

[For more information](#)

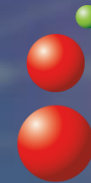


Unleashing the Power of Cell-Free: PUREfrex® for Protein Engineering and Discovery

Reconstituted cell-free protein synthesis kit

PUREfrex®

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



GeneFrontier

BIO-Asia-Taiwan
24-27 of July, 2025

Corporate Summary



GeneFrontier

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

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

People: ***17 (Ph.D. 8, MS 1)***

Place: ***Chiba, Japan***

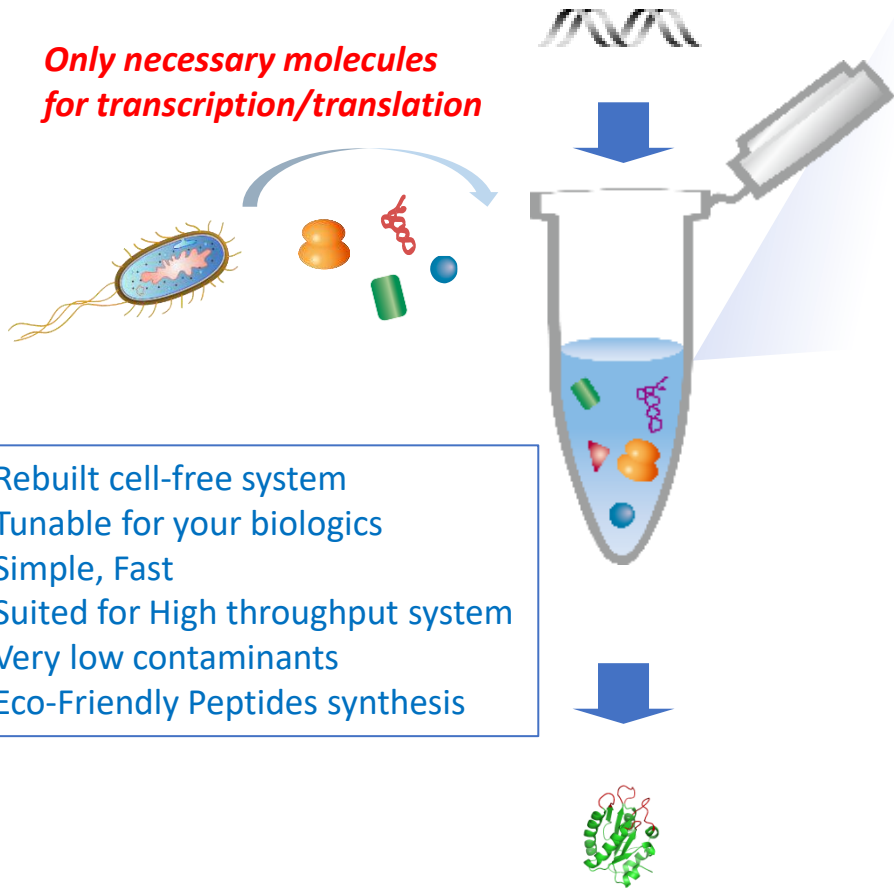
Mission: ***Rebuilding and Manipulating Biological system
for Inspiring the world!***



PUREfres[®]

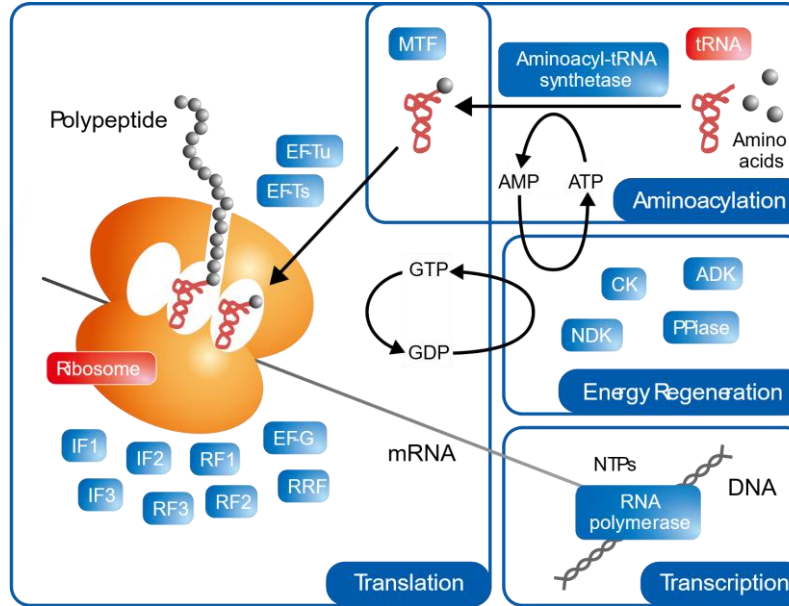
PURE (Protein synthesis Using Recombinant Elements) base cell-free expression

Only necessary molecules for transcription/translation

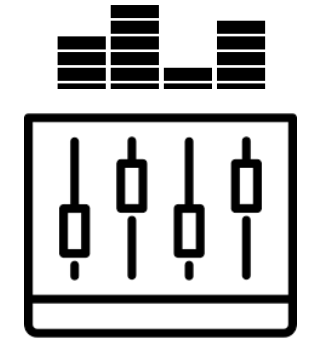
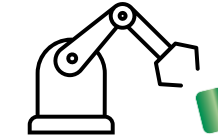
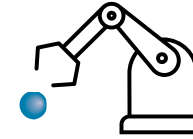


- ✓ Rebuilt cell-free system
- ✓ Tunable for your biologics
- ✓ Simple, Fast
- ✓ Suited for High throughput system
- ✓ Very low contaminants
- ✓ Eco-Friendly Peptides synthesis

Totally constructive, molecular based system



For more information

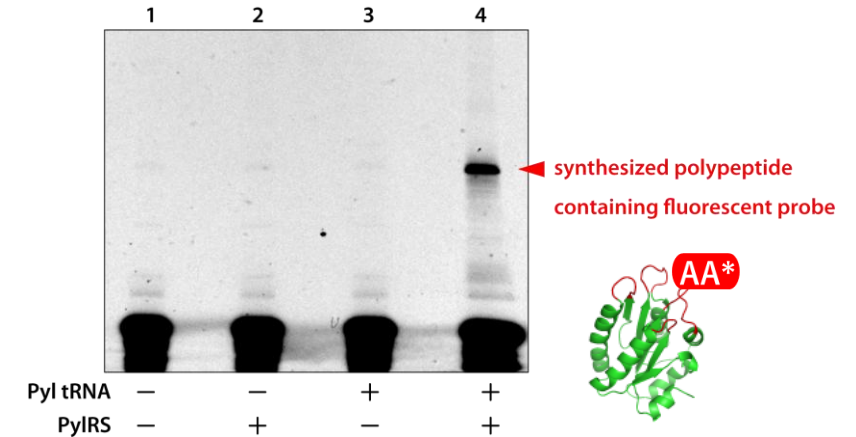
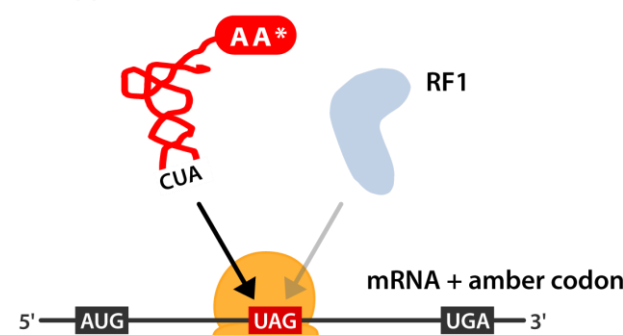


Translation factors
Chaperones
Detergent
Temperature/pH
Redox

<Ex, Non-natural AA introduction>

Translation - RF1

Suppressor tRNA + non-natural amino acid

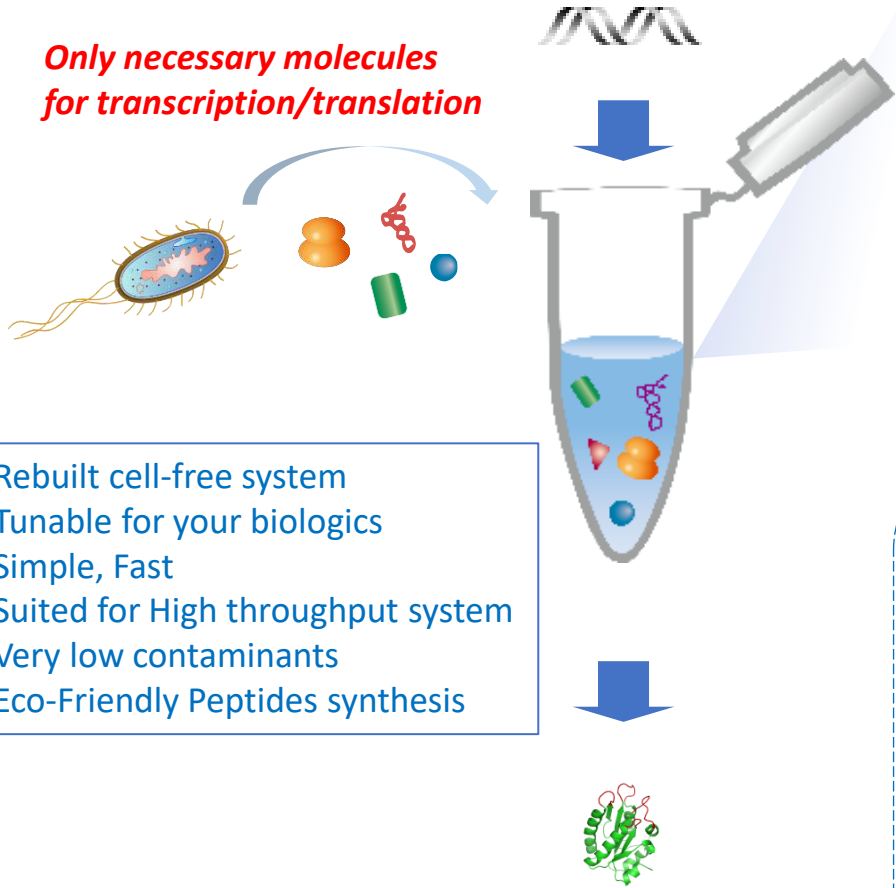


Ready-made kit is coming soon!

