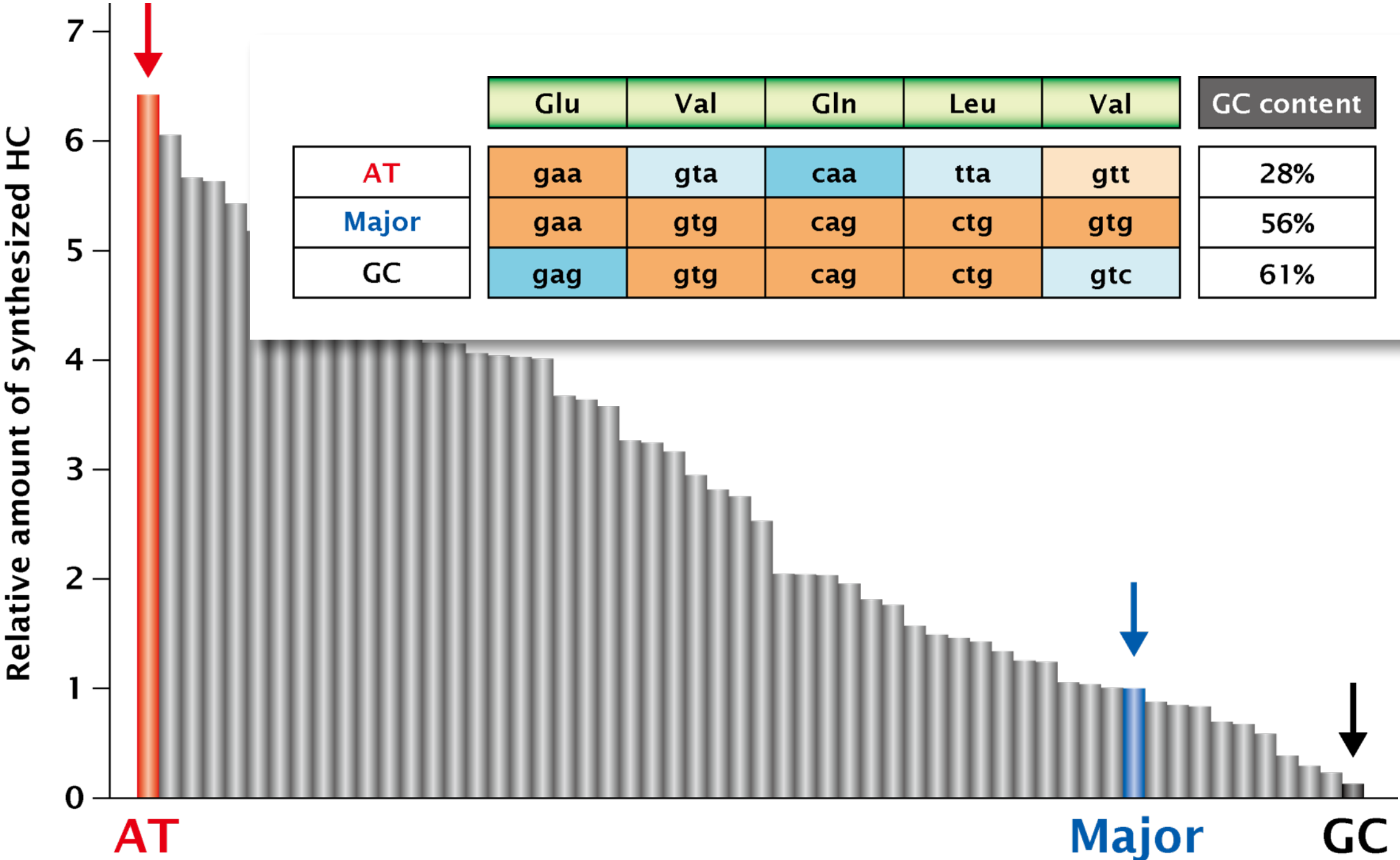
Tested clones : 56 clones



Effect of 5'-terminal nucleotide sequence on the yield

Trastuzumab Heavy chain (VH+CH1)

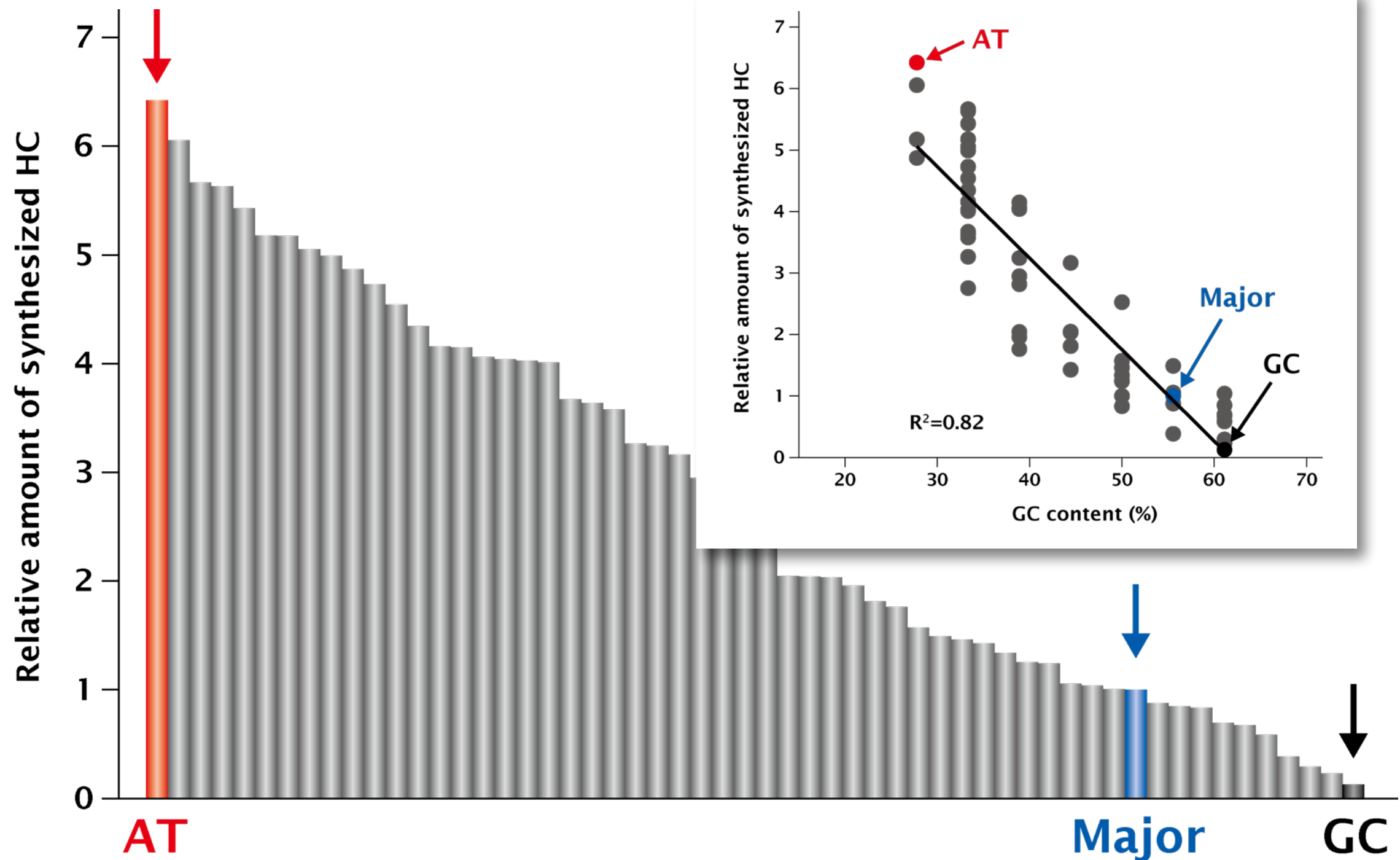
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Effect of 5'-terminal nucleotide sequence on the yield

