For more information

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

Reconstituted cell-free protein synthesis kit

PURE*frex*®

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

GeneFrontier

PEGS Boston
12-16 of May, 2025

Corporate Summary

GeneFrontier

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

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

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

Place : ***Chiba, Japan***

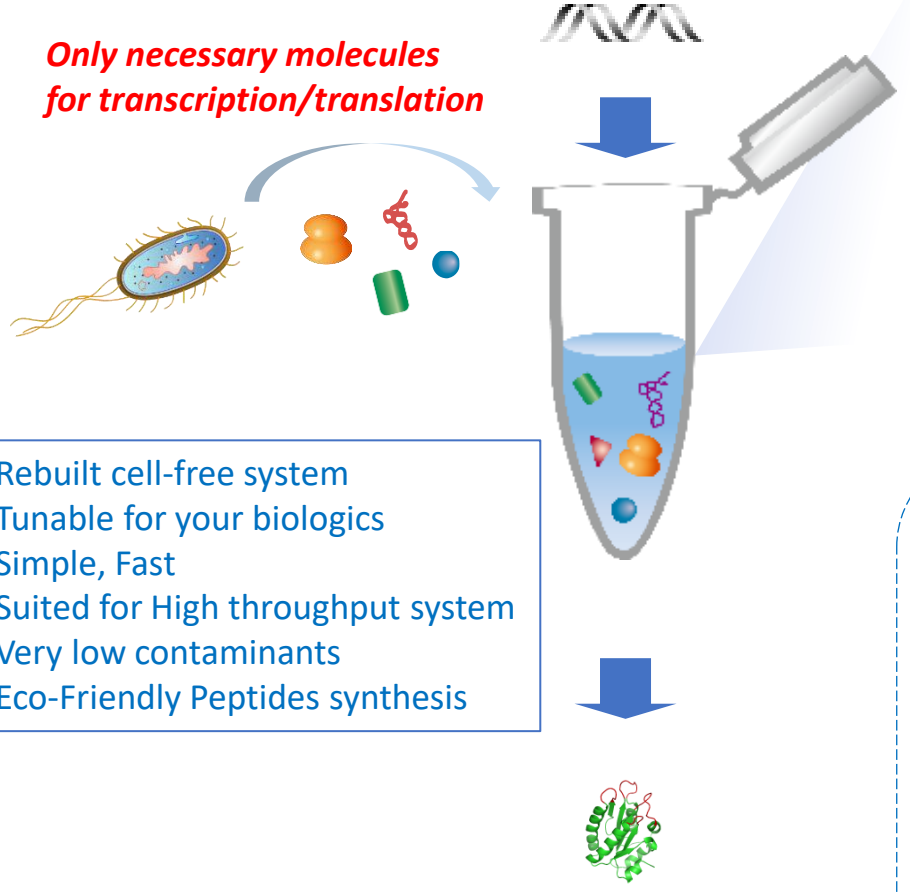
Mission : ***Rebuilding and Manipulating Biological system for Inspiring the world!***

Reconstituted cell-free protein synthesis kit

PURE*frex*®

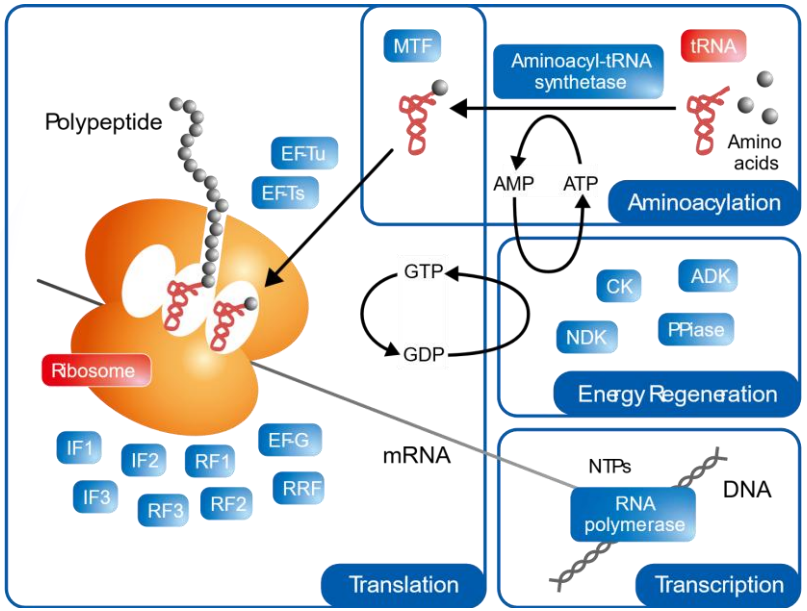
PURE (Protein synthesis Using Recombinant Elements) base cell-free expression

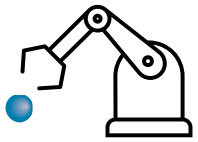
Only necessary molecules for transcription/translation

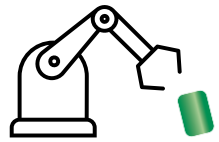


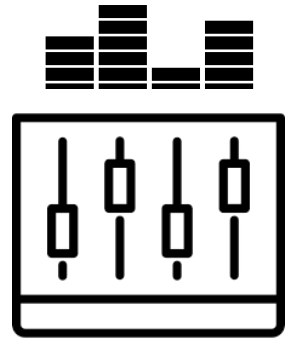
- ✓ Rebuilt cell-free system
- ✓ Tunable for your biologics
- ✓ Simple, Fast
- ✓ Suited for High throughput system
- ✓ Very low contaminants
- ✓ Eco-Friendly Peptides synthesis

Totally constructive, molecular based system









For more information

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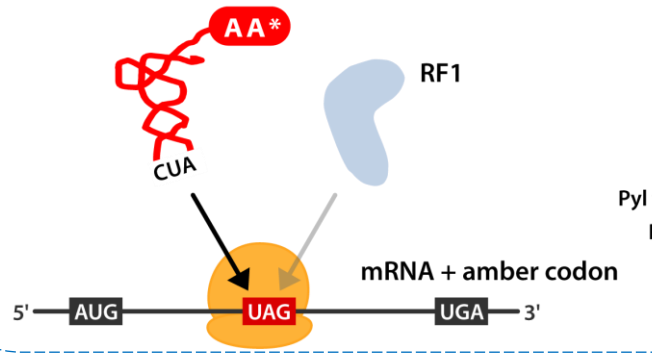
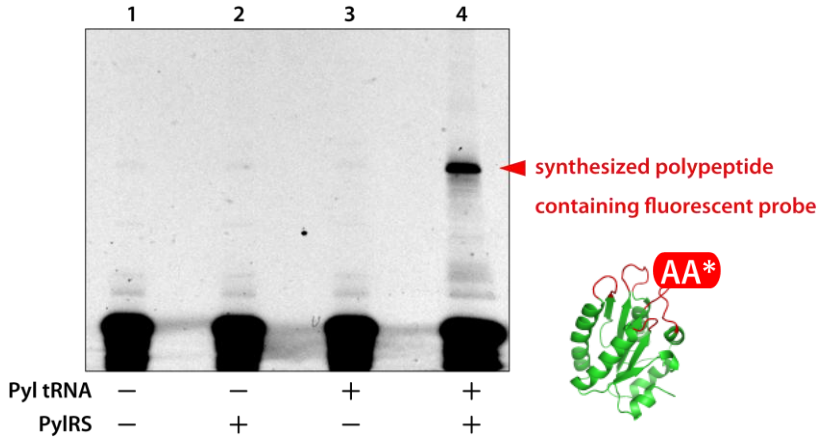
<Ex, Non-natural AA introduction>

Translation - RF1

suppressor tRNA + non-natural amino acid





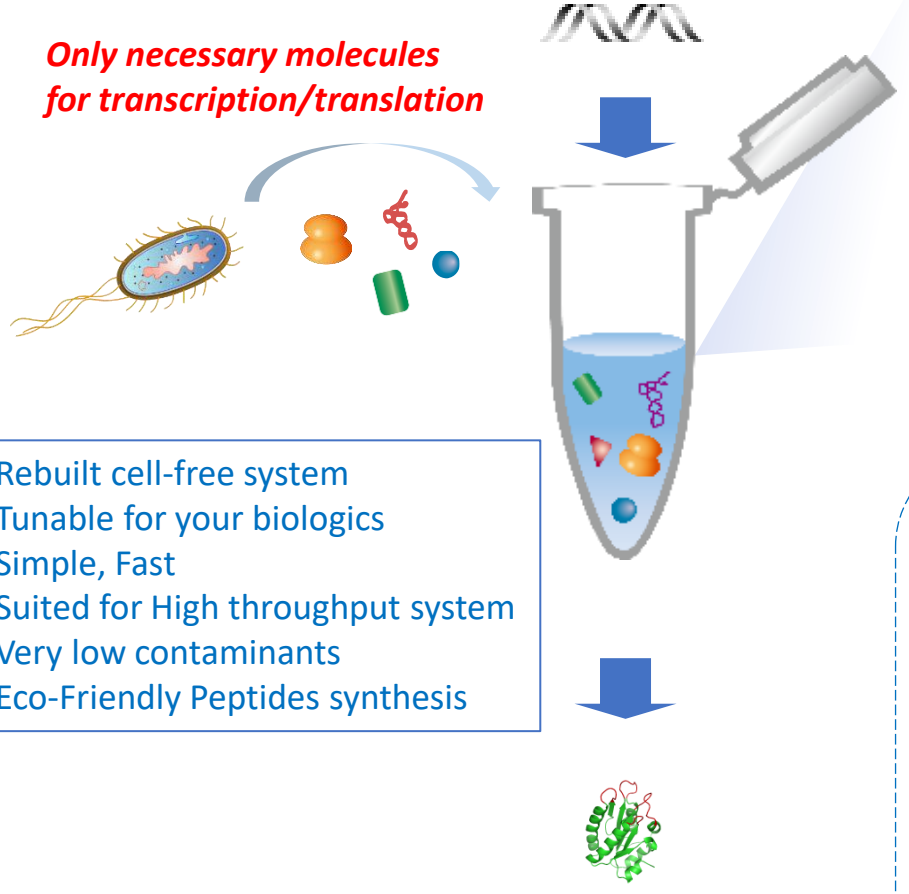
Ready-made kit is coming soon!

Reconstituted cell-free protein synthesis kit

PURE*frex*®

PURE (*Protein synthesis Using Recombinant Elements*) base cell-*free* *ex*pression

Only necessary molecules for transcription/translation



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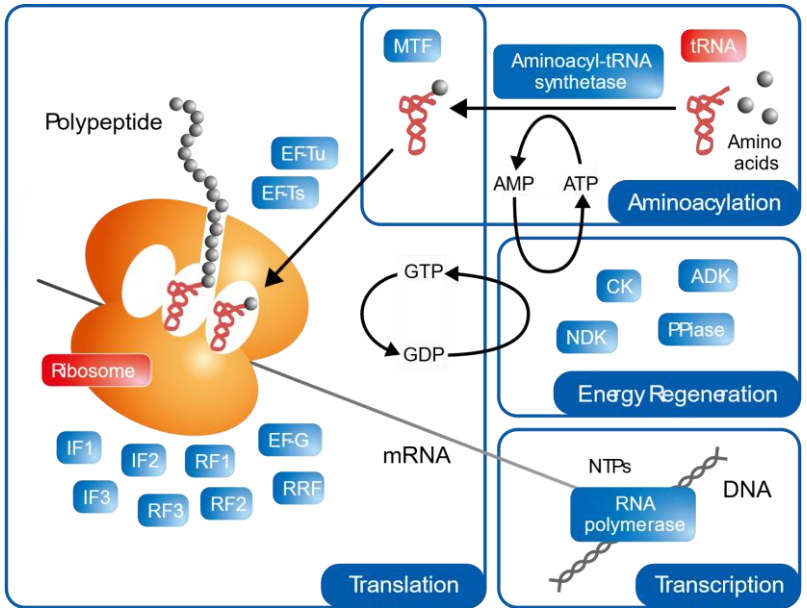

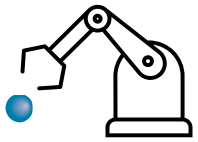

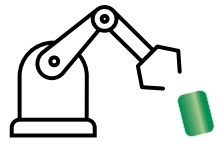

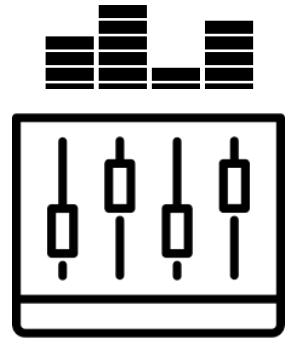

For more information

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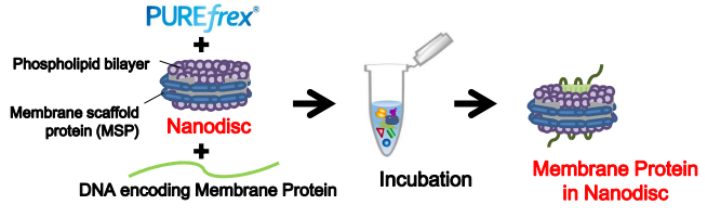
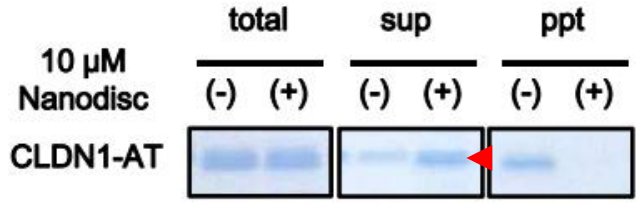
<Ex, Membrane protein with Nanodisc; artificial membrane-like structure>





The condition of membrane protein synthesis

Reaction mix	Template DNA	Incubation
PURE*frex*® *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.