PUREfres[®]

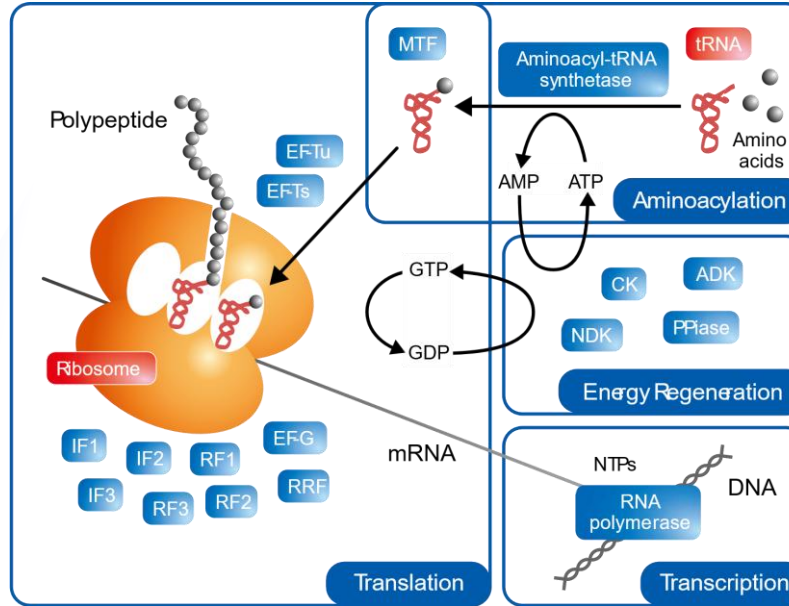
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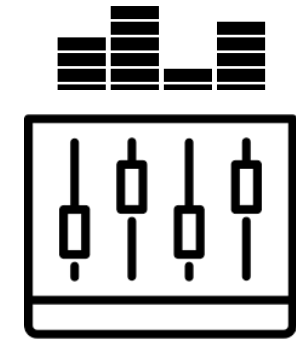
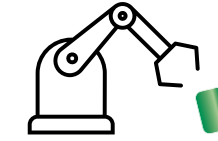
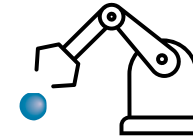


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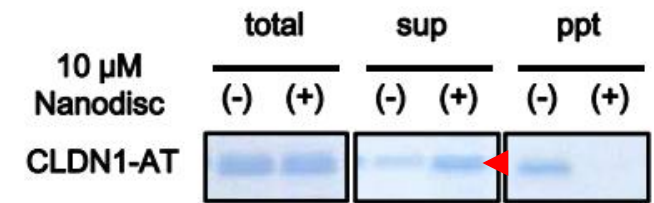
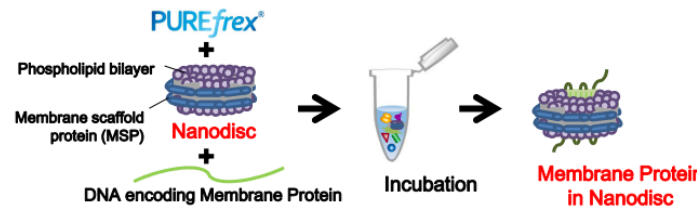


For more information



Translation factors
Chaperones
Detergent
Temperature/pH
Redox

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



Solubilized hCLDN1 was synthesized using PUREfres[®] and Nanodisc.

The condition of membrane protein synthesis

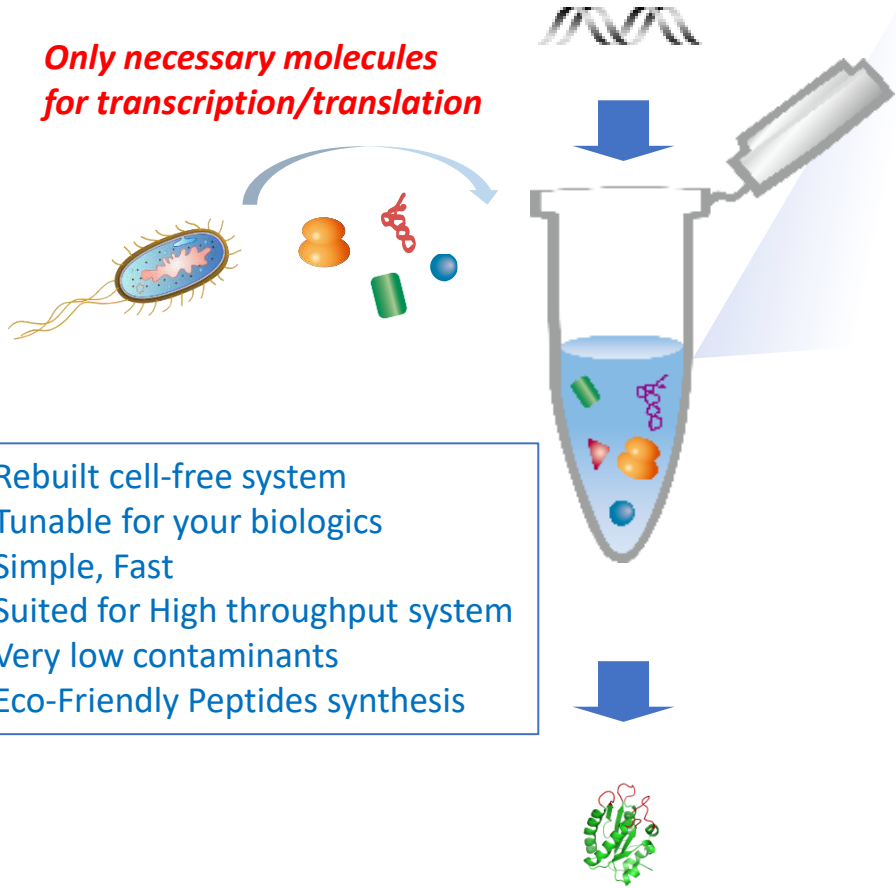
Reaction mix	Template DNA	Incubation
PUREfres [®] 2.0 +Nanodisc (MSP1E3D1-His POPC*, final 10 μM)	PCR product	37°C, 4 h

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

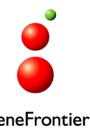
PUREfres[®]

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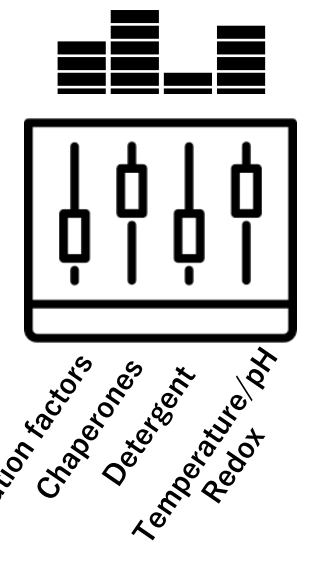
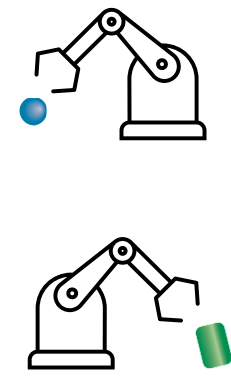
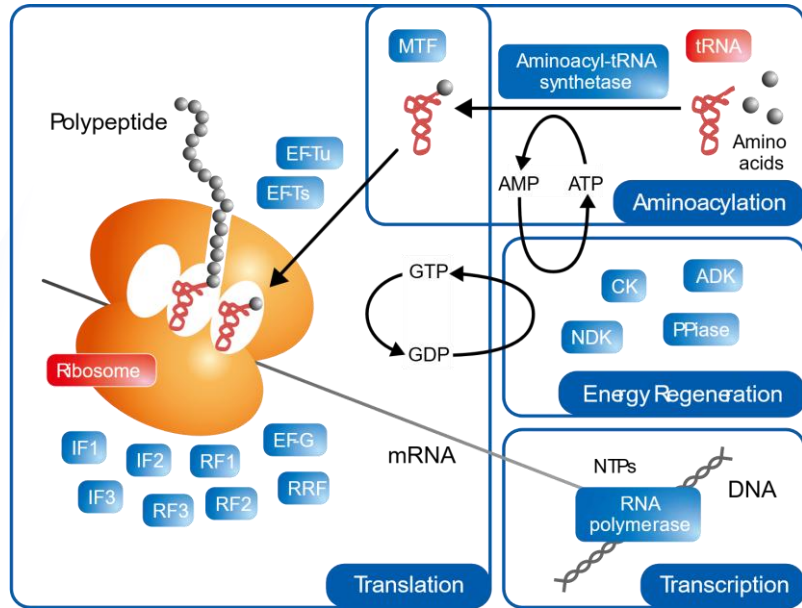
Only necessary molecules for transcription/translation



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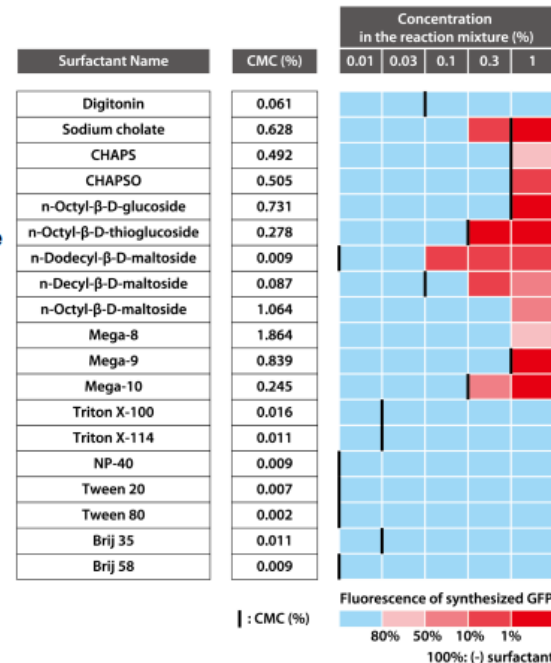