Trastuzumab Heavy chain (VH+CH1)

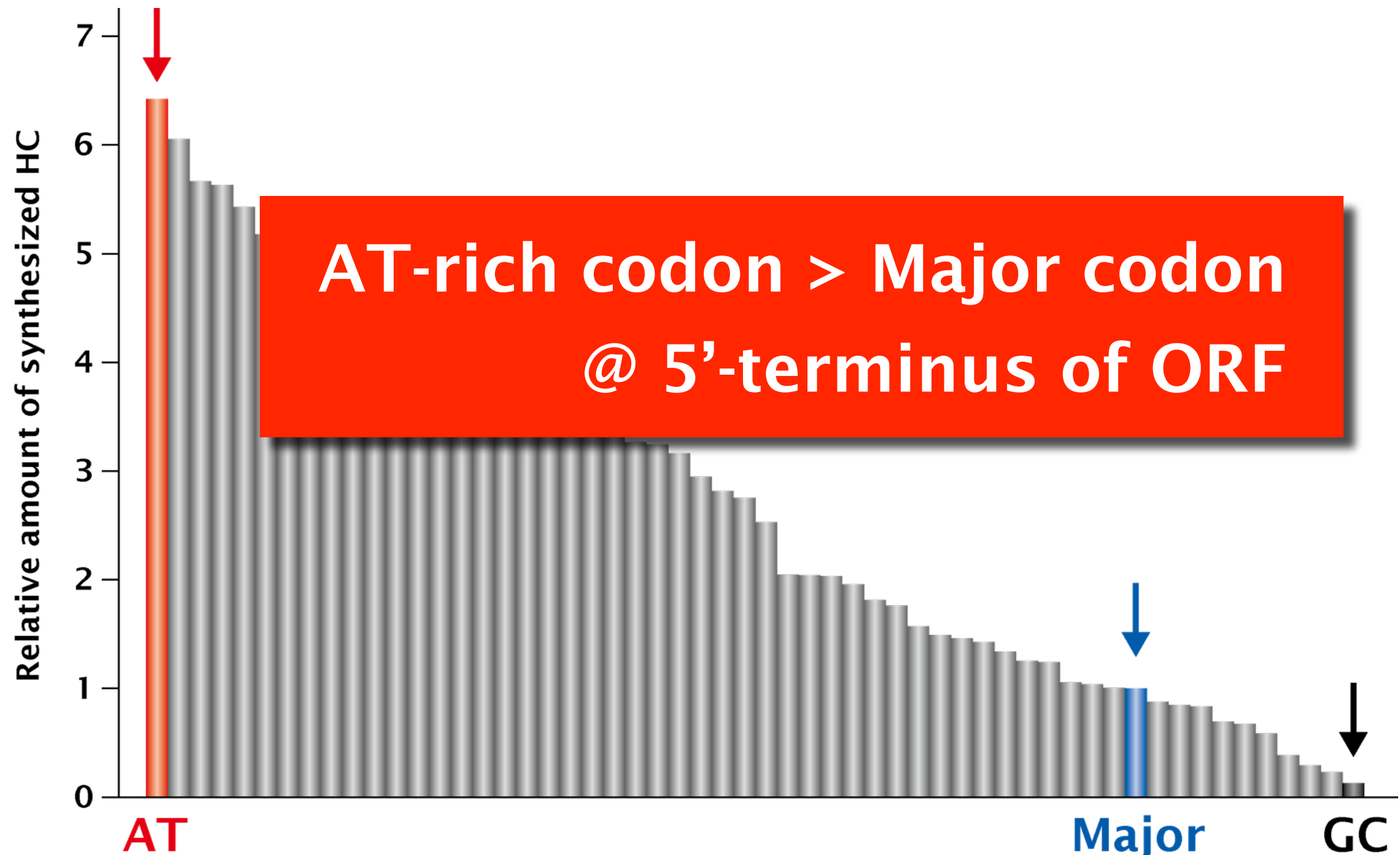
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Synthesis of functional proteins using PURE^{frex}[®]

