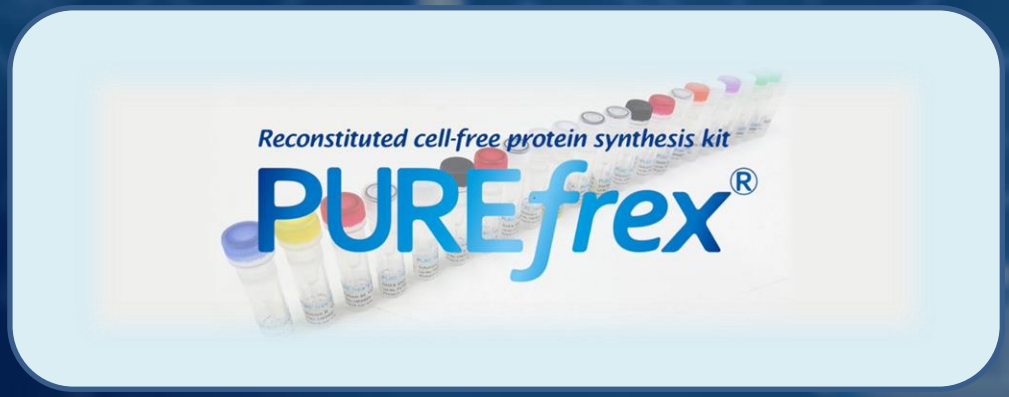
Cell Free Antibody Engineering:

Accelerating Discovery And Screening With PURExpress®



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

GeneFrontier

Discovery & Development US
12-13 of October, 2026

Corporate Summary

GeneFrontier

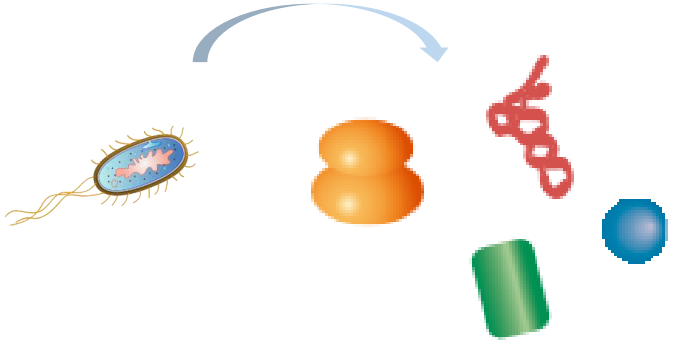
Founded :	***Oct 13th, 2010 (renewed)***
Shareholder :	***KANEKA Corporation (100%)***
People :	***18 (Ph.D. 9, MS 1)***
Place :	***Chiba, Japan***

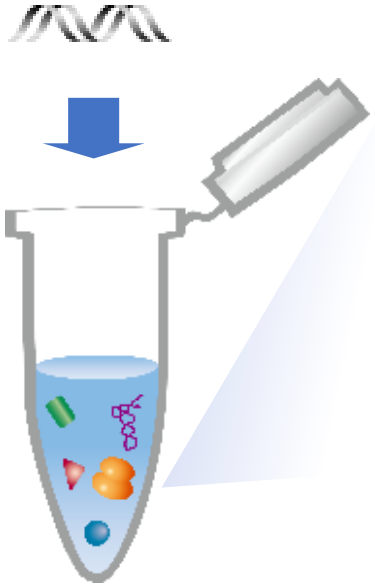


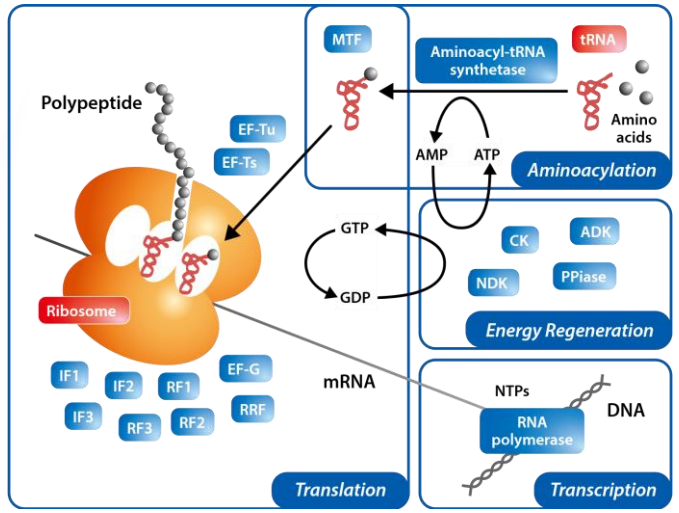


Mission : ***Rebuilding and Manipulating Biological system for Inspiring the world!***

Only necessary molecules
for transcription/translation







=Breaking down & Building up

PURE system

(Protein synthesis Using Recombinant Elements)



Shimizu Y. et al. Nature Biotechnology
vol 19, p751–755 (2001)