Experimental conditions for protein synthesis

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

→ Measurement of GFP fluorescence

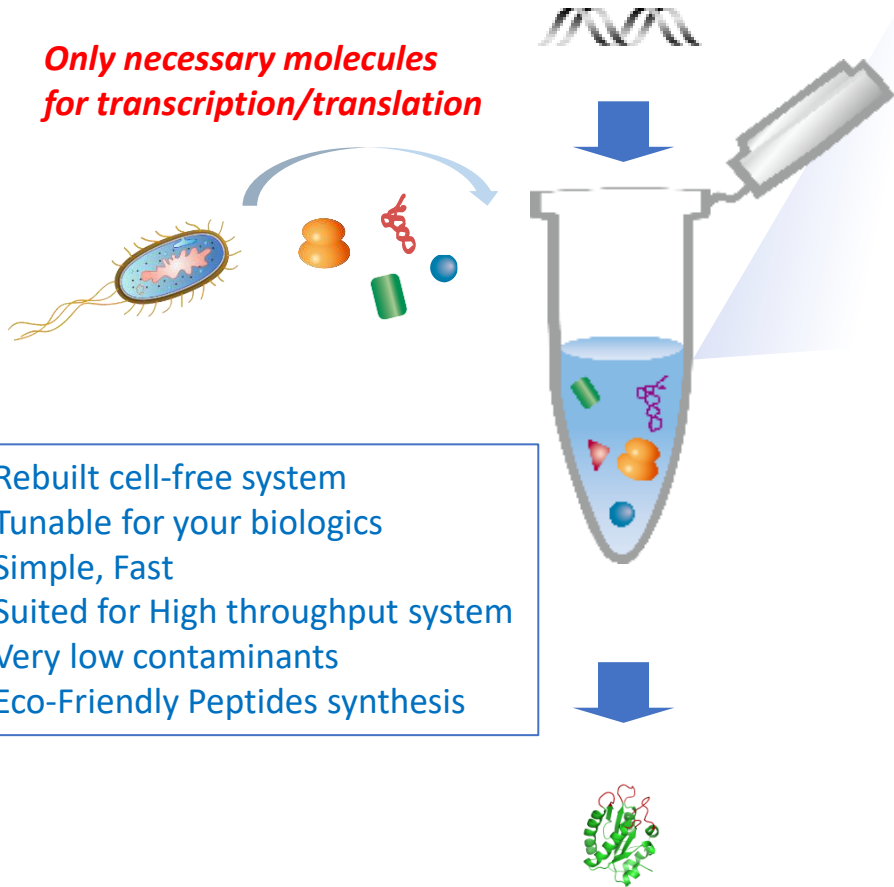
- Most surfactants did not inhibit the protein synthesis reaction by PUREfres[®] below the CMC.
- Some surfactants such as Triton X-100 and Tween 20 could be used even above the CMC.



PUREfres[®]

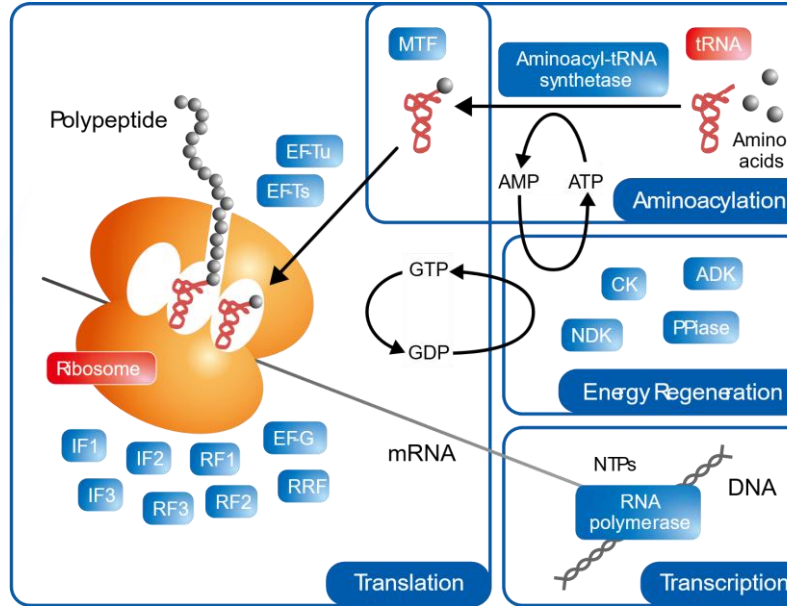
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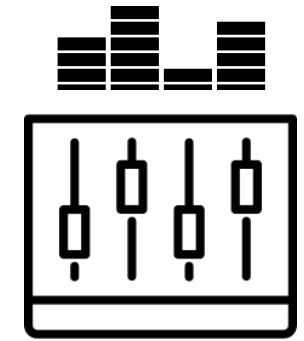
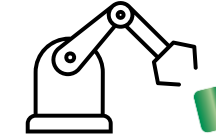
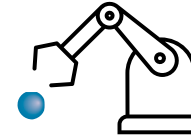


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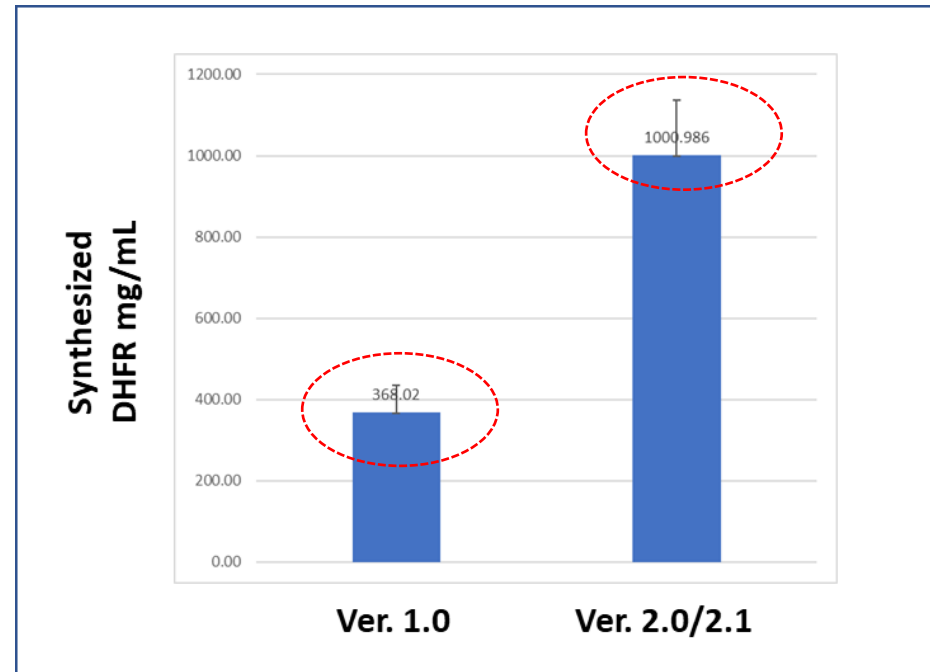
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For more information



Translation factors
Chaperones
Detergent
Temperature/pH
Redox



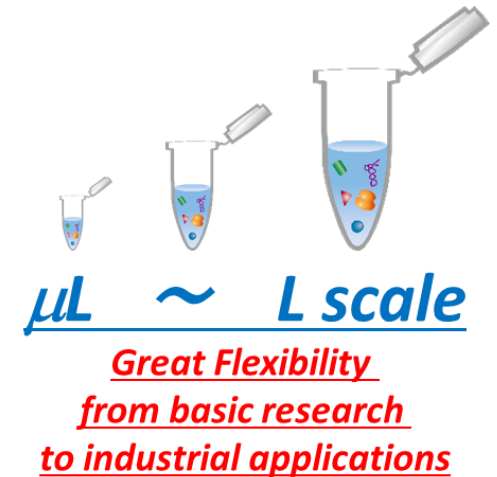
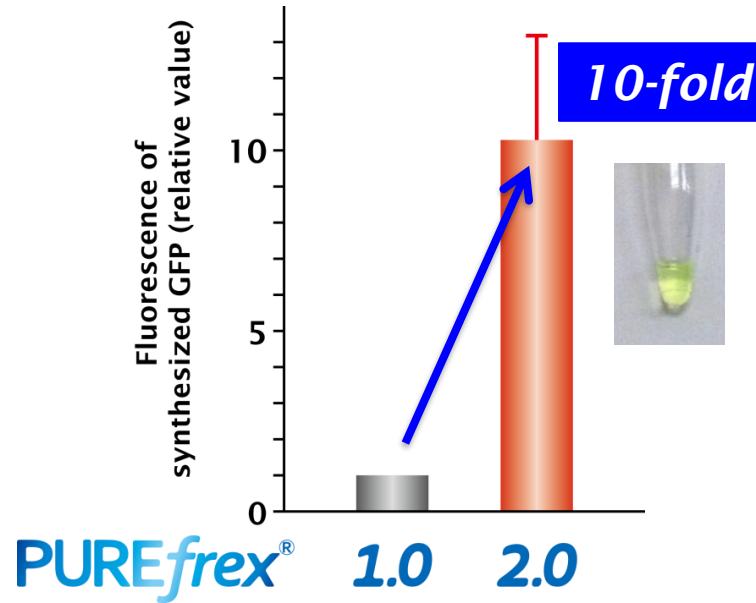
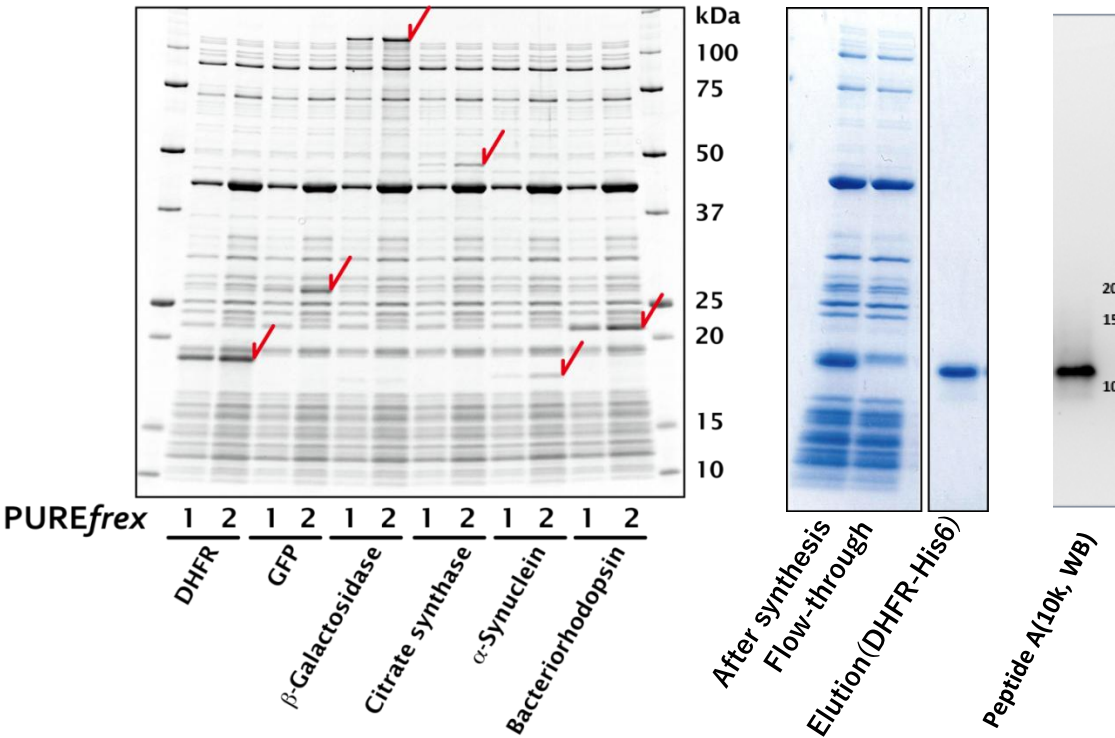
Versatile and Robust
Platform for protein synthesis