Translation process

Synthesis of
polypeptide chain

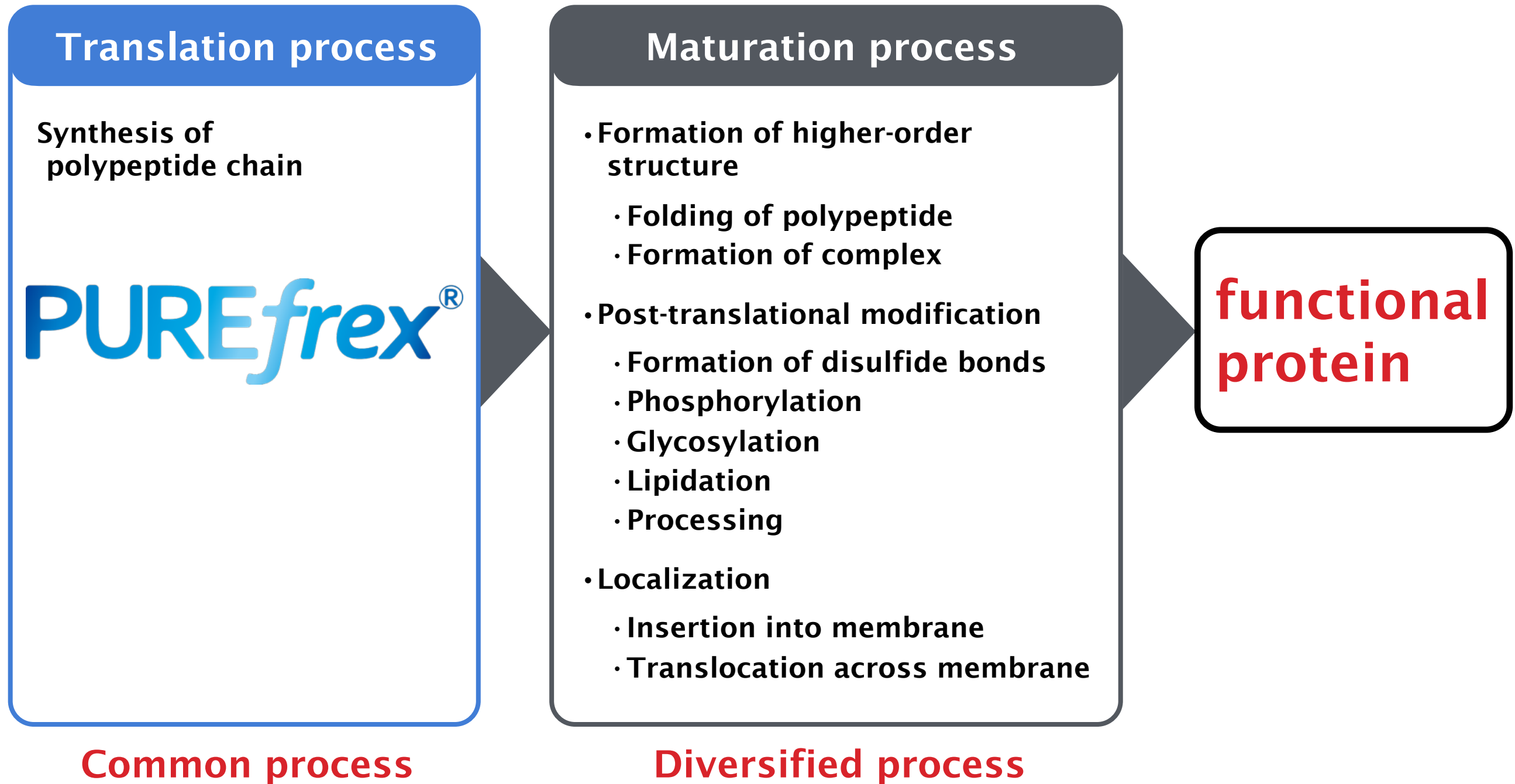
PURE^{frex}[®]

Polypeptide



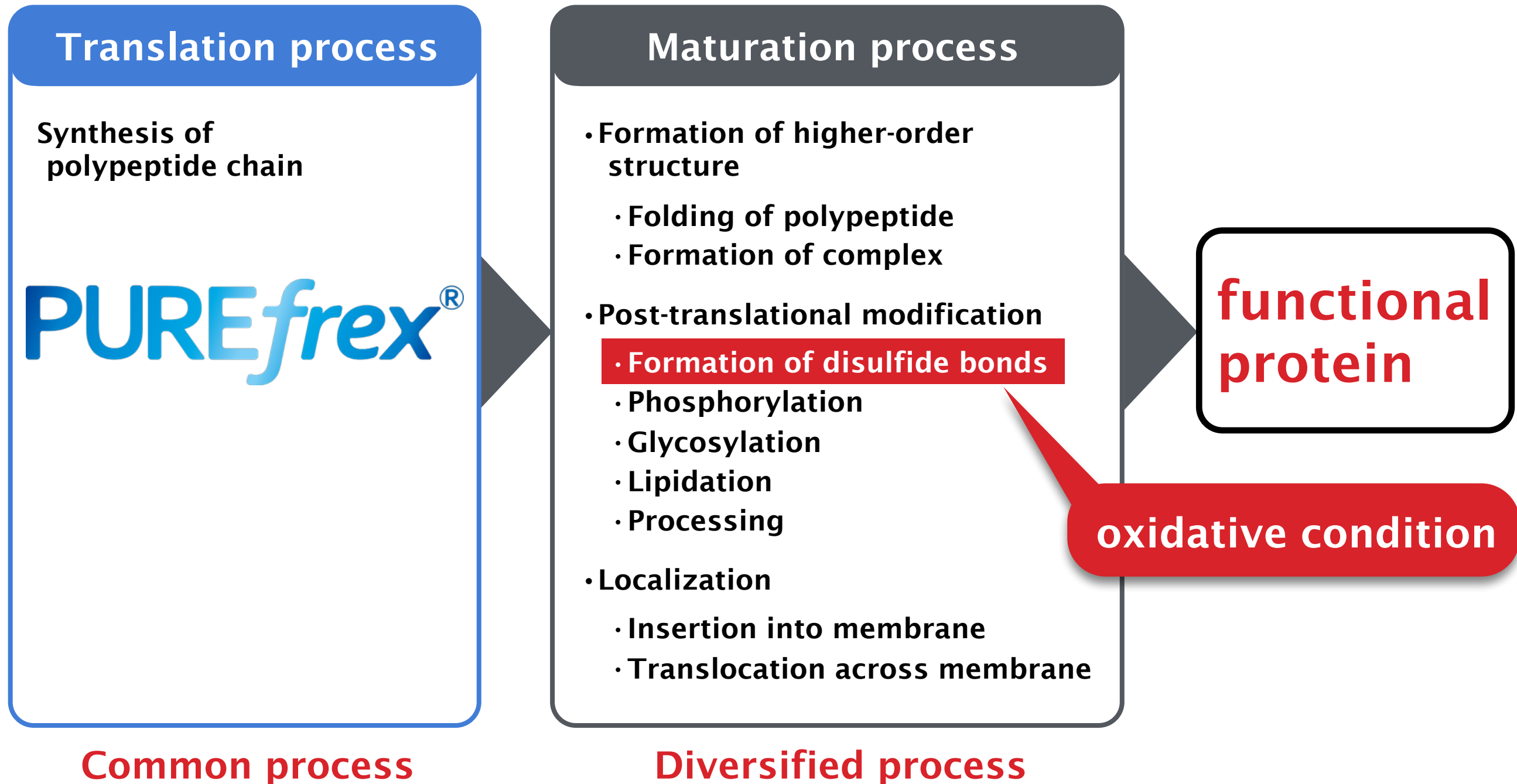
Synthesis of functional proteins using PUREfrex[®]

Polypeptide ≠ Functional protein



Synthesis of functional proteins containing disulfide bonds

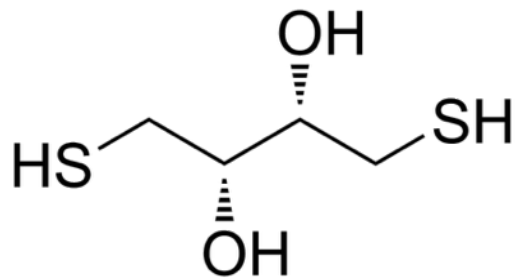
Polypeptide \neq Functional protein



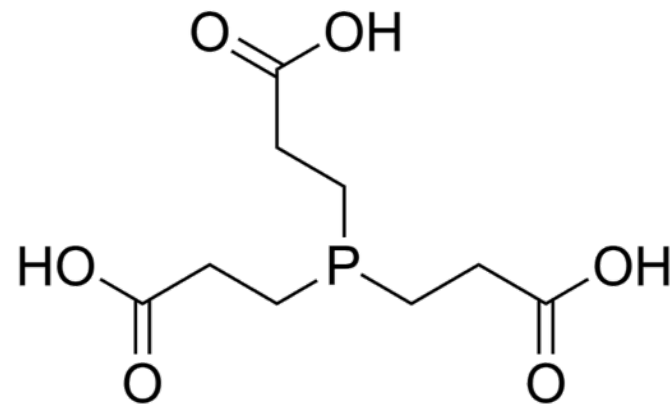
Synthesis of functional proteins containing disulfide bonds

