

For more information

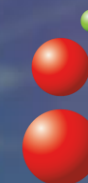


Unleashing the Power of Cell-Free: PUREfrex® for Protein Engineering and Discovery

Reconstituted cell-free protein synthesis kit

PUREfrex®

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



GeneFrontier

NextGenBiomed
12-14 of March, 2025

Corporate Summary



GeneFrontier

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

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

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

Place: ***Chiba, Japan***

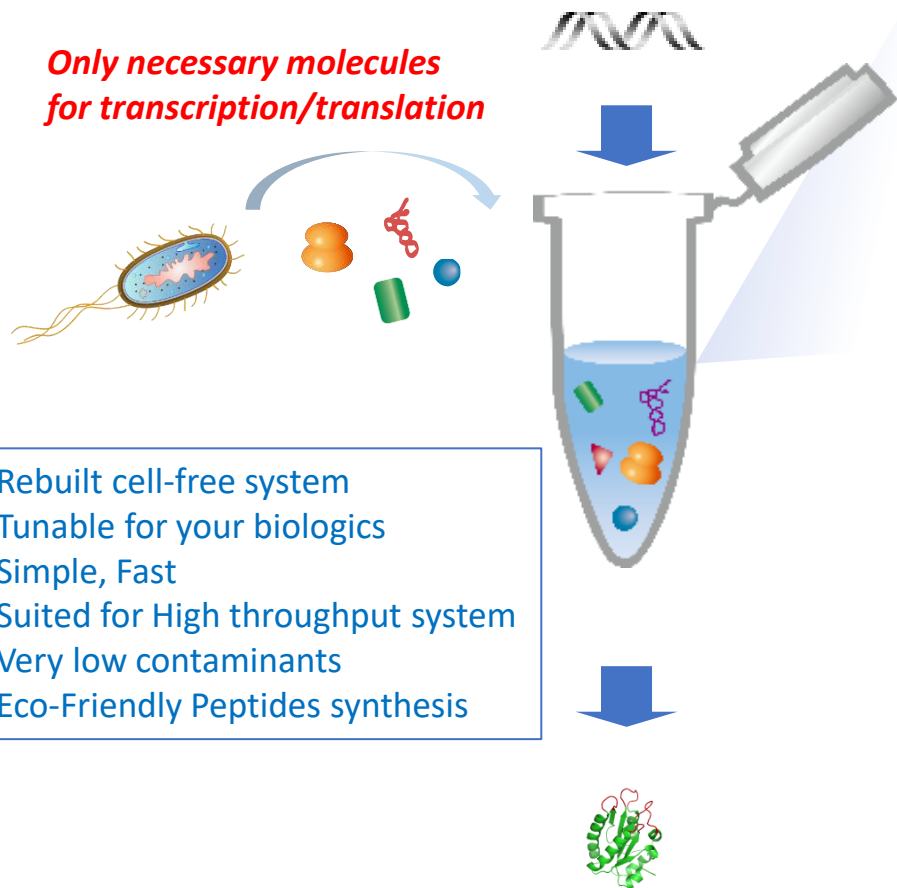


Mission: ***Rebuilding and Manipulating Biological system
for Inspiring the world!***

PUREfres[®]

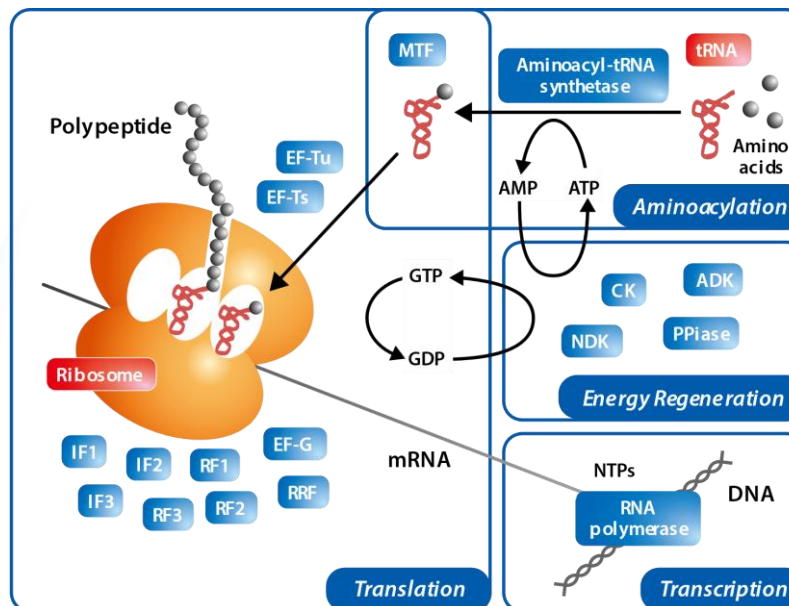
PURE (Protein synthesis Using Recombinant Elements) base cell-free expression

Only necessary molecules for transcription/translation

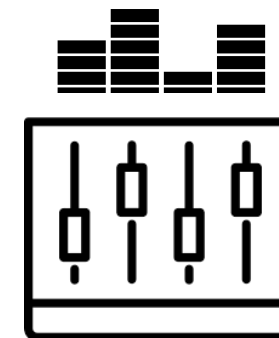
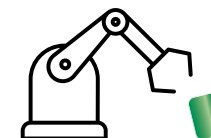
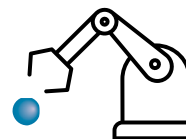


- ✓ Rebuilt cell-free system
- ✓ Tunable for your biologics
- ✓ Simple, Fast
- ✓ Suited for High throughput system
- ✓ Very low contaminants
- ✓ Eco-Friendly Peptides synthesis

Totally constructive, molecular based system



For more information

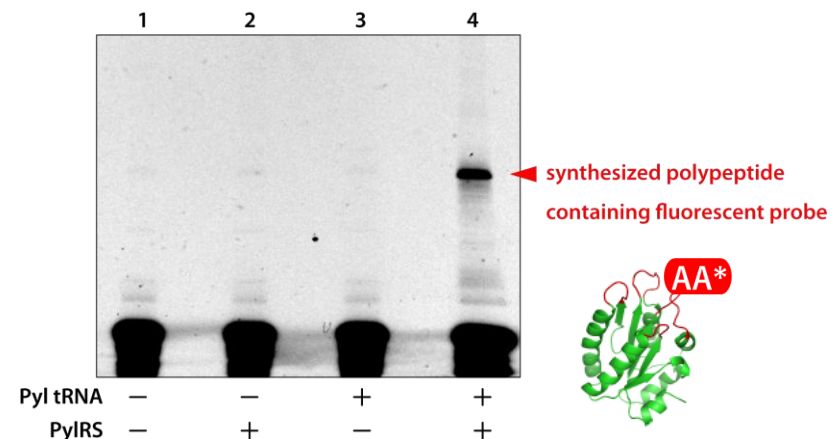
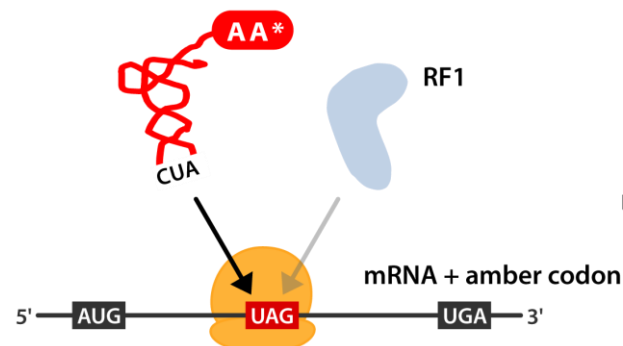


Translation factors
Chaperones
Detergent
Temperature/pH
Redox

<Ex, Non-natural AA introduction>

Translation - RF1

suppressor tRNA + non-natural amino acid

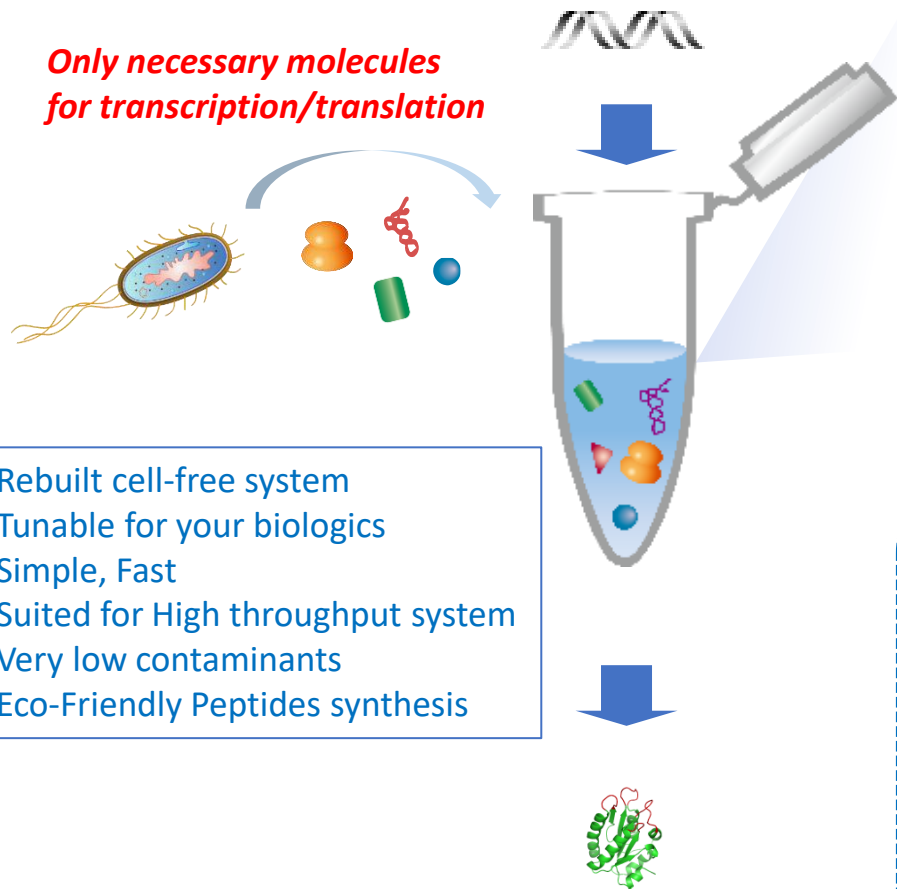


Ready-made kit is coming soon!

PUREfrex®

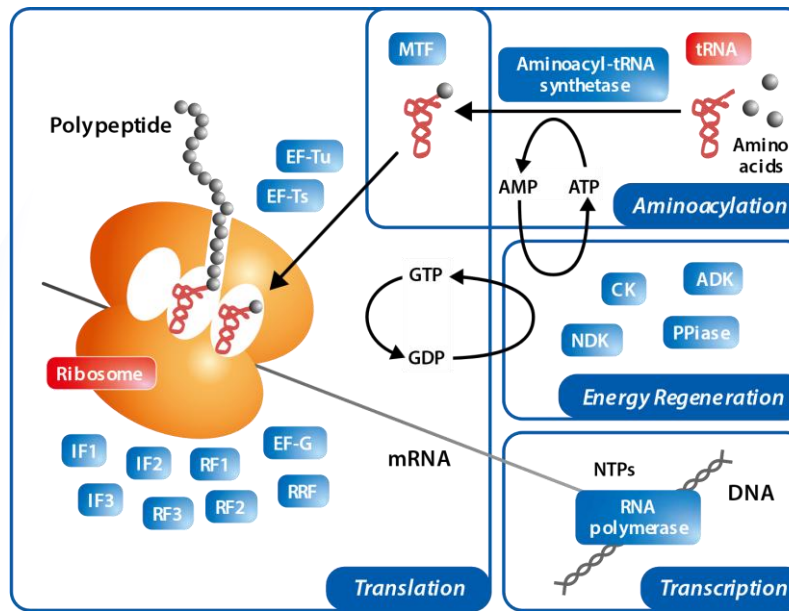
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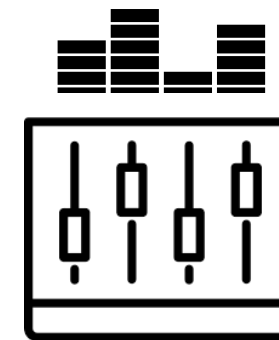
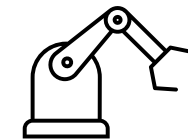
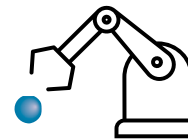


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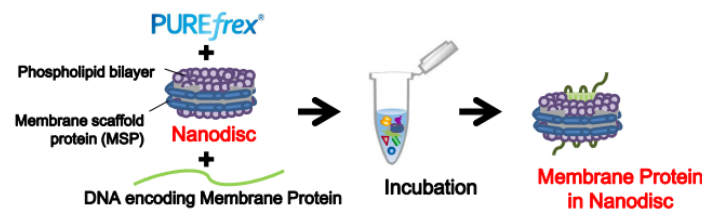


For more information



Translation factors
Chaperones
Detergent
Temperature/pH
Redox

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



Solubilized hCLDN1 was synthesized using PUREfrex® and Nanodisc.

