

[For more information](#)

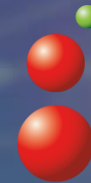


Rebuilding Expression System & Its Applications For R&D Of Biologics.

Reconstituted cell-free protein synthesis kit

PUREfrex[®]

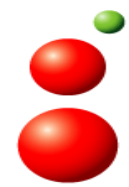
Takashi (Ebi) Ebihara, Ph.D.
COO
GeneFrontier Corporation



GeneFrontier

Biologics US 2024
3-4 of Oct, 2024

Corporate Summary



GeneFrontier

Founded: ***Oct 13th, 2010*** (renewed)

Shareholder: ***KANEKA Corporation*** (100%)

People: ***14*** (Ph.D. 8, MS 1)

Place: ***Chiba, Japan***

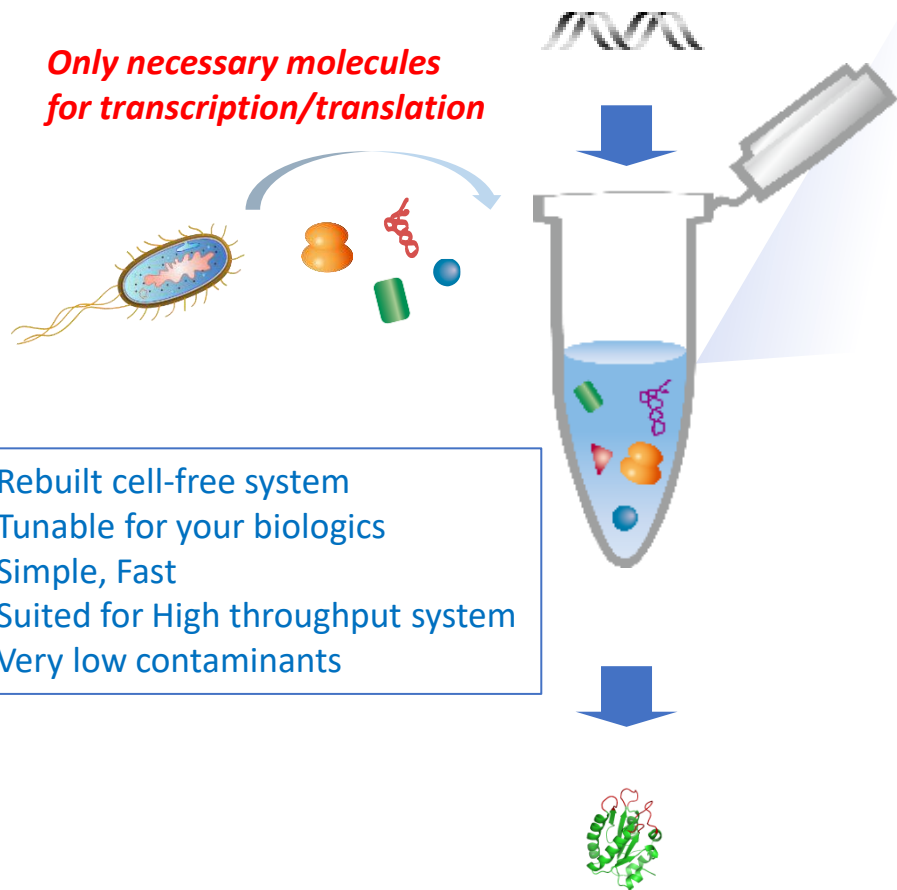


Mission: ***Rebuilding and Manipulating Biological system
for Inspiring the world!***

PUREfres[®]

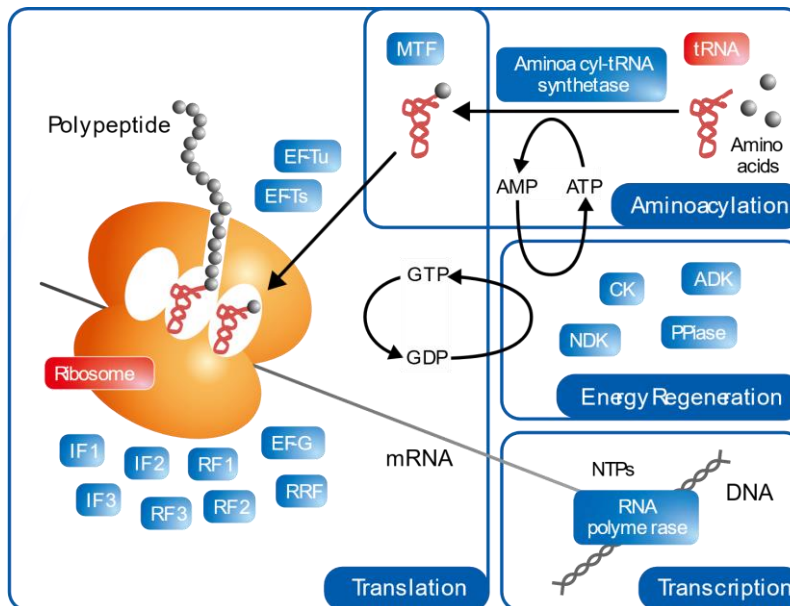
-Customize expression toolbox for your research-

Only necessary molecules for transcription/translation

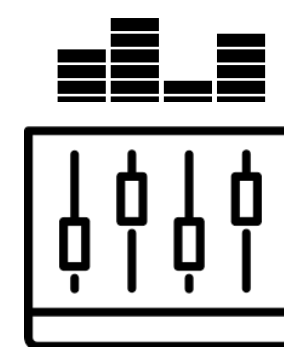
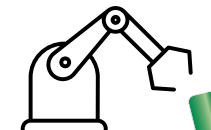
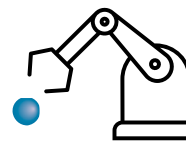


- ✓ Rebuilt cell-free system
- ✓ Tunable for your biologics
- ✓ Simple, Fast
- ✓ Suited for High throughput system
- ✓ Very low contaminants

Totally constructive, molecular based system



For more information

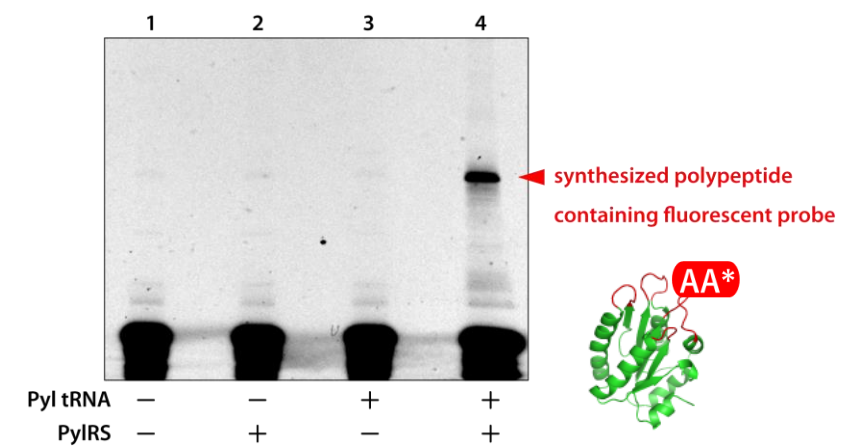
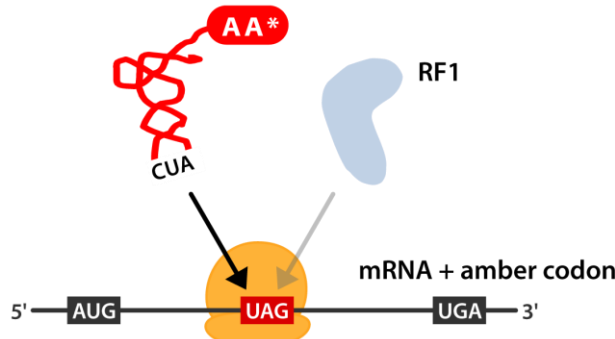


Translation factors
Chaperones
Detergent
Temperature/pH
Redox

<Ex, Non-natural AA introduction>

Translation - RF1

Suppressor tRNA + non-natural amino acid

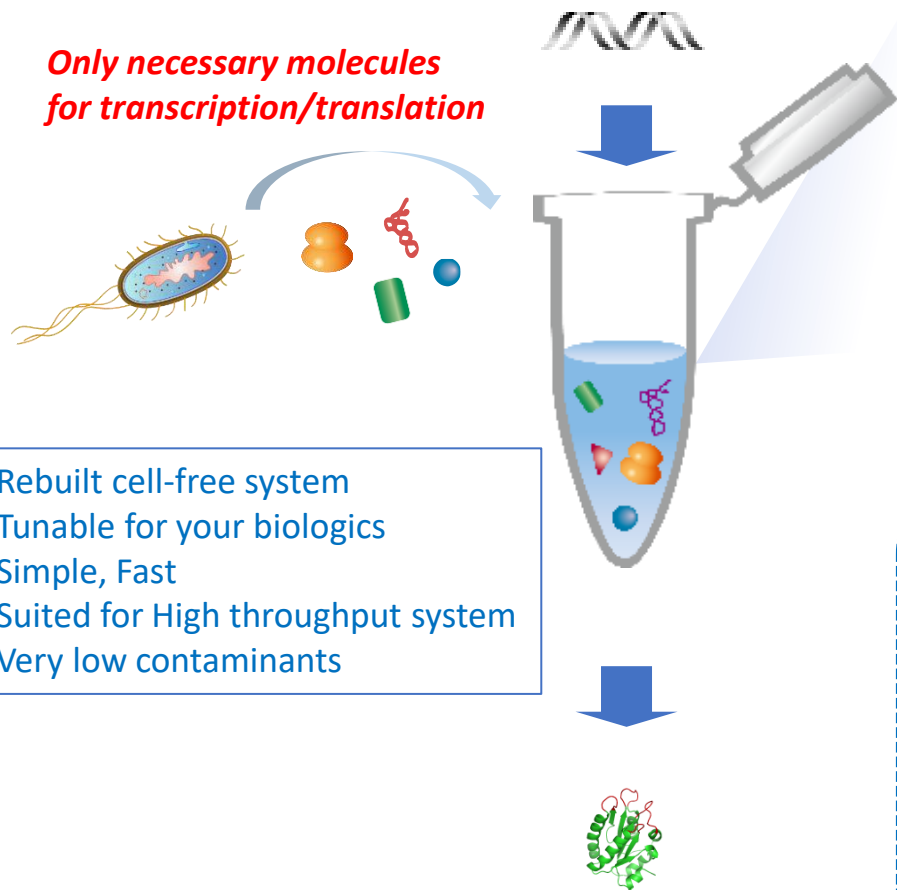


Ready-made kit is coming soon!