Reductant

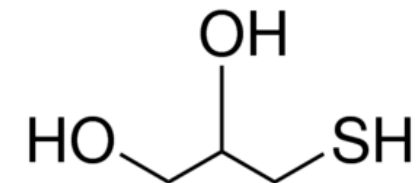
Dithiothreitol (DTT)



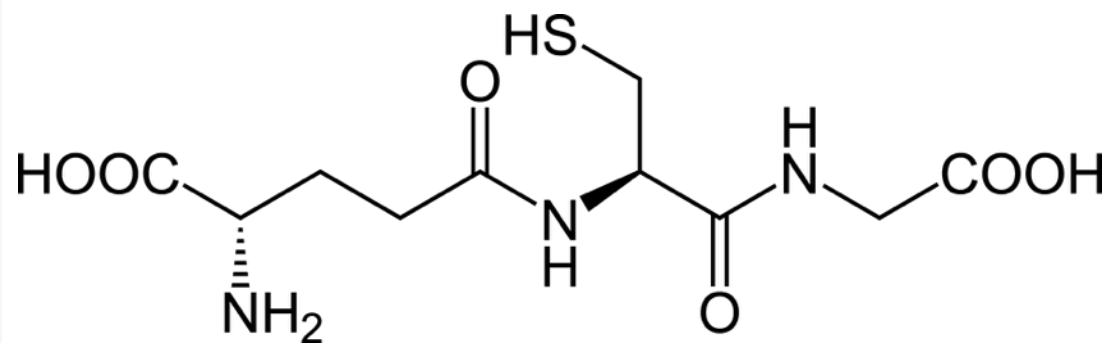
Tris(2-carboxyethyl)phosphine (TCEP)



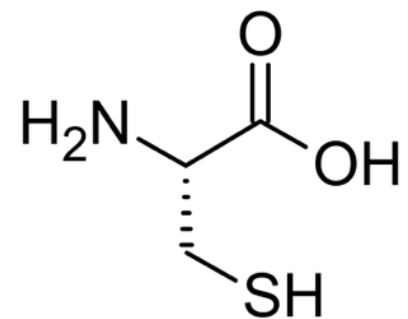
3-Mercapto-1,2-propanediol (MPD)



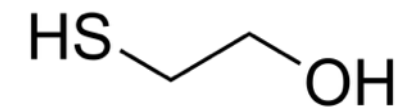
Glutathione (GSH)



Cysteine (Cys)



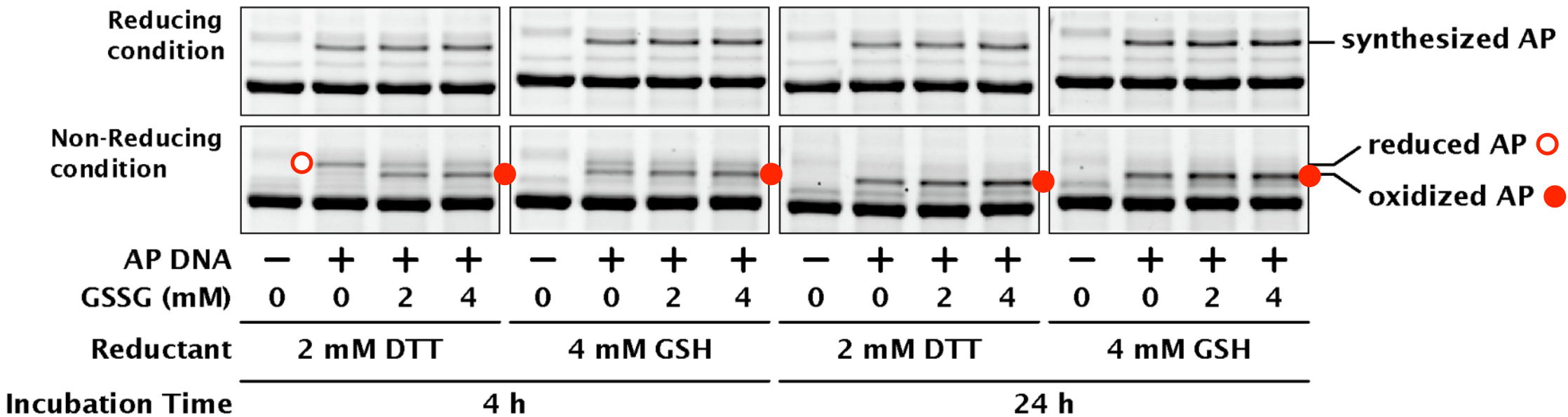
2-Mercaptoethanol (2-ME)



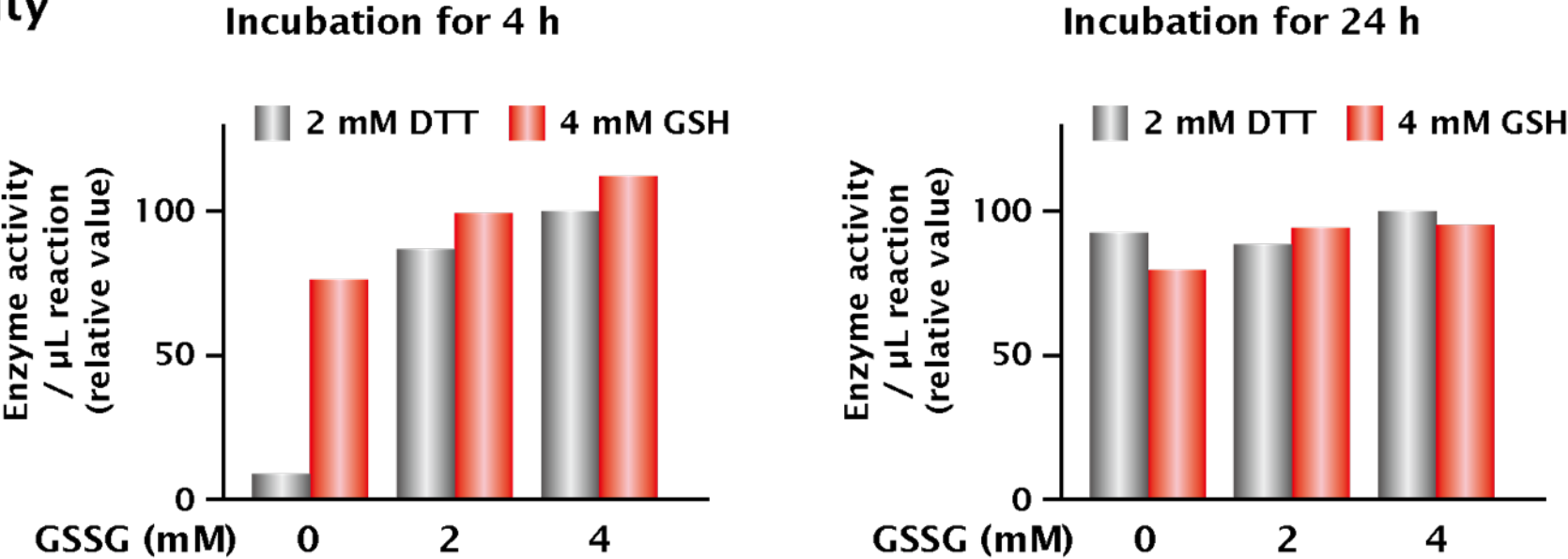
Synthesis of functional proteins containing disulfide bonds