Solubilized hCLDN1 was synthesized using PURE*frex*® and Nanodisc.

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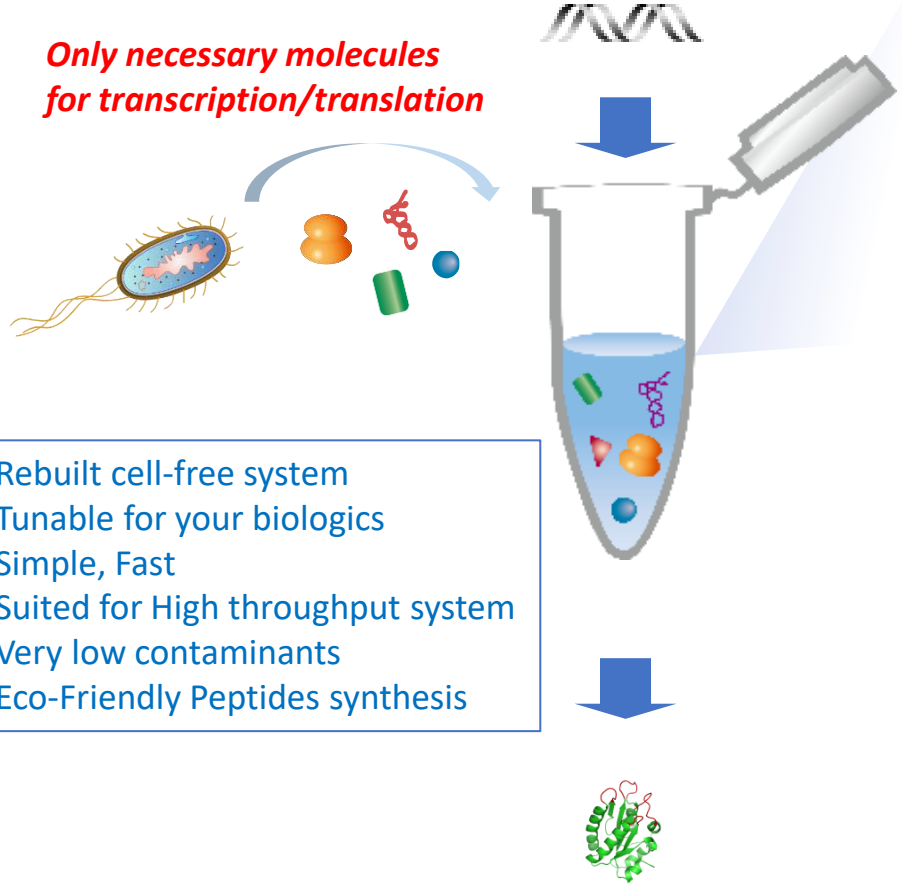

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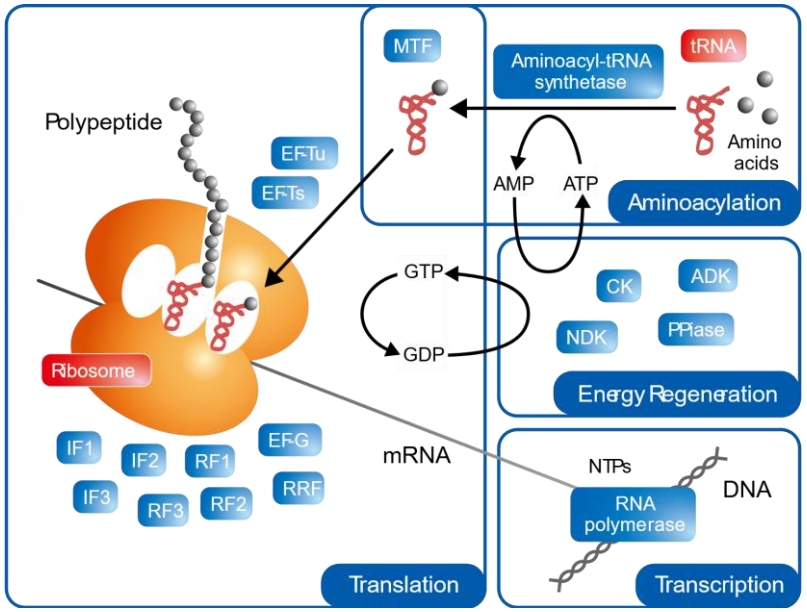
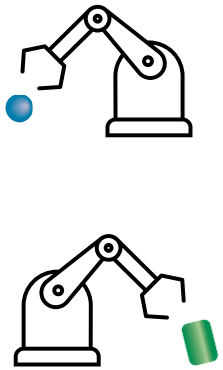
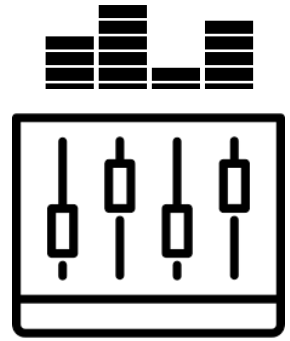






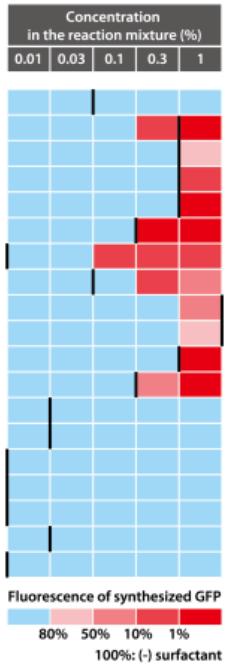
Experimental conditions for protein synthesis

Reaction mixture	Incubation	Template DNA
PURE*frex*®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 PURE*frex*® below the CMC.
- Some surfactants such as Triton X-100 and Tween 20 could be used even above the CMC.

Surfactant Name	CMC (%)
Digitonin	0.061
Sodium cholate	0.628
CHAPS	0.492
CHAPSO	0.505
n-Octyl-β-D-glucoside	0.731
n-Octyl-β-D-thioglucoside	0.278
n-Dodecyl-β-D-maltoside	0.009
n-Decyl-β-D-maltoside	0.087
n-Octyl-β-D-maltoside	1.064
Mega-8	1.864
Mega-9	0.839
Mega-10	0.245
Triton X-100	0.016
Triton X-114	0.011
NP-40	0.009
Tween 20	0.007
Tween 80	0.002
Brij 35	0.011
Brij 58	0.009



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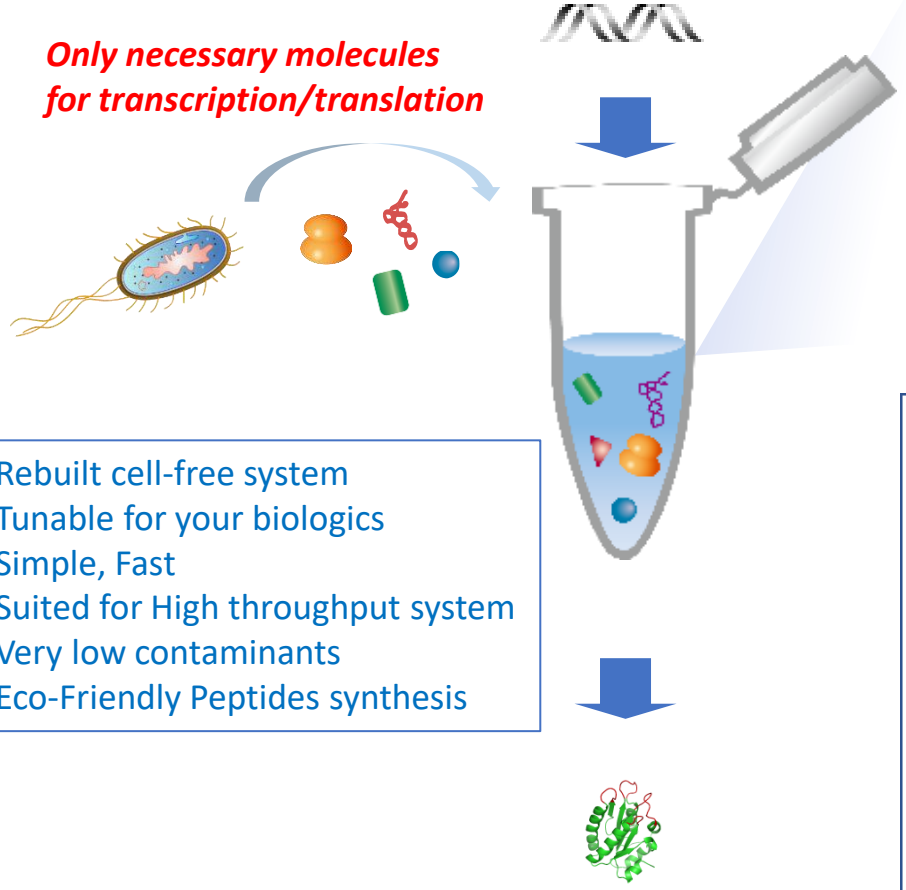

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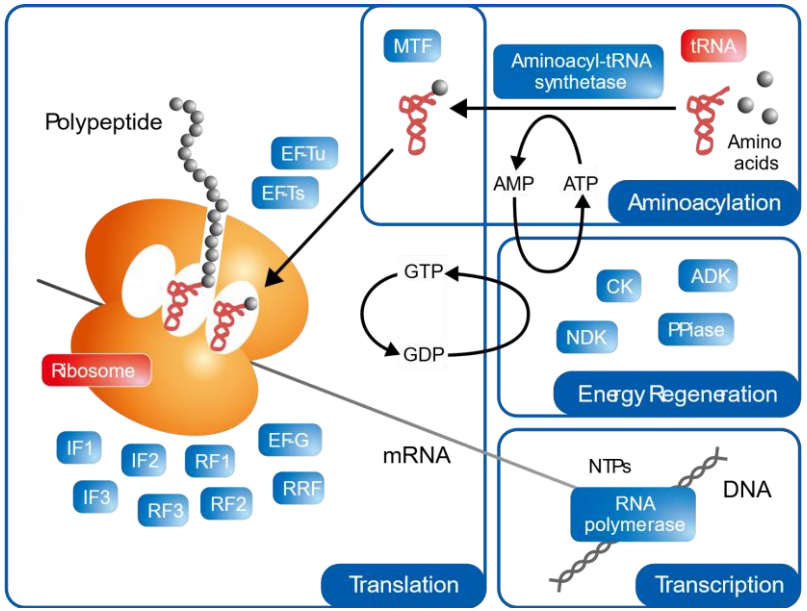