PUREfrefx®

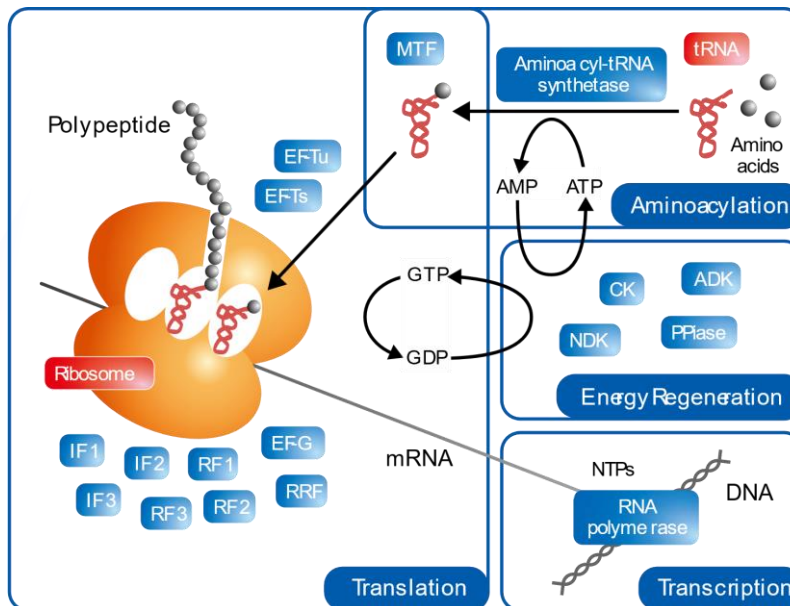
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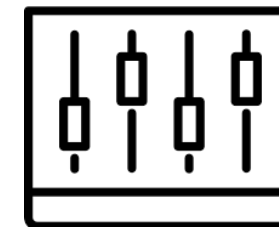
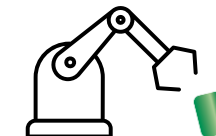
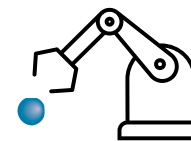


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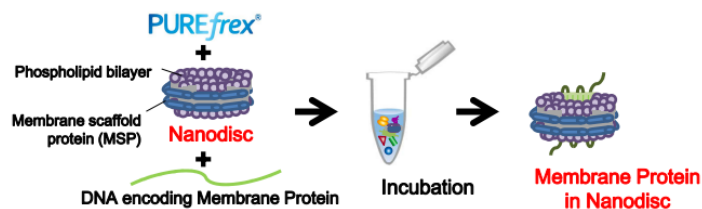


For more information



Translation factors
Chaperones
Detergent
Temperature/pH
Redox

<Ex, Membrane protein with Nanodisc; artificial membrane-like structure>



Solubilized hCLDN1 was synthesized using PUREfrefx® and Nanodisc.

The condition of membrane protein synthesis

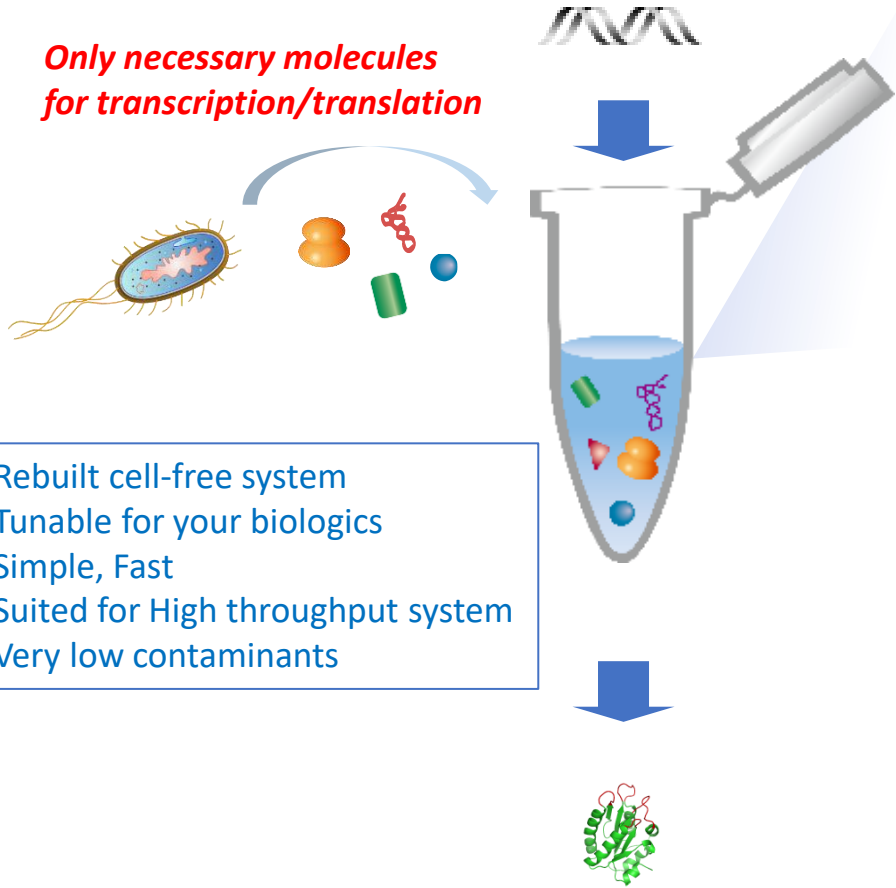
Reaction mix	Template DNA	Incubation
PUREfrefx® 2.0 +Nanodisc (MSP1E3D1-His POPC*, final 10 μM)	PCR product	37°C, 4 h

*Ref: Denisov et al. (2007) J.Biol.Chem., vol. 282, p. 7066.

PUREfres[®]

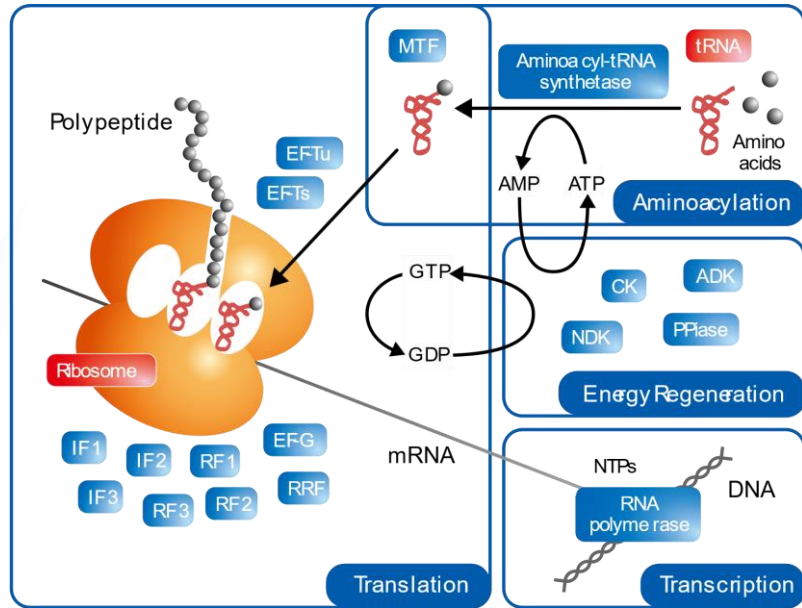
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Only necessary molecules for transcription/translation

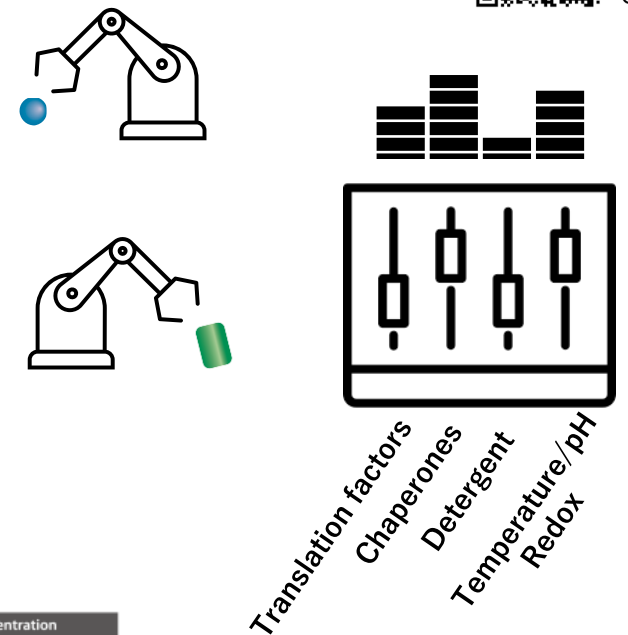


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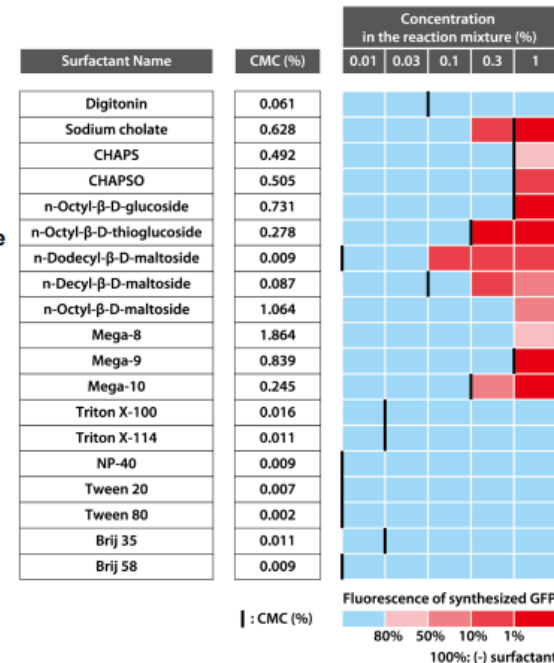


Experimental conditions for protein synthesis

Reaction mixture	Incubation	Template DNA
PUREfres [®] 2.1 (4 mM GSH) + Surfactants	37°C 4 h	sfGFP PCR product (1 ng/μL)

→ Measurement of GFP fluorescence

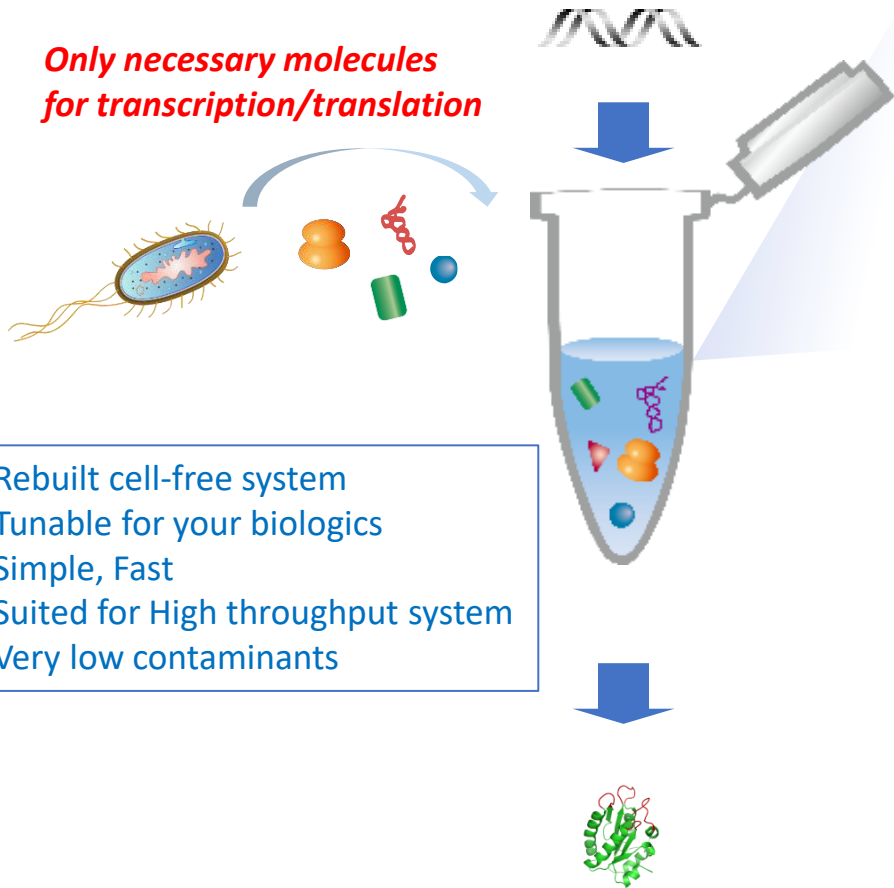
- Most surfactants did not inhibit the protein synthesis reaction by PUREfres[®] below the CMC.
- Some surfactants such as Triton X-100 and Tween 20 could be used even above the CMC.