The condition of membrane protein synthesis

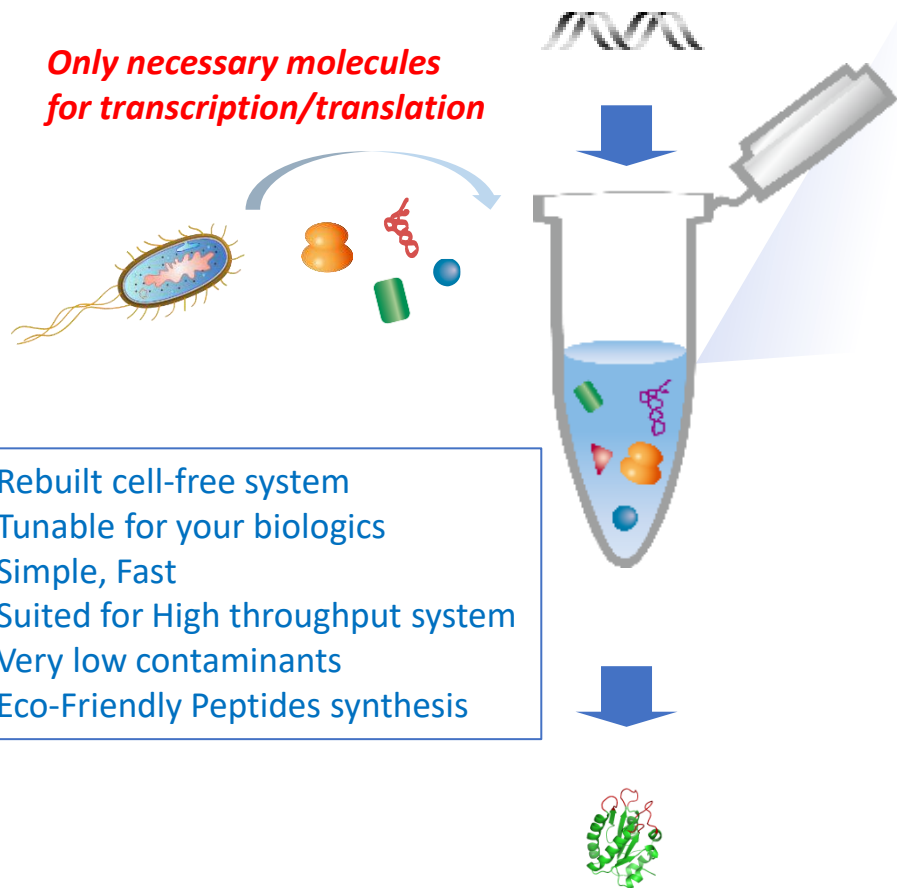
Reaction mix	Template DNA	Incubation
PUREfrex® 2.0 +Nanodisc (MSP1E3D1-His POPC*, final 10 μ M)	PCR product	37°C, 4 h

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

PUREfres[®]

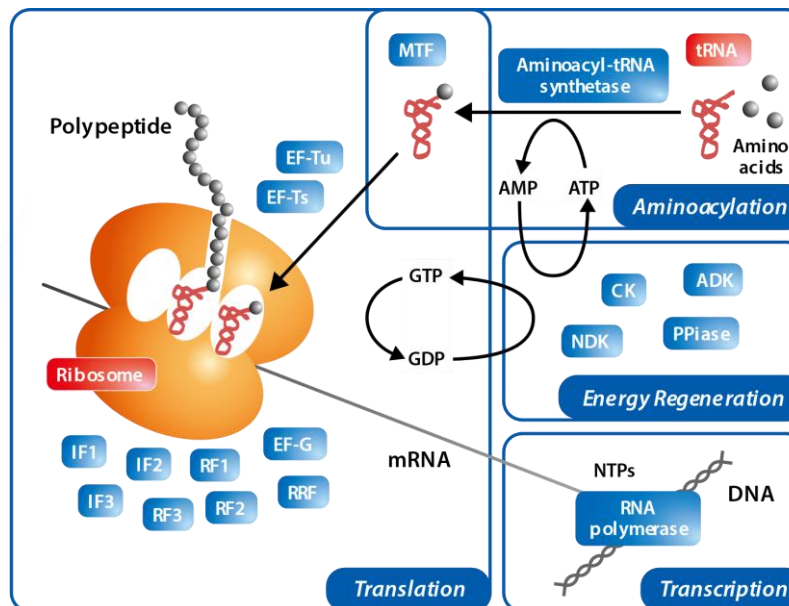
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Only necessary molecules for transcription/translation

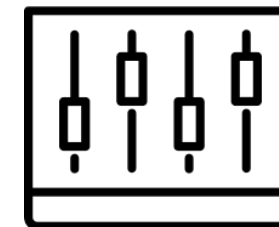
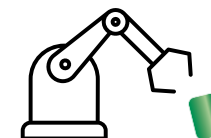
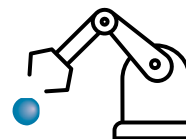


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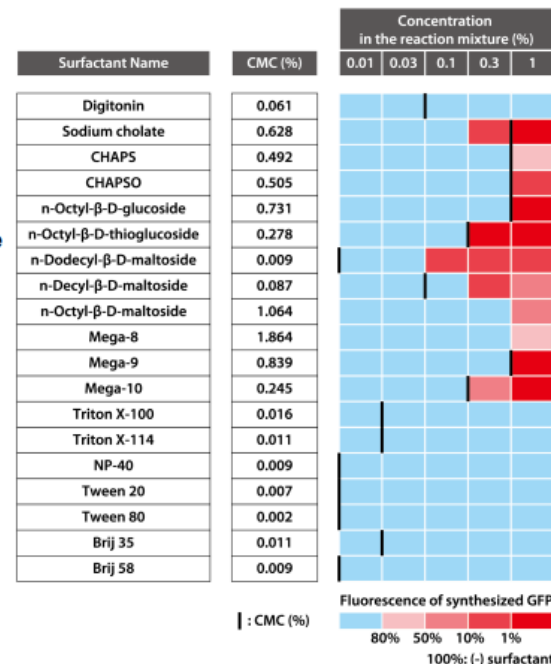
Translation factors
Chaperones
Detergent
Temperature/pH
Redox

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

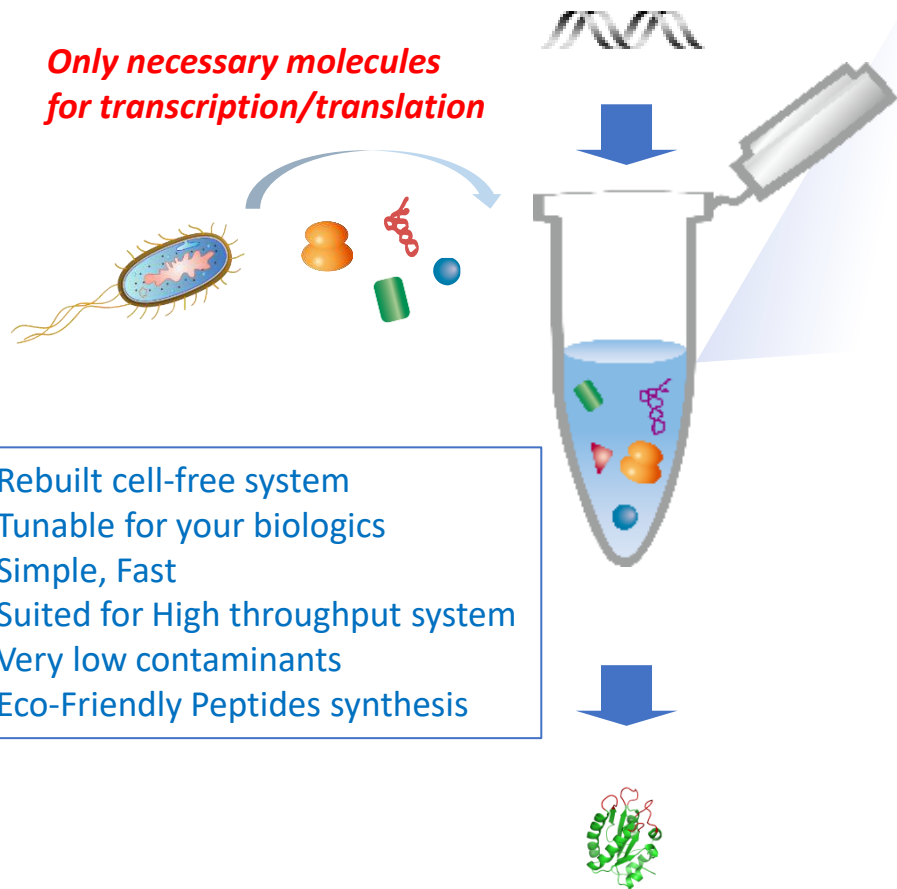
- Most surfactants did not inhibit the protein synthesis reaction by PUREfres[®] below the CMC.
- Some surfactants such as Triton X-100 and Tween 20 could be used even above the CMC.



PUREfres[®]

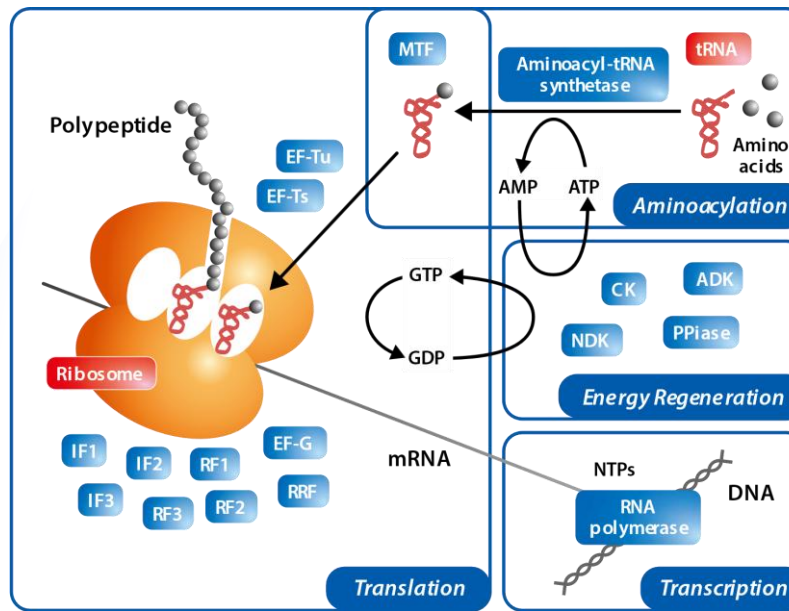
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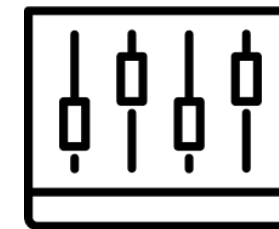
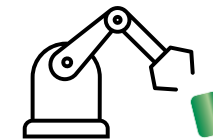
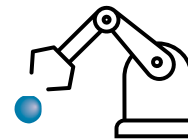