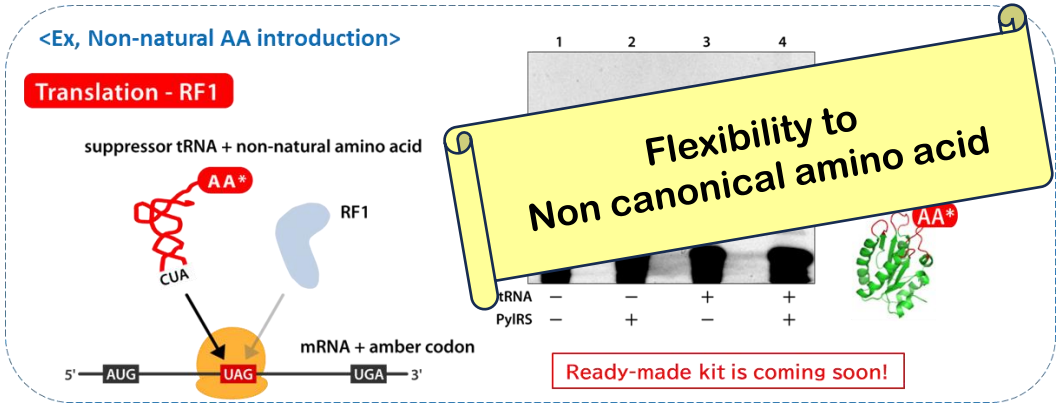


PURE system

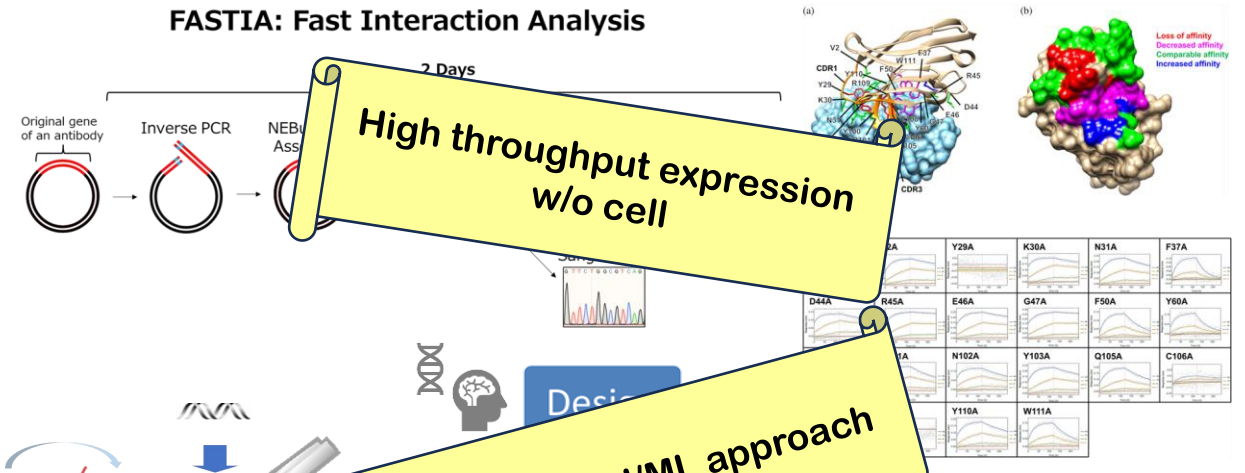
Reconstituted cell-free protein synthesis kit

PURE*frex*®

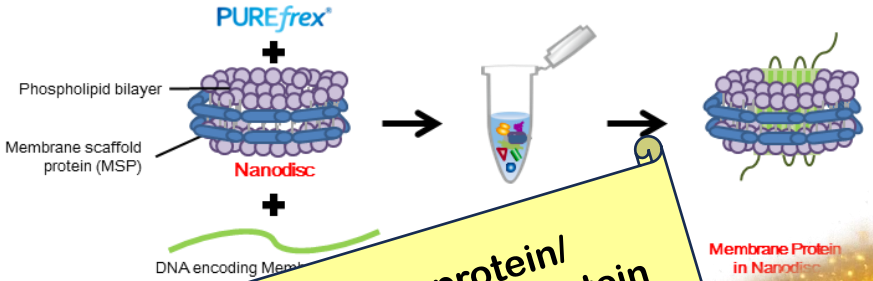
For Designing Central Dogma



Flexibility to
Non canonical amino acid



High throughput expression
w/o cell



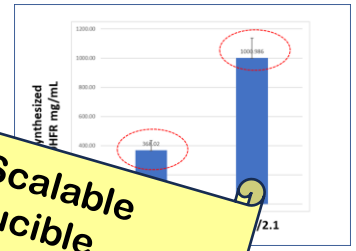
Membrane protein/
Difficult-to-express protein



Suitable for AI/ML approach

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

Express → Check



Speedy & Scalable
& Reproducible
reaction

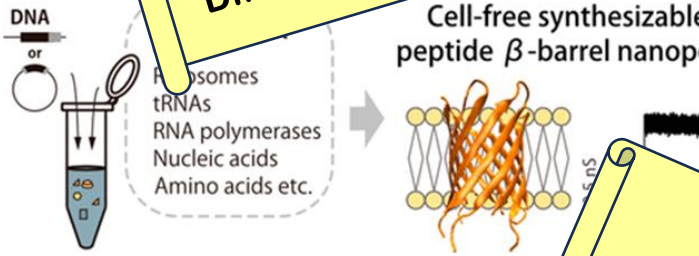
Membrane Protein in Nanodisc

Reconstituted cell-free protein synthesis kit

PURE*frex*®

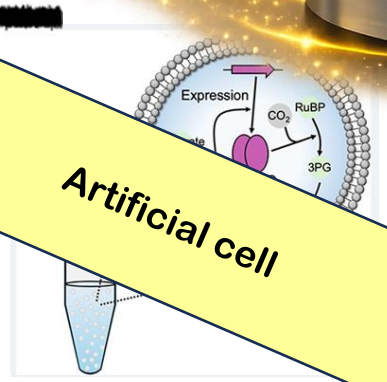
For Designing Central Dogma

BIOFOUNDARY PLATFORM



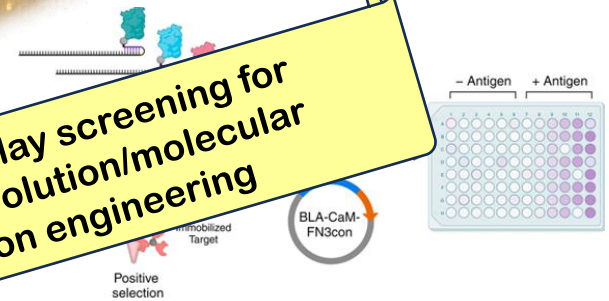
Fujita et al. (2023) ACS Nano. vol.17(4) p.33

Artificial cell



Sugii et al. (2023) Synth. Biol. vol.8, p1

μL ～ L scale



In vitro display screening for
Directed evolution/molecular
evolution engineering