PUREfres[®]

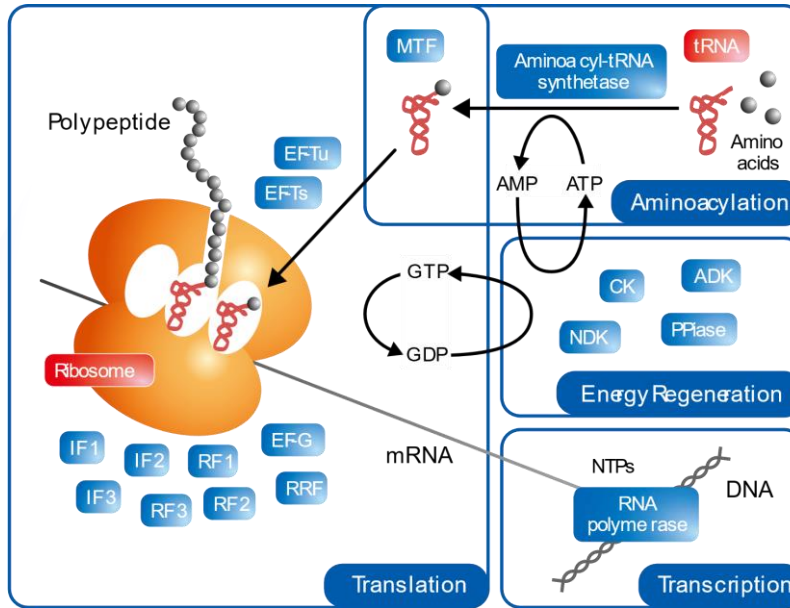
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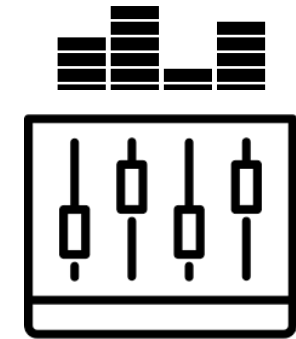
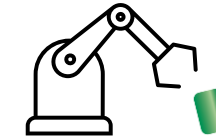
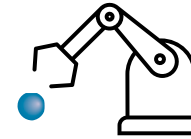


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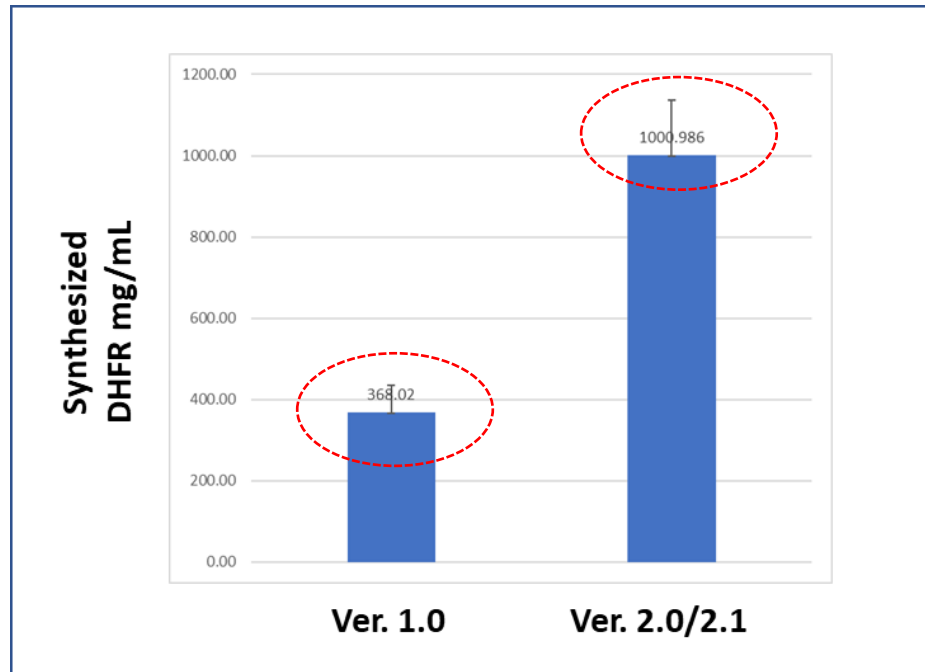
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For more information



Translation factors
Chaperones
Detergent
Temperature/pH
Redox



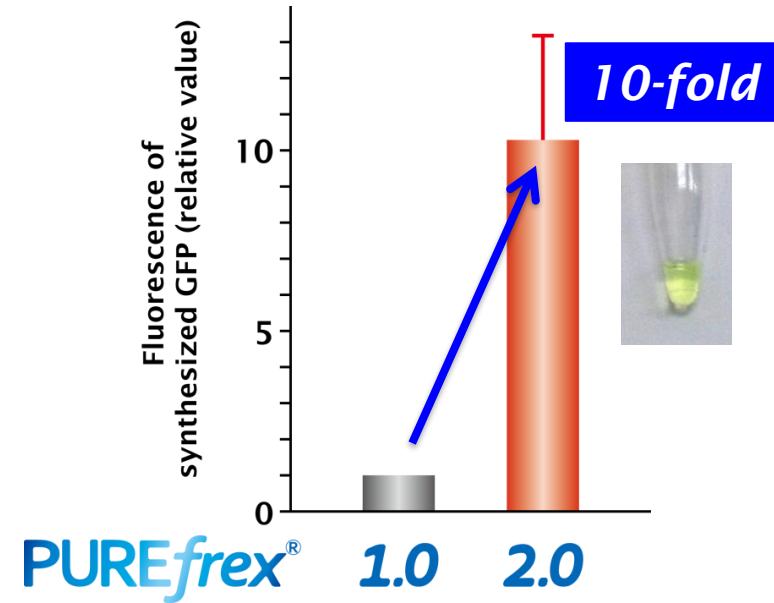
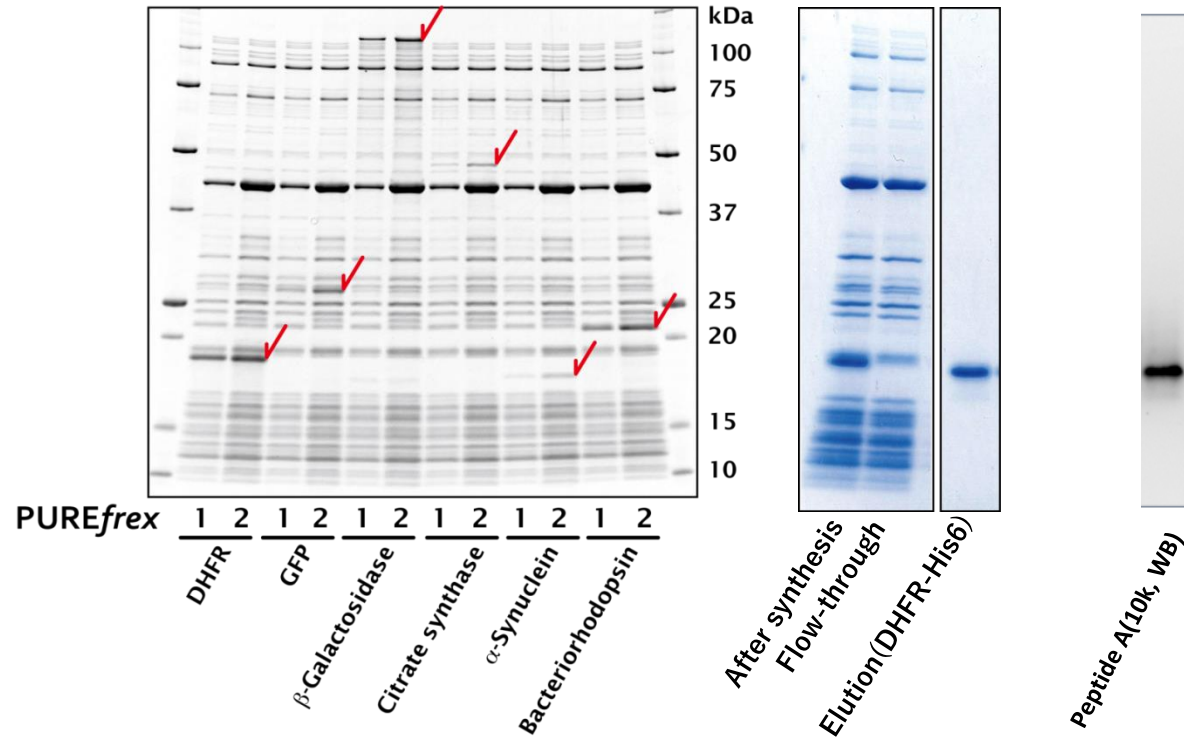
Versatile and Robust
Platform for protein synthesis

Huge potential as
New platform in Biotech industry





-Having good productivity-



- Reaction at 37°C for 4 h
- 0.5 μL of reaction mix/ lane
- stained with Oriole (Bio-Rad) and analyzed with an image analyzer (LAS)

- ✓ Good expression for many proteins, small to large.
- ✓ Good purity with simple purification.
- ✓ Good productivity, ~g/L.