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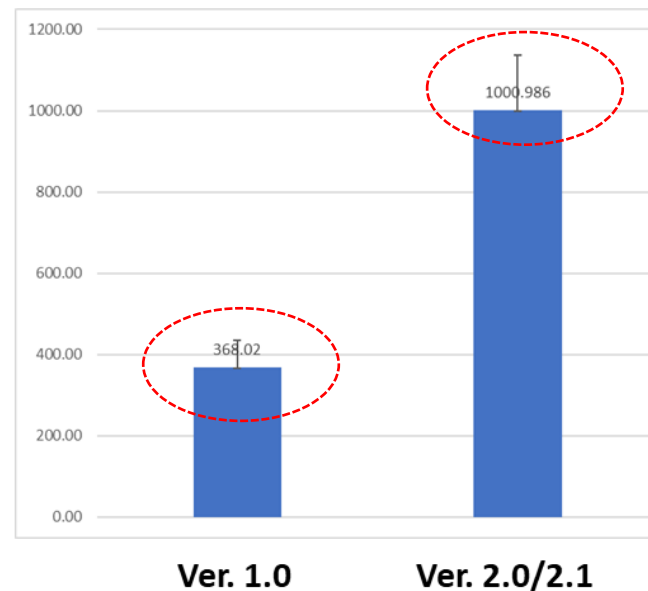


For more information



Translation factors
Chaperones
Detergent
Temperature/pH
Redox

Synthesized
DHFR mg/mL



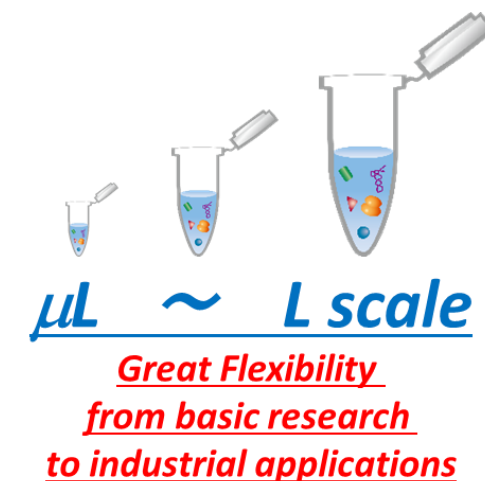
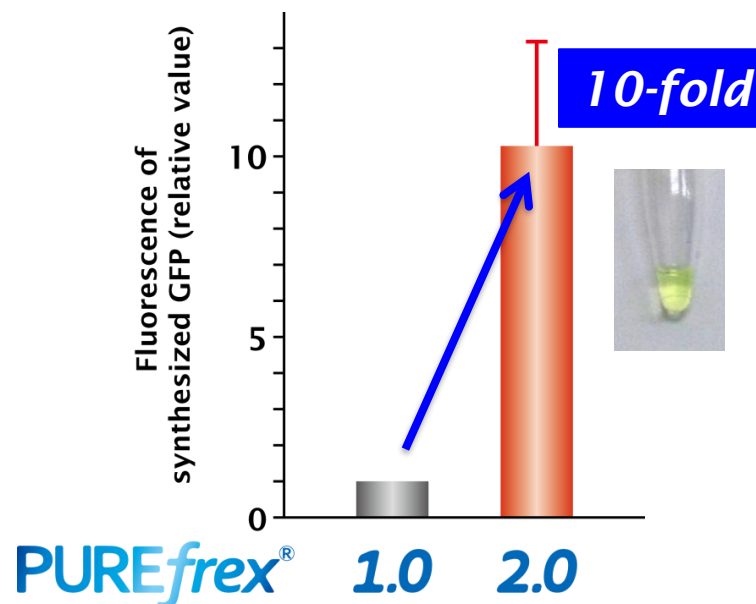
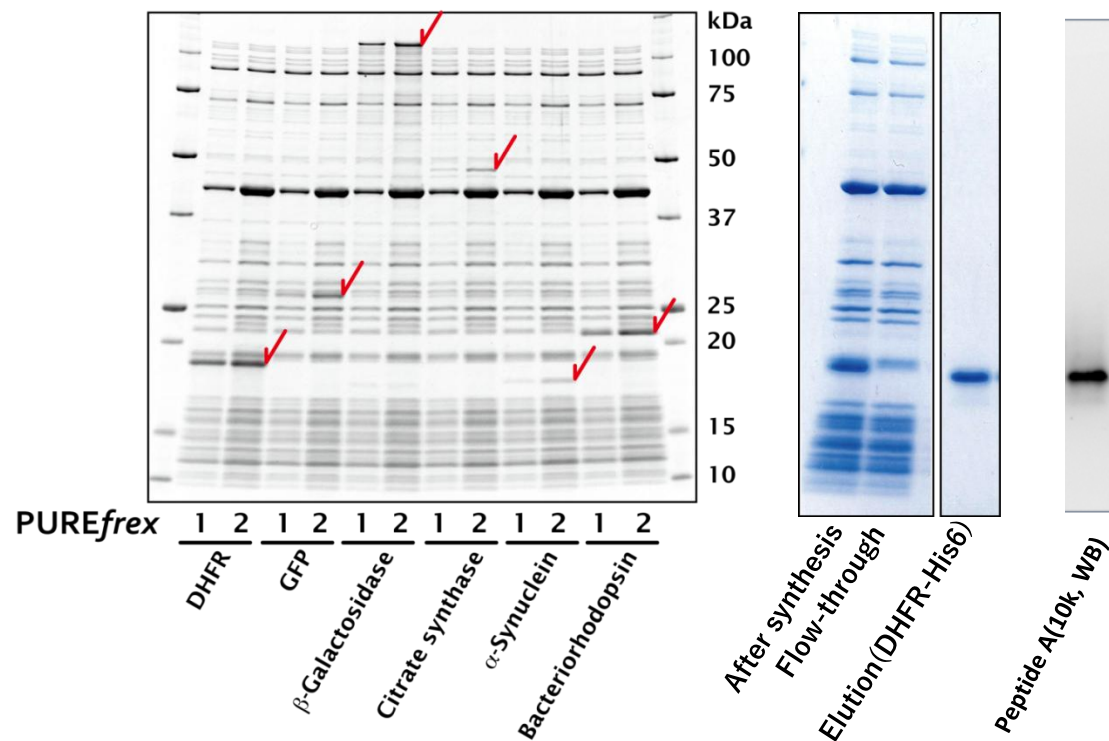
Versatile and Robust
Platform for protein synthesis

Huge potential as
New platform in Biotech industry





-Having good productivity-



- Reaction at 37°C for 4 h
- 0.5 μ L 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

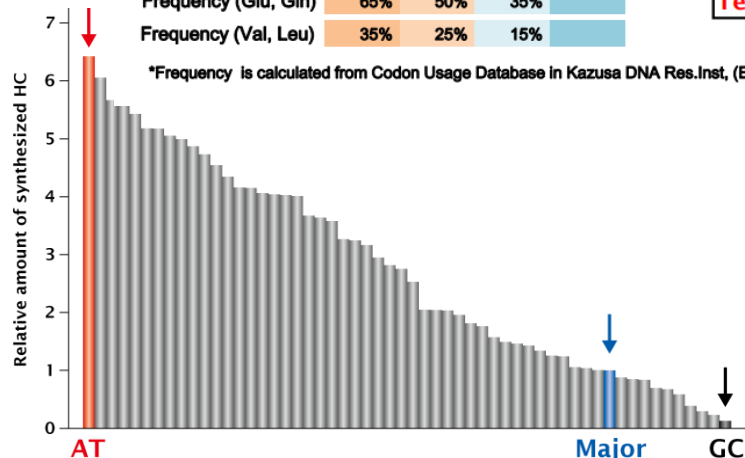
Met- **Glu-Val-Gln-Leu-Val-** FLAG

2		3		4		5		6	
Glu		Val		Gln		Leu		Val	
codon	freq (%)	codon	freq (%)	codon	freq (%)	codon	freq (%)	codon	freq (%)
gaa	70	gtt	25	caa	30	tgt	15	gtt	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.

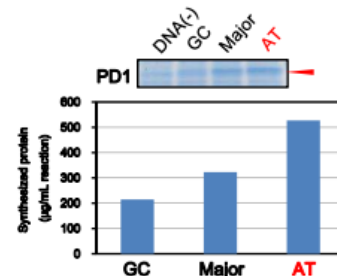
Manual is Free to download from our Web site here.



PD1

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

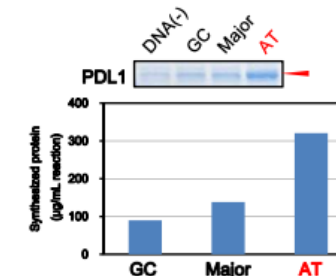
N-term type	1	2(38)	3(37)	4(38)	5(39)	6(40)	GC(%)
Met	Met	Thr	Phe	Ser	Pro	Ala	1-6 a.a.
GC	atg	acc	ttc	tcc	cgc	gcg	67%
major	atg	acc	ttt	tct	cgc	gcg	56%
AT	atg	act	ttt	tca	cgc	gct	39%



PDL1

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

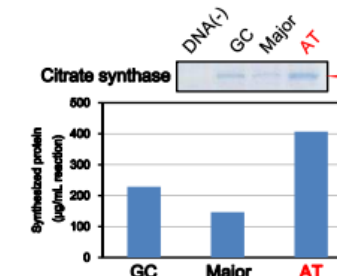
N-term type	1	2(18)	3(19)	4(20)	5(21)	6(22)	GC(%)
Met	Met	Ala	Phe	Thr	Val	Thr	1-6 a.a.
GC	atg	gcg	tcc	acc	gtg	acc	61%
major	atg	gcg	ttt	acc	gtg	acc	56%
AT	atg	gct	ttt	act	gta	aca	33%



Citrate Synthase

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

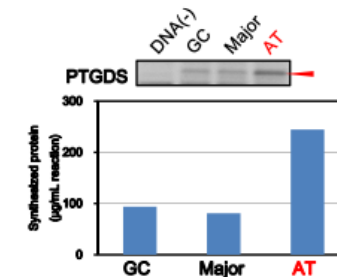
N-term type	1	2(38)	3(39)	4(40)	5(41)	6(42)	GC(%)
Met	Met	Ser	Ser	Ala	Ser	Glu	1-6 a.a.
GC	atg	tcc	tcc	gcg	tcc	gag	67%
major	atg	tct	tct	gcg	tct	gaa	44%
AT	atg	tca	tca	gct	tca	gaa	39%



PTGDS

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

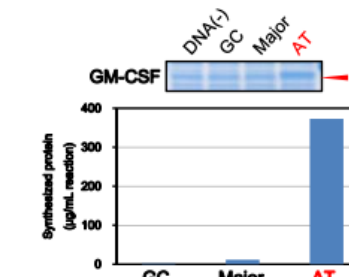
N-term type	1	2(23)	3(24)	4(25)	5(26)	6(27)	GC(%)
Met	Met	Ala	Pro	Glu	Ala	Gln	1-6 a.a.
GC	atg	gca	cgc	gaa	gca	cag	61%
major	atg	gcg	cgc	gaa	gcg	cag	72%
AT	atg	gca	cct	gaa	gct	caa	50%



GM-CSF

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

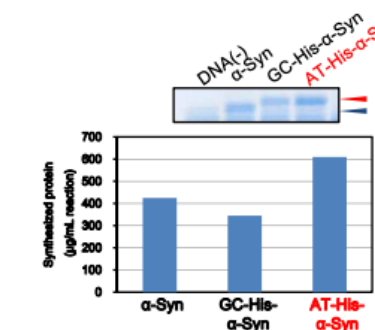
N-term type	1	2(18)	3(19)	4(20)	5(21)	6(22)	GC(%)
Met	Met	Ala	Pro	Ala	Arg	Ser	1-6 a.a.
GC	atg	gcg	cgc	cgc	cgc	tcc	83%
major	atg	gcg	cgc	cgc	cgc	tct	78%
AT	atg	gca	cct	gct	aga	tca	50%