Huge potential as
New platform in Biotech industry





-Having good productivity-



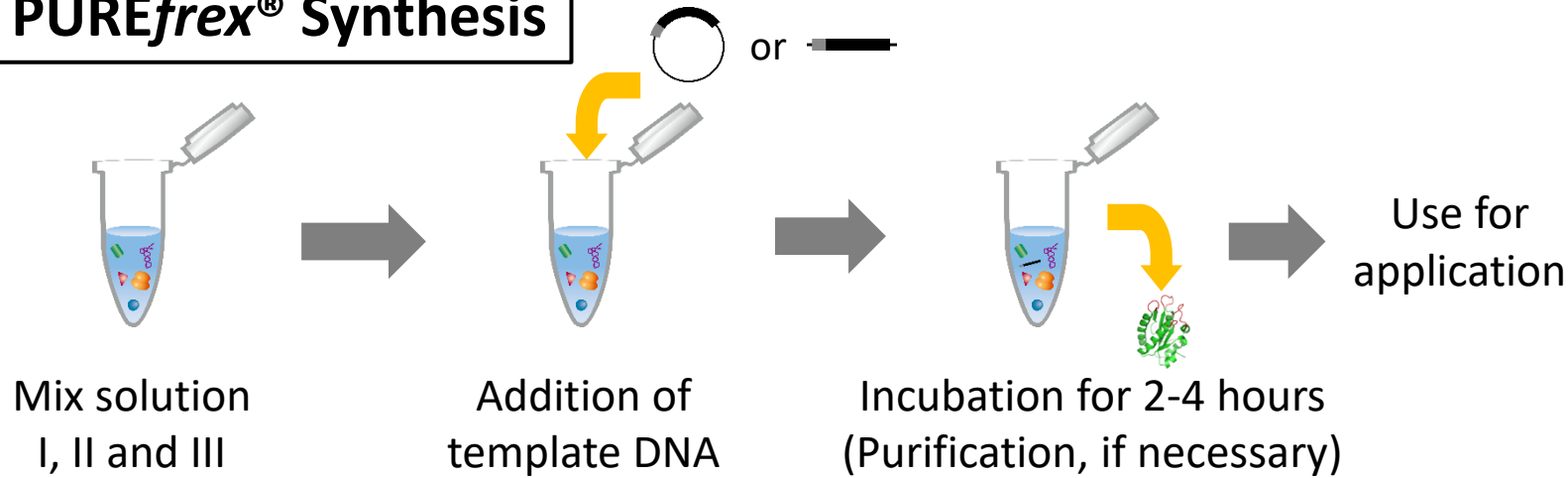
- Reaction at 37°C for 4 h
- 0.5 μ L of reaction mix/ lane
- stained with Oriole (Bio-Rad) and analyzed with an image analyzer (LAS)

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



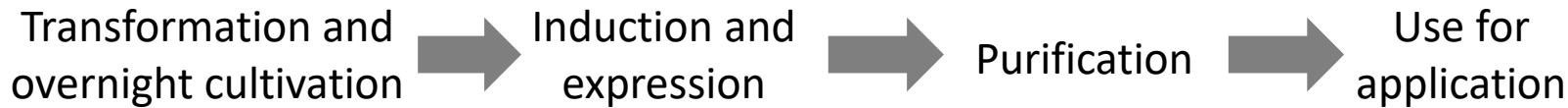
-Improve Expression from Days to Hours-

PUREfres[®] Synthesis

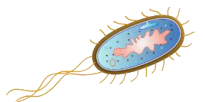
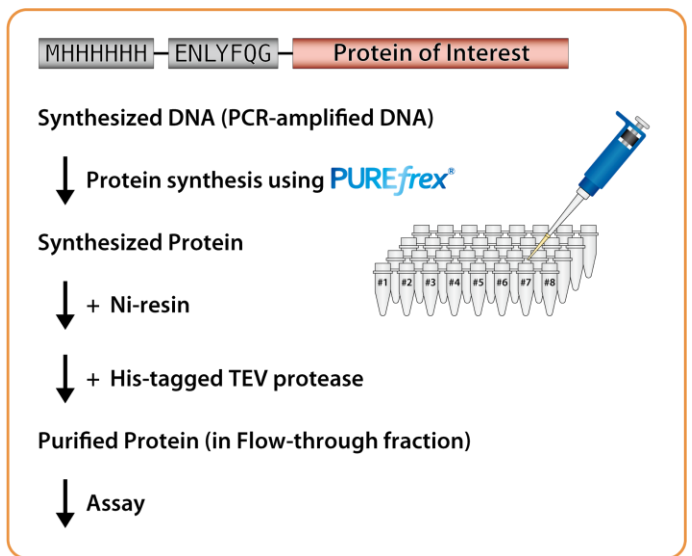
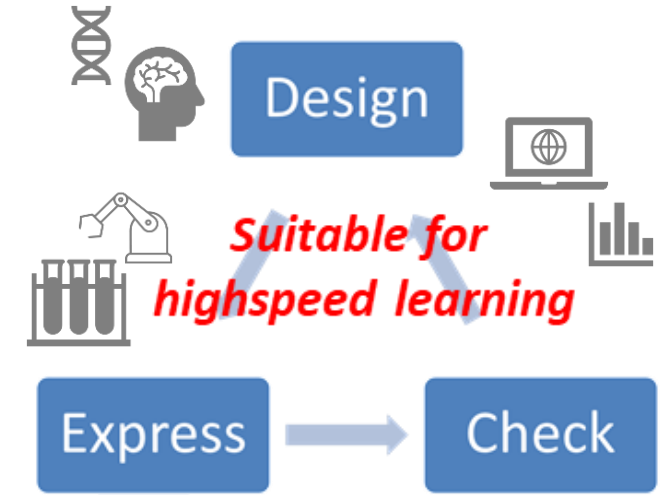


Total: 2-4 hours

E. coli Expression



Total: 3-4 days





-KSF; AT rich codon on N-term-

Fab Heavy Chain (Herceptin)

Herceptin Fab HC

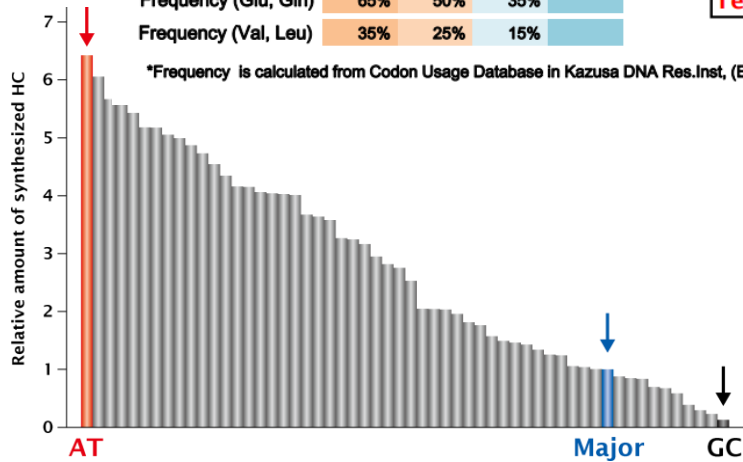
Met- **Glu-Val-Gln-Leu-Val-** FLAG

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.

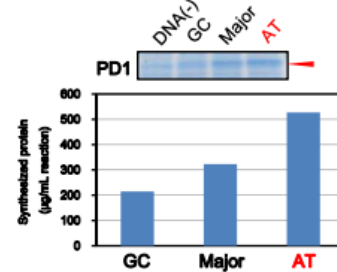
Manual is Free to download from our Web site here.