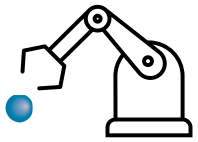

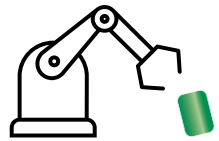

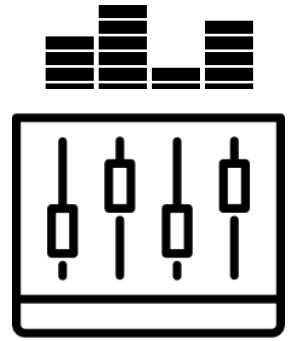

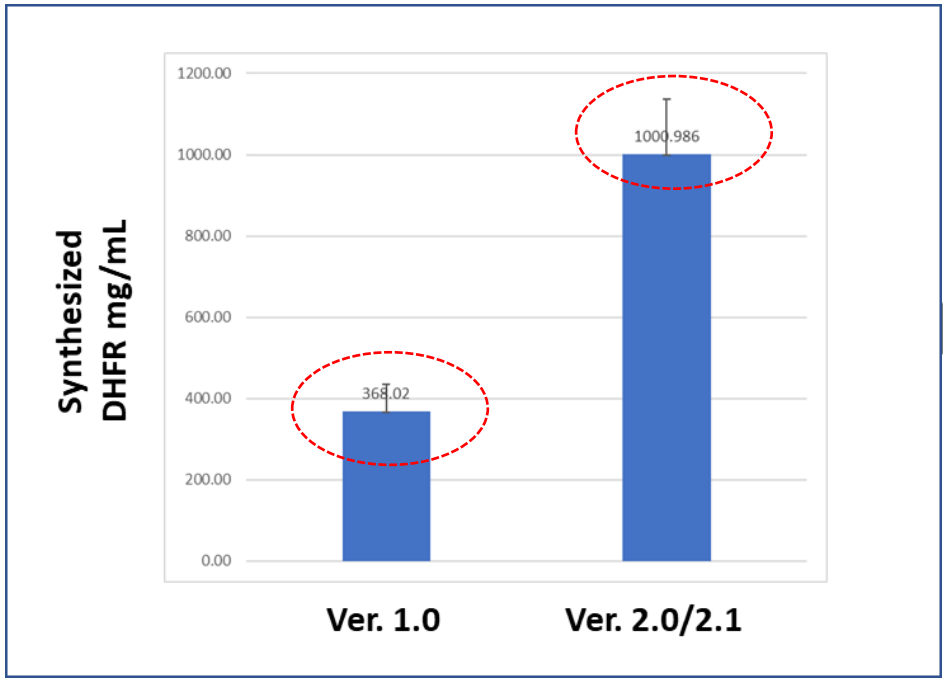

Versatile and Robust Platform for protein synthesis

Huge potential as New platform in Biotech industry

For more information

GeneFrontier

Reconstituted cell-free protein synthesis kit

PURE*frex*®

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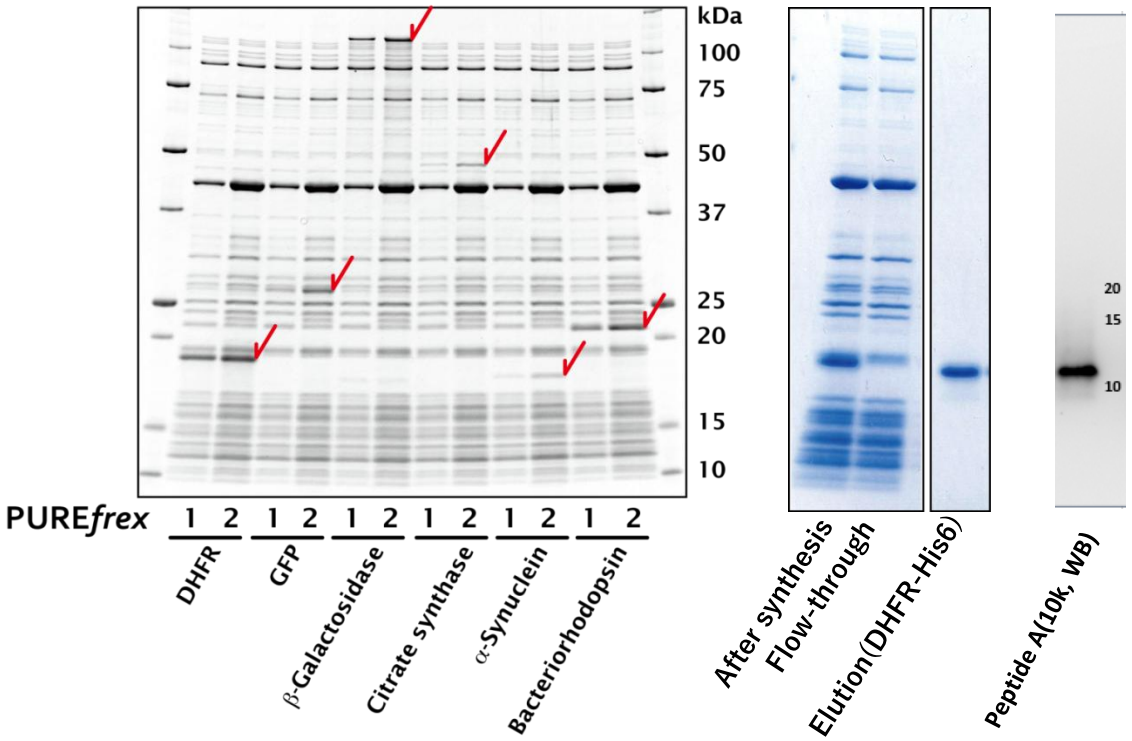
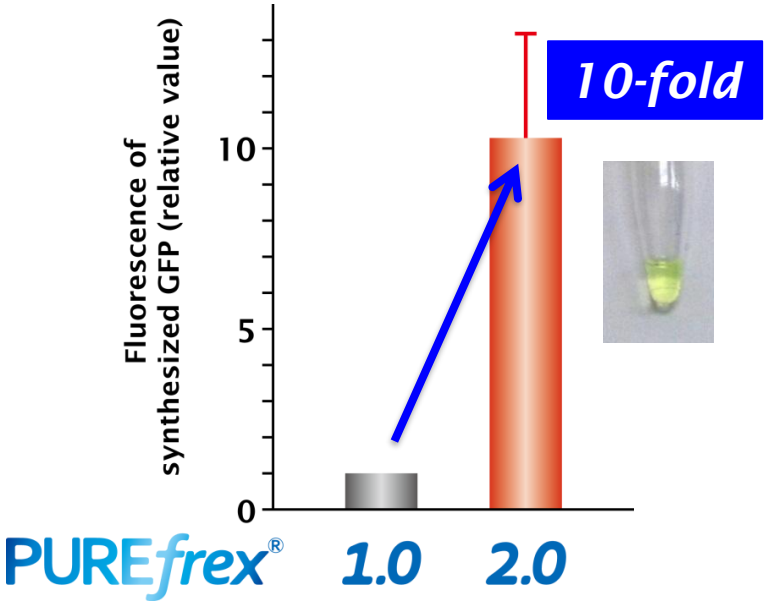
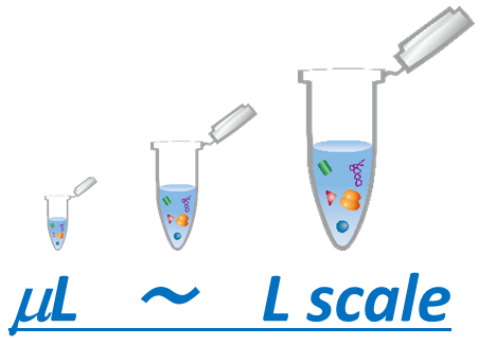
-Having good productivity-







Great Flexibility
from basic research
to industrial applications

- Reaction at 37ºC 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.

Reconstituted cell-free protein synthesis kit

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-KSF; AT rich codon on N-term-

Fab Heavy Chain (Herceptin)

Herceptin Fab HC

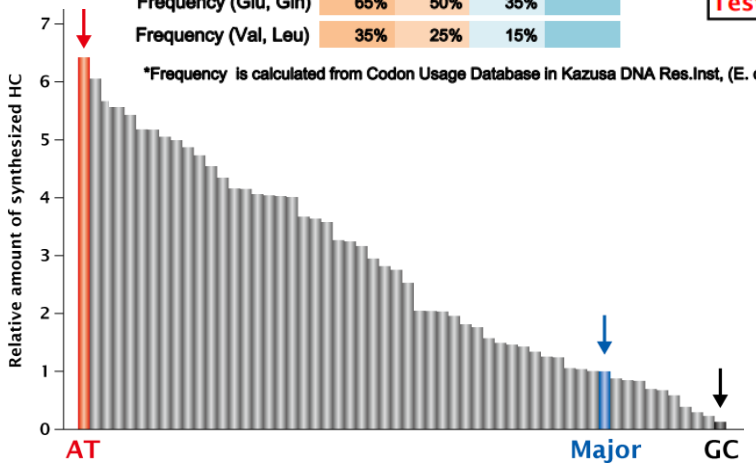
Met- Glu-Val-Gln-Leu-Val- FLAG

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	ttg	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		

All clones; 384
Tested clones; 56

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

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

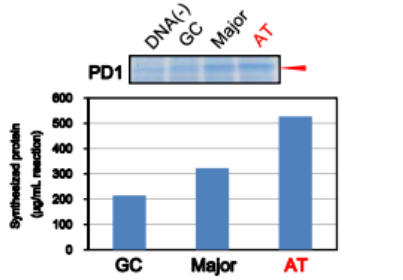


Design of DNA template is important.
Manual is Free to download from our Web site here.

PD1

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

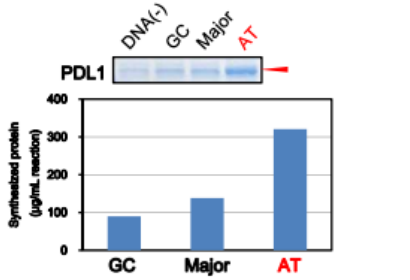
N-term type	1 Met	2(36) Thr	3(37) Phe	4(38) Ser	5(39) Pro	6(40) Ala	GC(%) 1-6 a.a.
GC	atg	acc	ttc	tcc	ccg	gcg	67%
Major	atg	acc	ttt	tct	ccg	gcg	56%
AT	atg	act	ttt	tca	cca	gct	39%



PDL1

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

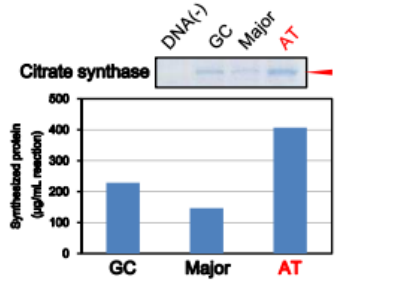
N-term type	1 Met	2(18) Ala	3(19) Phe	4(20) Thr	5(21) Val	6(22) Thr	GC(%) 1-6 a.a.
GC	atg	gcg	ttc	acc	gtg	acc	61%
major	atg	gcg	ttt	acc	gtg	acc	56%
AT	atg	gct	ttt	act	gta	aca	33%



Citrate Synthase

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

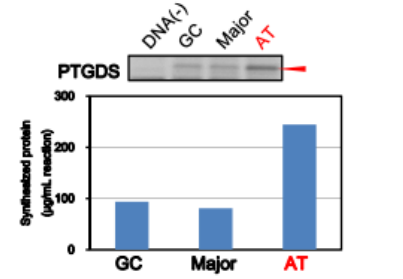
N-term type	1 Met	2(38) Ser	3(39) Ser	4(40) Ala	5(41) Ser	6(42) Glu	GC(%) 1-6 a.a.
GC	atg	tcc	tcc	gcg	tcc	gag	67%
major	atg	tct	tct	gcg	tct	gaa	44%
AT	atg	tca	tca	gct	tca	gaa	39%