His-α-Synuclein

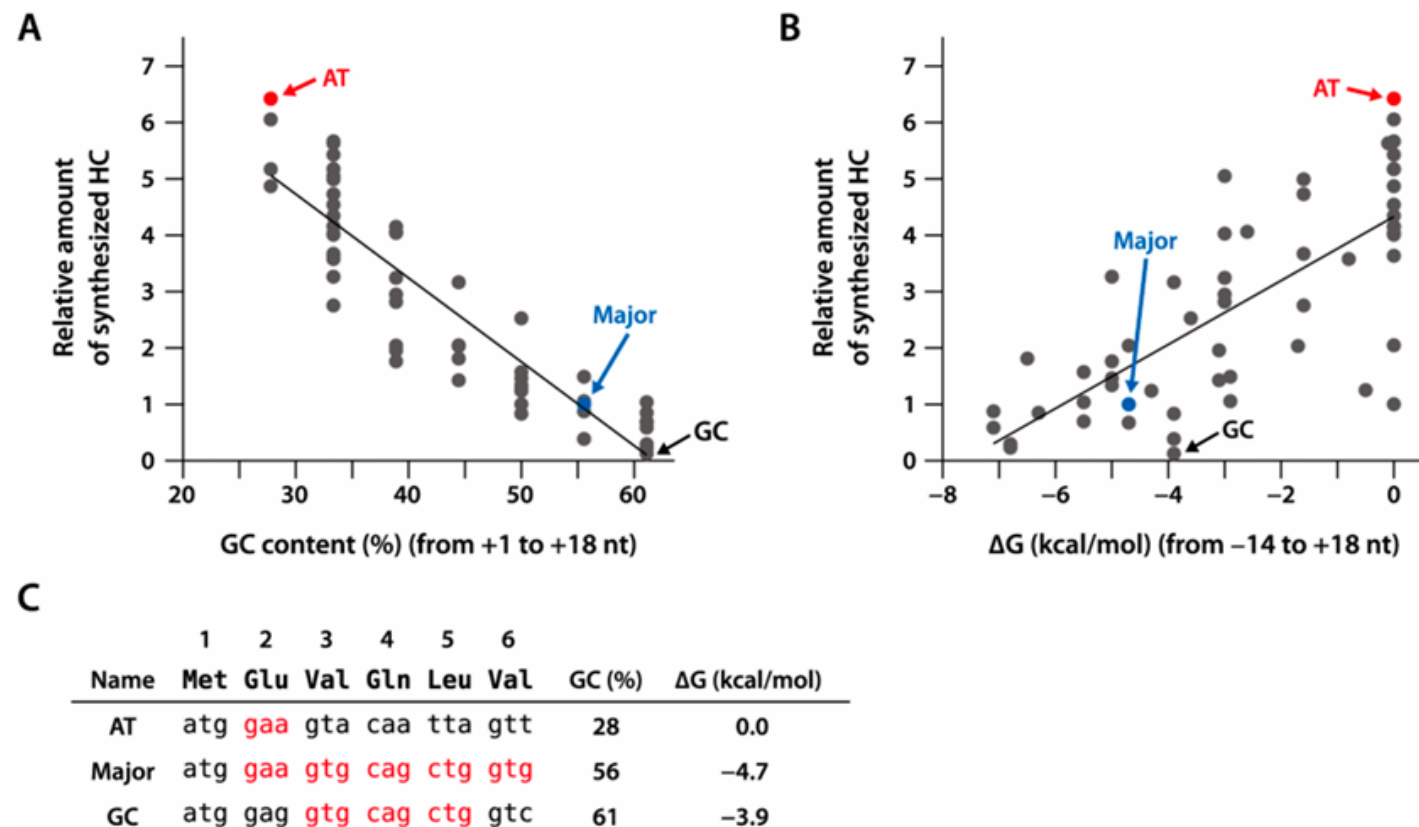
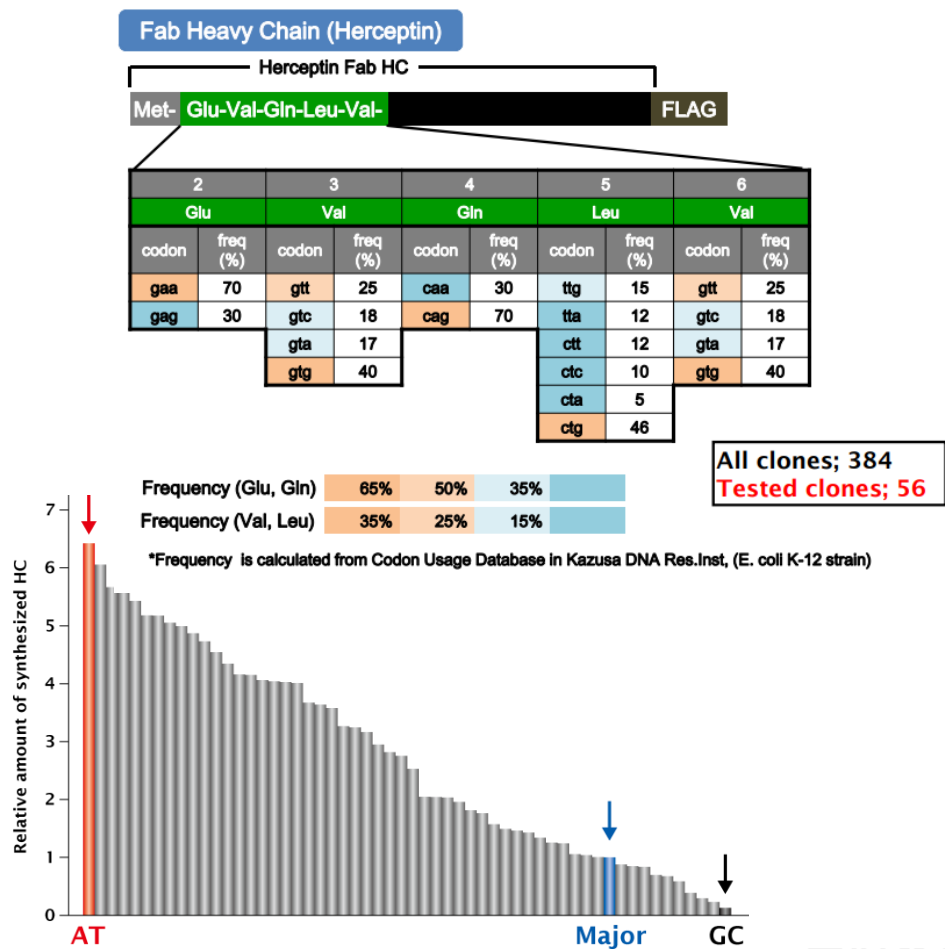
Organism: *Homo sapiens*
Synthesized region: (Hisx6)-(Gly-Ser)-2(10)Asp-140(148)Ala
Length: 148 a.a.
Molecular weight: 15,427 Da

Tag	1	2	3	4	5	6	7	8	9	GC(%)
Met	Met	His	His	His	His	His	His	Gly	Ser	1-9 a.a.
GC	atg	cac	cac	cac	cac	cac	cac	ggt	tct	59%
AT	atg	cat	cat	cat	cat	cat	cat	ggt	tct	37%





-KSF; AT rich codon on N-term-



Murakami et al. (2024) Int. J. Mol. Sci. 2024, 25(10), 5264

Design of DNA template is important.

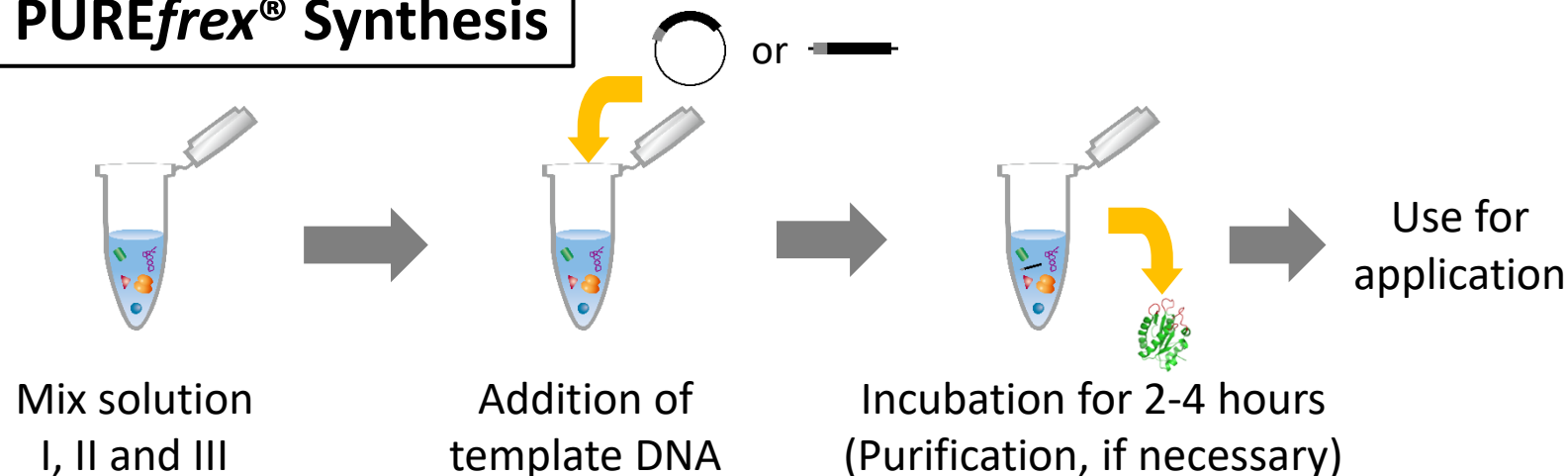
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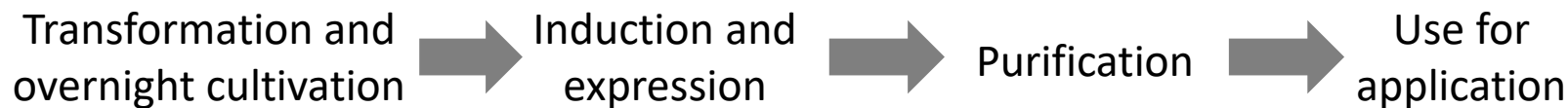
-Improve Expression from Days to Hours-

PUREfres[®] Synthesis



Total: 2-4 hours

E. coli Expression



Total: 3-4 days



MHHHHH—ENLYFQG—Protein of Interest

Synthesized DNA (PCR-amplified DNA)

↓ Protein synthesis using PUREfres[®]

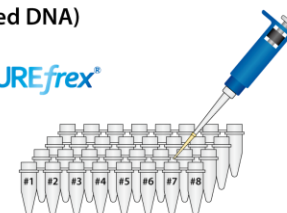
Synthesized Protein

↓ + Ni-resin

↓ + His-tagged TEV protease

Purified Protein (in Flow-through fraction)