-KSF; AT rich codon on N-term-

Fab Heavy Chain (Herceptin)

Herceptin Fab HC

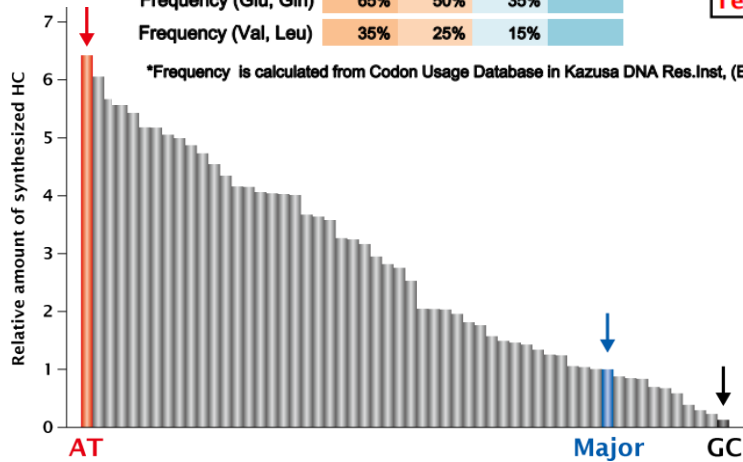
Met- **Glu-Val-Gln-Leu-Val-** FLAG

2		3		4		5		6	
Glu		Val		Gln		Leu		Val	
codon	freq (%)	codon	freq (%)	codon	freq (%)	codon	freq (%)	codon	freq (%)
gaa	70	ggt	25	caa	30	ttg	15	ggt	25
gag	30	gtc	18	cag	70	tta	12	gtc	18
		gta	17			ctt	12	gta	17
		gtg	40			ctc	10	gtg	40
						cta	5		
						ctg	46		

Frequency (Glu, Gln)	65%	50%	35%
Frequency (Val, Leu)	35%	25%	15%

All clones; 384
Tested clones; 56

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



Design of DNA template is important.

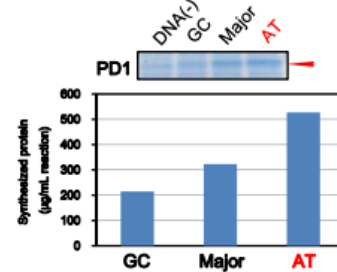
Manual is Free to download from our Web site here.



PD1

Organism: *Homo sapiens*
Synthesized region: 36Thr-150Glu-(Hisx8)
Length: 124 a.a.
Molecular weight: 14,148 Da

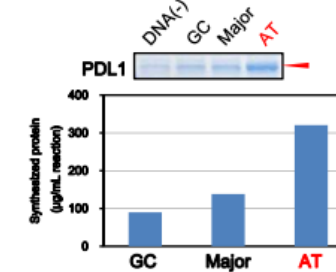
N-term type	1	2(38)	3(37)	4(38)	5(39)	6(40)	GC(%) 1-6 a.a.
GC	atg	acc	ttc	toc	cog	gog	67%
Major	atg	acc	ttt	tct	cog	gog	56%
AT	atg	act	ttt	tca	cca	gct	39%



PDL1

Organism: *Homo sapiens*
Synthesized region: 18Ala-239Thr-(Hisx8)
Length: 231 a.a.
Molecular weight: 26,593 Da

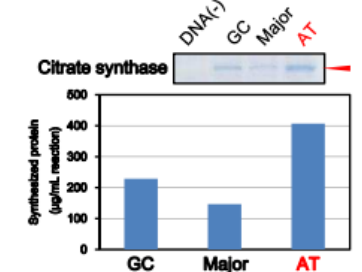
N-term type	1	2(18)	3(19)	4(20)	5(21)	6(22)	GC(%) 1-6 a.a.
GC	atg	gog	ttc	acc	gtg	acc	61%
Major	atg	gog	ttt	acc	gtg	acc	56%
AT	atg	gct	ttt	act	gta	aca	33%



Citrate Synthase

Organism: *Saccharomyces cerevisiae*
Synthesized region: 38Ser-479Asn
Length: 443 a.a.
Molecular weight: 49,346 Da

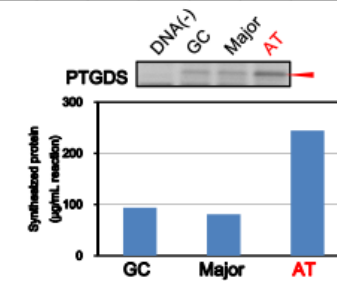
N-term type	1	2(38)	3(39)	4(40)	5(41)	6(42)	GC(%) 1-6 a.a.
GC	atg	toc	toc	gog	toc	gag	67%
Major	atg	tct	tct	gog	tct	gaa	44%
AT	atg	tca	tca	gct	tca	gaa	39%



PTGDS

Organism: *Homo sapiens*
Synthesized region: 23Ala-190Gln
Length: 169 a.a.
Molecular weight: 18,829 Da

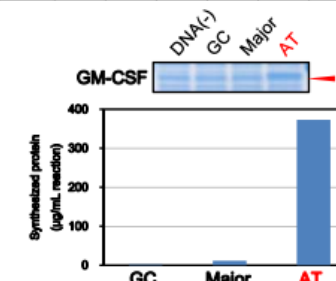
N-term type	1	2(23)	3(24)	4(25)	5(26)	6(27)	GC(%) 1-6 a.a.
GC	atg	gca	cog	gaa	gca	cag	61%
Major	atg	gca	cog	gaa	gca	cag	72%
AT	atg	gca	cct	gaa	gct	caa	50%



GM-CSF

Organism: *Homo sapiens*
Synthesized region: 18Ala-144Glu
Length: 128 a.a.
Molecular weight: 14,608 Da

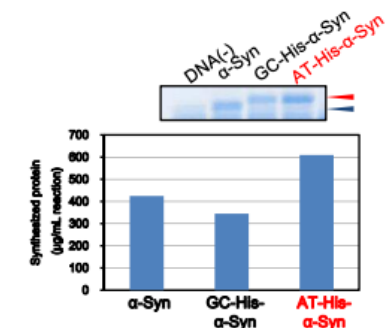
N-term type	1	2(18)	3(19)	4(20)	5(21)	6(22)	GC(%) 1-6 a.a.
GC	atg	gca	cog	gca	cgc	toc	83%
Major	atg	gca	cog	gca	cgc	tct	78%
AT	atg	gca	cct	gct	aga	tca	50%



His-α-Synuclein

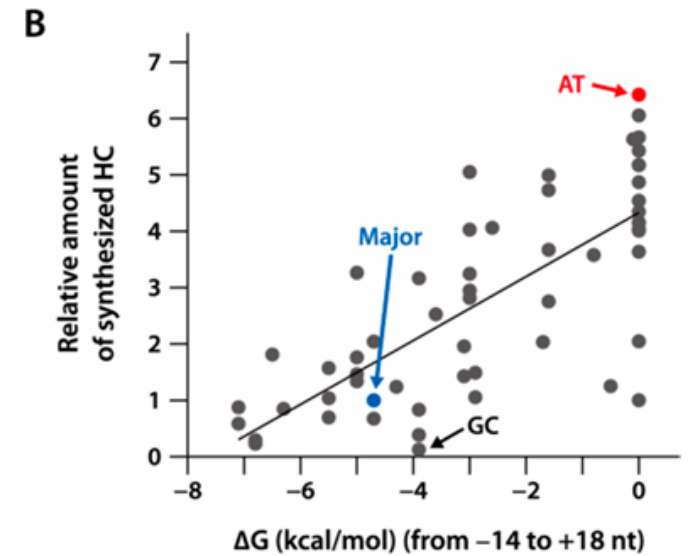
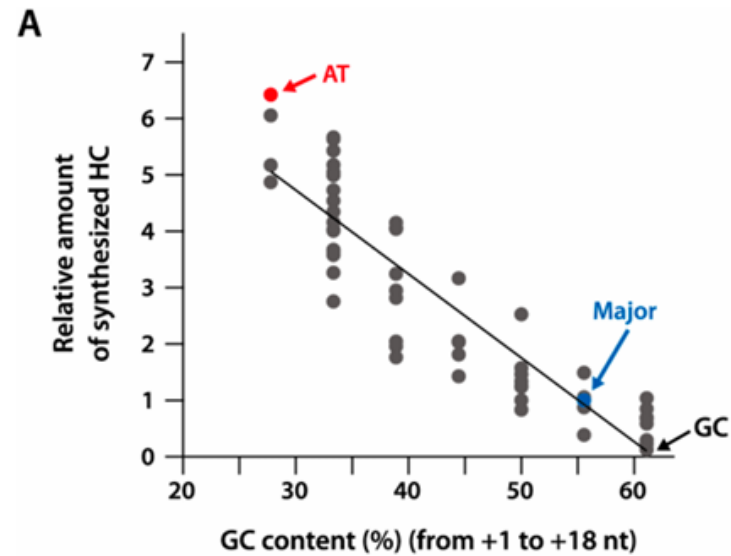
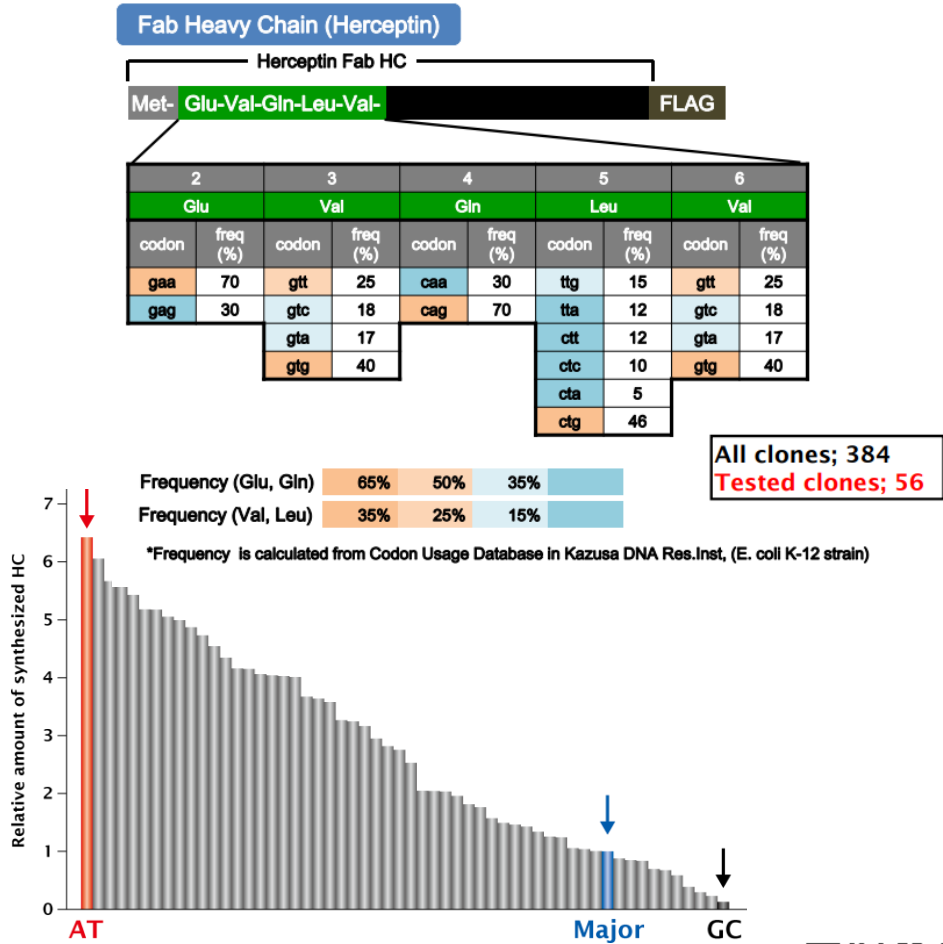
Organism: *Homo sapiens*
Synthesized region: (Hisx6)-(Gly-Ser)-2(10)Asp-140(148)Ala
Length: 148 a.a.
Molecular weight: 15,427 Da