E. coli alkaline phosphatase (AP) (2 disulfide bonds)

A) SDS-PAGE



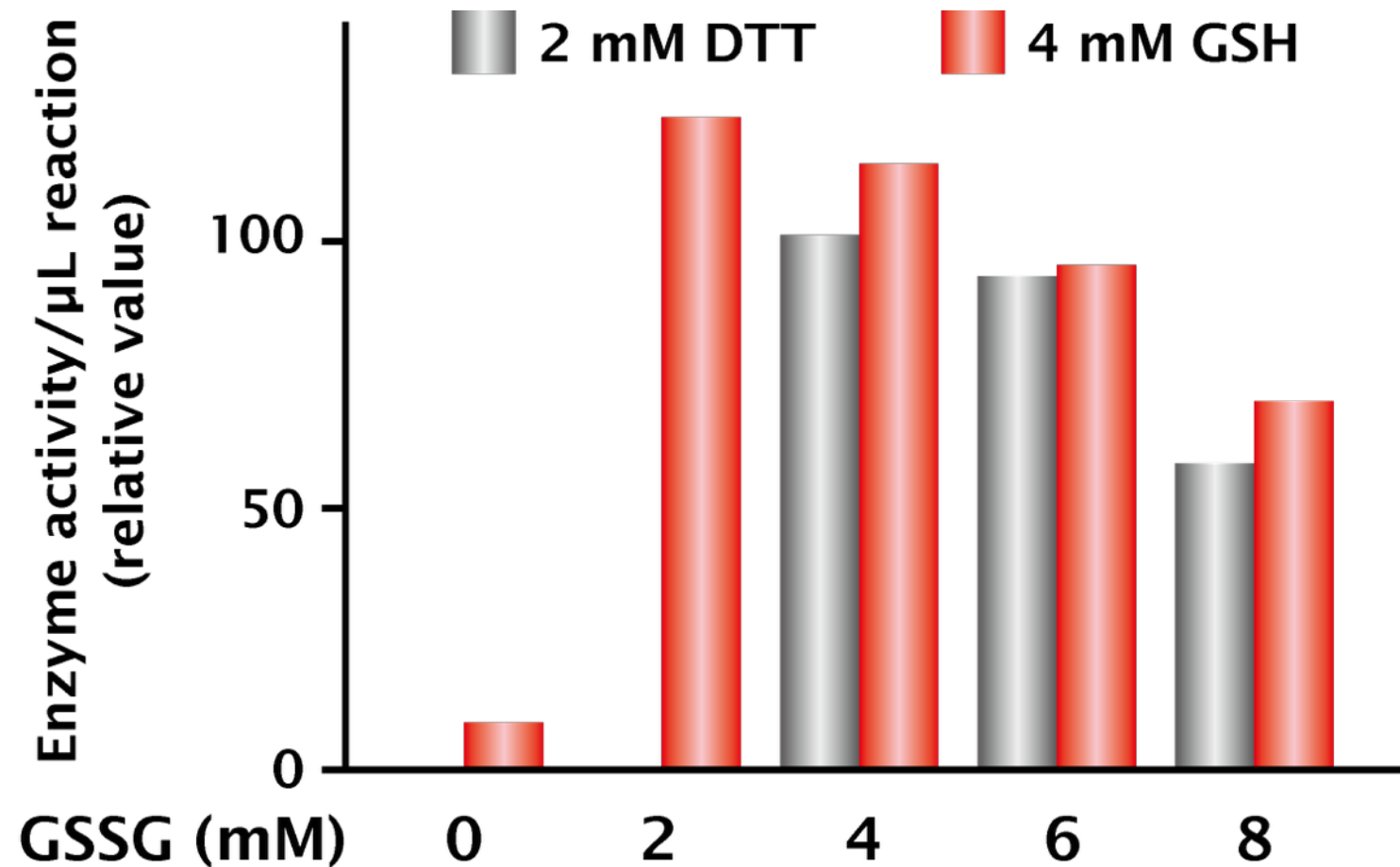
B) Activity



Synthesis of functional proteins containing disulfide bonds

E. coli acid phosphatase (AppA) (5 disulfide bonds)

+ DsbC (*E. coli* disulfide isomerase)



New product

PUREfrex[®] 2.0

Solution I
Solution II
Solution III
DHFR DNA



PUREfrex[®] 2.1

Solution I (-Cys, -DTT)
Solution II
Solution III
Cysteine
DTT
GSH
DHFR DNA



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Synthesis of scFv and Fab

Trastuzumab-derived scFv and Fab

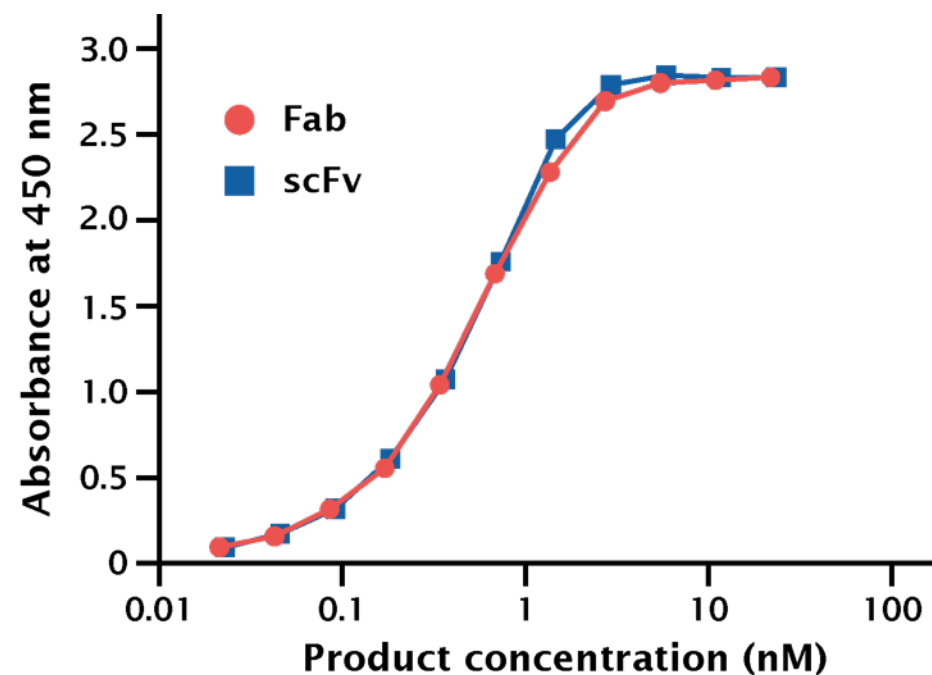
PUREfrex[®] 2.1 (+ DTT)