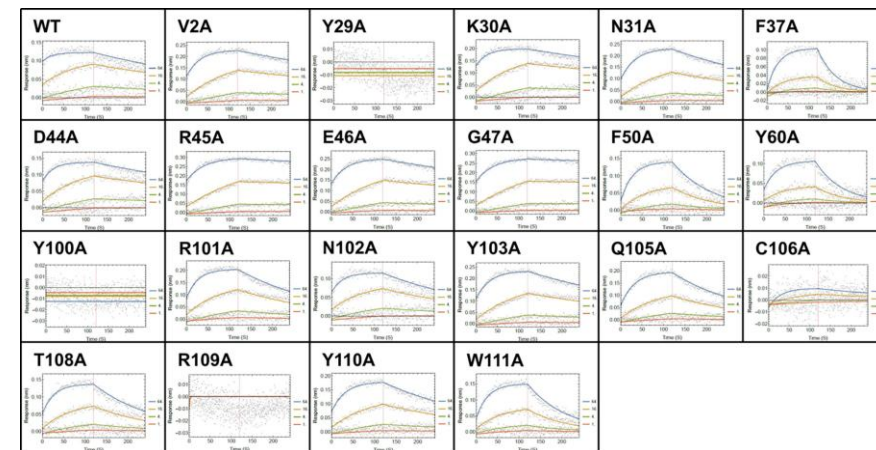
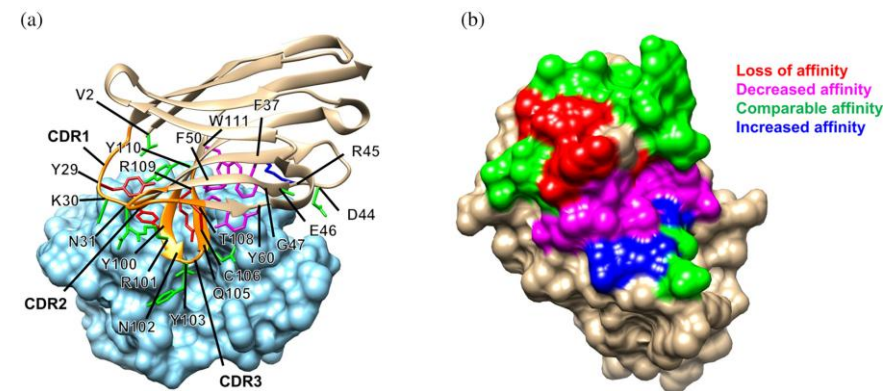
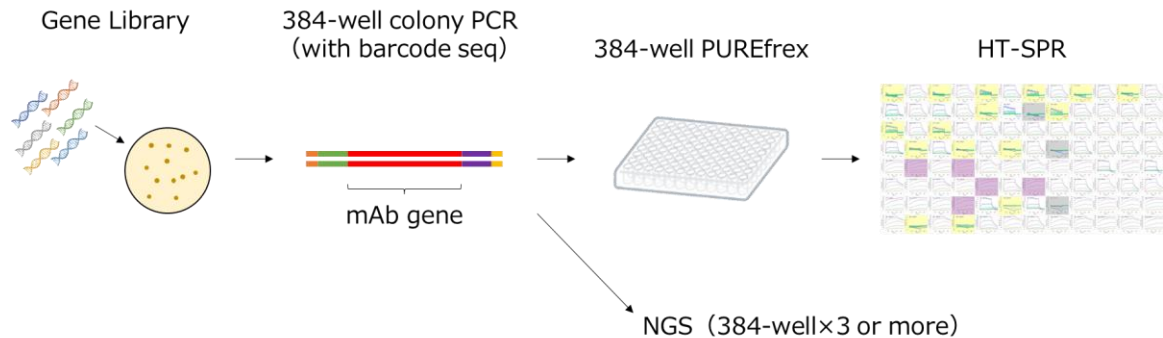
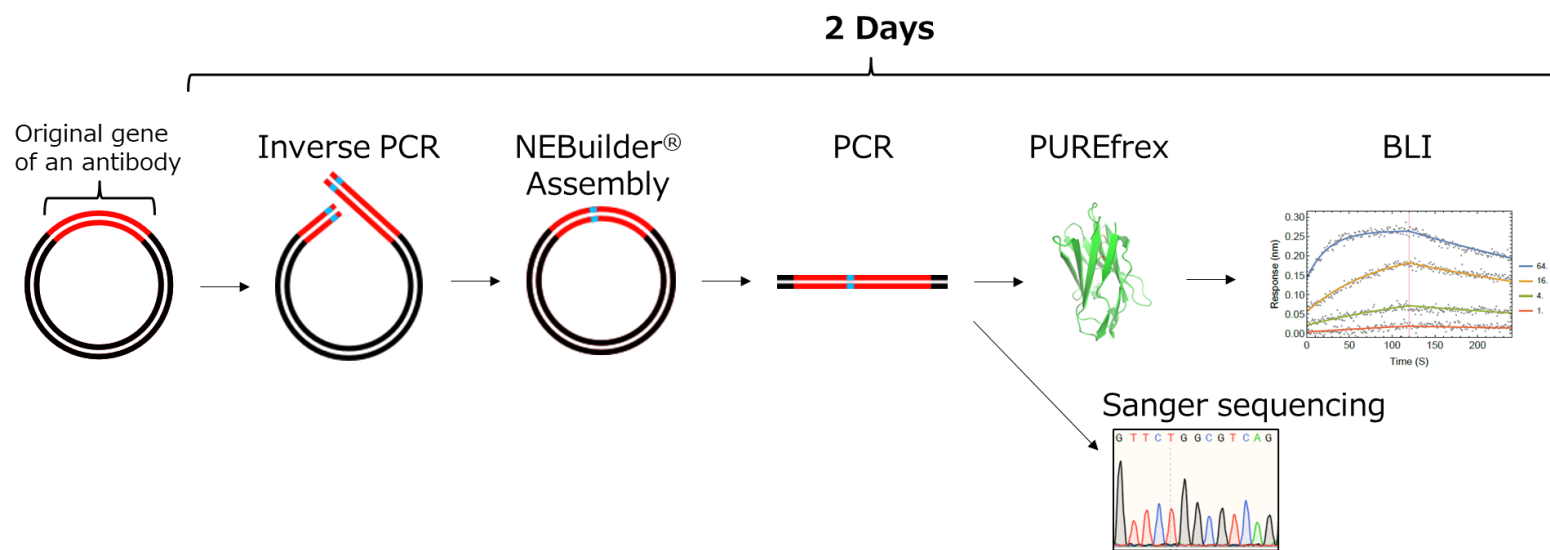
↓ Assay





-Improve Validation from Weeks to Days-

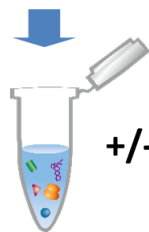
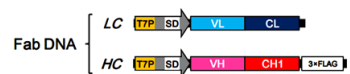
FASTIA: Fast Interaction Analysis



Matsunaga et al. (2025) Protein Sci. Mar;34(3):e70065. doi: 10.1002/pro.70065.

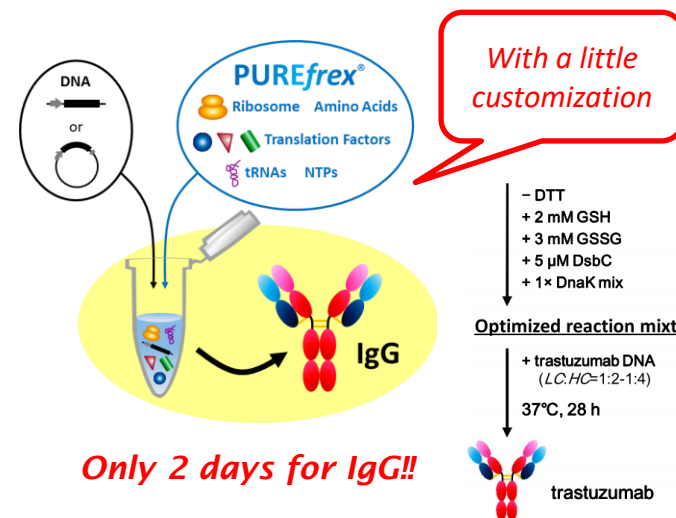
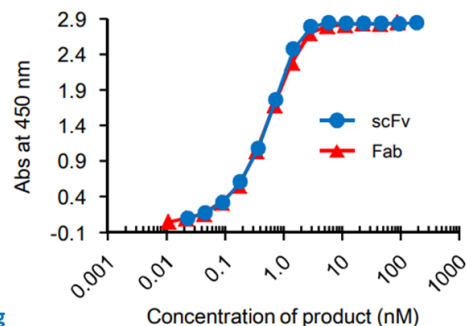


-Expression of scFv, Fab, IgG and more-

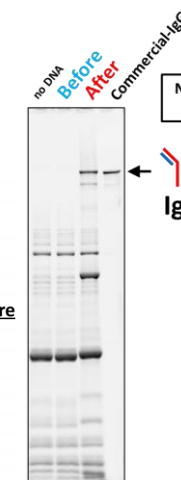


+/- DsbC Set
DnaK Mix
For correct folding

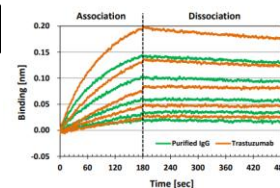
Activity



Only 2 days for IgG!!



Binding kinetics analysis

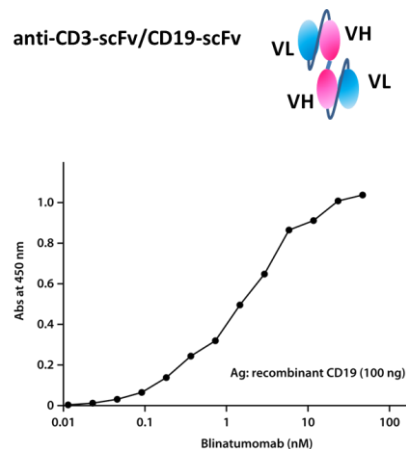


	K_D [M]	k_{on} [1/Ms]	k_{off} [1/s]
Purified IgG	4.24E-10	6.38E+05	2.70E-04
Trastuzumab	4.03E-10	6.29E+05	2.53E-04

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; k_{on} : Association rate constant; k_{off} : Dissociation rate constant.

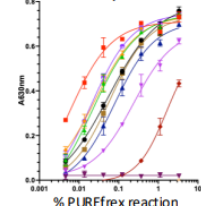
[Murakami et al. \(2019\) Sci. Rep. vol.9, p.671. \(Supplementary Information\)](#)

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

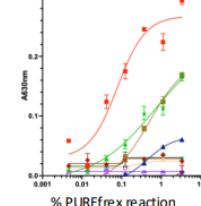


Round 1: IFN-α variants tested by in vitro transcription/translation

HEK293 Human IFNα-Reporter Cells

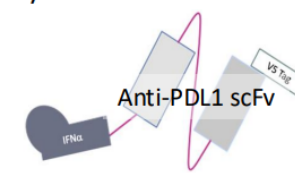


Mouse B16 Reporter Cells



- Universal IFN-α
- hIFNα_L152F
- R121K
- R121K_Q125R
- R121K_Q125R_K132T
- Y86C_R121K_Q125R
- Y86C_R121K_Q125R_K132T
- mIFN2
- IFNα2b
- scFv control
- Human IFNα2b (starts at 1nM)
- No DNA

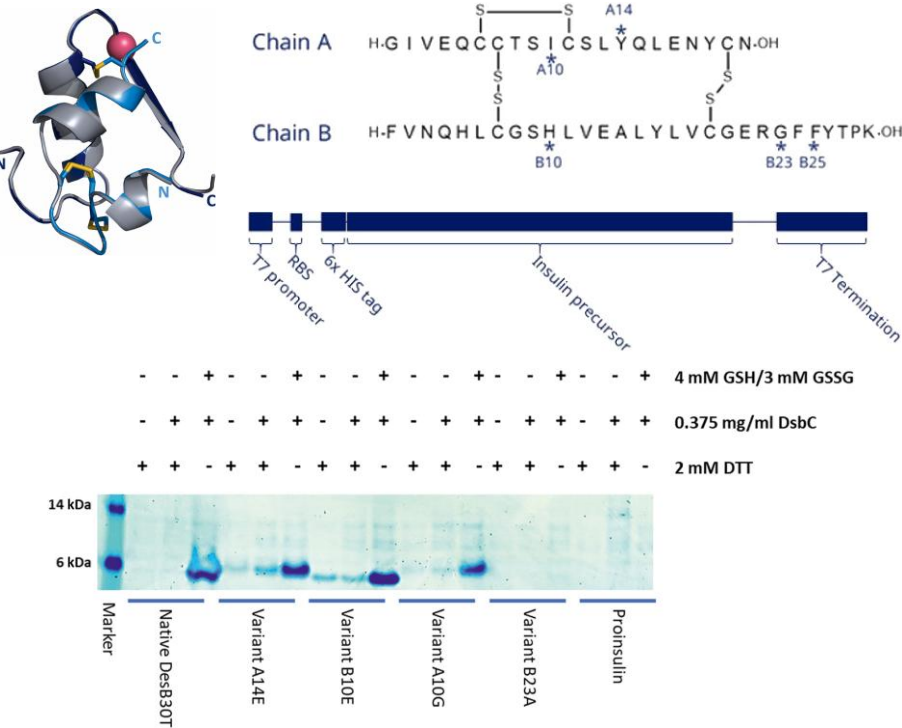
IFN-α variants were generated as IFNα-scFv fusions to identify mutations affecting activity in mouse and human cells