Tag type	1	2	3	4	5	6	7	8	9	GC(%) 1-9 a.a.
GC	atg	ccc	ccc	ccc	ccc	ccc	ccc	ggg	tct	59%
AT	atg	cat	cat	cat	cat	cat	cat	ggt	tct	37%





-KSF; AT rich codon on N-term-



C

	1	2	3	4	5	6	GC (%)	ΔG (kcal/mol)
Name	Met	Glu	Val	Gln	Leu	Val		
AT	atg	gaa	gta	caa	tta	gtt	28	0.0
Major	atg	gaa	gtg	cag	ctg	gtg	56	-4.7
GC	atg	gag	gtg	cag	ctg	gtc	61	-3.9

Design of DNA template is important.
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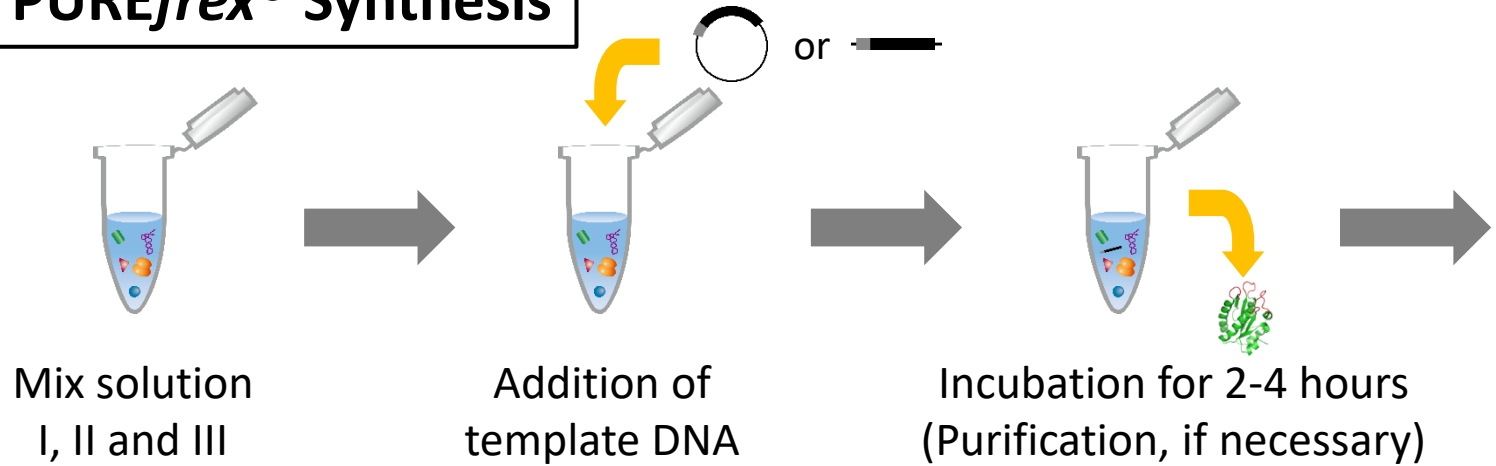




PUREfres[®]

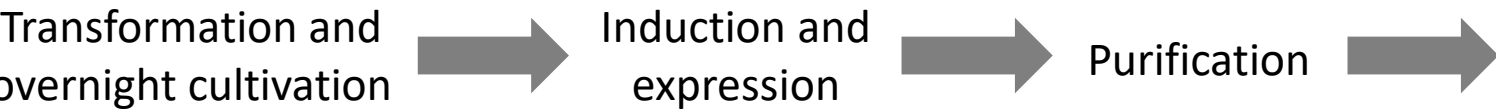
-Improve Expression from Days to Hours-

PUREfres[®] Synthesis

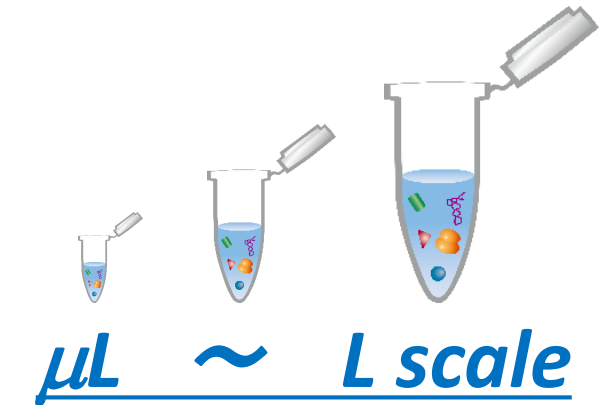
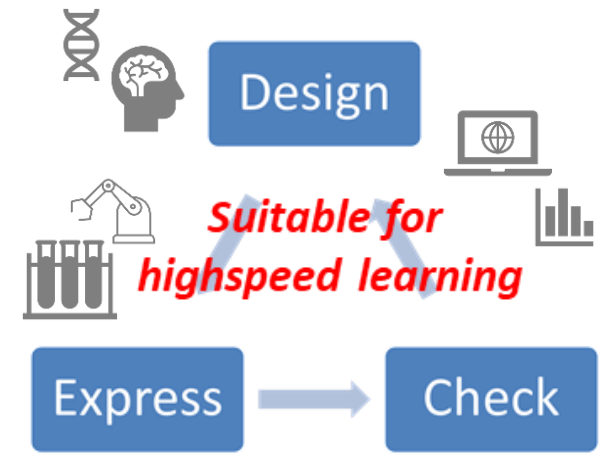


Total: 2-4 hours

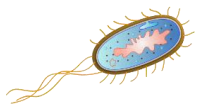
E. coli Expression



Total: 3-4 days

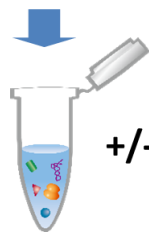
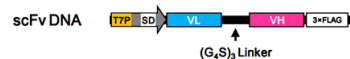
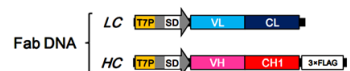


Great Flexibility from basic research to industrial applications



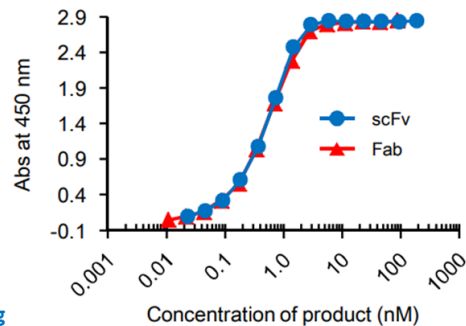


-Expression of scFv, Fab and more-

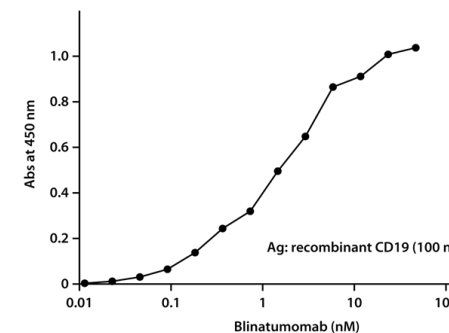
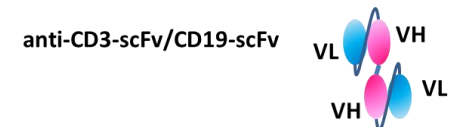


+/- DsbC Set
DnaK Mix
For correct folding

Activity



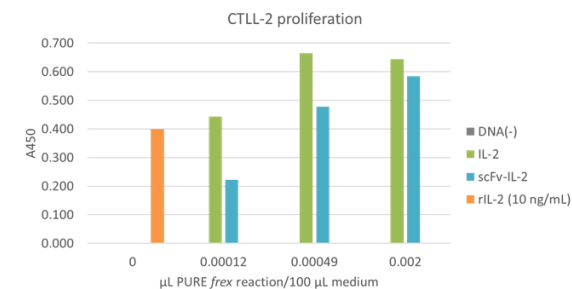
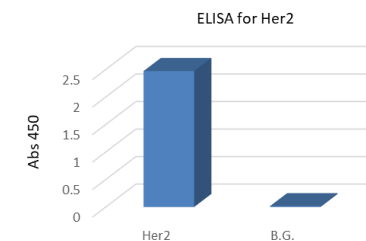
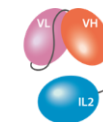
[Murakami et al. \(2019\) Sci. Rep. vol.9, p.671. \(Supplementary Information\)](#)



	1	2	3	4	5	6	7	8	9	10
	Proinsulin Aspart	Proinsulin Lispro	Proinsulin Glargine	Regular Proinsulin	Insulin A Chain	Insulin B Chain	Insulin A Chain Heterodimer	Insulin B Chain Heterodimer	Oxytocin	Glucagon
PURE	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
CIm24	✗	✗	✗	✗	✓	✗	✓	✓	✓	✓
BL21	✗	✗	✗	✗	✓	✗	✓	✓	✓	✗
759	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗
	11	12	13	14	15	16	17	18	19	20
	Glucagon Like Peptide 1 mutant (GLP-1 mut)	Glucagon Like Peptide 1 (GLP-1)	Insulin Like Growth Factor	Growth Hormone (GH)	Leptin	Vaso-pressin	Angiotensin II	Parathyroid Hormone (PTH)	Somato- statin	Leuprolide
PURE	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
CIm24	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓
BL21	✗	✗	✗	✗	✗	✗	✓	✓	✓	✓
759	✓	✓	✗	✓	✓	✓	✓	✗	✓	✓

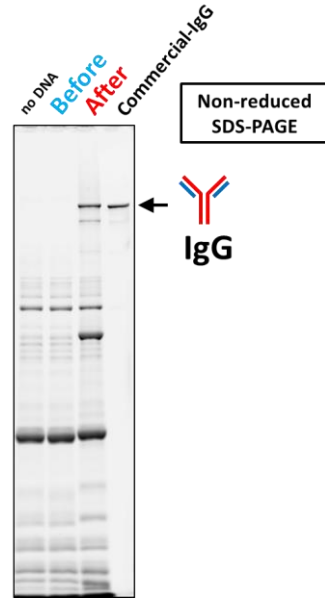
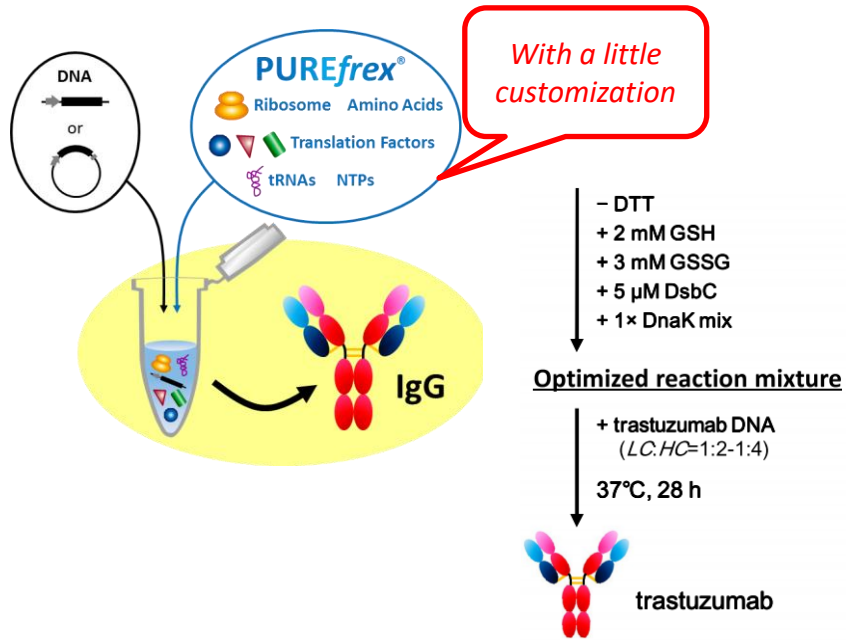
[DeWinter et al. \(2023\) ACS Synth. Biol. vol.12, 4, p1216. \(Supplementary Information\)](#)

scFv-IL-2



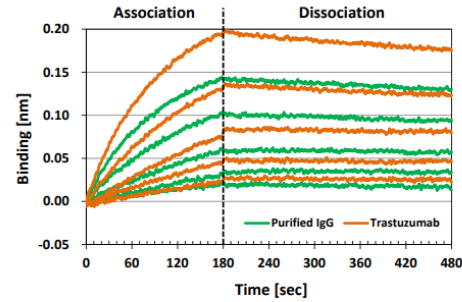


-Application for IgG-



Only 2 days for IgG!!