↓ + **DS supplement** + **DnaK Mix**

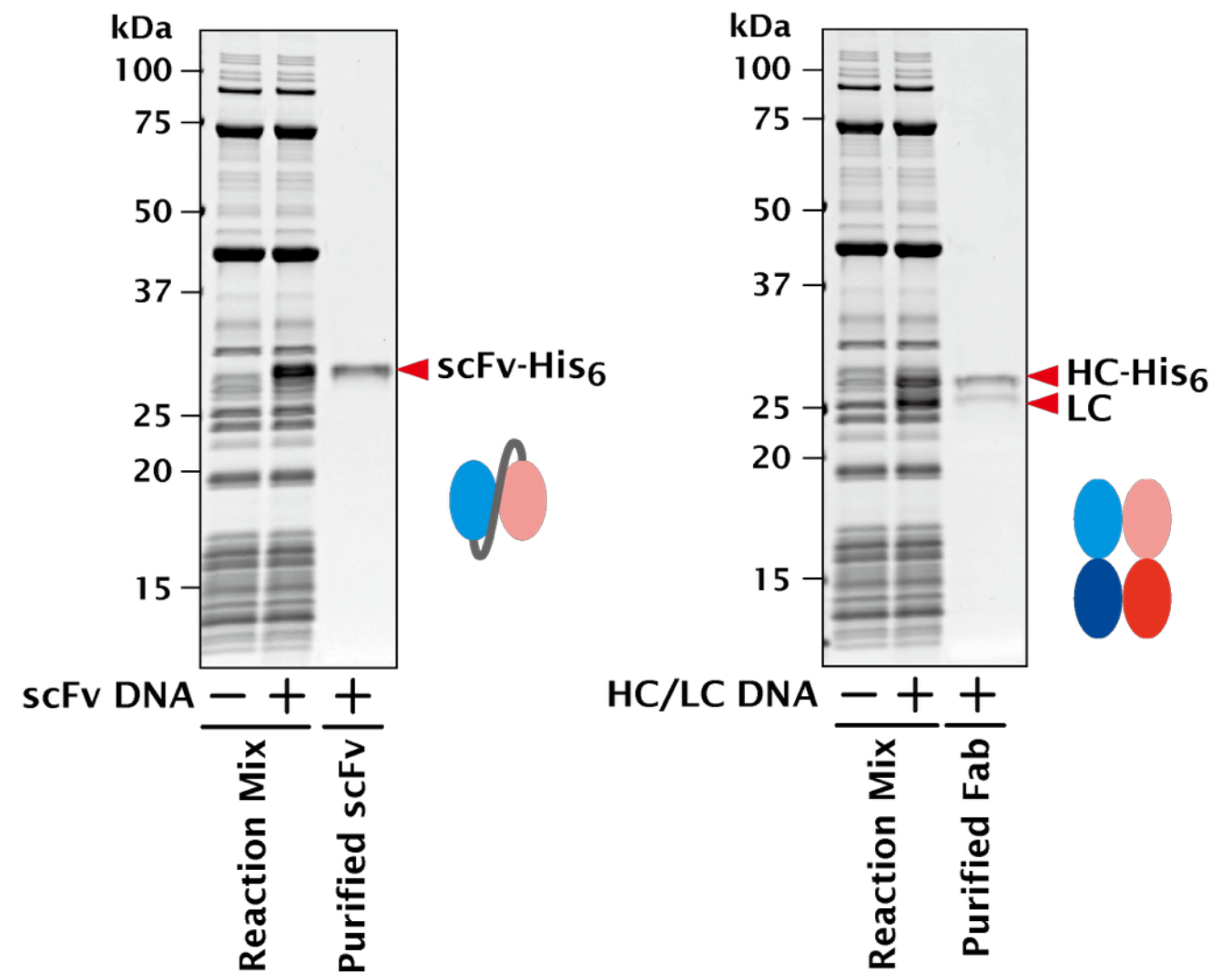
↓ + scFv or HC/LC DNA (PCR product)