IFNα-scFv protein was generated with an anti-PDL1 antibody by in vitro transcription/translation using the PUREfres[®] system (CosmoBIO USA). Protein mixture was serially diluted and protein was captured via an anti-V5 tag coated to the wells of the plate. Proteins were assayed for PD-L1 binding to assess expression (data not shown) or the ability to stimulate an IFN-α response in receptor reporter cell lines

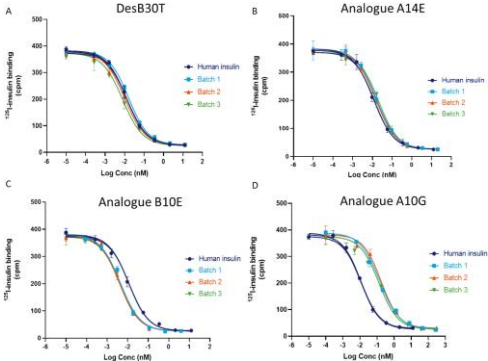
[Killebrew et al. \(2024\) SITC 2024 Annual Meeting \(Poster, BONUMTX.com\).](#)

-Application for complex molecule-



	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	X	X	X	X	✓	X	✓	✓	✓	✓
BL21	X	X	X	X	✓	X	✓	✓	✓	X
759	✓	✓	✓	✓	✓	✓	✓	✓	✓	X
	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	✓	✓	X	✓	✓	✓	✓	✓	✓	✓
BL21	X	X	X	X	X	X	✓	✓	✓	✓
759	✓	✓	X	✓	✓	✓	✓	X	✓	✓

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



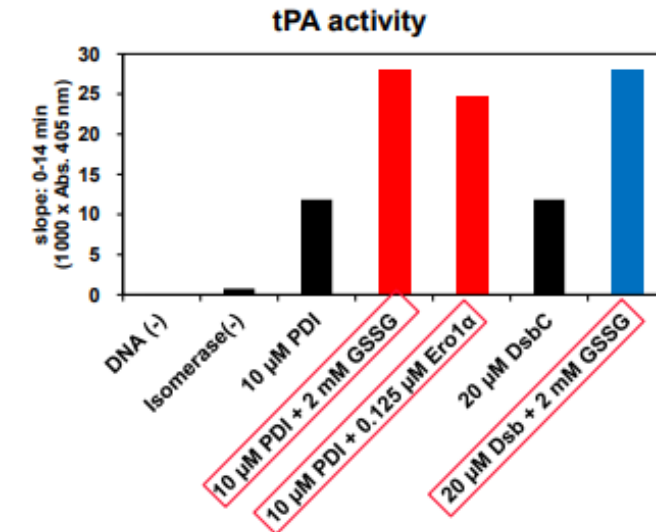
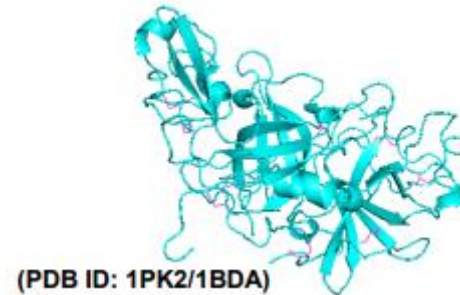
[Jensen et al. \(2021\) Protein Expr. Purif., 186, 105910.](#)



-Application for complex molecule-

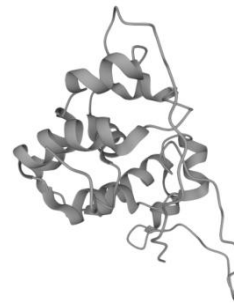
truncated version of tissue plasminogen activator (tPA)

Organism	<i>Homo sapiens</i>
Synthesized region	36Ser-40Ile/211Gly-562Pro (+FLAG)
Length	368 a.a.
Molecular weight	41,072 Da
No. of disulfide bonds	9

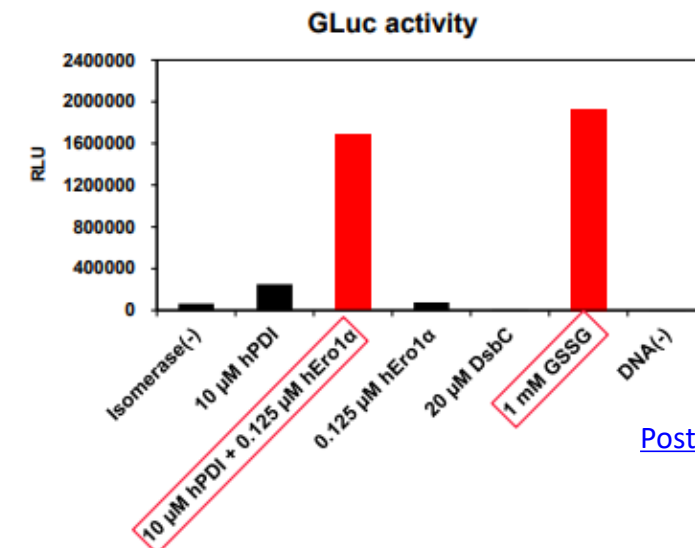


Gaussia Luciferase (GLuc)

Organism	<i>Gaussia princeps</i>
Synthesized region	18Lys-185Asp (+FLAG-His6)
Length	187 a.a.
Molecular weight	20,407 Da
No. of disulfide bonds	5

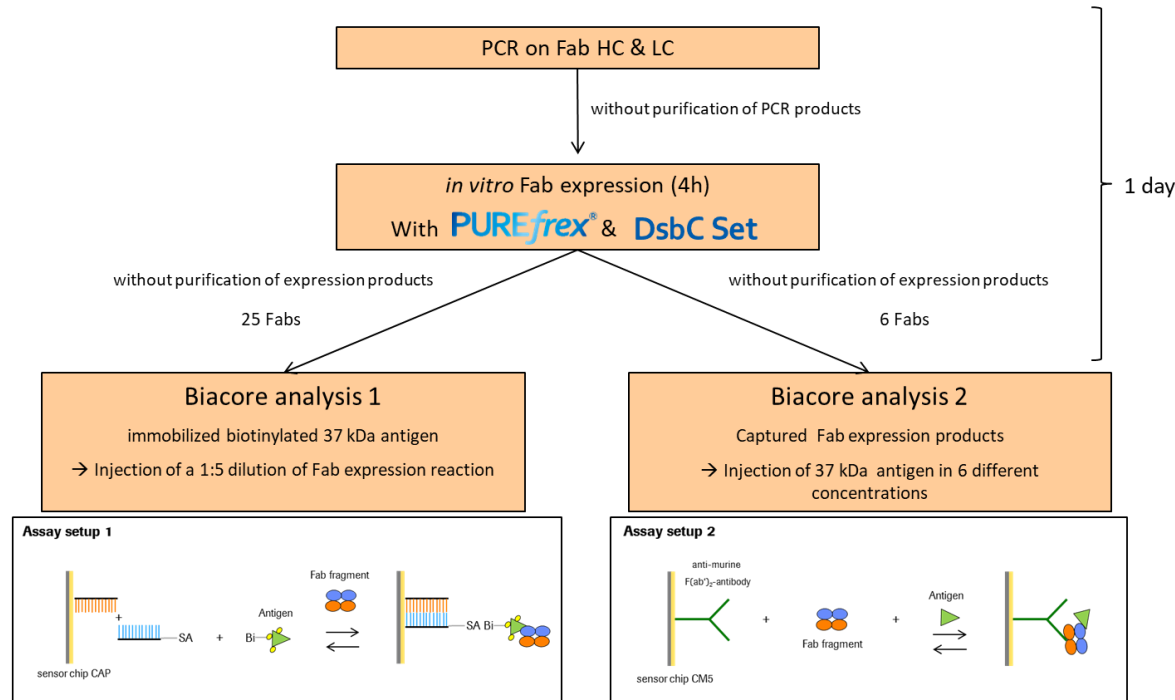


[Luciferase - Gaussia princeps \(uniprot.org\)](http://uniprot.org)

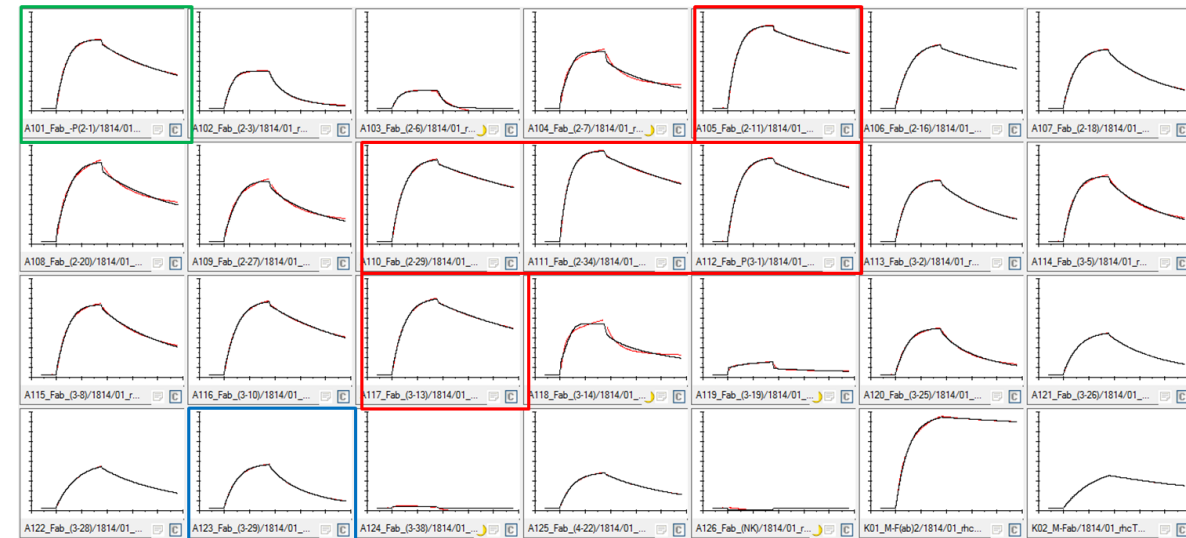




In vitro expression and Biacore analysis of Fab fragments



Kinetic analysis of 25 Fab binders



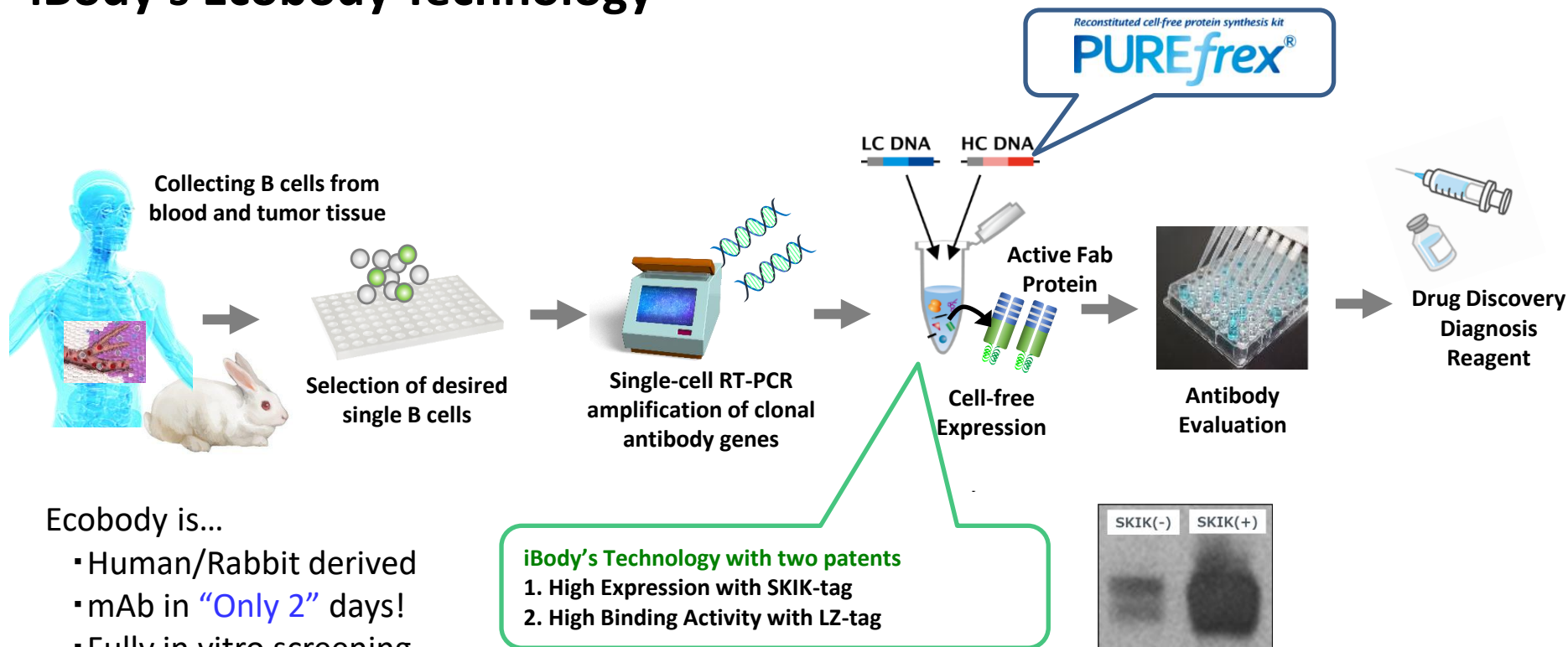
Data by Leopold von Proff

→ 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

iBody's Technology with two patents

1. High Expression with SKIK-tag
2. High Binding Activity with LZ-tag

Ojima-Kato et al. (2017) Sci. Rep., 7, 13979.