PD1

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

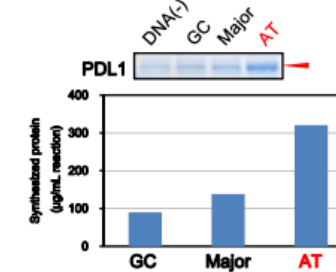
N-term type	1	2(38)	3(37)	4(38)	5(39)	6(40)	GC(%) 1-6 a.a.
GC	atg	acc	ttc	toc	cog	gog	67%
Major	atg	acc	ttt	tct	cog	gog	56%
AT	atg	act	ttt	tca	cca	gct	39%



PDL1

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

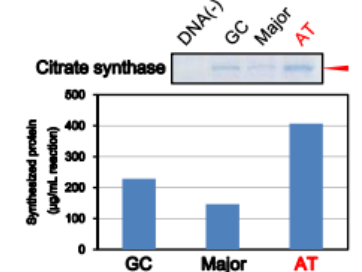
N-term type	1	2(18)	3(19)	4(20)	5(21)	6(22)	GC(%) 1-6 a.a.
GC	atg	gog	ttc	acc	gtg	acc	61%
Major	atg	gog	ttt	acc	gtg	acc	56%
AT	atg	gct	ttt	act	gta	aca	33%



Citrate Synthase

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

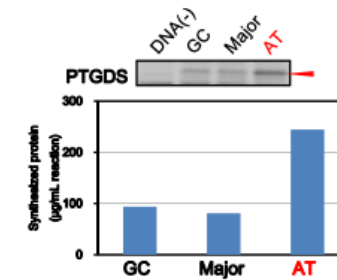
N-term type	1	2(38)	3(39)	4(40)	5(41)	6(42)	GC(%) 1-6 a.a.
GC	atg	toc	toc	gog	toc	gag	67%
Major	atg	tct	tct	gog	tct	gaa	44%
AT	atg	tca	tca	gct	tca	gaa	39%



PTGDS

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

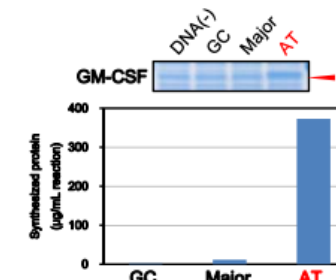
N-term type	1	2(23)	3(24)	4(25)	5(26)	6(27)	GC(%) 1-6 a.a.
GC	atg	gca	cog	gaa	gca	cag	61%
Major	atg	gca	cog	gaa	gca	cag	72%
AT	atg	gca	cct	gaa	gct	caa	50%



GM-CSF

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

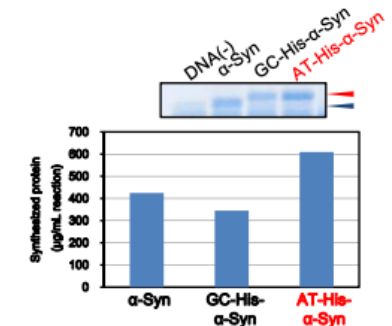
N-term type	1	2(18)	3(19)	4(20)	5(21)	6(22)	GC(%) 1-6 a.a.
GC	atg	gca	cog	gca	cgc	toc	83%
Major	atg	gca	cog	gca	cgc	tct	78%
AT	atg	gca	cct	gct	aga	tca	50%



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

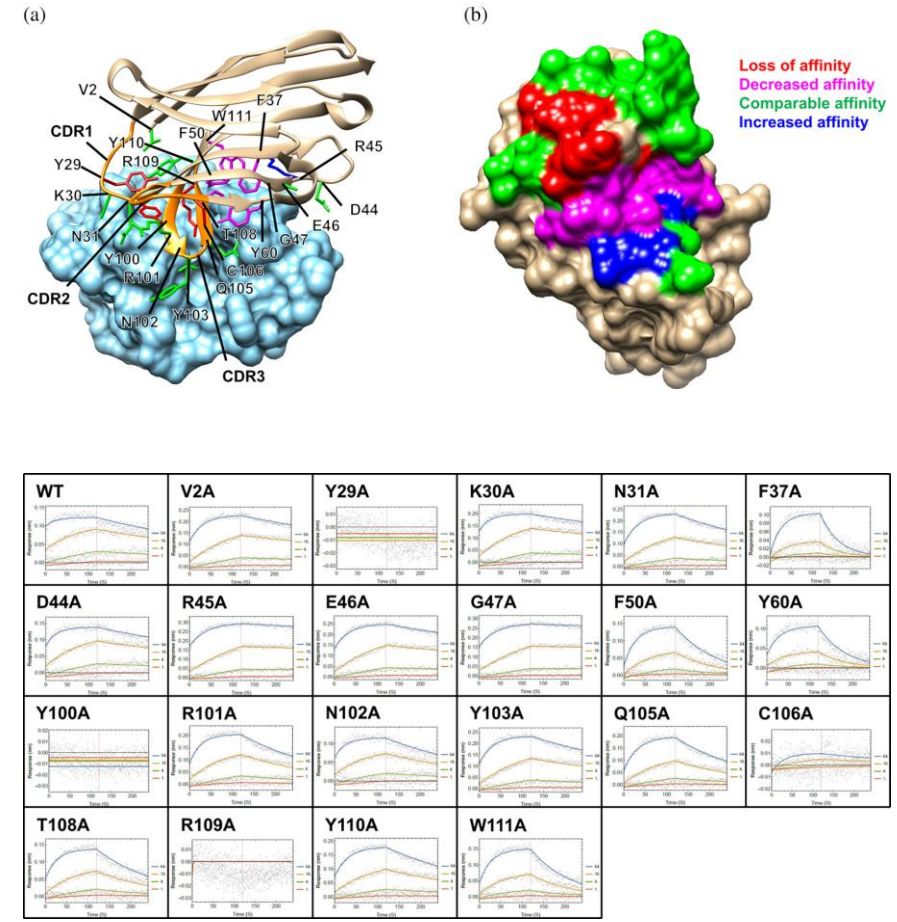
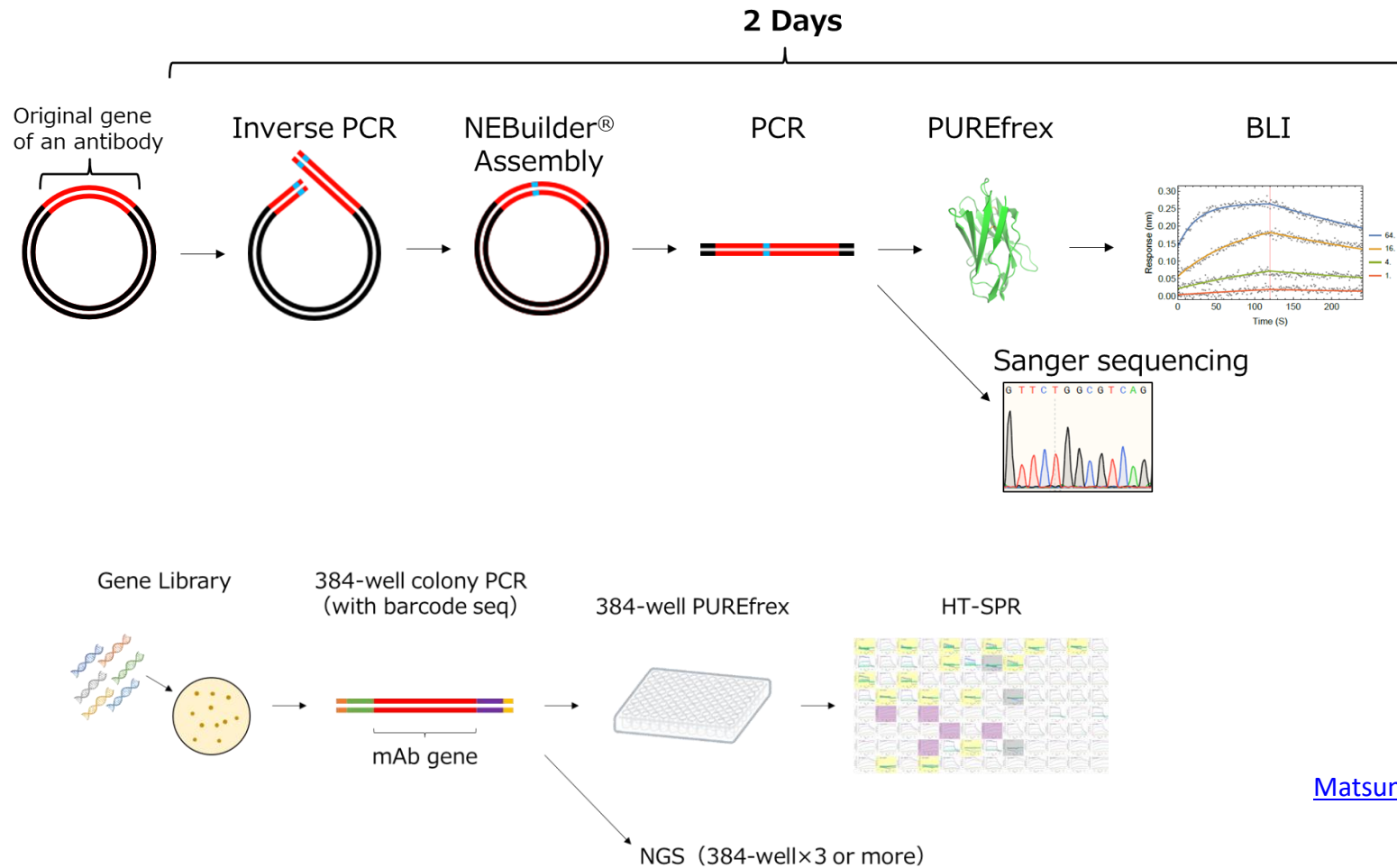
Tag type	1	2	3	4	5	6	7	8	9	GC(%) 1-9 a.a.
GC	atg	ccc	ccc	ccc	ccc	ccc	ccc	ggg	tct	59%
AT	atg	cat	cat	cat	cat	cat	cat	ggg	tct	37%





-Improve Validation from Weeks to Days-

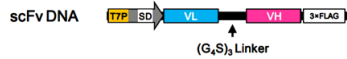
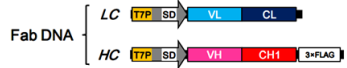
FASTIA: Fast Interaction Analysis