PTGDS

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

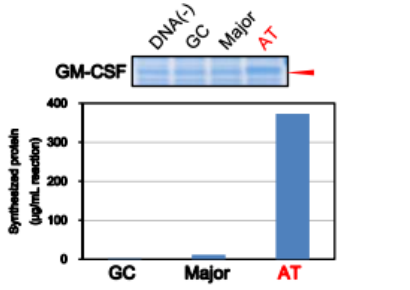
N-term type	1 Met	2(23) Ala	3(24) Pro	4(25) Glu	5(26) Ala	6(27) Gln	GC(%) 1-6 a.a.
GC	atg	gca	ccg	gaa	gca	cag	61%
major	atg	gcg	ccg	gaa	gcg	cag	72%
AT	atg	gca	cct	gaa	gct	caa	50%



GM-CSF

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

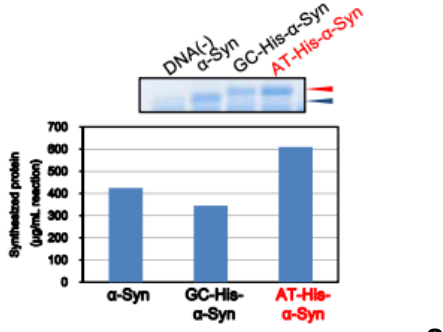
N-term type	1 Met	2(18) Ala	3(19) Pro	4(20) Ala	5(21) Arg	6(22) Ser	GC(%) 1-6 a.a.
GC	atg	gcg	ccg	gcg	cgc	tcc	83%
major	atg	gcg	ccg	gcg	cgc	tct	78%
AT	atg	gca	cct	gct	aga	tca	50%



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 Met	2 His	3 His	4 His	5 His	6 His	7 His	8 Gly	9 Ser	GC(%) 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-

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	ttg	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		

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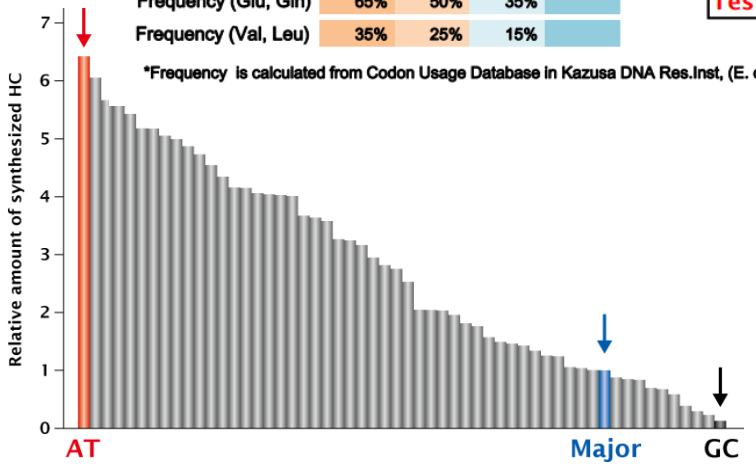
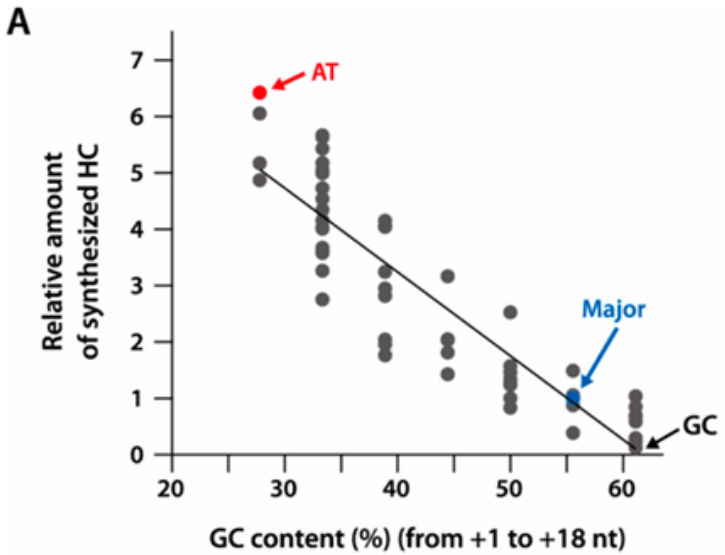
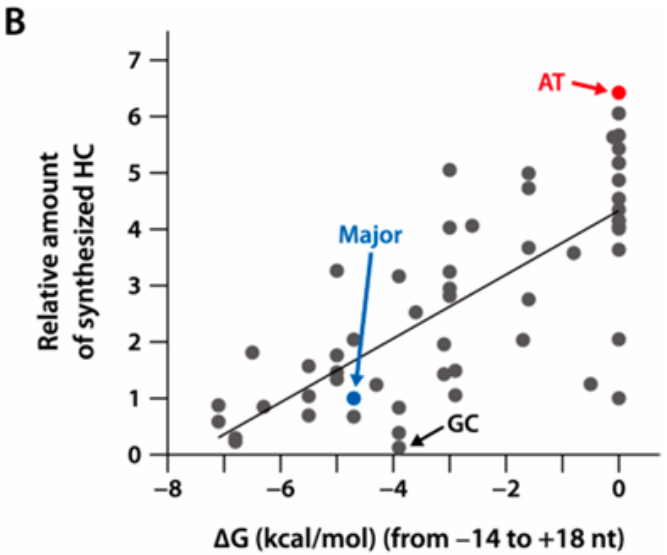






C

	1	2	3	4	5	6		
Name	Met	Glu	Val	Gln	Leu	Val	GC (%)	ΔG (kcal/mol)
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

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

Design of DNA template is important.
Manual is Free to download from our Web site here.

Reconstituted cell-free protein synthesis kit

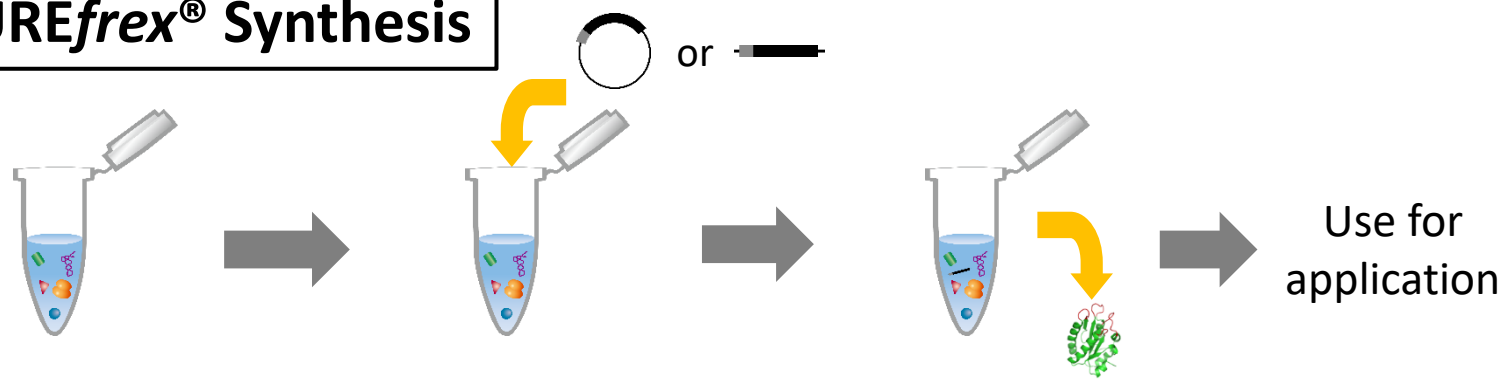
PURE*frex*®

For more information

GeneFrontier

-Improve Expression from Days to Hours-

PURE*frex*® Synthesis

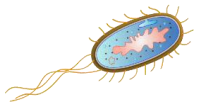


Mix solution I, II and III → Addition of template DNA → Incubation for 2-4 hours (Purification, if necessary) → Use for application

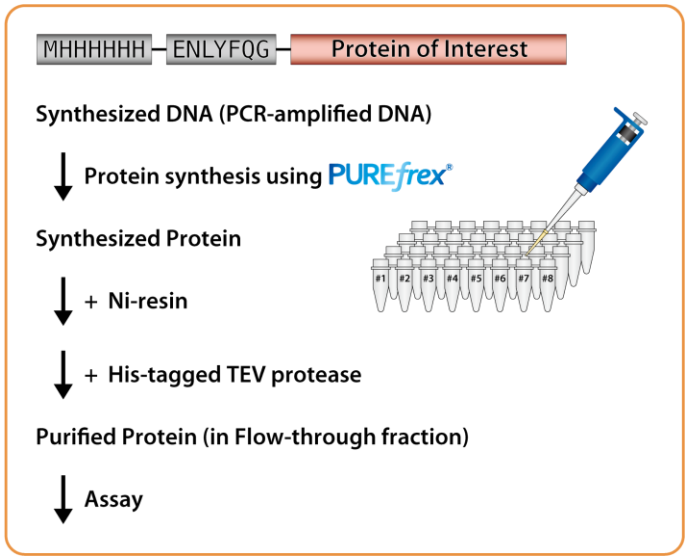
Total: 2-4 hours

E. coli Expression

Transformation and overnight cultivation → Induction and expression → Purification → Use for application