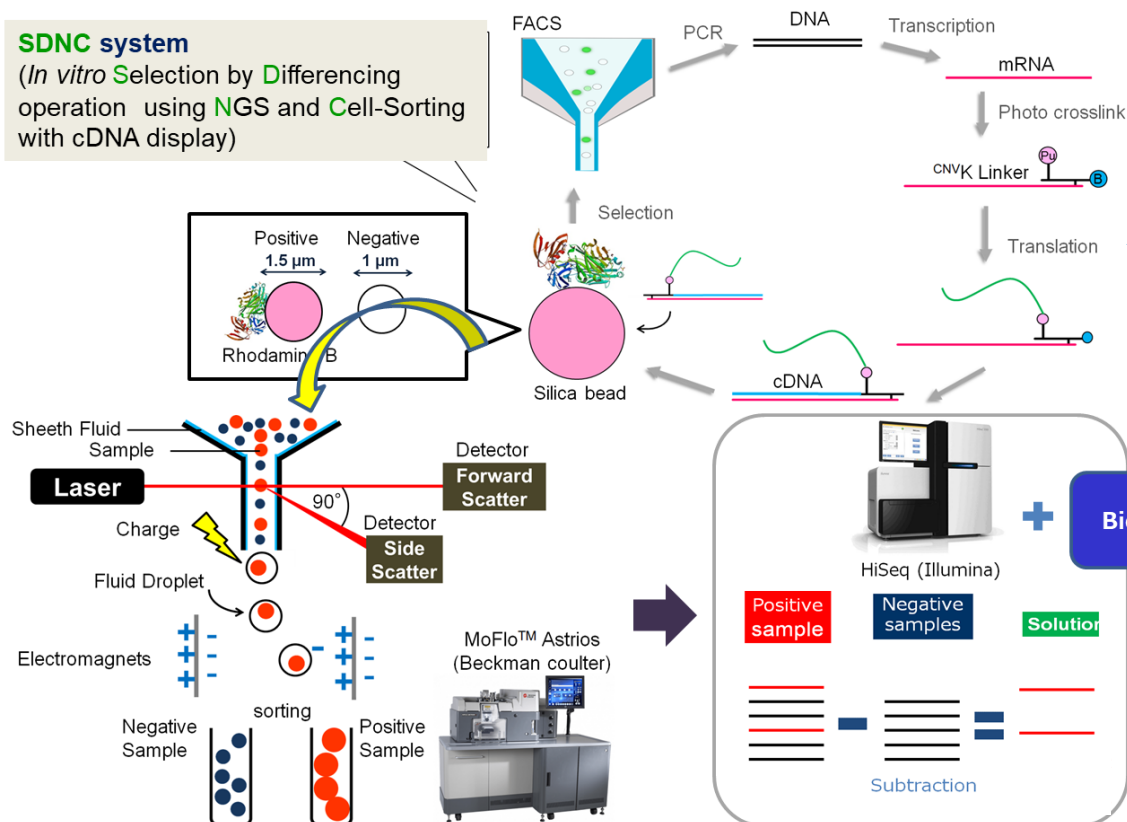
<https://www.ibody.co.jp/en/>

✓ Active Fab is expressed/screened in HT manner.



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

Reconstituted cell-free protein synthesis kit
PUREfres[®]

Evaluation

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

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

✓ **PUREfres is applied for cDNA display based screening.**





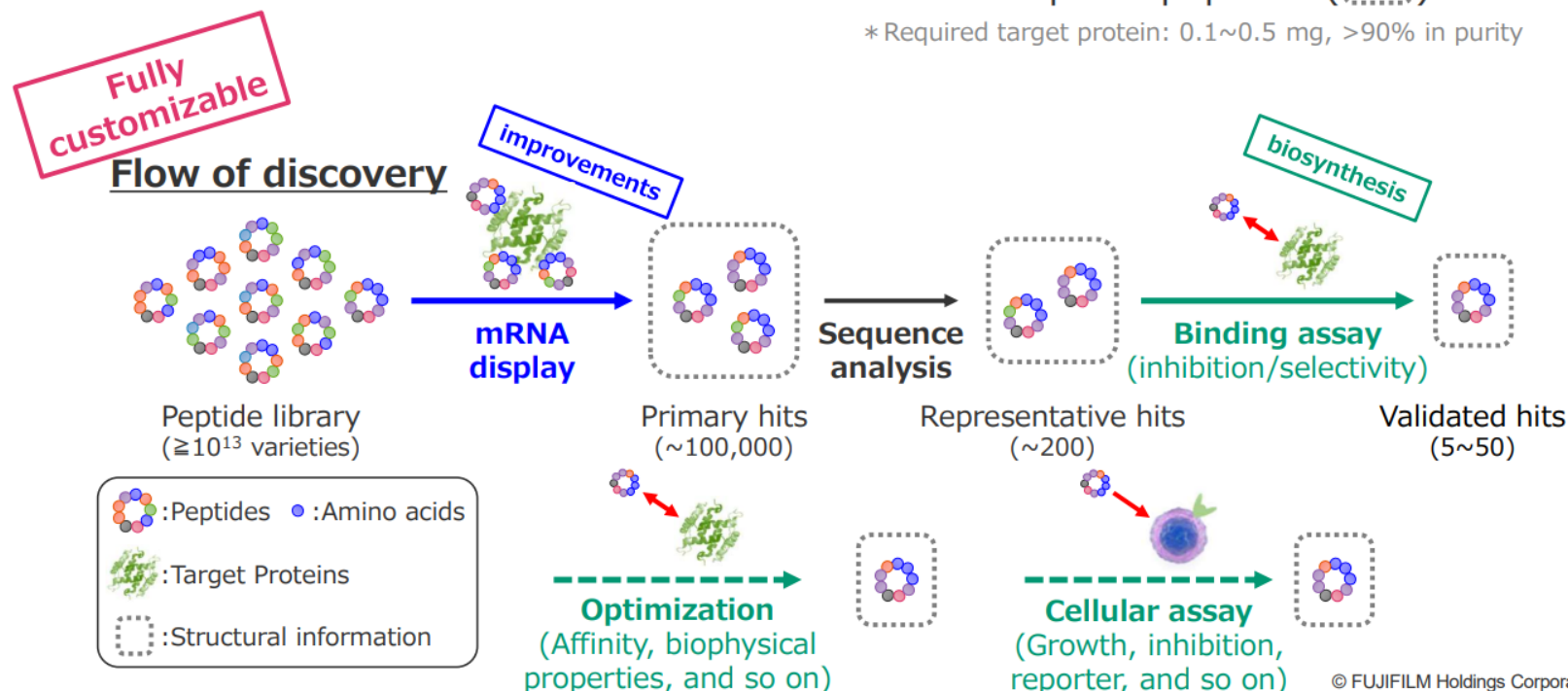
PUREfrex[®] -For display & HTS-

FUJIFILM peptide discovery services collaborated with PUREfrex[®]

- ✓ **Innovative improvements** in **mRNA display** enable screening from $>10^{13}$ peptides
 - ✓ **Practical biosynthesis & assays** enable rapid selection and activity explorations.
- Peptides hits with wide varieties and high-affinities can be obtained.

We provide a CRO service, in which we receive target () from the customer* and return the structural information of the acquired peptides ().

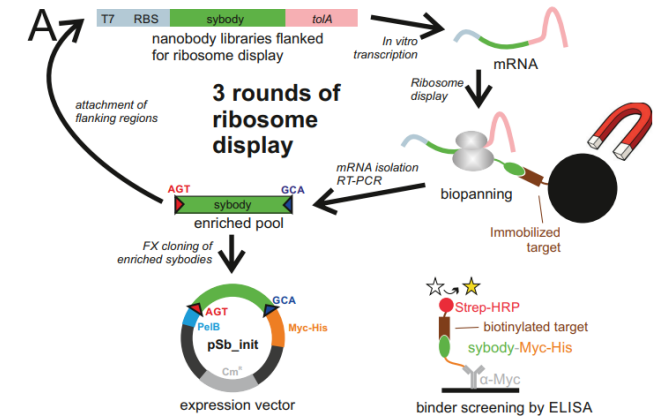
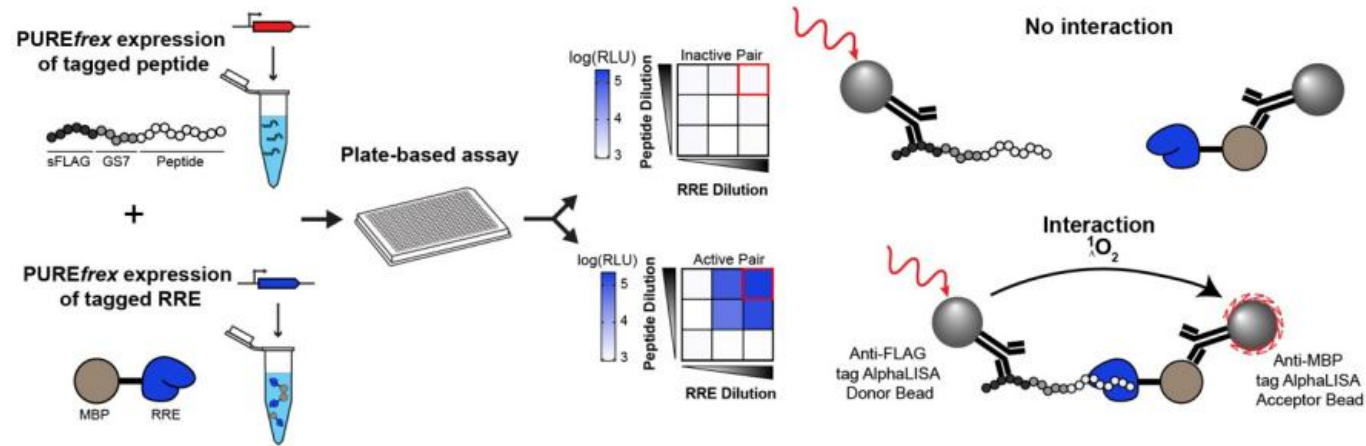
* Required target protein: 0.1~0.5 mg, >90% in purity