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

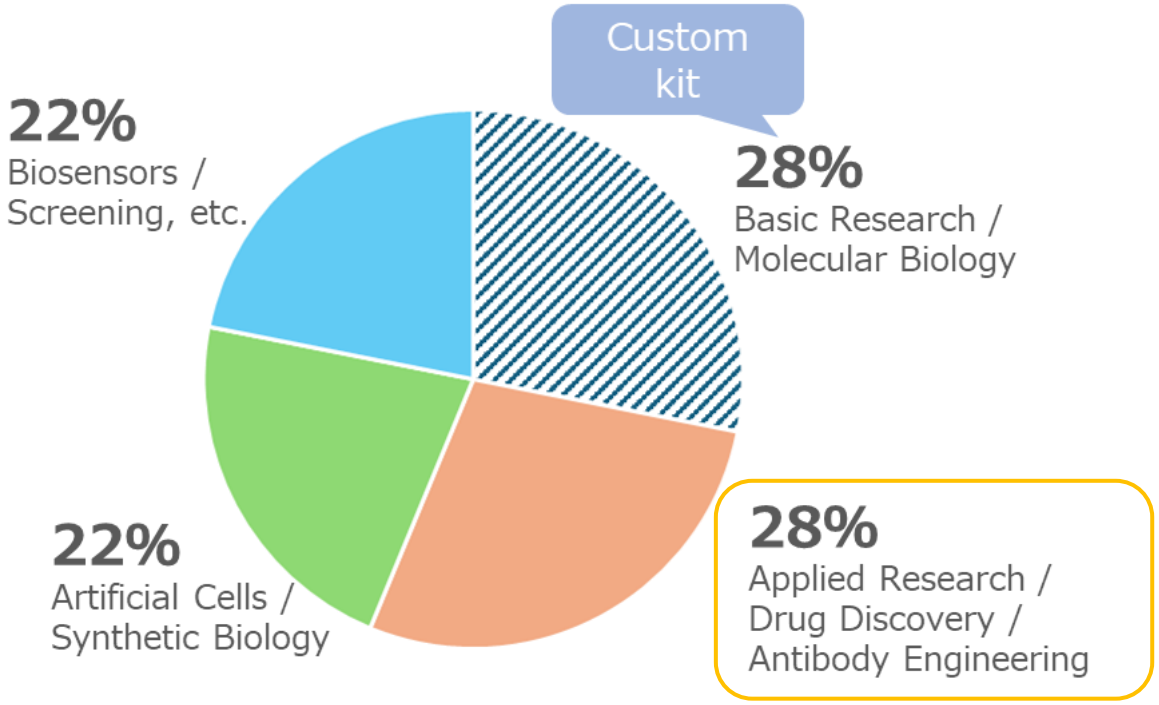
Reconstituted cell-free protein synthesis kit

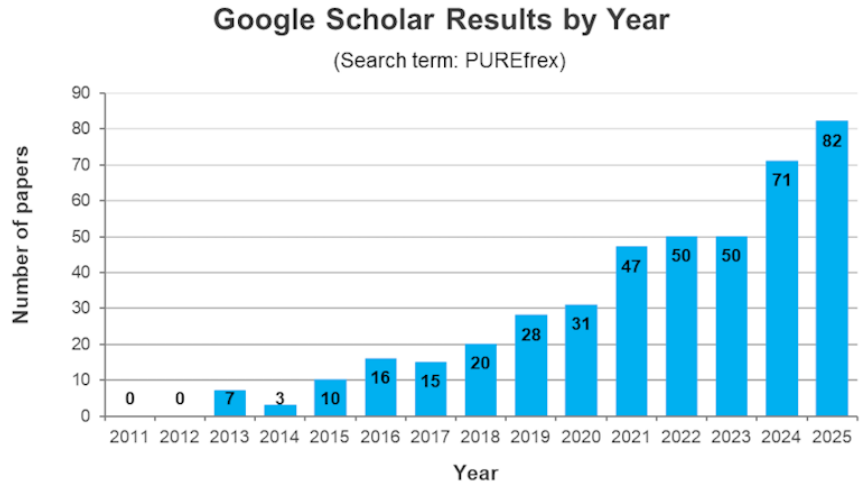
PURE*frex*®

–Primary Applications（analysis of papers in 2025）–

GeneFrontier

Applications	Screening Structural Analysis（X-ray、NMR、Cryo-EM） Functional Analysis Enzyme Engineering and Protein Modification





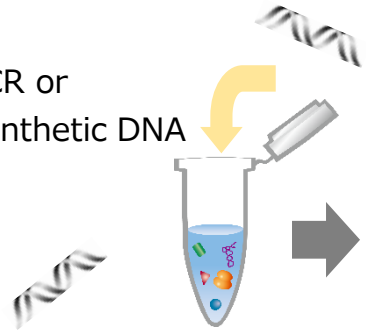
PURExpress Is Recommended If You:

- Require only a few hundred μg
- Want to synthesize multiple proteins
  (including **for AI/ML**)
- Drug discovery/Screening especially for
  antibody-related protein expression
- Want to skip purification

Advantages of Cell-Free Systems

Cell-Free

PCR or
Synthetic DNA



Add DNA to the kit and incubate

Ready for the next assay
(no purification required)

2～6 hours

- ✓ No cell culture;
  Easy operation
- ✓ Ideal for small-scale,
  High-throughput
- ✓ Cytotoxic proteins
  OK



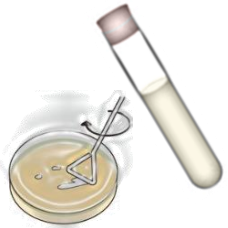


Design of DNA template **Expression** **Assay**

Living cells (ex, *E. coli*)

Cloning into a
plasmid vector





Transformation &
overnight culture



Induction &
Expression



Purification



Next assay

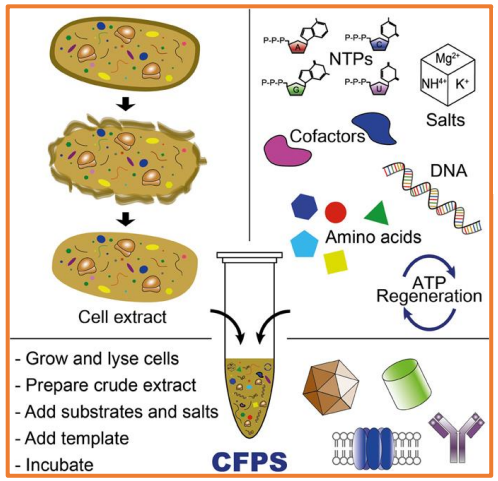
3～4 days

GeneFrontier
www.genefrontier.com

Cell-Free Systems (by Preparation Method)

• Cell Extract–Based System

E.coli, Wheat, Human, Insect, Tobacco etc.



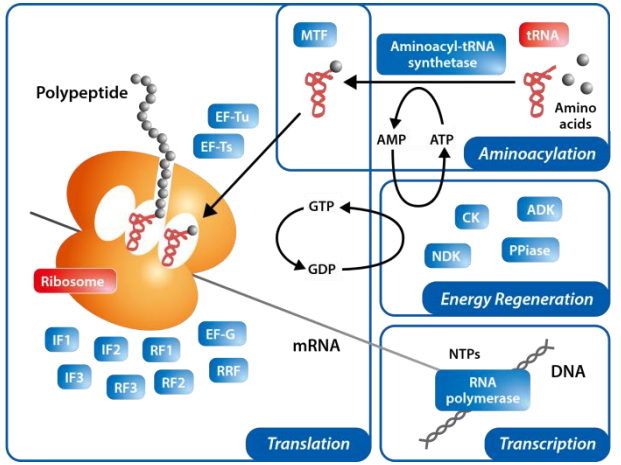
→ Break the cells

→ Extract fraction containing translation factors

Ref : Hong SH, Kwon YC, Jewett MC. (2014). Non standard amino acid incorporation into proteins using Escherichia coli cell free protein synthesis.
Front Chem. 2, Fig.2.