Total: 3-4 days



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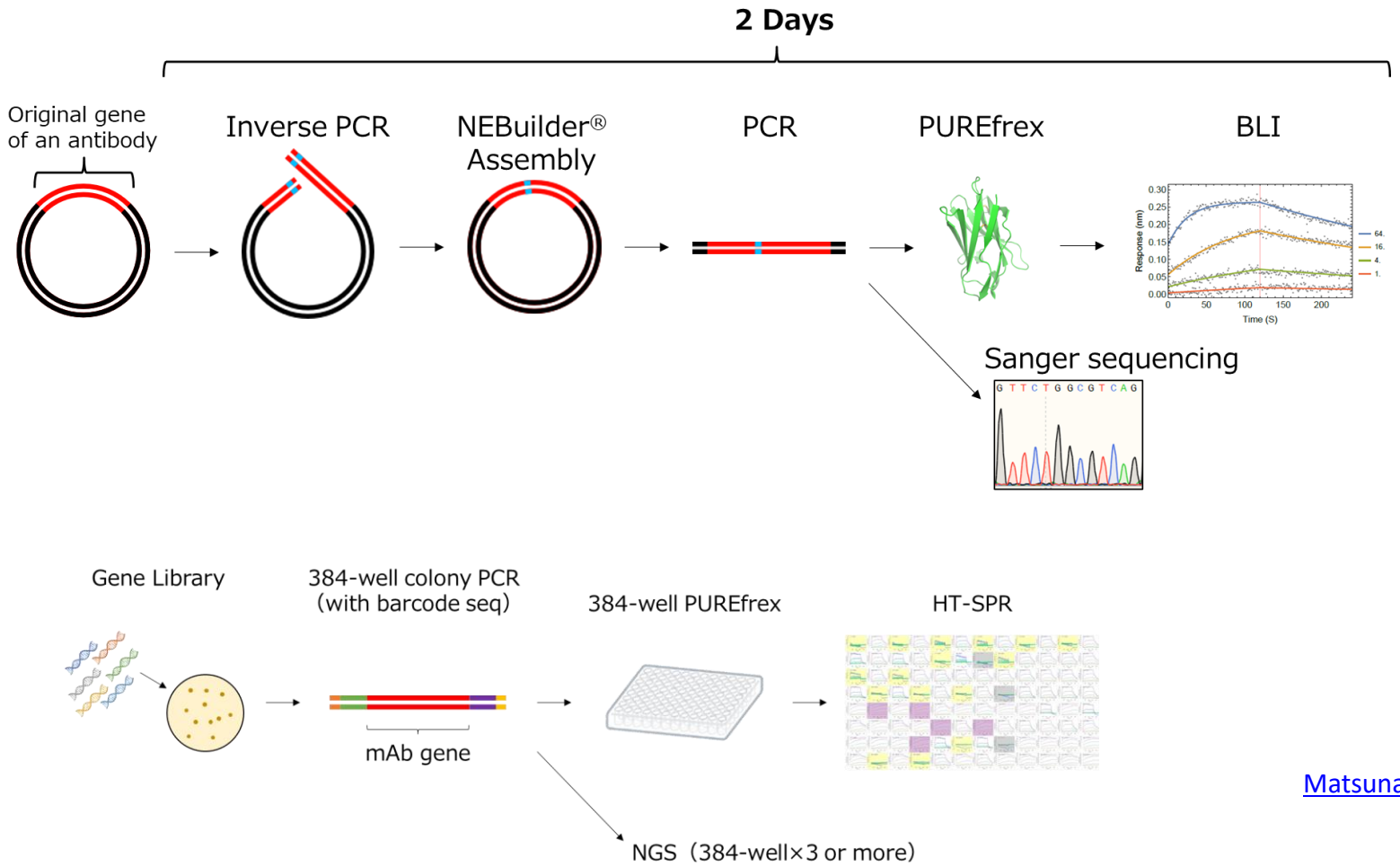
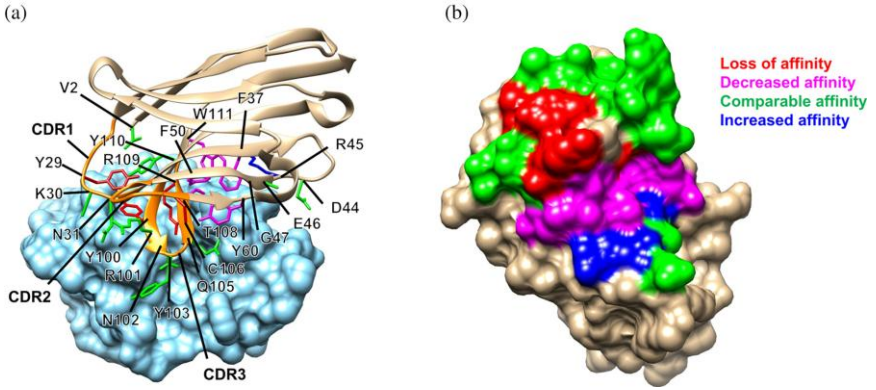
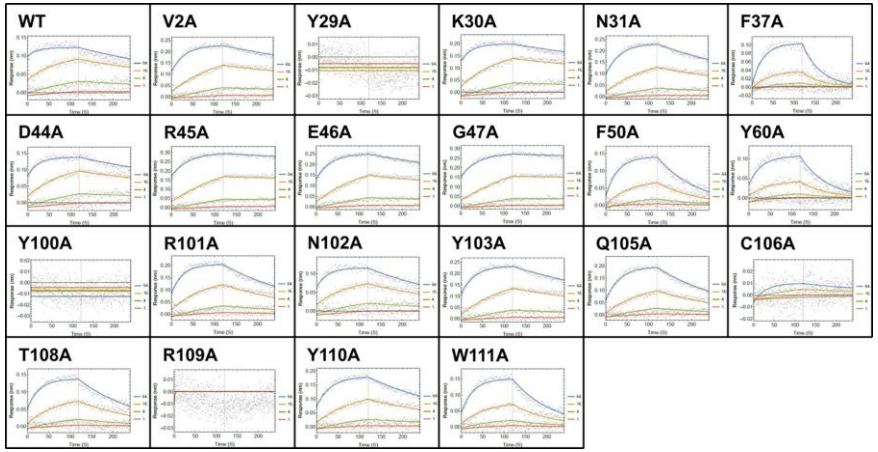
-Improve Validation from Weeks to Days-

FASTIA: Fast Interaction Analysis







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

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For more information

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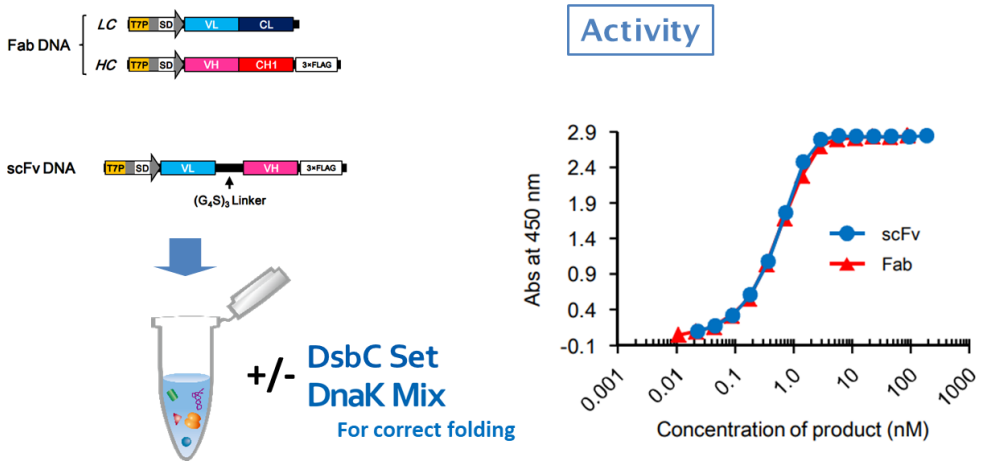
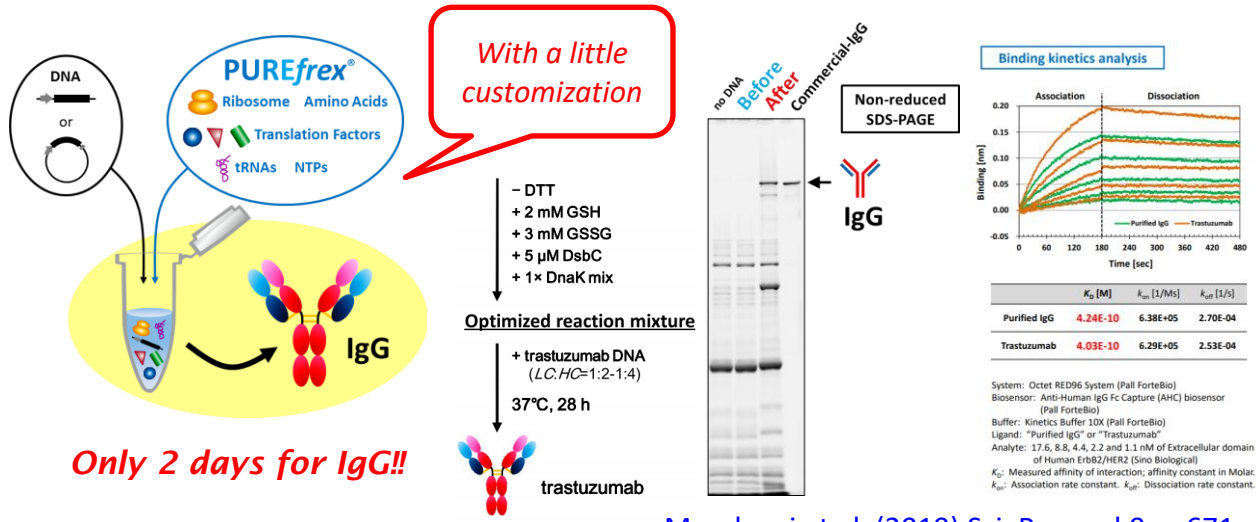
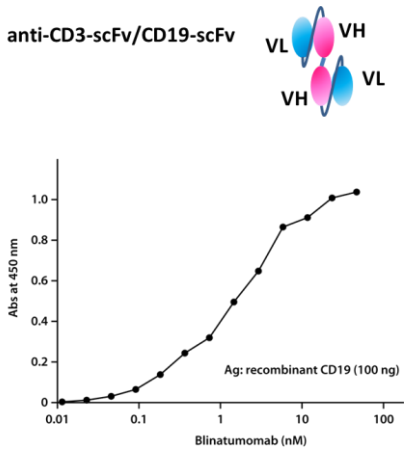
-Expression of scFv, Fab, IgG and more-



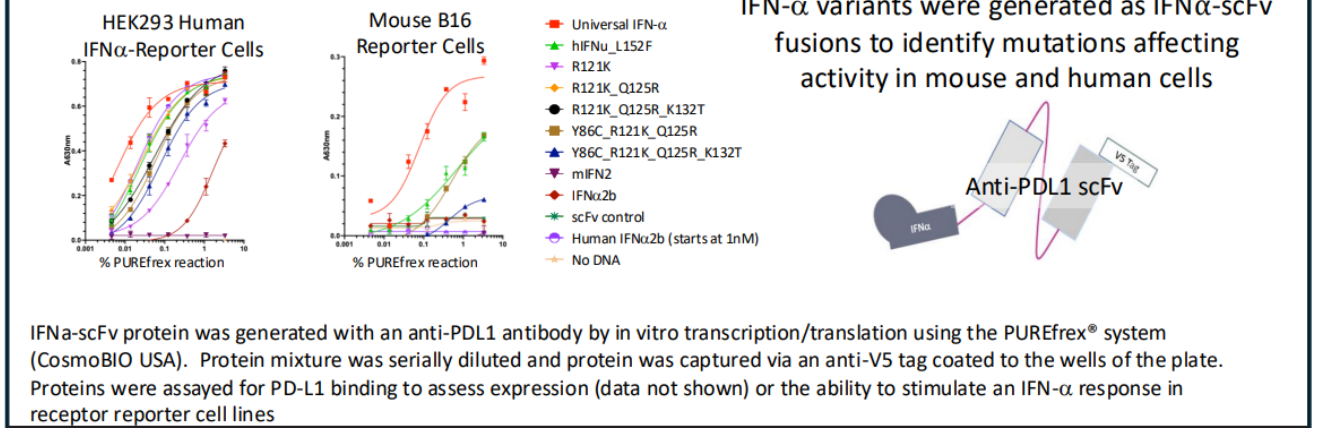
Murakami et al. (2019) Sci. Rep. vol.9, p.671. (Supplementary Information)



Murakami et al. (2019) Sci. Rep. vol.9, p.671.



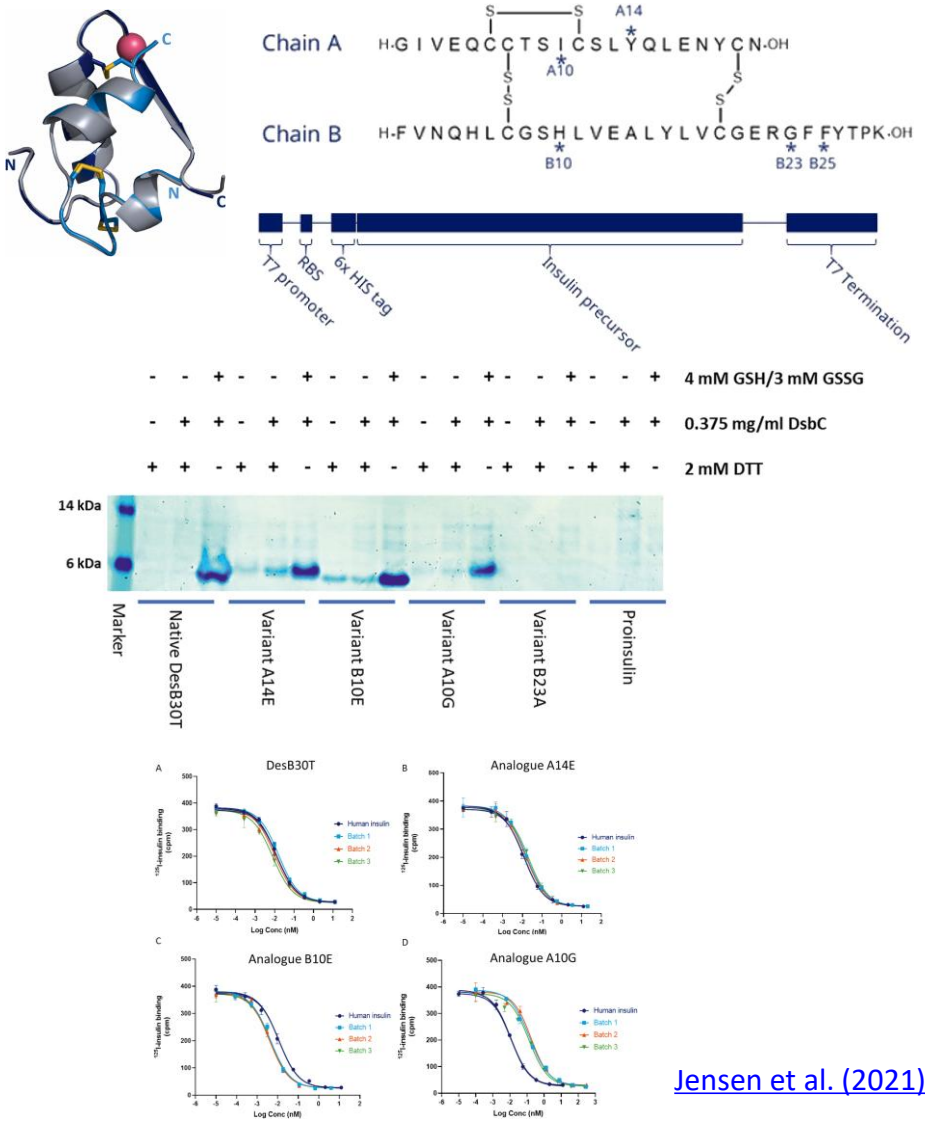
Round 1: IFN-α variants tested by in vitro transcription/translation