↓ incubation at 37°C for 6 h

B) ELISA



A) SDS-PAGE



Yield: 0.4 mg/mL

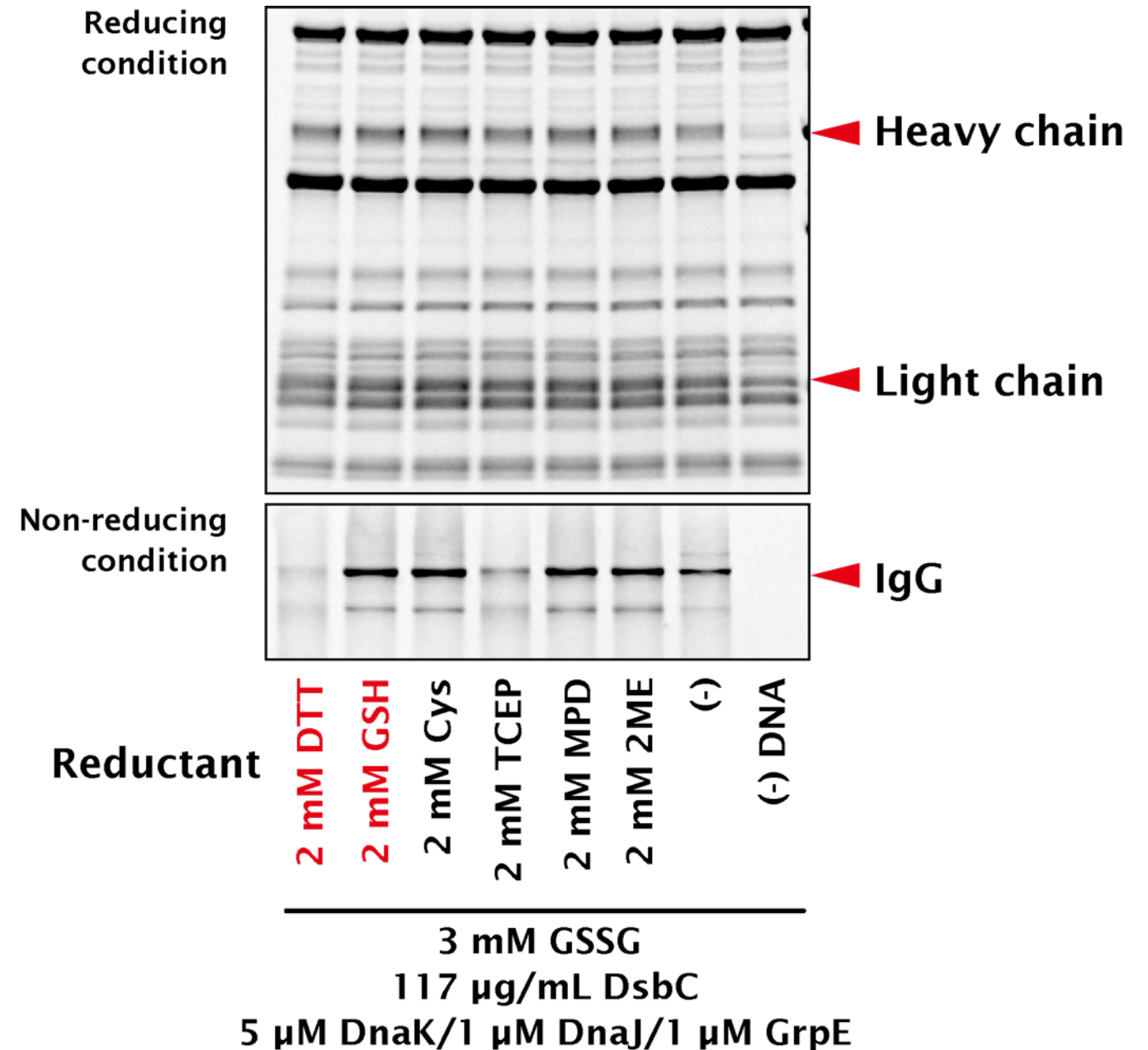
0.5 mg/mL

Synthesis of aglycosylated IgG

Trastuzumab-derived aglycosylated IgG

Key Factors

- HC/LC DNA ratio
- Reductant
- Molecular chaperones
- Incubation Temperature
- Incubation Time

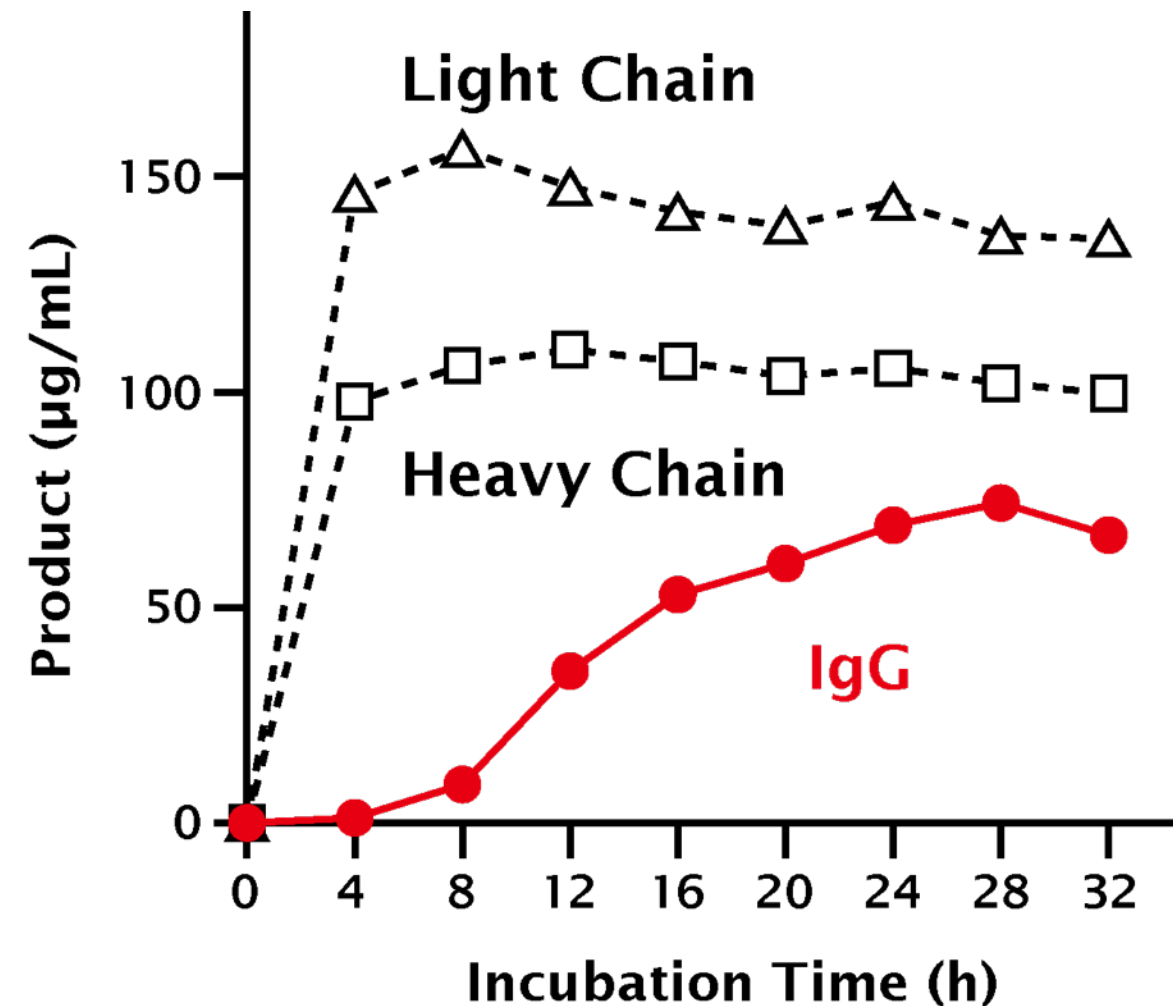


Synthesis of aglycosylated IgG

Trastuzumab-derived aglycosylated IgG

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Synthesis of aglycosylated IgG

Trastuzumab-derived aglycosylated IgG

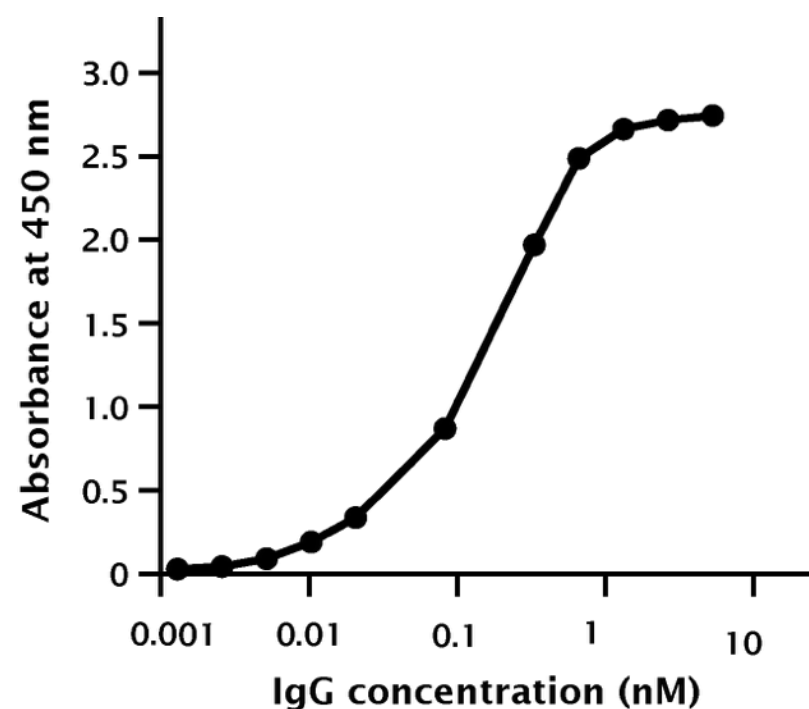
PUREfrex[®] 2.1 (+Reduced glutathione)