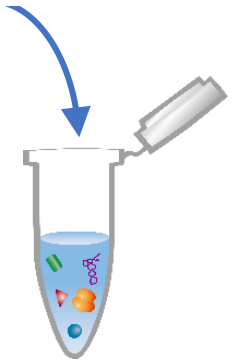
• Reconstituted (PURE) System

Hybrid of purified E. coli and eukaryotic factors



→ ~40 factors individually purified

→ Quantified and mixed

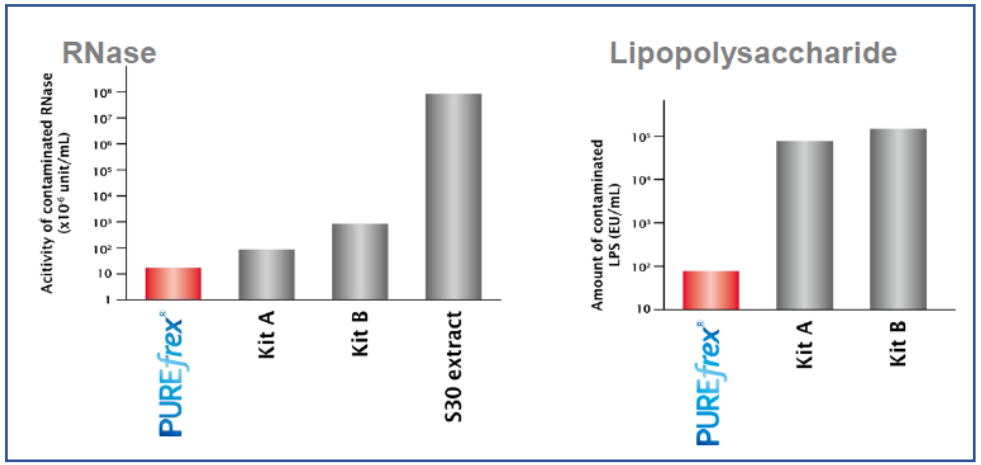


Challenges

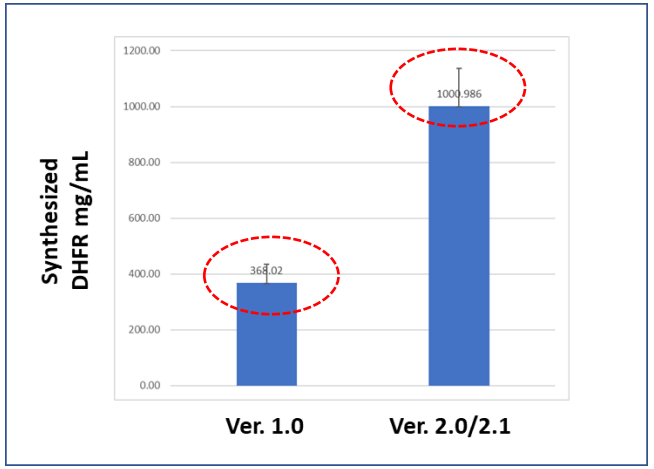
- Lot-to-lot variability
- Nuclease / protease contamination
- Unwanted side reactions (e.g., metabolism)

Advantages of PUREfrex

Individually purified
→ fewer contaminants



Quantified and mixed
→ low lot-to-lot variability



Challenges

- Lot-to-lot variability
- Nuclease / protease contamination
- Unwanted side reactions (e.g., metabolism)

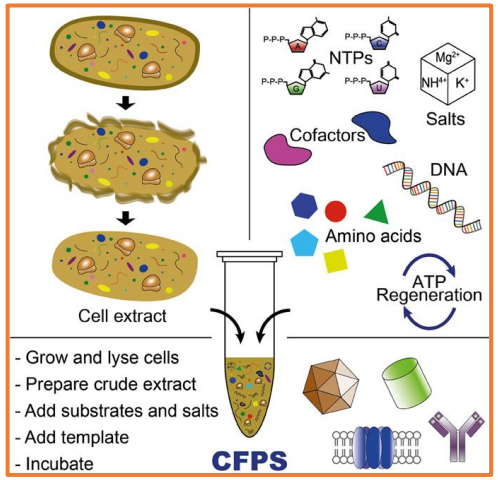
Solution

Why Recommended for Antibody Production

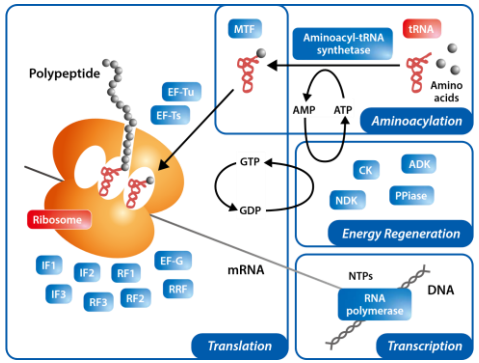
The intracellular environment is **highly reducing** to protect proteins from oxidative damage.

vs

Antibody structure depends on disulfide bonds formed in an **oxidative environment**.



Living cells are reducing, and extract-based cell-free systems also tend to be reducing due to intracellular reducing factors.



Because PUREfrex uses only purified factors, reducing components are removed, allowing easy oxidation upon addition of an oxidizing agent.

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

PUREfrex 2.1 does **not contain reducing agents** (GSH or DTT); they are supplied separately.

By adjusting the mixing ratio of the supplemental oxidizing agent (GSSG) and reducing agent, the **desired redox state can be maintained**.