IFNa-scFv protein was generated with an anti-PDL1 antibody by in vitro transcription/translation using the PURExpress® 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	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Clm24	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	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Clm24	✓	✓	X	✓	✓	✓	✓	✓	✓	✓
BL21	X	X	X	X	X	X	✓	✓	✓	✓
759	✓	✓	X	✓	✓	✓	✓	X	✓	✓

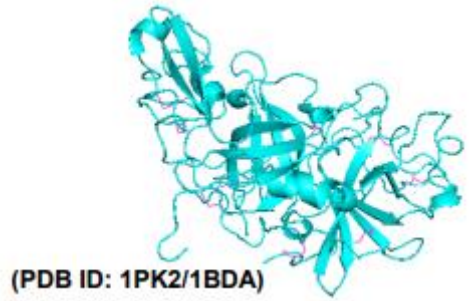
DeWinter et al. (2023) ACS Synth. Biol. vol.12, 4, p1216. (Supplementary Information)

Jensen et al. (2021) Protein Expr. Purif., 186, 105910.

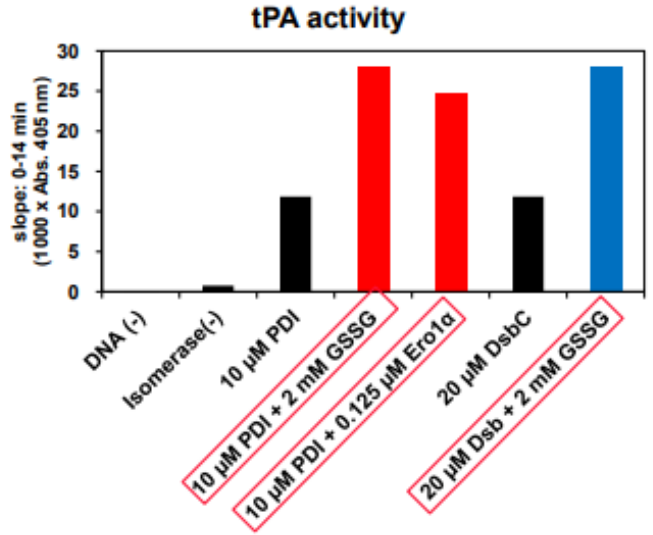
-Application for complex molecule-

truncated version of tissue plasminogen activator (tPA)

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

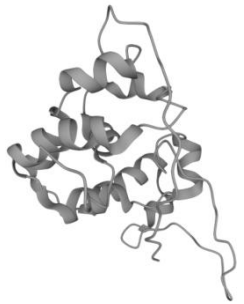


(PDB ID: 1PK2/1BDA)

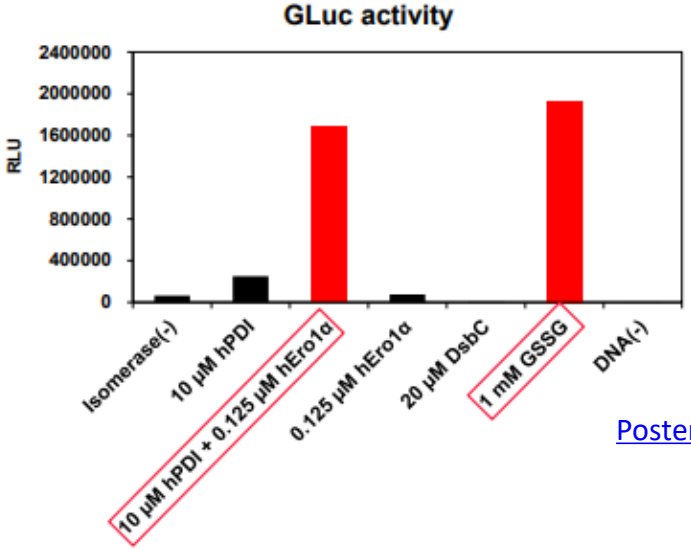


Gaussia Luciferase (GLuc)

Organism	*Gaussia princeps*
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)



Poster_PSSJ 2018

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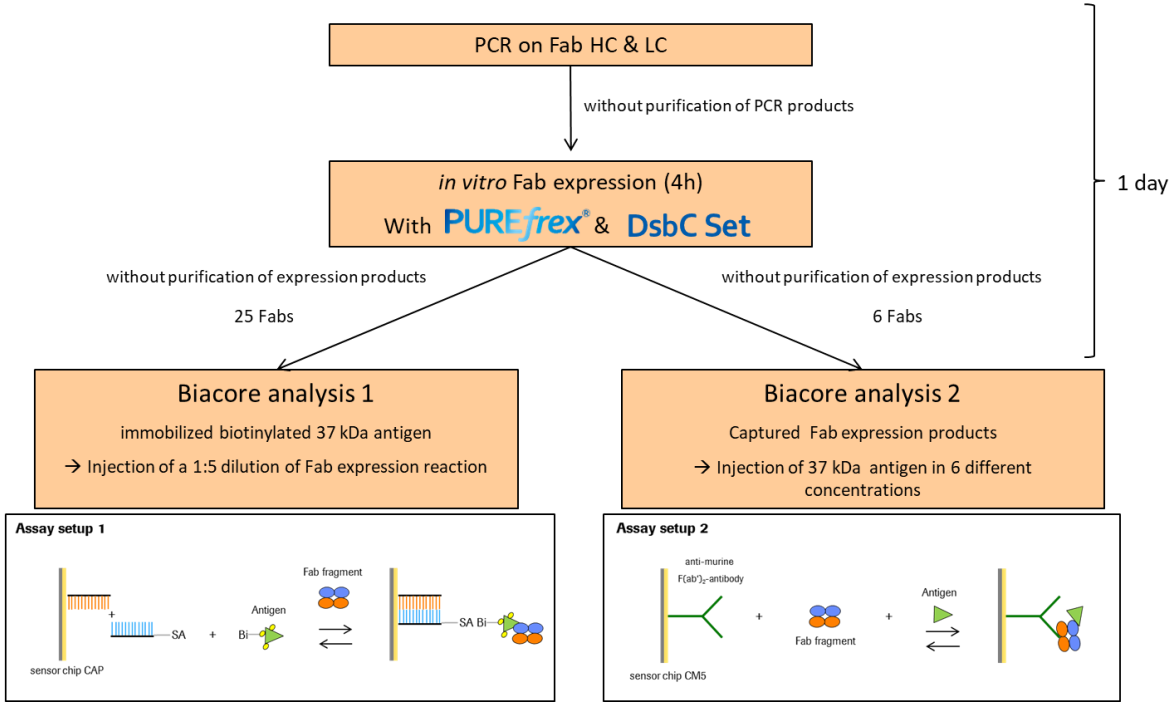
-For HTS of mAb-