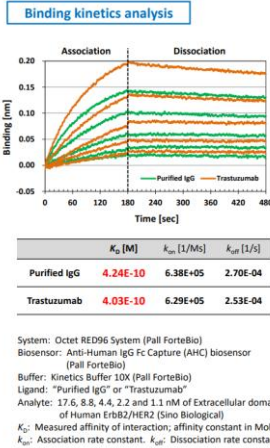
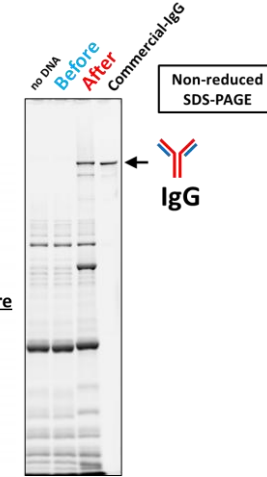
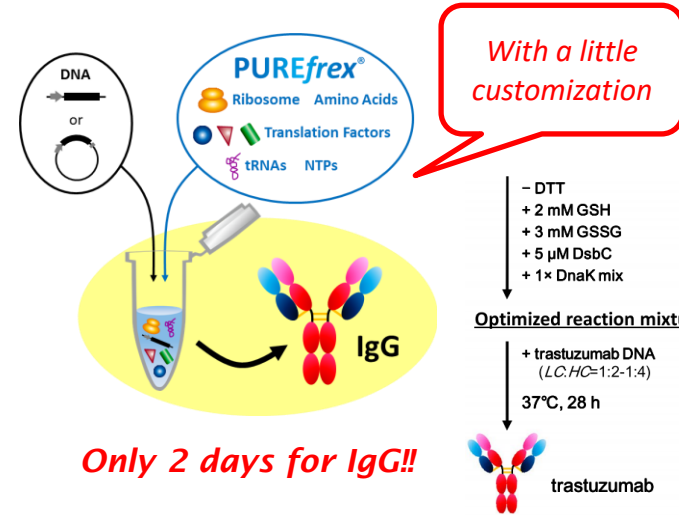
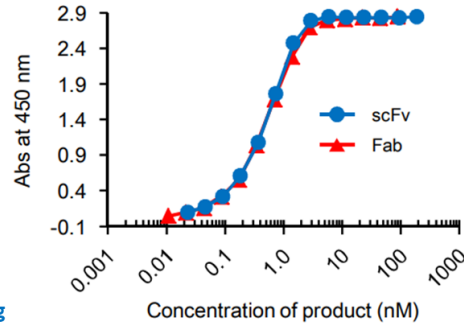
Matsunaga et al. (2025) Protein Sci. Mar;34(3):e70065. doi: 10.1002/pro.70065.



-Expression of scFv, Fab, IgG and more-

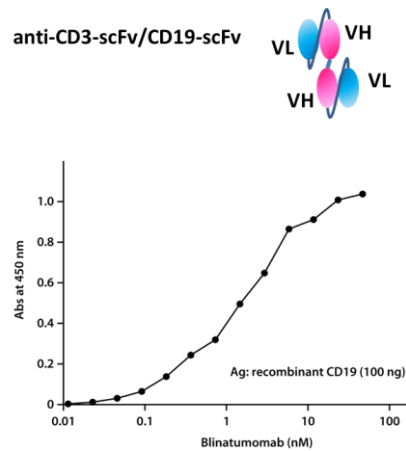


Activity

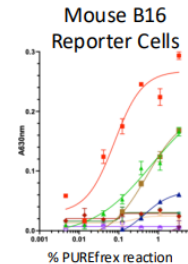
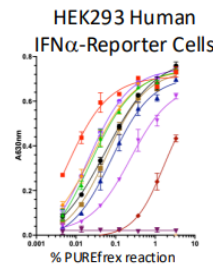


[Murakami et al. \(2019\) Sci. Rep. vol.9, p.671. \(Supplementary Information\)](#)

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

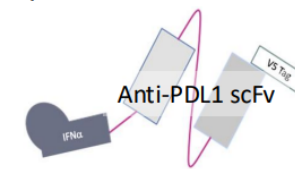


Round 1: IFN-α variants tested by in vitro transcription/translation



- Universal IFN-α
- hIFN_u_L152F
- R121K
- R121K_Q125R
- R121K_Q125R_K132T
- Y86C_R121K_Q125R
- Y86C_R121K_Q125R_K132T
- mIFN2
- IFN_u2b
- scFv control
- Human IFN_u2b (starts at 1nM)
- No DNA

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

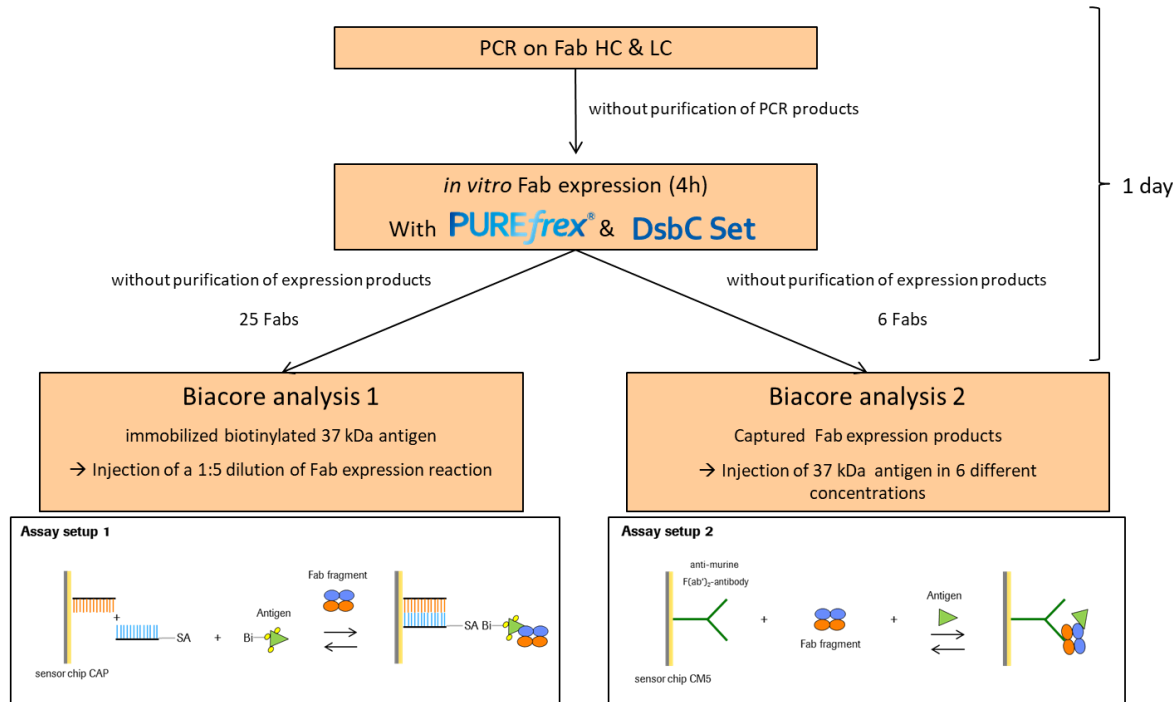


IFNα-scFv protein was generated with an anti-PDL1 antibody by in vitro transcription/translation using the PUREfres[®] system (CosmoBIO USA). Protein mixture was serially diluted and protein was captured via an anti-V5 tag coated to the wells of the plate. Proteins were assayed for PD-L1 binding to assess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

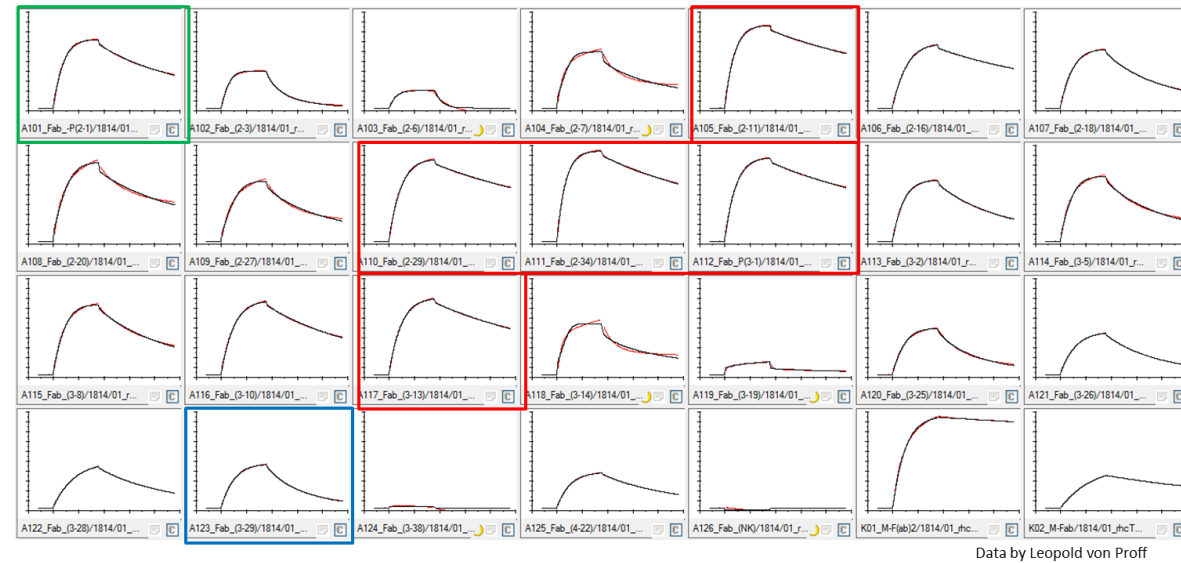
[Killebrew et al. \(2024\) SITC 2024 Annual Meeting \(Poster, BONUMTX.com\).](#)