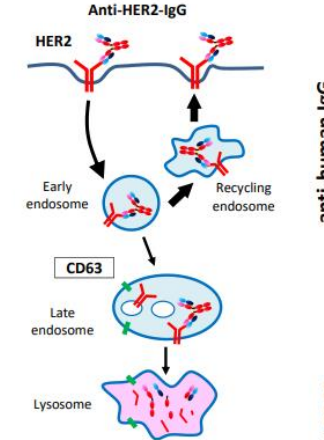
Binding kinetics analysis



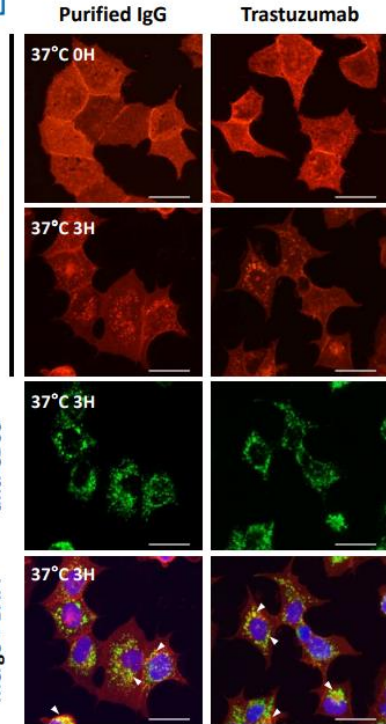
	K_D [M]	k_{on} [1/Ms]	k_{off} [1/s]
Purified IgG	4.24E-10	6.38E+05	2.70E-04
Trastuzumab	4.03E-10	6.29E+05	2.53E-04

System: Octet RED96 System (Pall ForteBio)
 Biosensor: Anti-Human IgG Fc Capture (AHC) biosensor (Pall ForteBio)
 Buffer: Kinetics Buffer 10X (Pall ForteBio)
 Ligand: "Purified IgG" or "Trastuzumab"
 Analyte: 17.6, 8.8, 4.4, 2.2 and 1.1 nM of Extracellular domain of Human ErbB2/HER2 (Sino Biological)
 K_D : Measured affinity of interaction; affinity constant in Molar.
 k_{on} : Association rate constant. k_{off} : Dissociation rate constant.

Internalization analysis



BT-474 cells were surface-labeled at 4°C for 60 min with the binding medium (DMEM, 3% BSA, 20 mM HEPES (pH 7.4)) containing 10 nM of "purified IgG" or "Trastuzumab". Cells were washed five times with the binding medium and incubated at 37°C for 3h. Cells were then fixed and processed for dual-label indirect immunofluorescence microscopy. CD63 (Lamp3) is marker of late endosomes and lysosomes. White arrows indicate partial co-localization of anti-HER2-IgG with CD63. Bar indicates 20 μm.

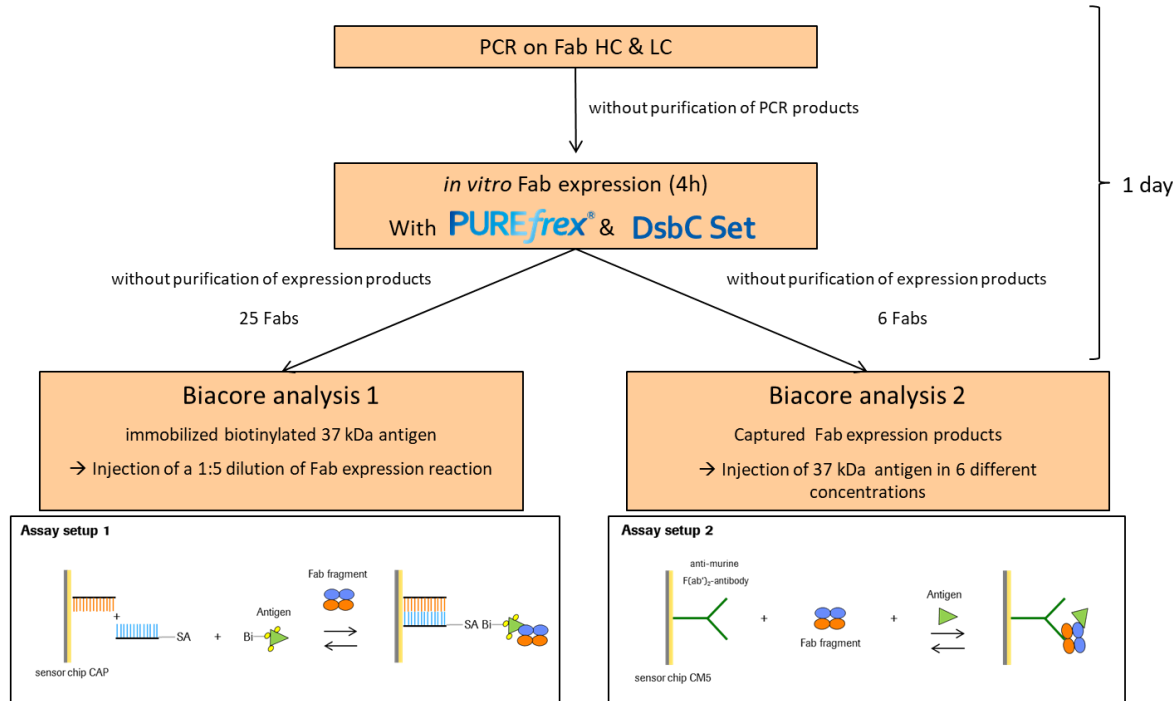


[Murakami et al. \(2019\) Sci. Rep. vol.9, p.671.](#)
[Poster PSSJ 2017](#)

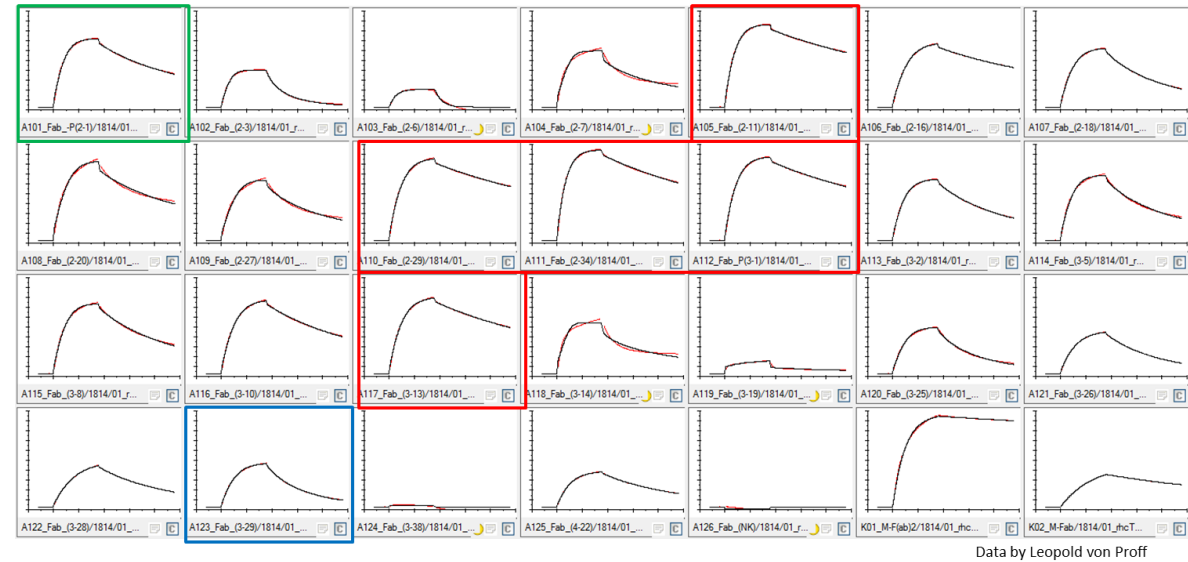
✓ Full size IgG can be synthesized.