Roche

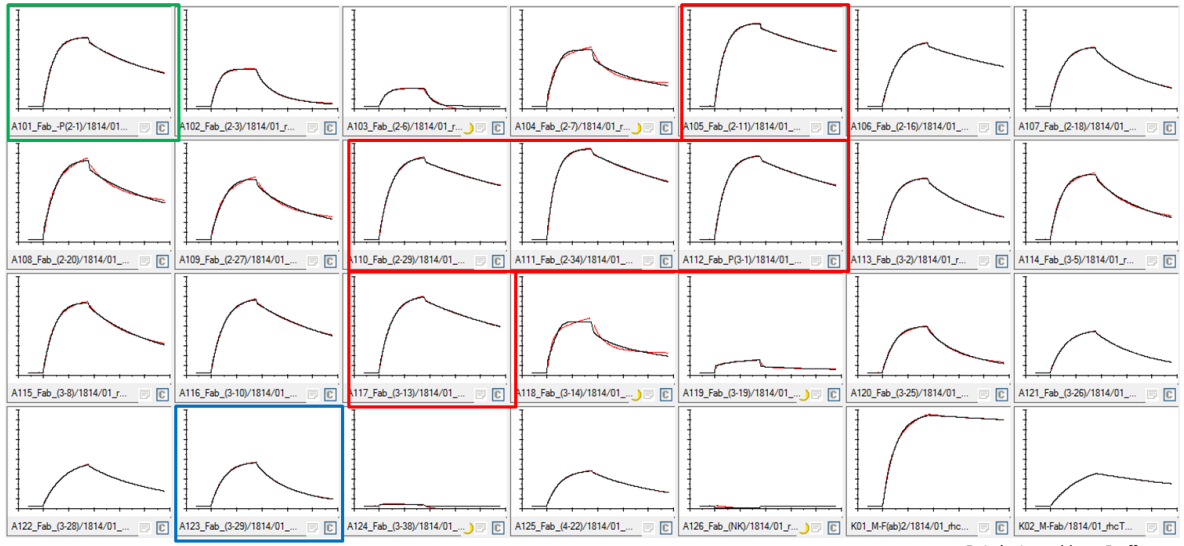
For more information

GeneFrontier

In vitro expression and Biacore analysis of Fab fragments



Kinetic analysis of 25 Fab binders

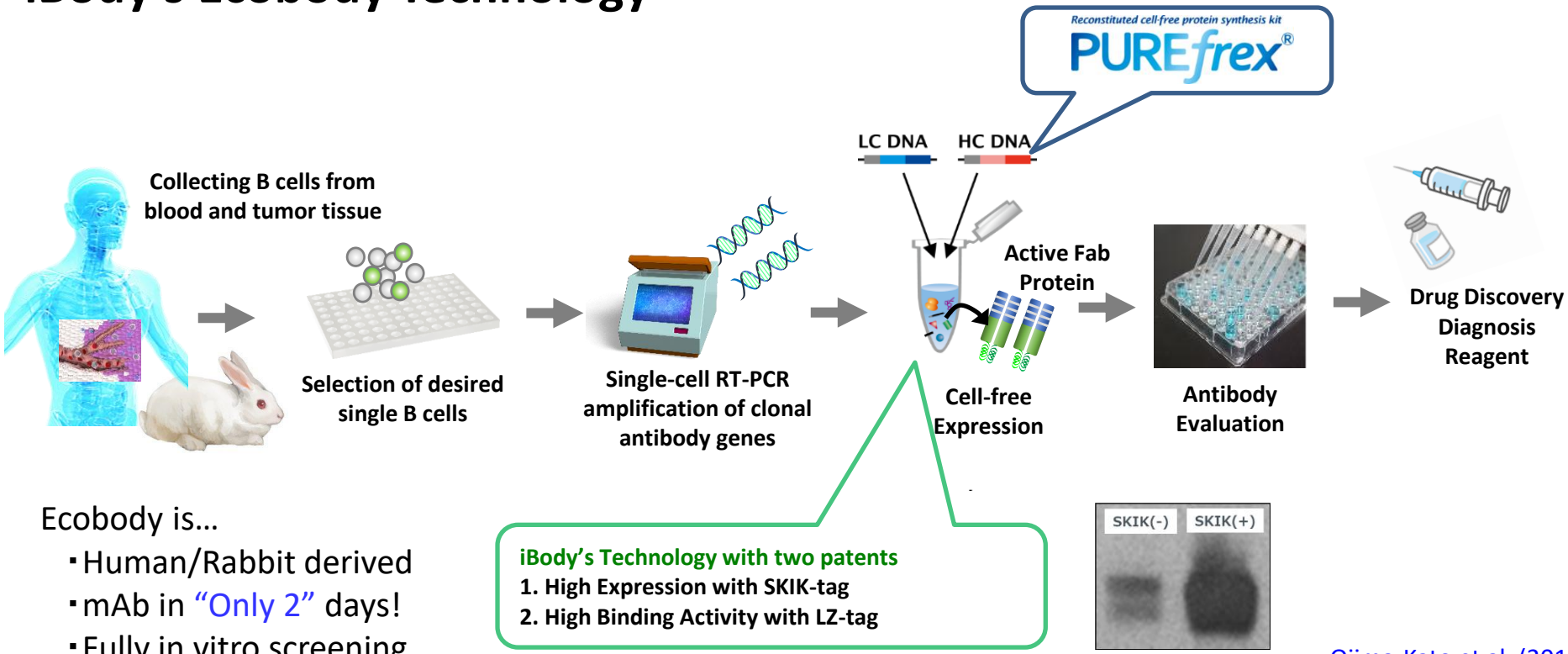


Data by Leopold von Proff

→ 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

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

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

✓ **Active Fab is expressed/screened in HT manner.**

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-For display technology-

Epsilon Molecular Engineering
Molecular Design for Human Life

For more information

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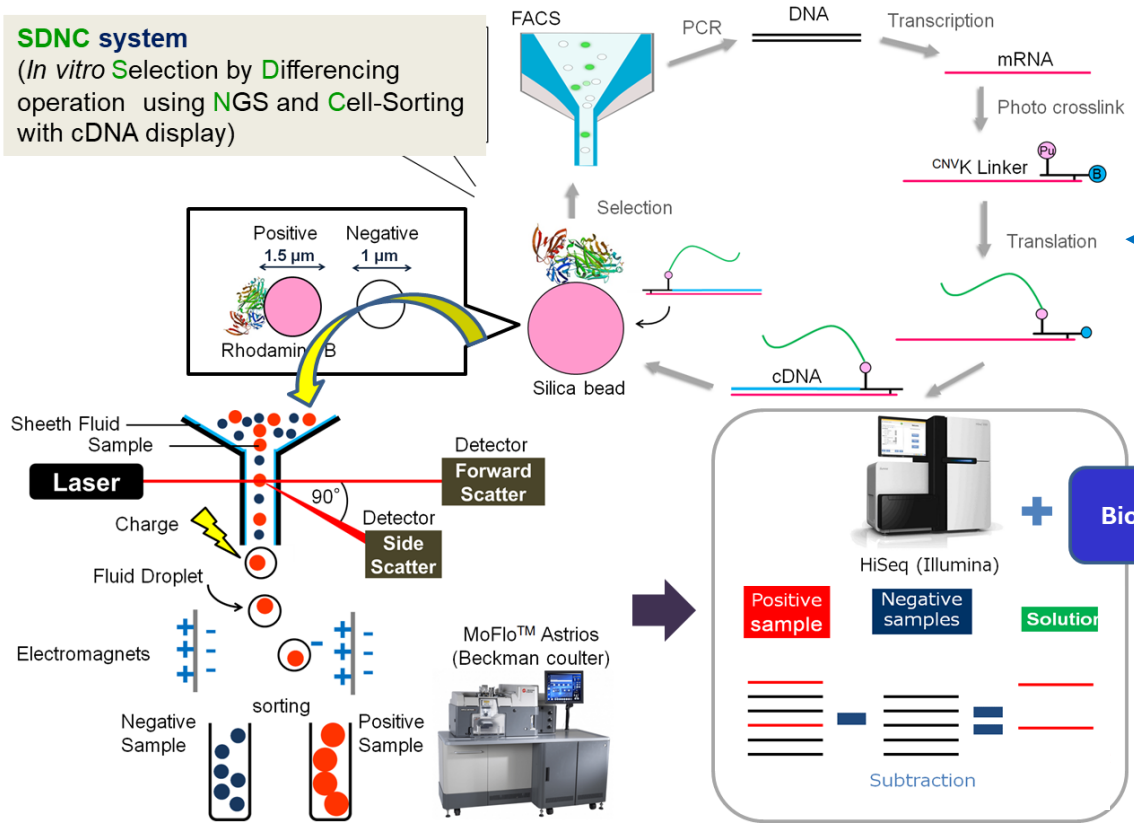
SDNC system
(*In vitro* Selection by Differencing operation using NGS and Cell-Sorting with cDNA display)



SDNC system is…
- for VHH/cyclic peptide
- with High diversity
- Fully in vitro screening

Reconstituted cell-free protein synthesis kit
PURE*frex*®

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

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

✓ PURE*frex* is applied for cDNA display based screening.

Reconstituted cell-free protein synthesis kit

PURE*frex*® -For display & HTS-

For more information

GeneFrontier

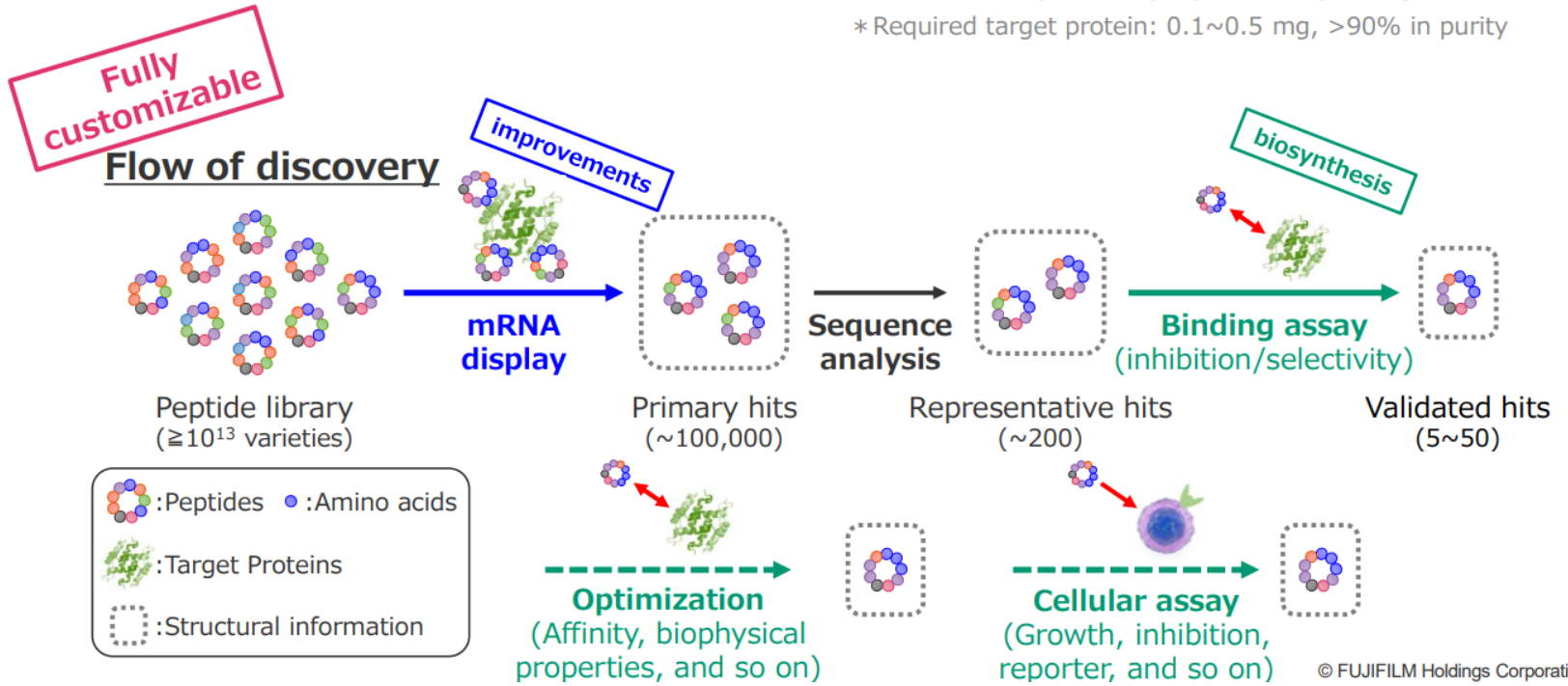
FUJIFILM peptide discovery services collaborated with PURE*frex*®

- ✓ **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.

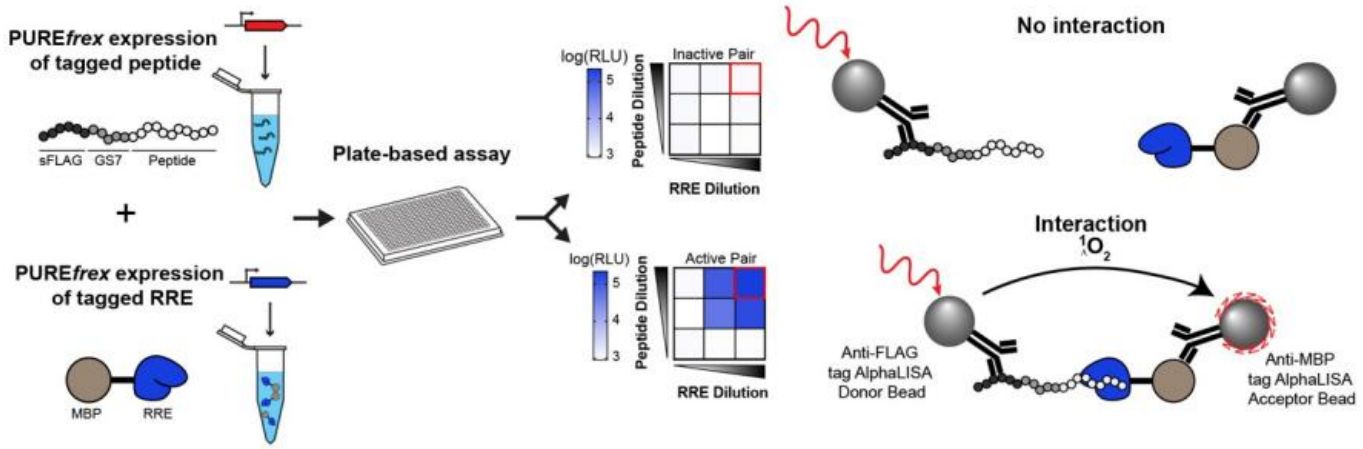
We provide a CRO service, in which we receive target ( ) from the customer* and return the structural information of the acquired peptides ( ).