In vitro expression and Biacore analysis of Fab fragments



Kinetic analysis of 25 Fab binders

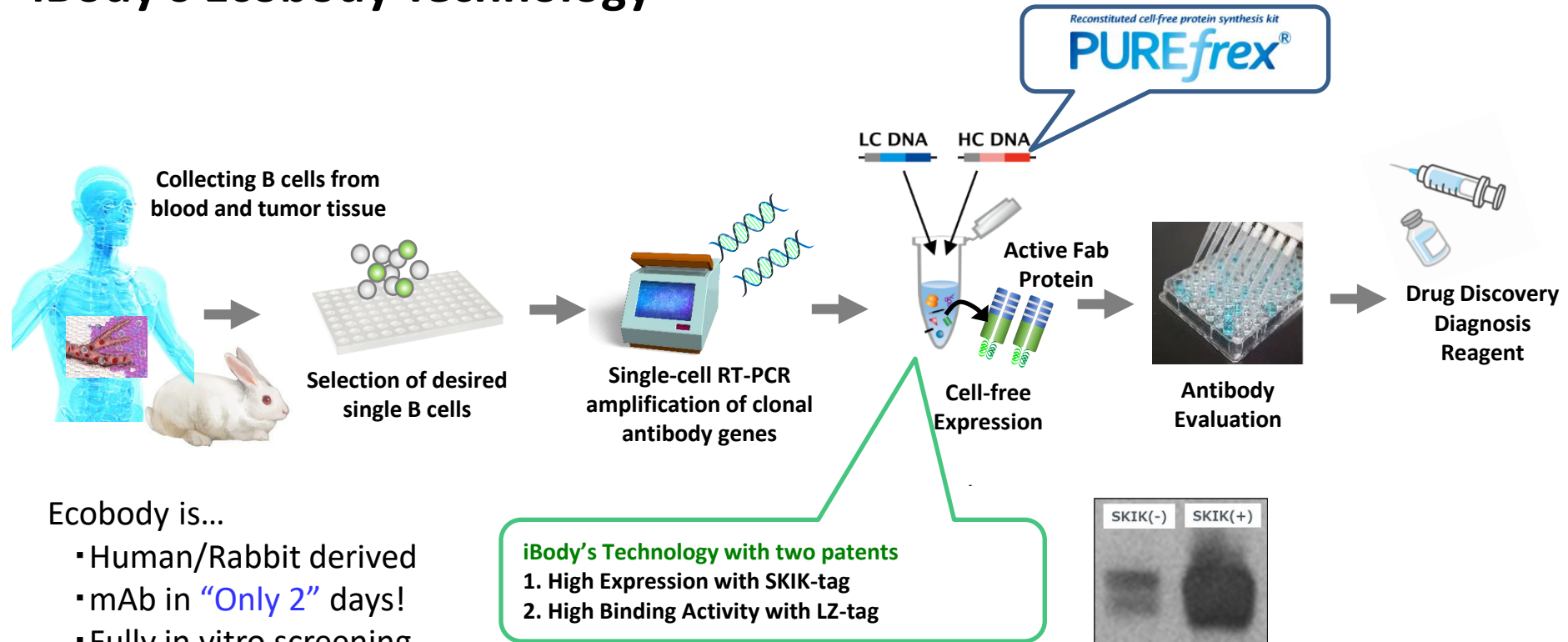


→ Selection of Fabs for further kinetic analysis

✓ 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

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

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

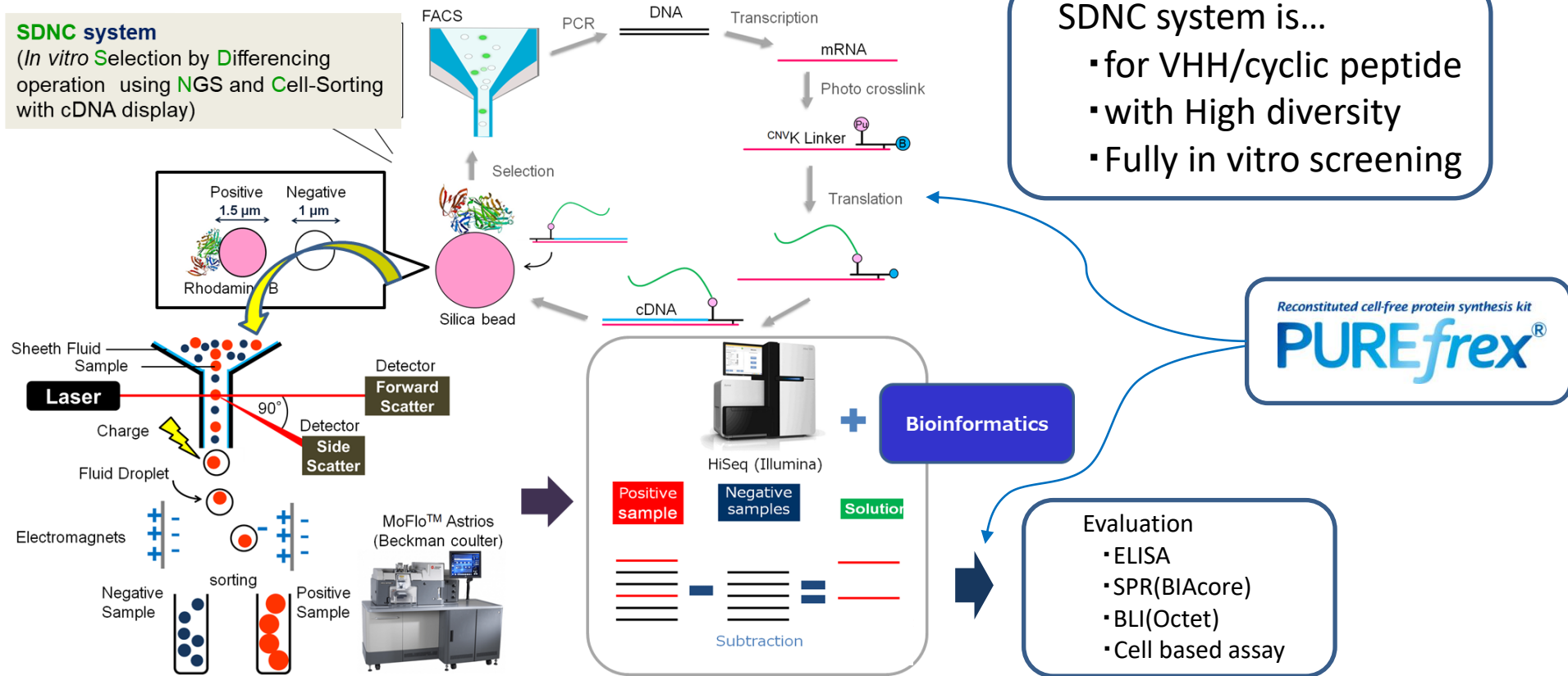


✓ Active Fab is expressed/screened in HT manner.



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



Evaluation

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

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

✓ PUREfres is applied for cDNA display based screening.







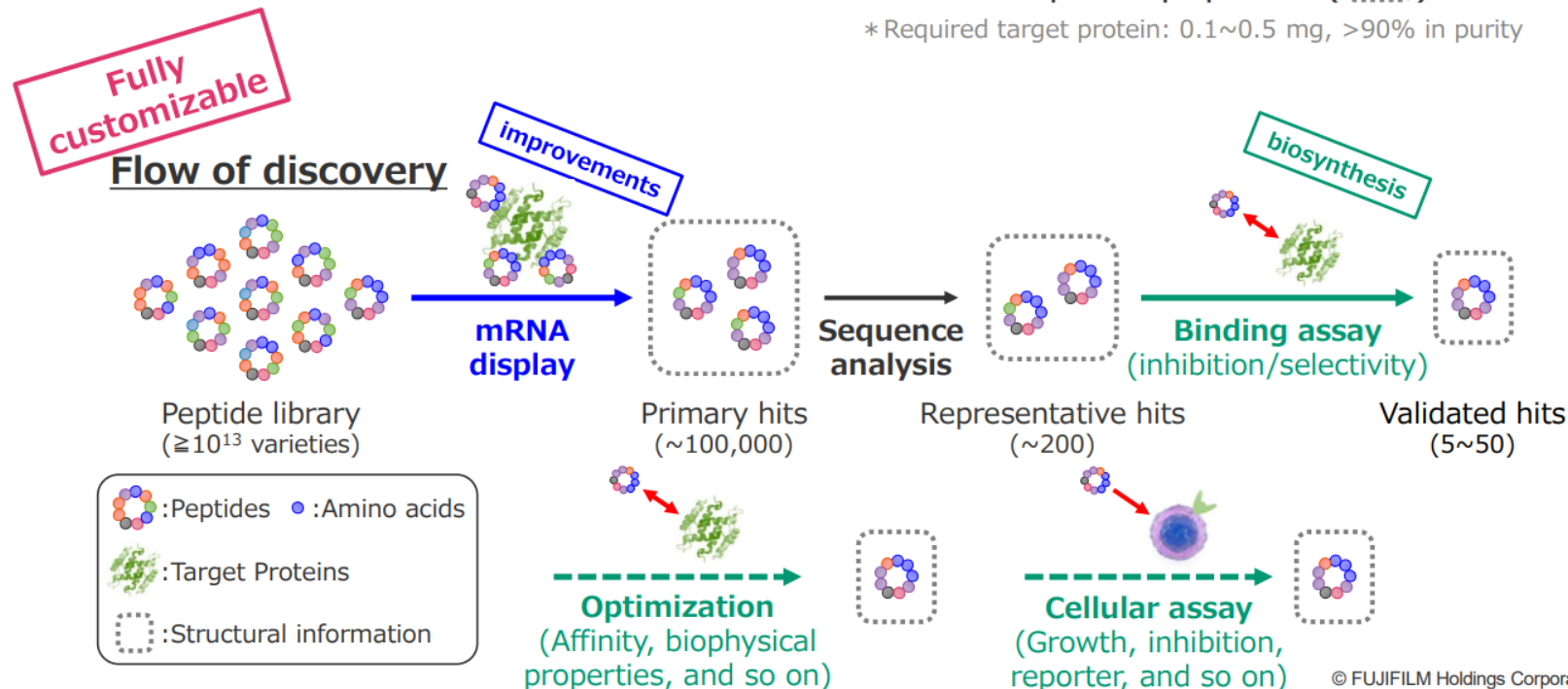
PUREfres[®] -For display & HTS-

FUJIFILM peptide discovery services collaborated with PUREfres[®]

- ✓ **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 ().