In vitro expression and Biacore analysis of Fab fragments



Kinetic analysis of 25 Fab binders

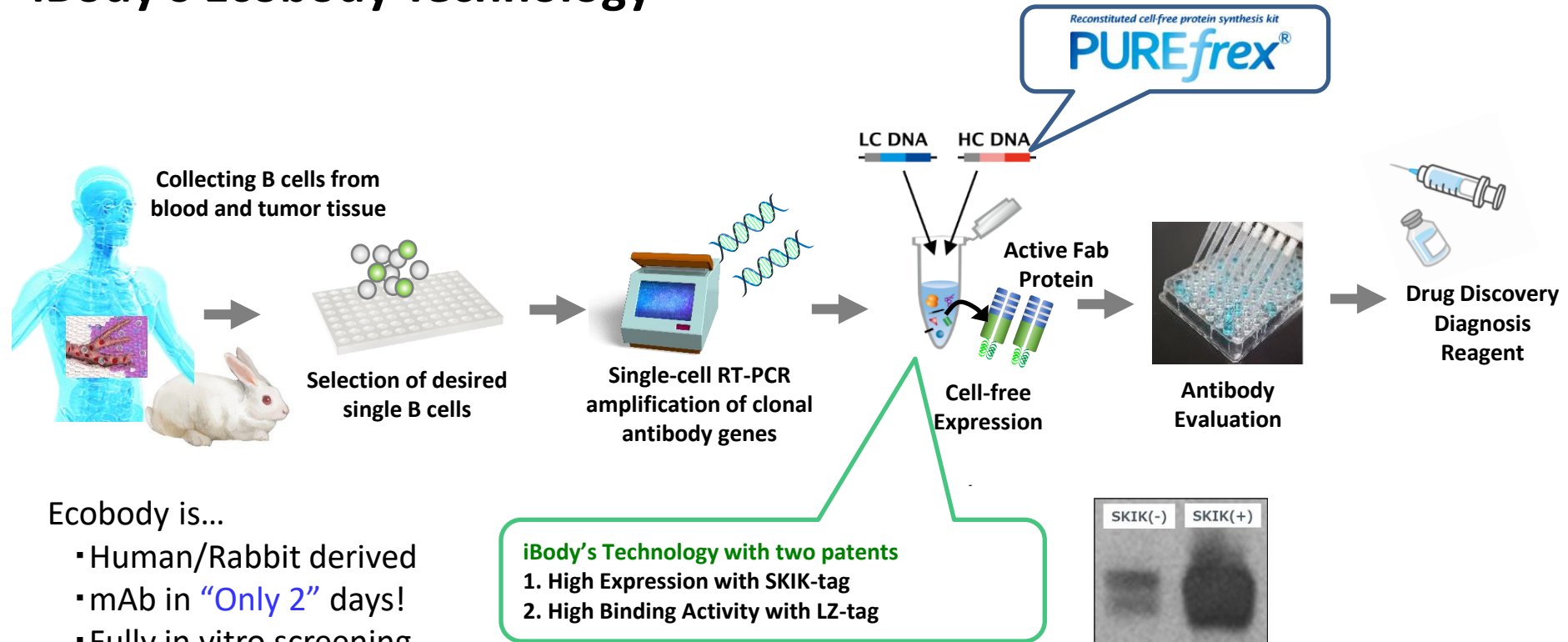


→ Selection of Fabs for further kinetic analysis

✓ Active Fabs are expressed/screened in HT manner.



iBody's Ecobody Technology



Ecobody is...

- Human/Rabbit derived
- mAb in "Only 2" days!
- Fully in vitro screening
- No culture

✓ Active Fab is expressed/screened in HT manner.

[Ojima-Kato et al. \(2017\) Sci. Rep., 7, 13979.](#)

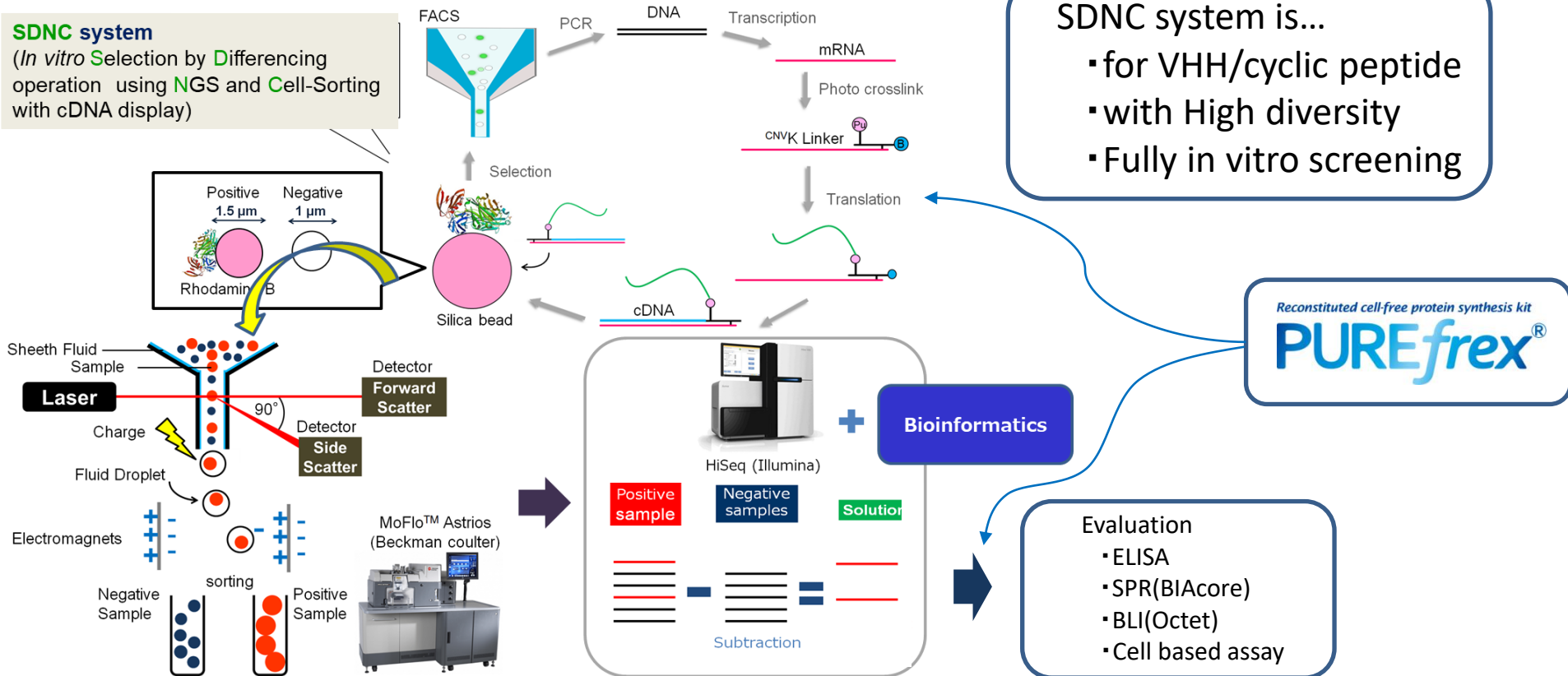
<https://www.ibody.co.jp/en/>





SDNC system

(In vitro Selection by Differencing operation using NGS and Cell-Sorting with cDNA display)



SDNC system is...

- for VHH/cyclic peptide
- with High diversity
- Fully in vitro screening



- Evaluation
- ELISA
 - SPR(BIAcore)
 - BLI(Octet)
 - Cell based assay

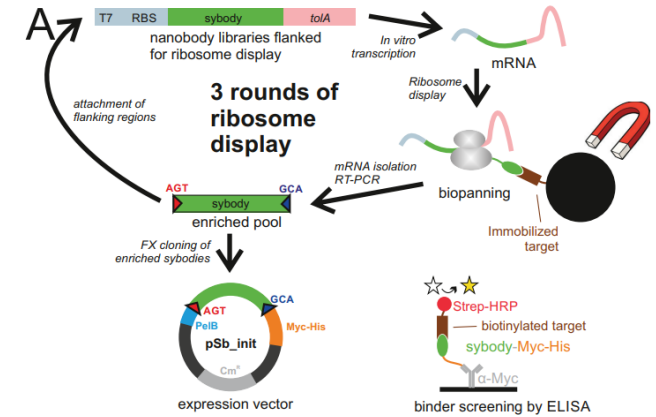
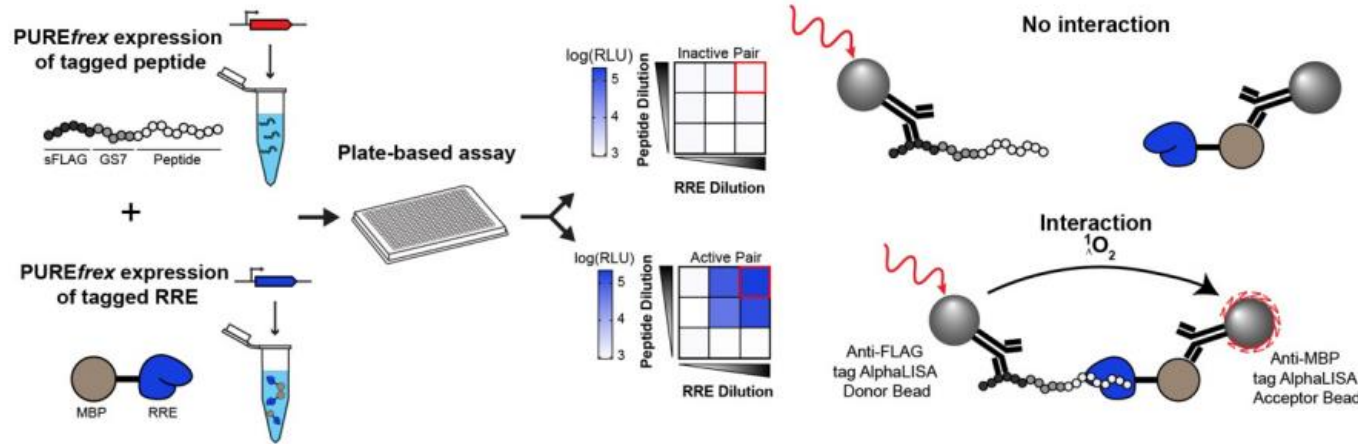
<https://www.epsilon-mol.co.jp/eng/>

✓ PUREfres is applied for cDNA display based screening.





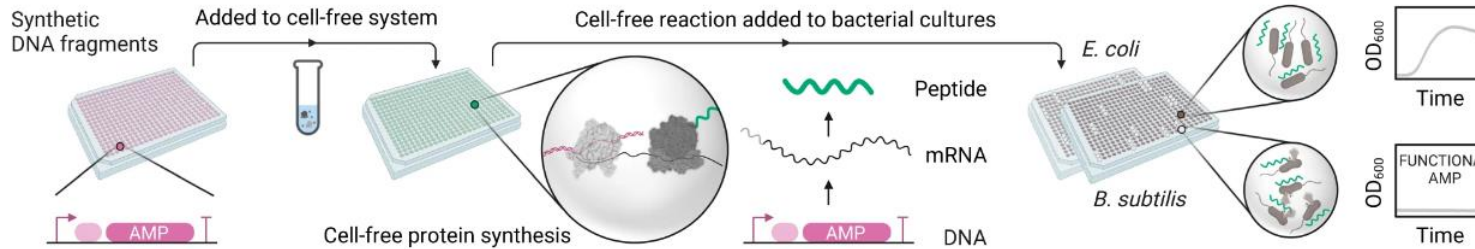
-Broad applications, yet to come!-



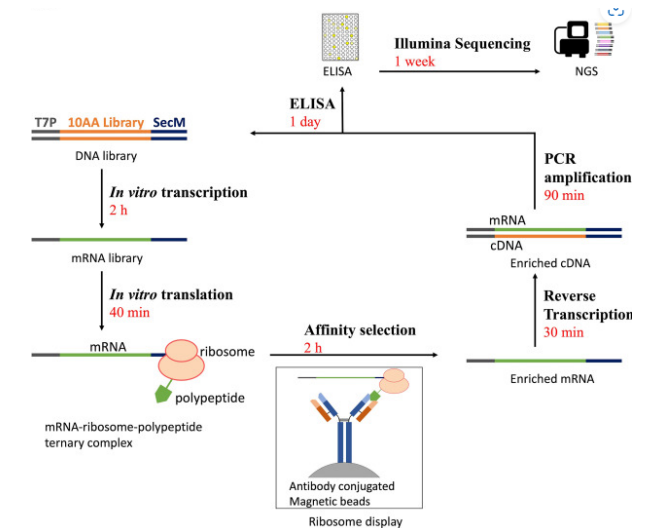
Zimmermann I. et al. (2018) eLife, 7, e34317.