<https://labchem-wako.fujifilm.com/europe/category/95372.html>



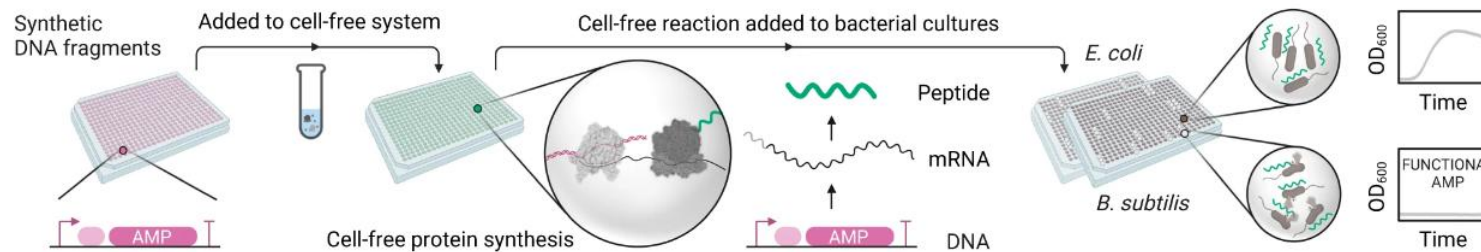
-Broad applications, yet to come!-



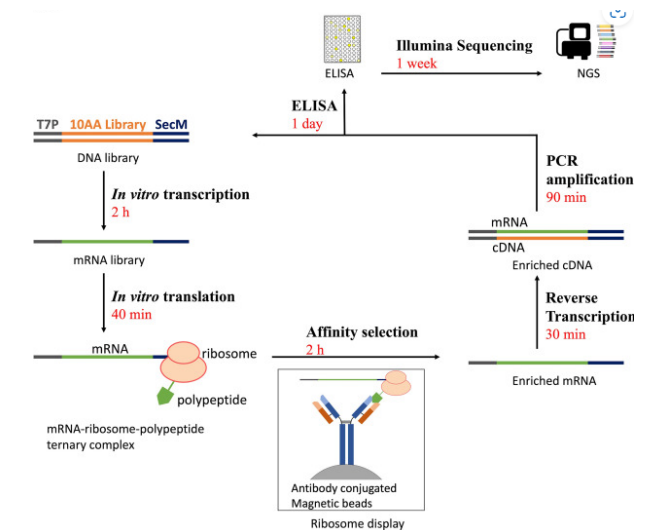
[Zimmermann I. et al. \(2018\) eLife, 7, e34317.](#)

[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)



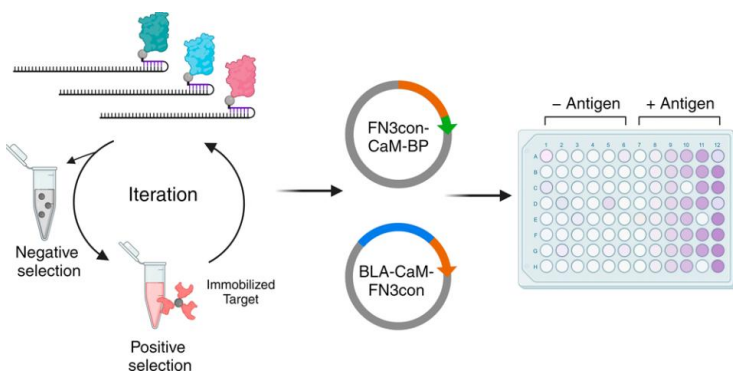
[Pandi et al. \(2023\) Nat Communications. vol.14\(7197\).](#)



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



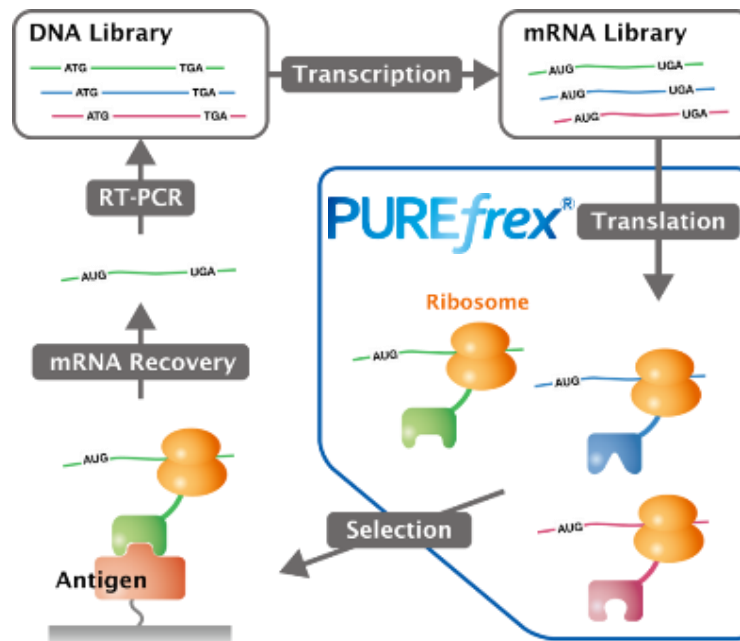
-Broad applications, yet to come!-



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

in vitro protein selection technology

PUREfres[®] RD



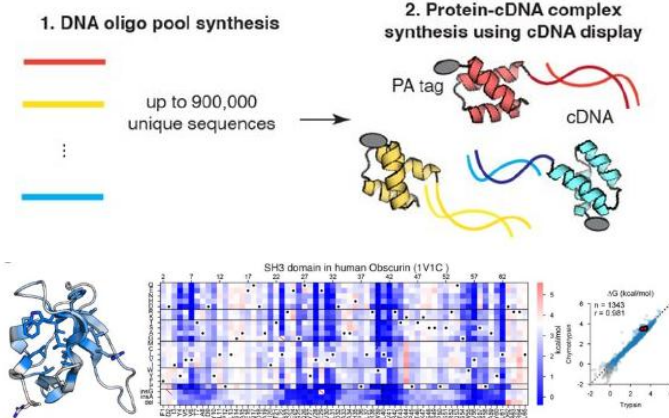
Licensed technology under JP4931135 etc.

◆ Advanced screening system for Biologics

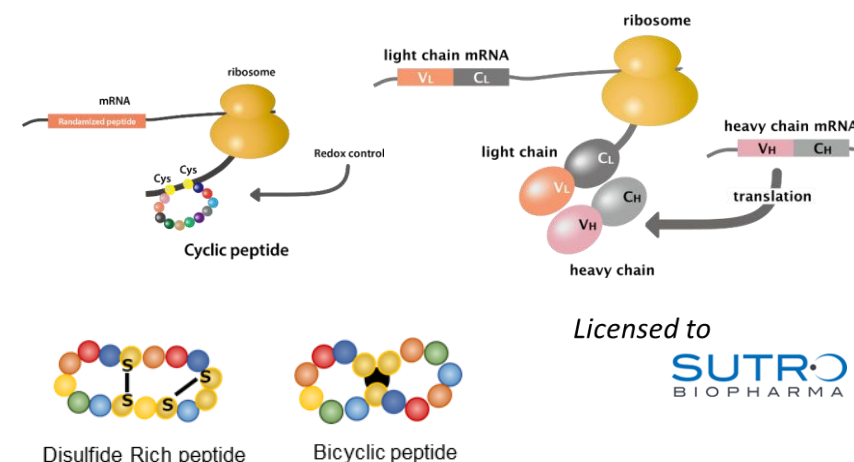
- **mAb (scFv / Fab)**
- **VHH**
- **Cyclic peptide**

◆ High Selection Efficiency

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

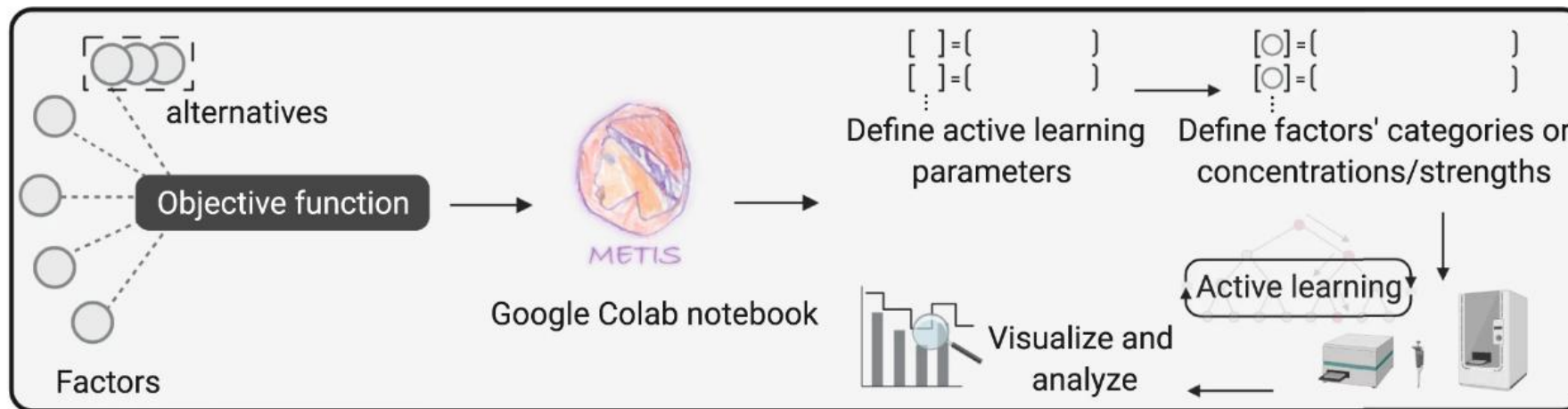


[Tsuboyama et al. \(2023\) Nature, 620, p434.](#)

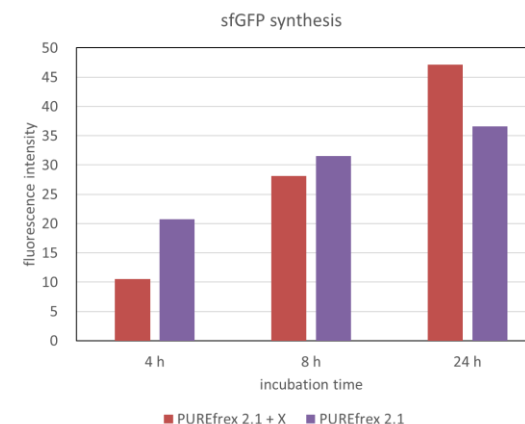
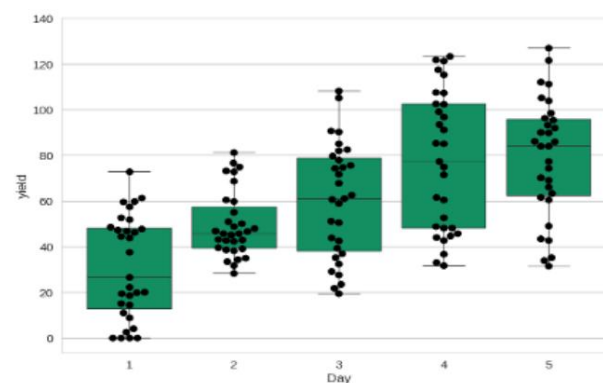
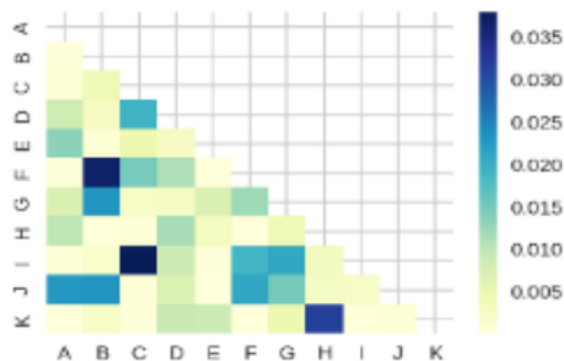




-Broad applications, yet to come!-



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



- ✓ Perfect fit to AI/ML approach with great controllability & reproducibility.
- ✓ Unique expression platform will give you great advantage in R&D.

Contact information

Reconstituted cell-free protein synthesis kit

PUREfrefx[®]

For reagent use for expression / screening of biologics

GeneFrontier

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



in vitro protein selection technology

PUREfrefx[®]RD

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

Takashi Ebihara, Ph.D., COO, GeneFrontier

E-mail: ebihara@genefrontier.com