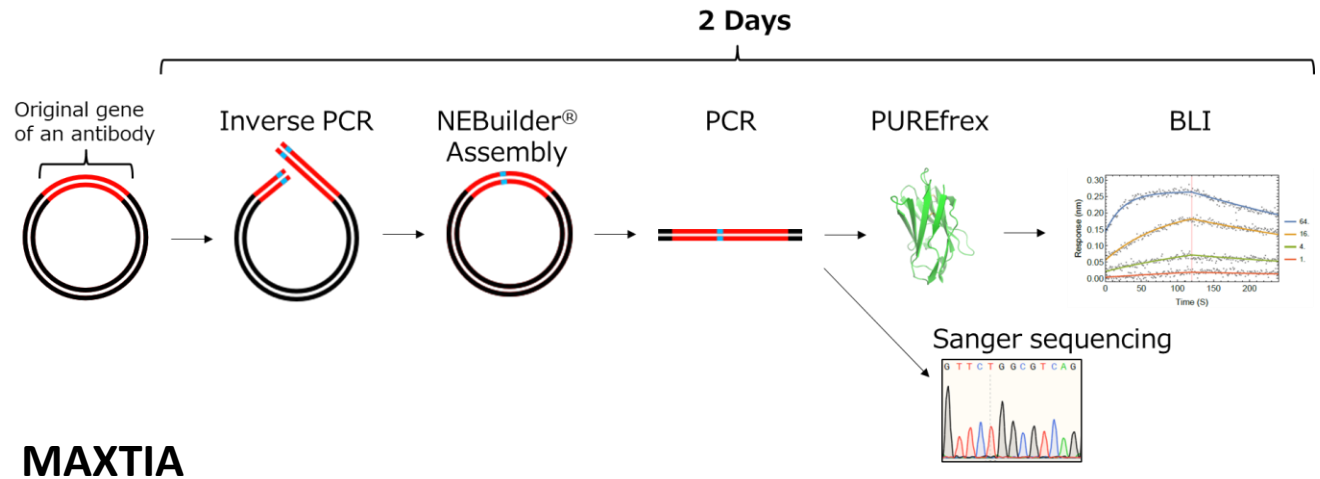
Reconstituted cell-free protein synthesis kit

PURE*frex*®

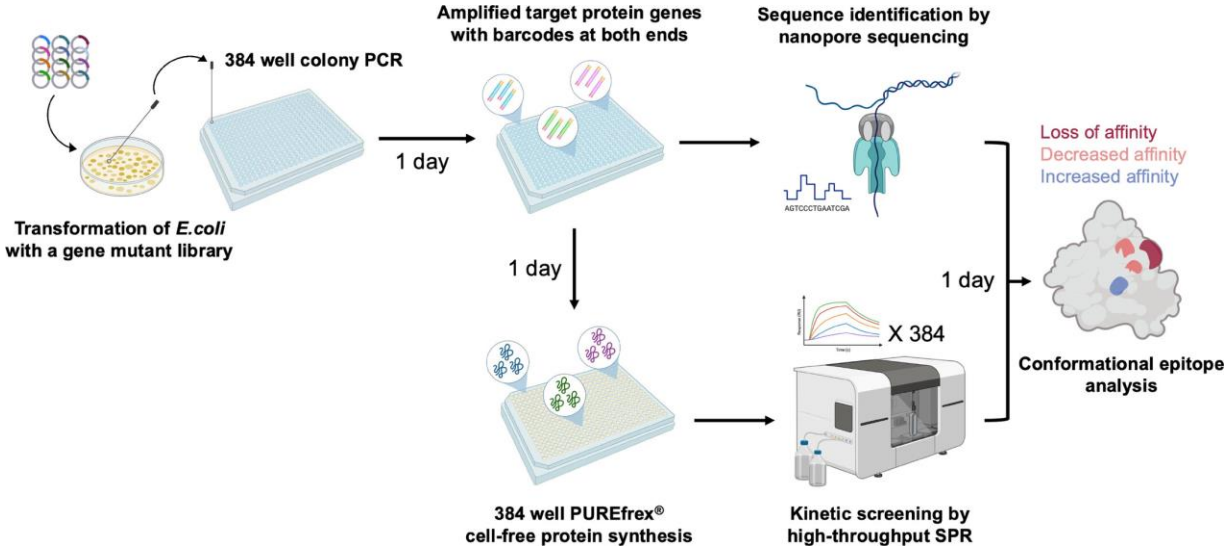
-Improve Validation from Weeks to Days-

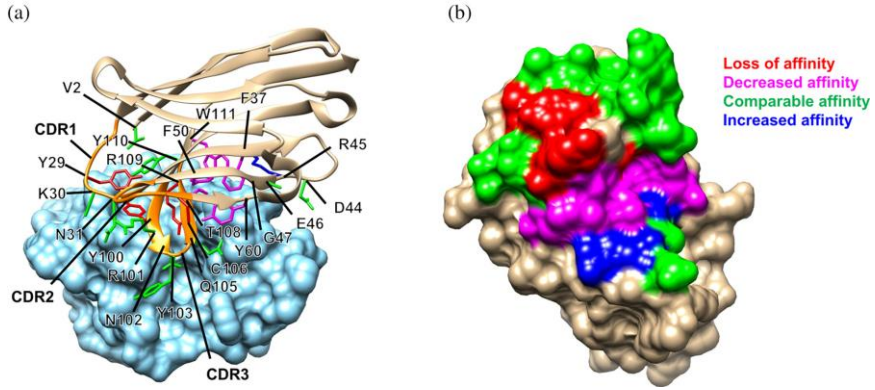
GeneFrontier

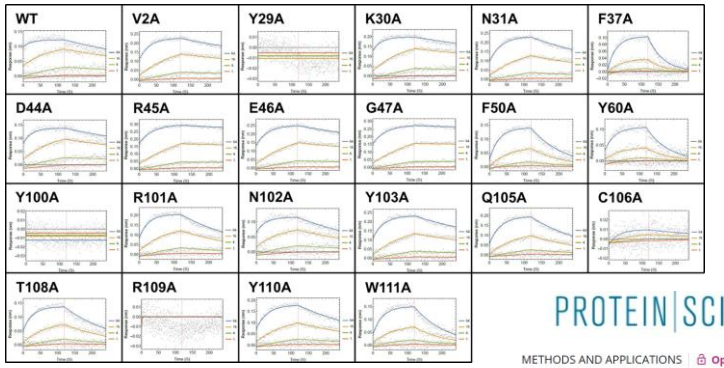
FASTIA: Fast Interaction Analysis



MAXTIA







PROTEIN SCIENCE

METHODS AND APPLICATIONS | Open Access

FASTIA: A rapid and accessible platform for protein variant interaction analysis demonstrated with a single-domain antibody

Ryo Matsunaga, Kouhei Tsumoto

First published: 21 February 2025 | https://doi.org/10.1002/pro.70065 | VIEW METRICS