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

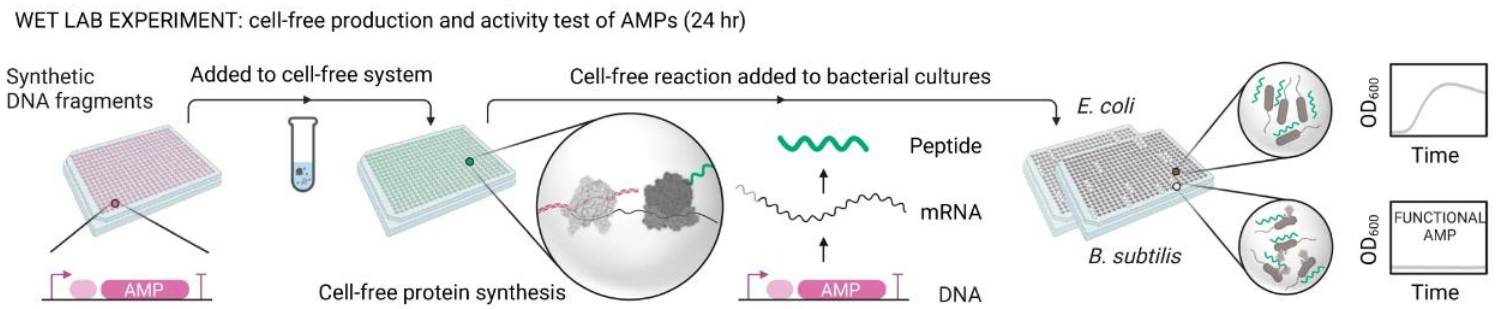


https://labchem-wako.fujifilm.com/europe/category/95372.html

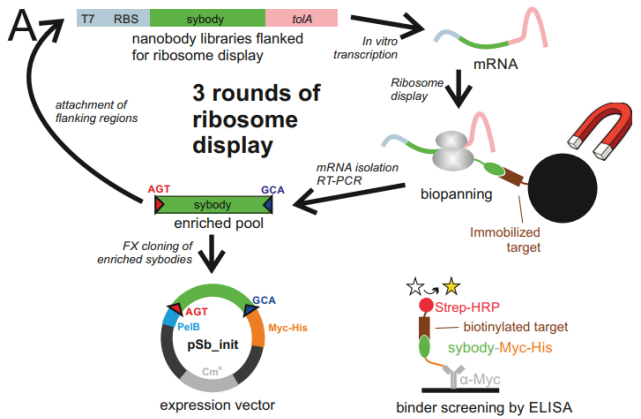
-Broad applications, yet to come!-



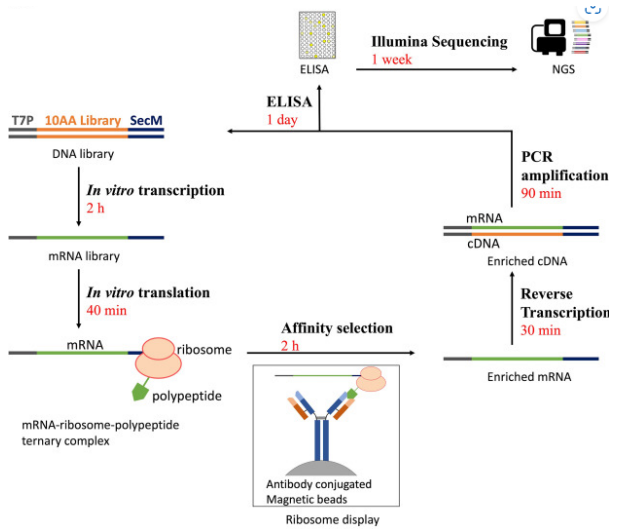
Wong et al. (2024) bioRxiv https://doi.org/10.1101/2024.03.25.586624.



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



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



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

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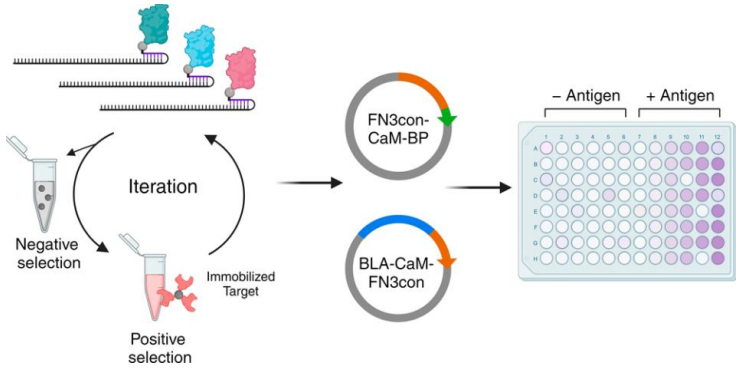
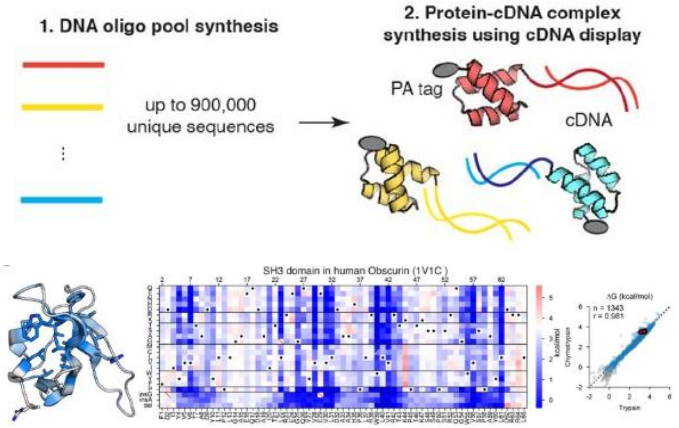
-Broad applications, yet to come!-



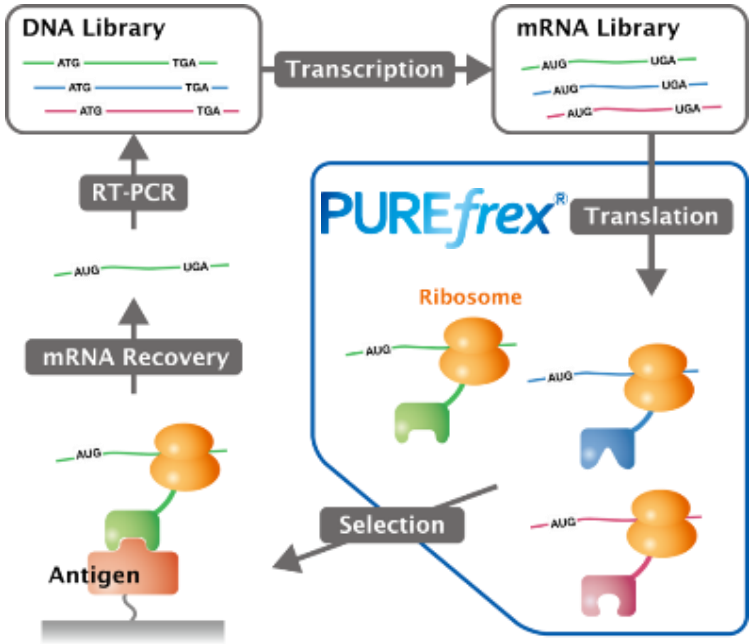
Chui Z. et al. (2024) ACS Sens, 9(6):2846-2857.



Tsuboyama et al. (2023) Nature, 620, p434.

in vitro protein selection technology

PURE*frex*® RD



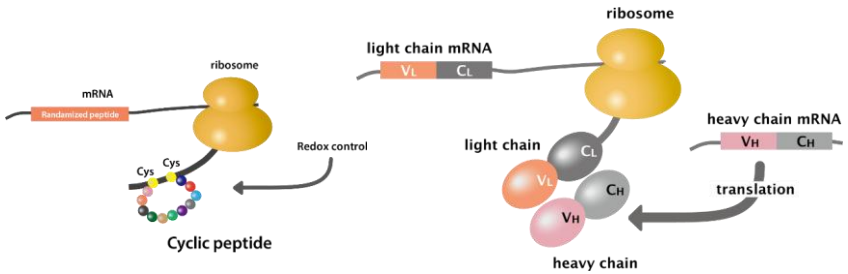
Licensed technology under JP4931135 etc.

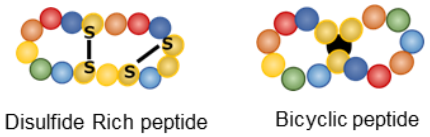
◆ **Advanced screening system for Biologics**

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

◆ **High Selection Efficiency**

- **Completely molecular based system**
- **>10^{12} diversity**

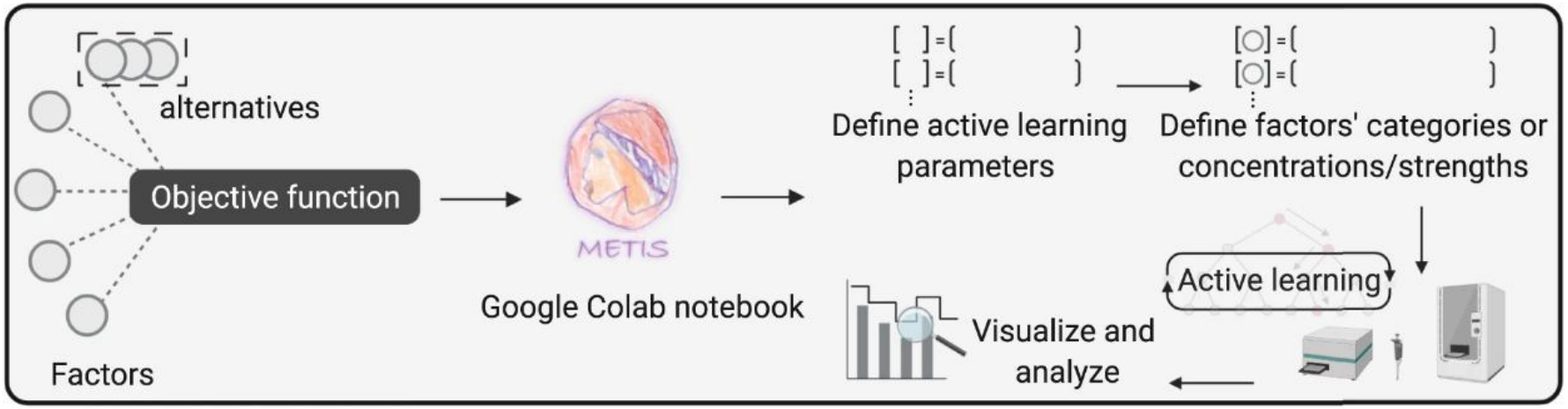




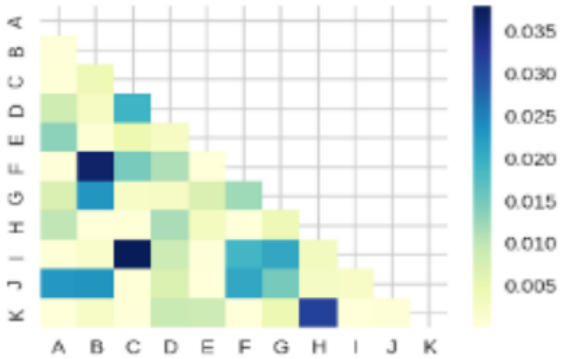
Licensed to

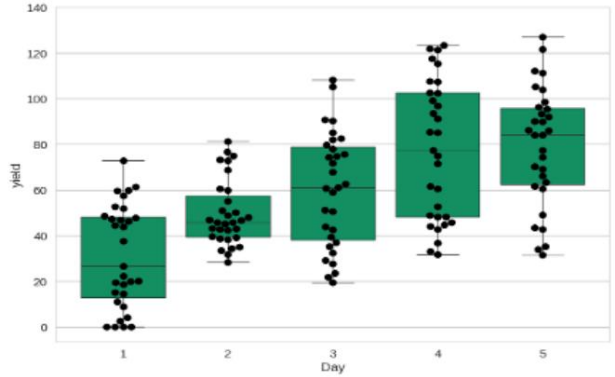
SUTRO BIOPHARMA

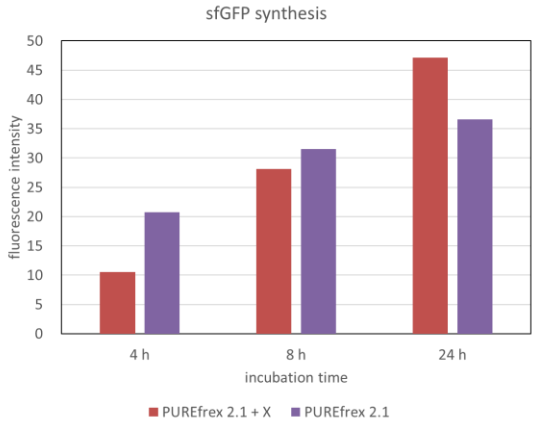
-Broad applications, yet to come!-



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

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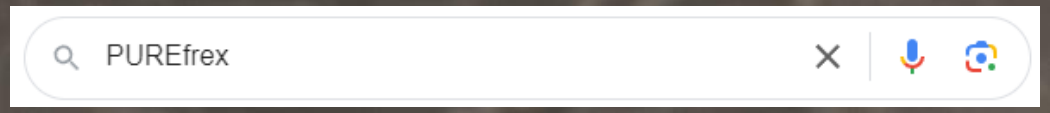
For reagent use for expression / screening of biologics

https://purefrex.genefrontier.com/



in vitro protein selection technology

PURE*frex*® RD

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

Takashi Ebihara, Ph.D., COO, GeneFrontier

E-mail: ebihara@genefrontier.com