Wong et al. (2024) bioRxiv <https://doi.org/10.1101/2024.03.25.586624>.

WET LAB EXPERIMENT: cell-free production and activity test of AMPs (24 hr)



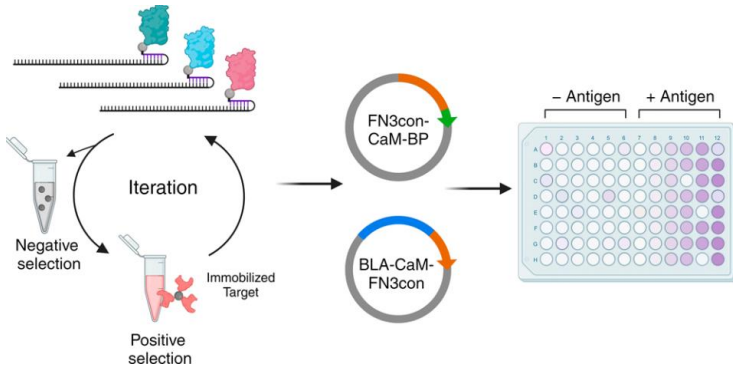
Pandi et al. (2023) Nat Communications. vol.14(7197).



Jia B. et al. (2024) J Biosci Bioeng, 137(4):321-328.



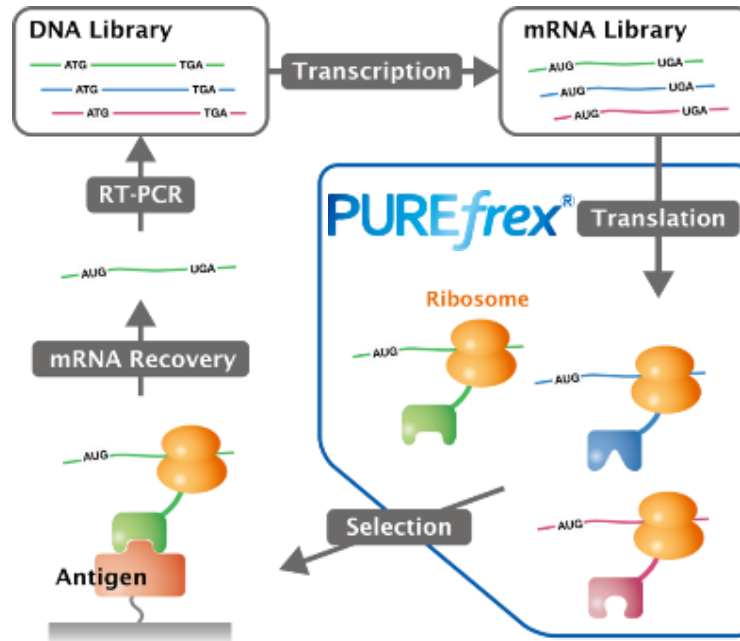
-Broad applications, yet to come!-



[Chui Z. et al. \(2024\) ACS Sens, 9\(6\):2846-2857.](#)

in vitro protein selection technology

PUREfres[®] RD



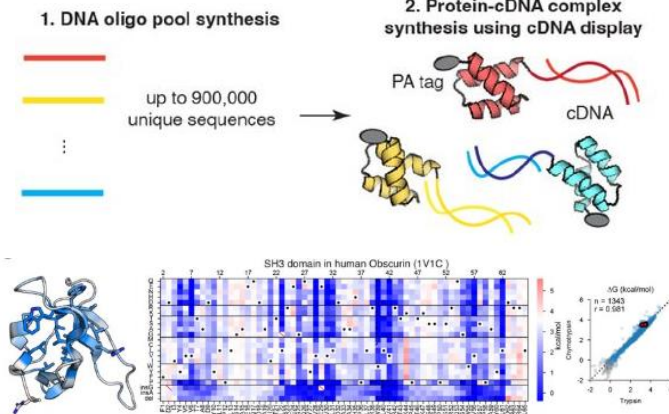
Licensed technology under JP4931135 etc.

◆ Advanced screening system for Biologics

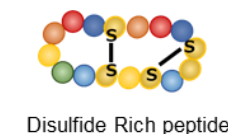
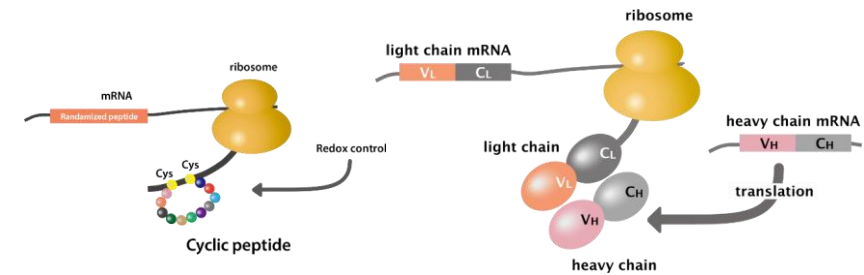
- **mAb (scFv / Fab)**
- **VHH**
- **Cyclic peptide**

◆ High Selection Efficiency

- **Completely molecular based system**
- **>10¹² diversity**



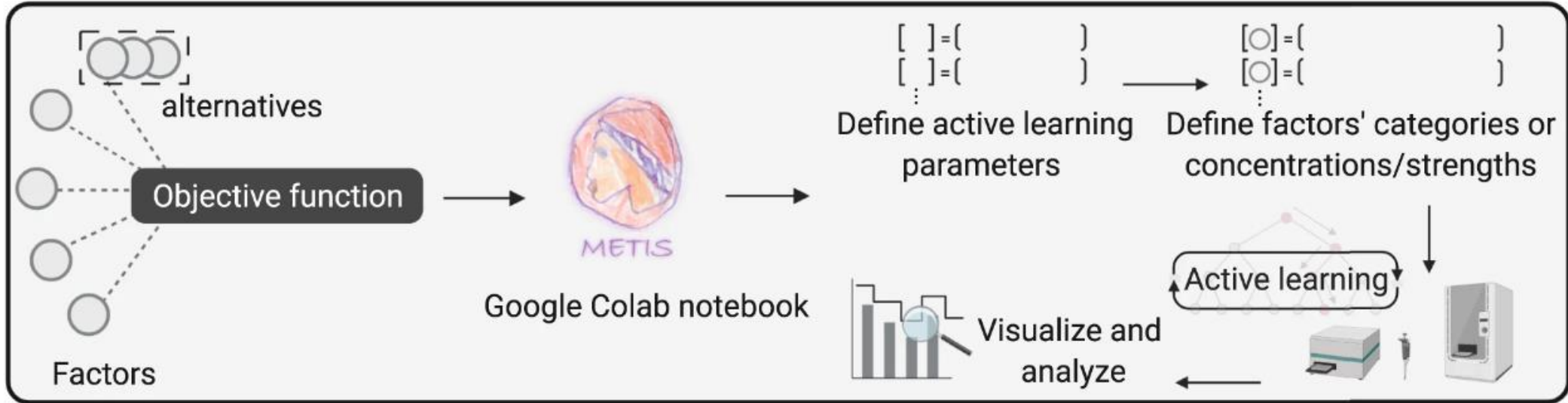
[Tsuboyama et al. \(2023\) Nature, 620, p434.](#)



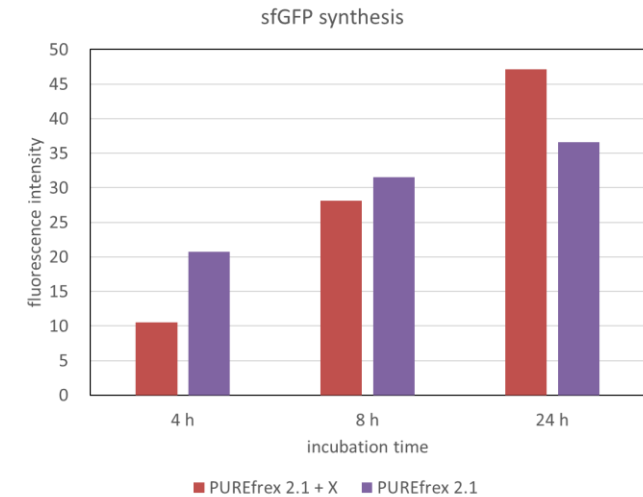
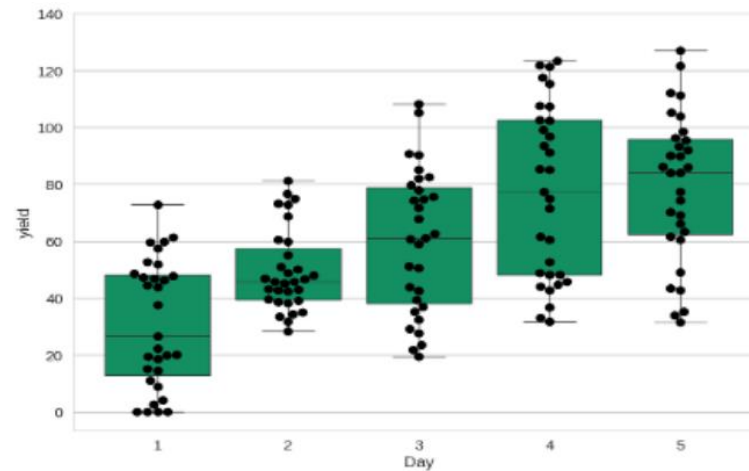
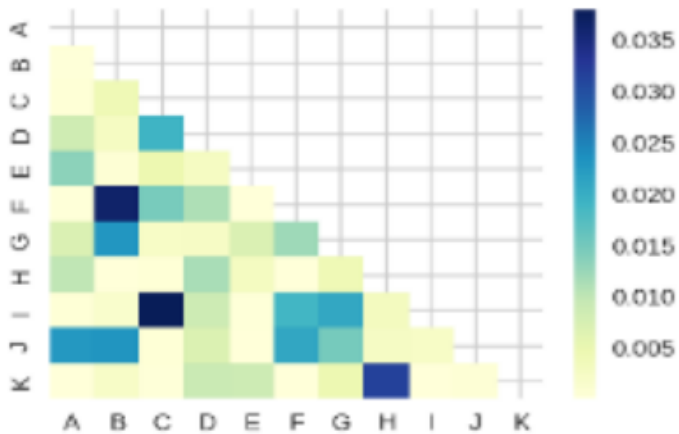
Licensed to
SUTRO
BIOPHARMA



-Broad applications, yet to come!-



[Pandi A et al. \(2022\) Nature Communications, 13, 3876.](#)



Contact information



Reconstituted cell-free protein synthesis kit

PUREfrex[®]

For reagent use for expression / screening of biologics

<https://purefrex.genefrontier.com/>



in vitro protein selection technology

PUREfrex[®]RD

*For screening service / collaboration / technology transfer
for generation of new biologics*

Takashi Ebihara, Ph.D., COO, GeneFrontier

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