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

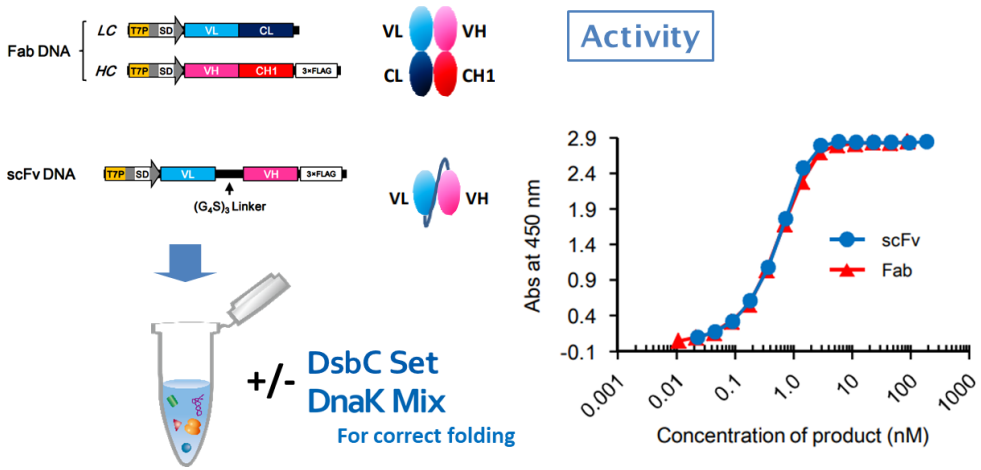
Kim et al. (2026) Protein Sci. Sep; 35(9):e70769. doi.org/10.1002/pro.70769

Reconstituted cell-free protein synthesis kit

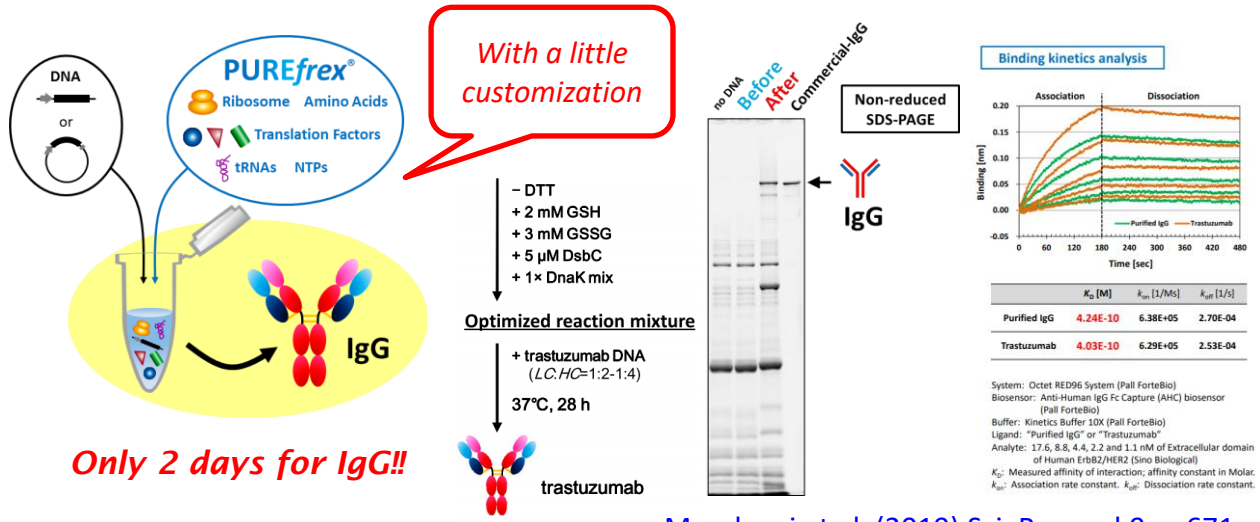
PURE*frex*®

-Expression of scFv, Fab, IgG and more-

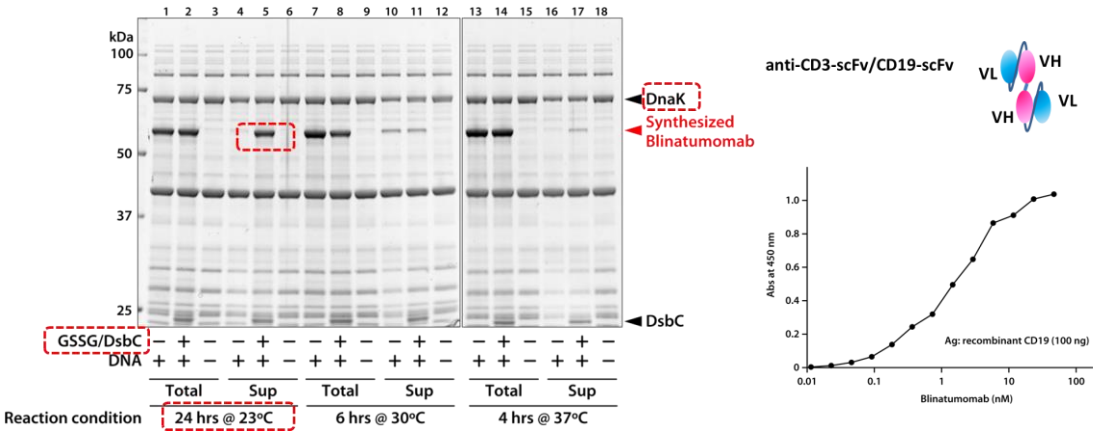
GeneFrontier



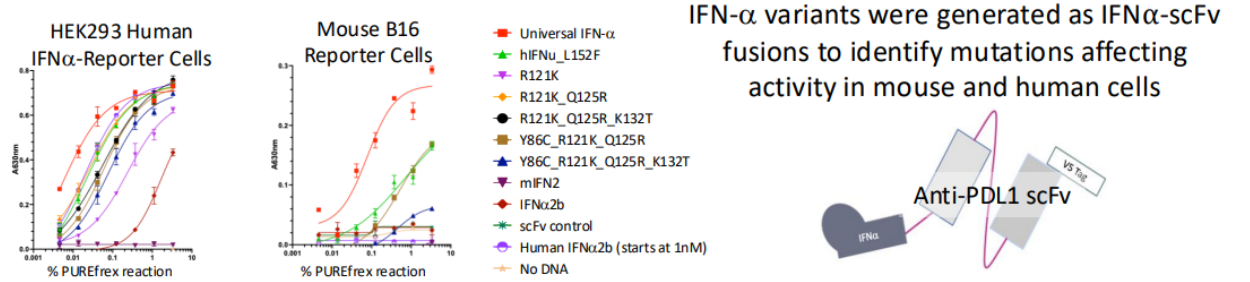
Murakami et al. (2019) Sci. Rep. vol.9, p.671. (Supplementary Information)



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

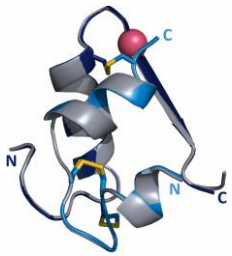
Killebrew et al. (2024) SITC 2024 Annual Meeting (Poster, BONUMTX.com).