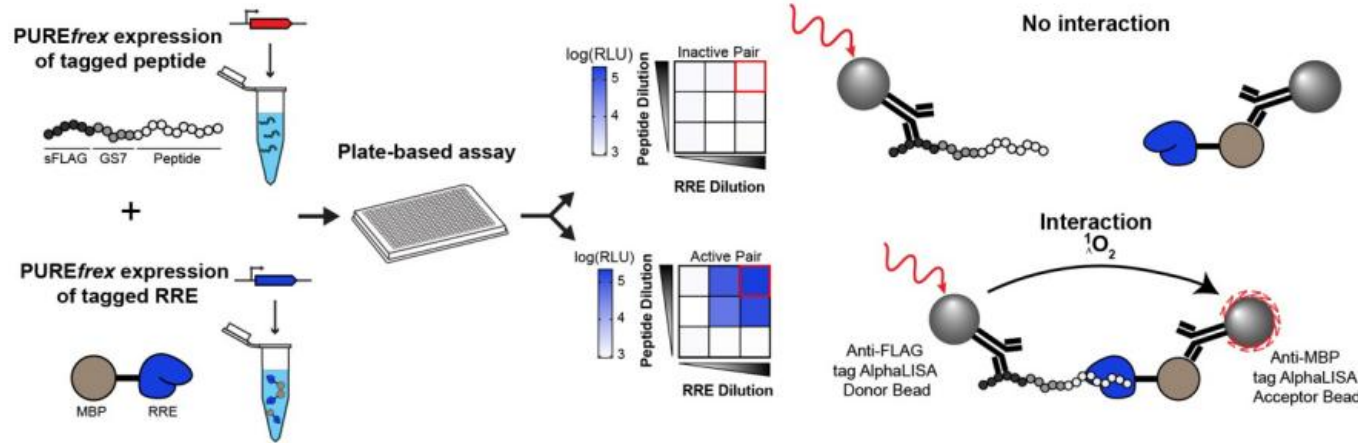
* Required target protein: 0.1~0.5 mg, >90% in purity



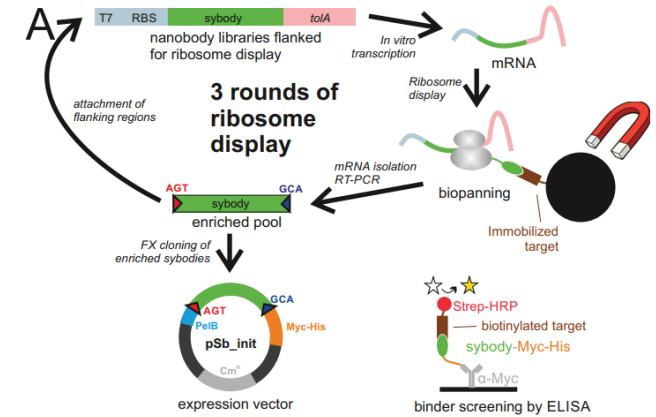
<https://labchem-wako.fujifilm.com/europe/category/95372.html>



-Broad applications, yet to come!-

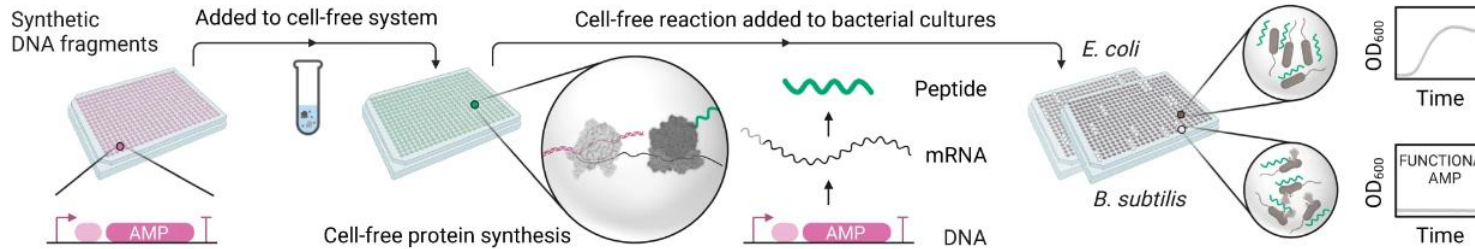


[Wong et al. \(2024\) bioRxiv https://doi.org/10.1101/2024.03.25.586624.](https://doi.org/10.1101/2024.03.25.586624)

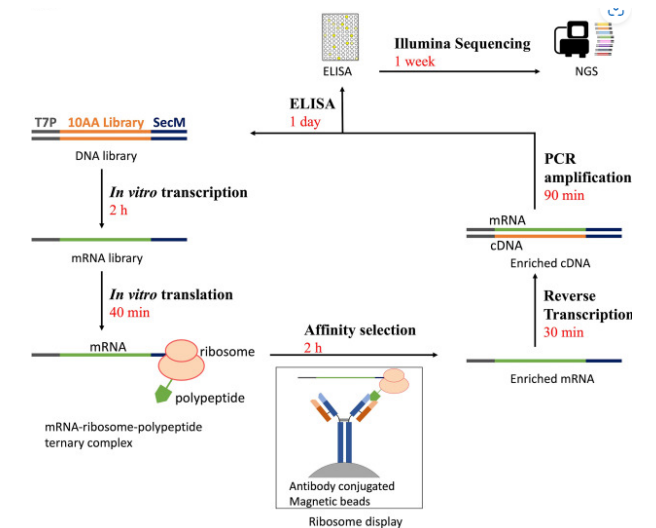


[Zimmermann I. et al. \(2018\) eLife, 7, e34317.](https://doi.org/10.1101/2024.03.25.586624)

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



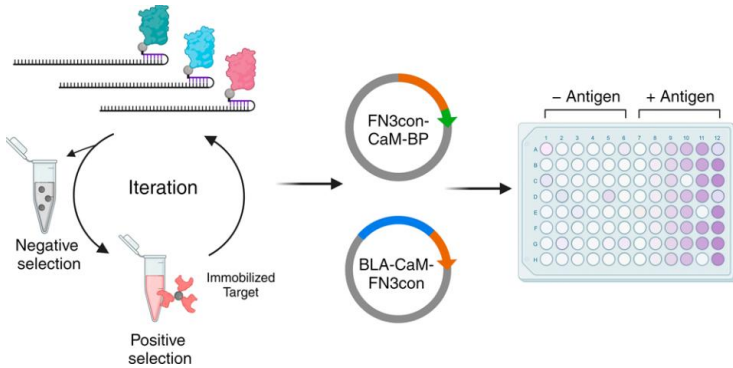
[Pandi et al. \(2023\) Nat Communications. vol.14\(7197\).](https://doi.org/10.1101/2024.03.25.586624)



[Jia B. et al. \(2024\) J Biosci Bioeng, 137\(4\):321-328.](https://doi.org/10.1101/2024.03.25.586624)



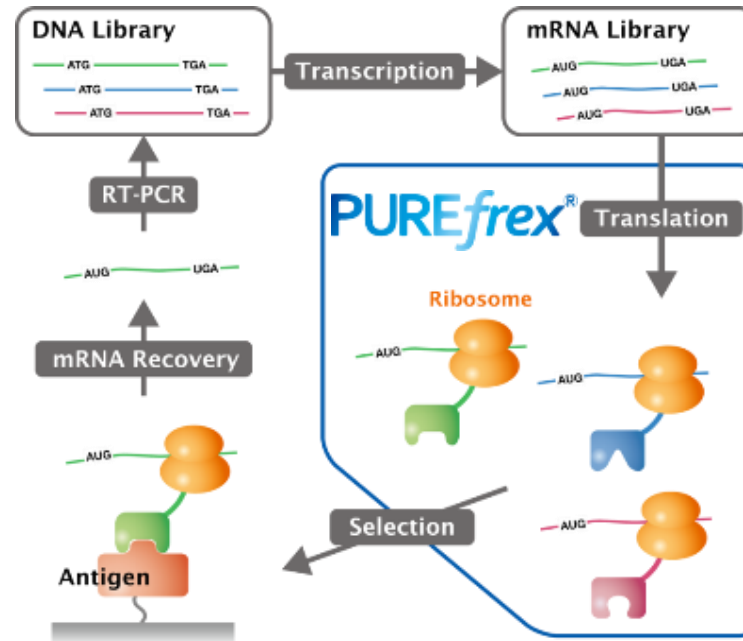
-Broad applications, yet to come!-



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

in vitro protein selection technology

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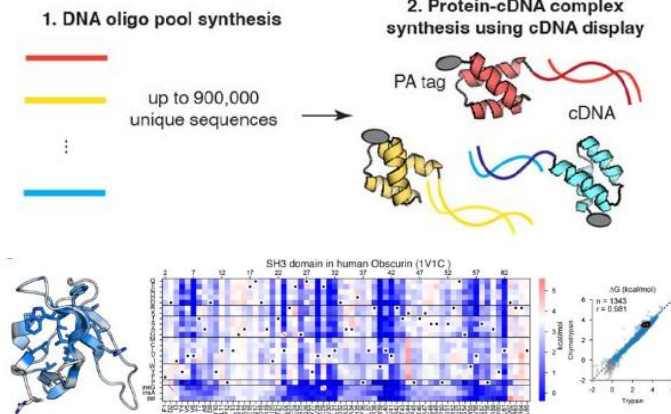
Licensed technology under JP4931135 etc.

◆ Advanced screening system for Biologics

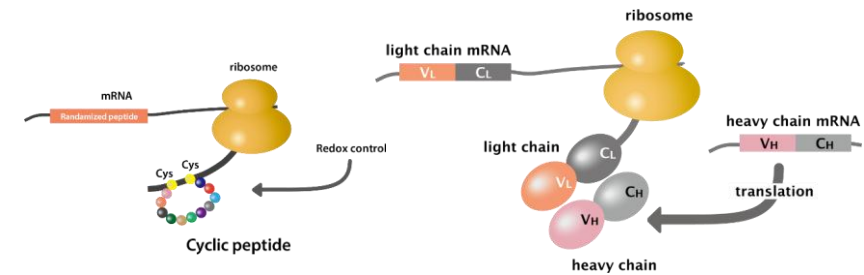
- **mAb (scFv / Fab)**
- **VHH**
- **Cyclic peptide**

◆ High Selection Efficiency

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



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



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Contact information

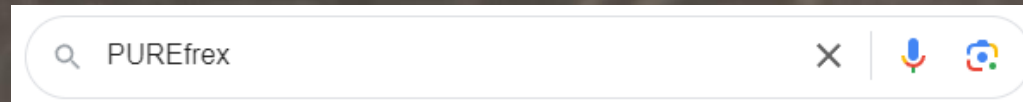
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- *E. coli*



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- High-cell density fed-batch
- Control of critical fermentation parameters
- Animal-free media



Purification types

- Ion-Exchange
- Hydrophobic Interaction Chromatography (HIC)
- Mix mode
- Affinity



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- Additional custom QC development available
- QP release