↓ + **DS supplement** + **DnaK Mix**

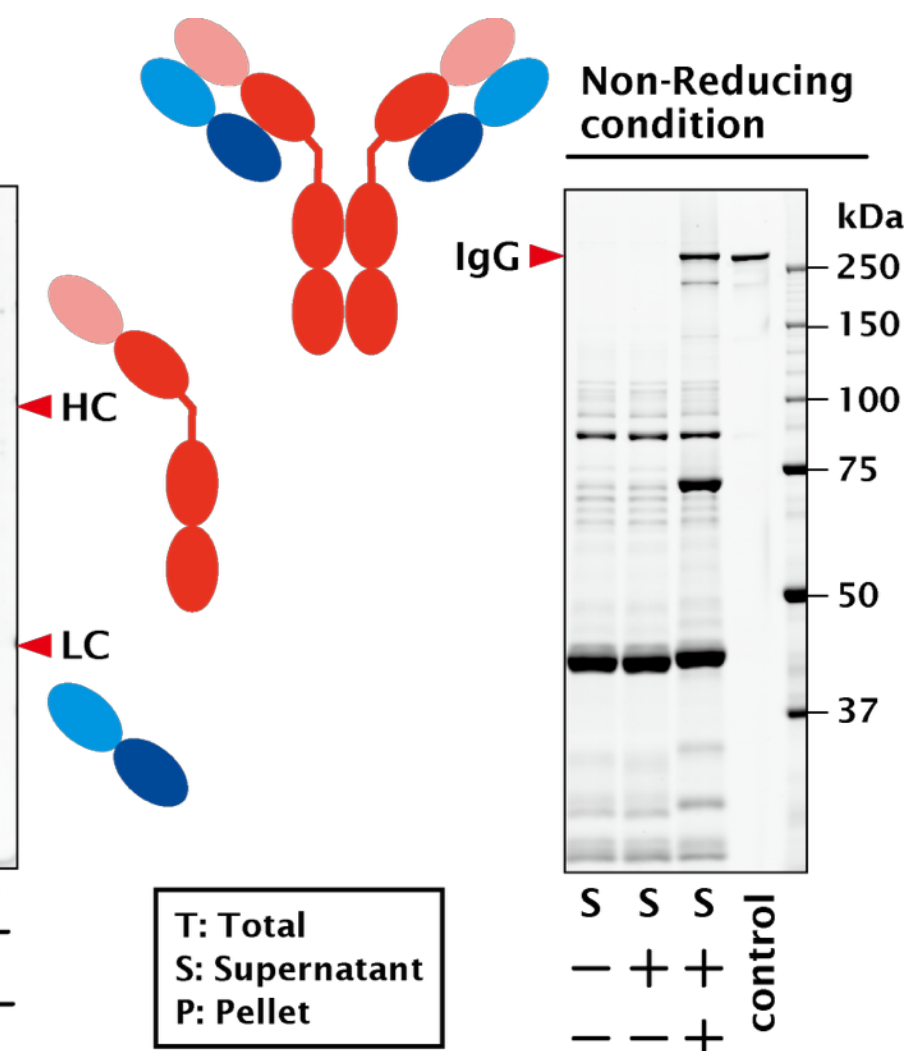
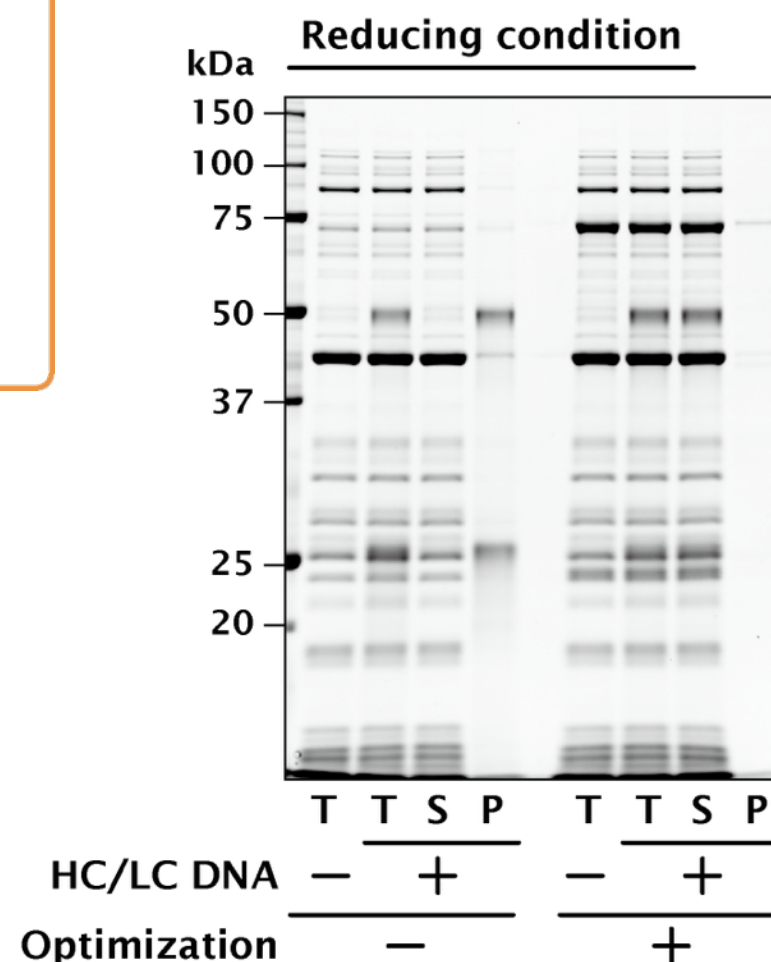
↓ + HC/LC DNA (PCR product)

↓ incubation at 37°C for 28 h

B) ELISA



A) SDS-PAGE



Yield: 0.12 mg/mL

Product List

タンパク質合成反応液

PUREfrex[®] 1.0

PUREfrex[®] 2.0

New

PUREfrex[®] 2.1

添加剤

DnaK Mix

DnaK/DnaJ/GrpE

GroE Mix

GroEL/GroES

DS supplement

Oxidized Glutathione, DsbC