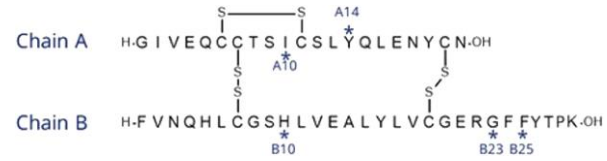
Reconstituted cell-free protein synthesis kit

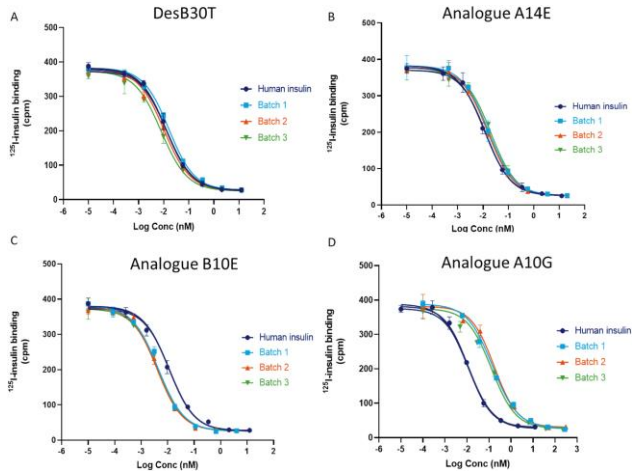
PURE*frex*®

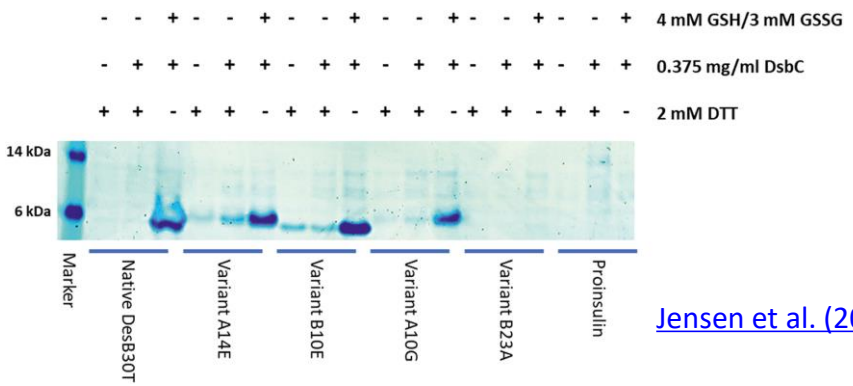
-Application for complex molecule-

GeneFrontier









	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.

Reconstituted cell-free protein synthesis kit

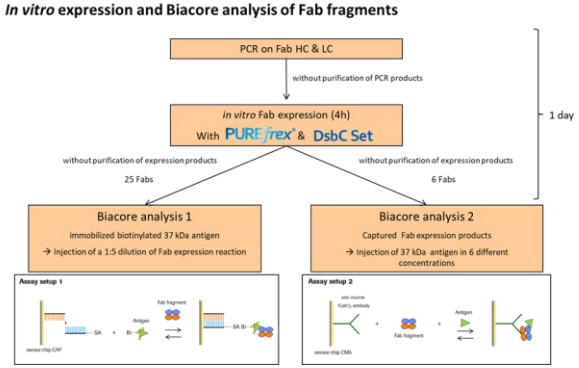
PURE*frex*®

-as a platform for many companies-

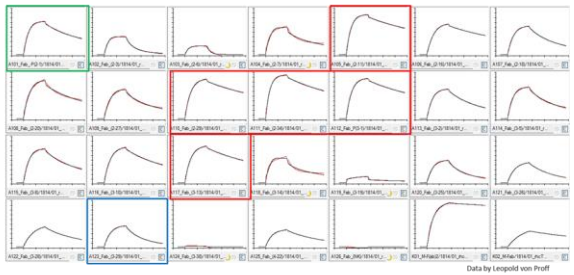
GeneFrontier

Roche

In vitro expression and Biacore analysis of Fab fragments



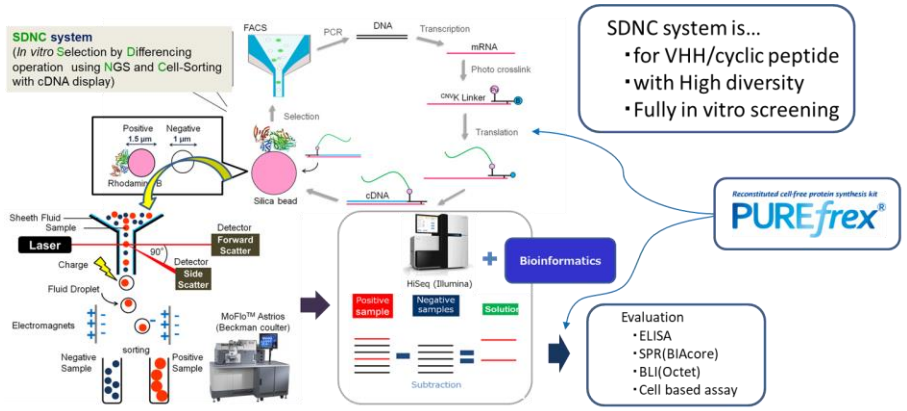
Kinetic analysis of 25 Fab binders



→ Selection of Fabs for further kinetic analysis

Epsilon Molecular Engineering

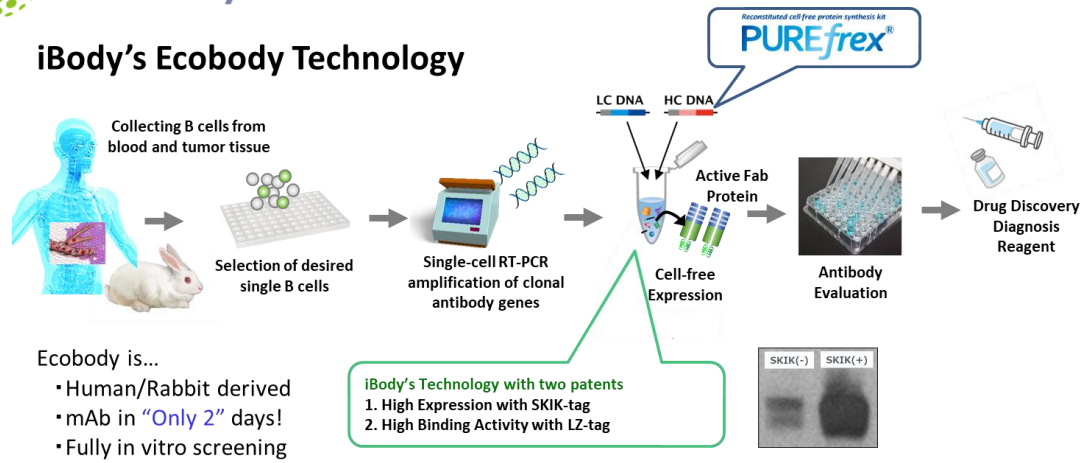
Molecular Design for Human Life



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

iBody

iBody's Ecobody Technology



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

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

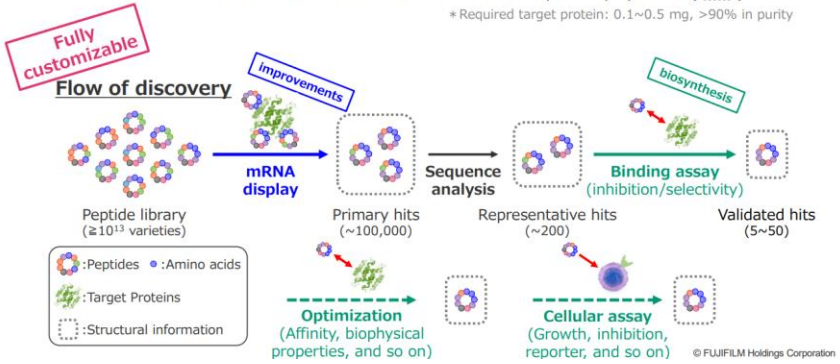
FUJIFILM peptide discovery services

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



Reconstituted cell-free protein synthesis kit

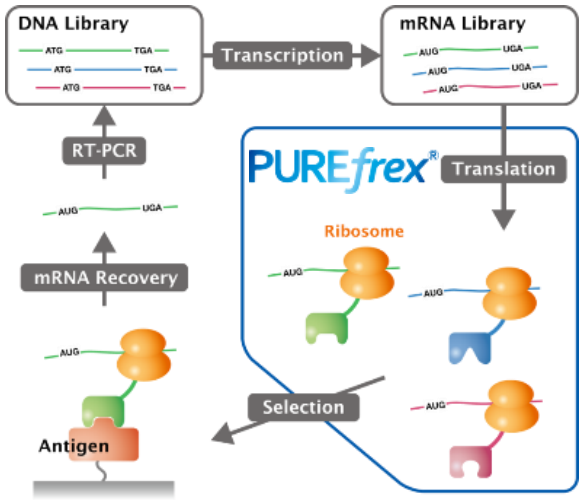
PURE*frex*®

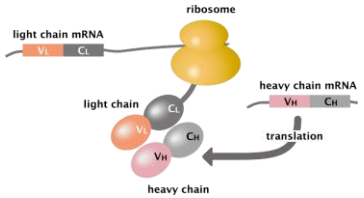
-as a platform for in vitro display-

GeneFrontier

in vitro protein selection technology

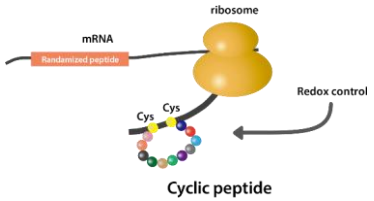
PURE*frex*® RD

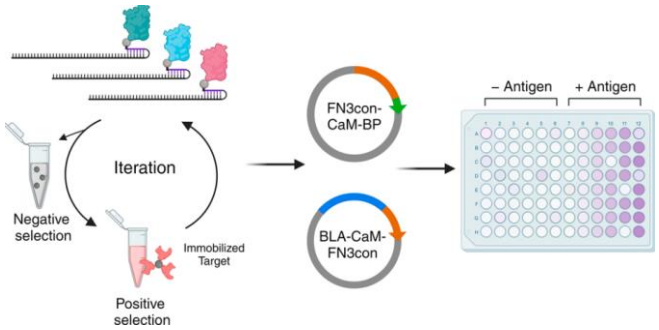




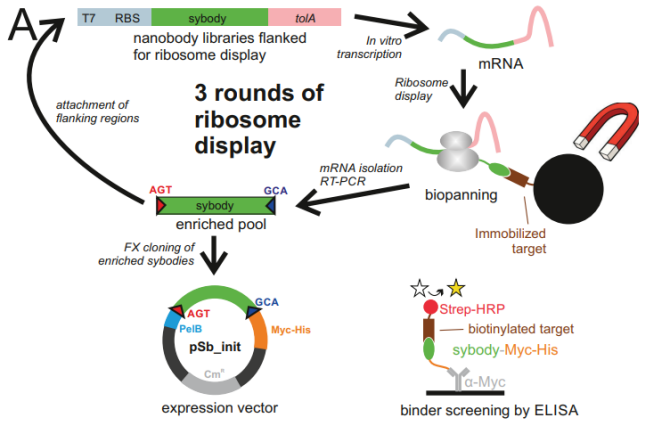
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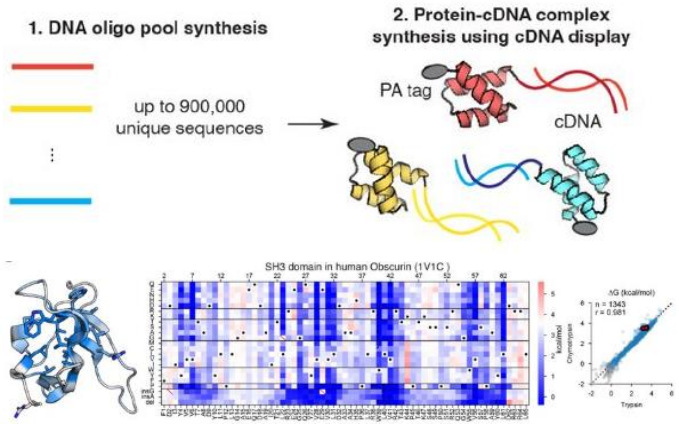




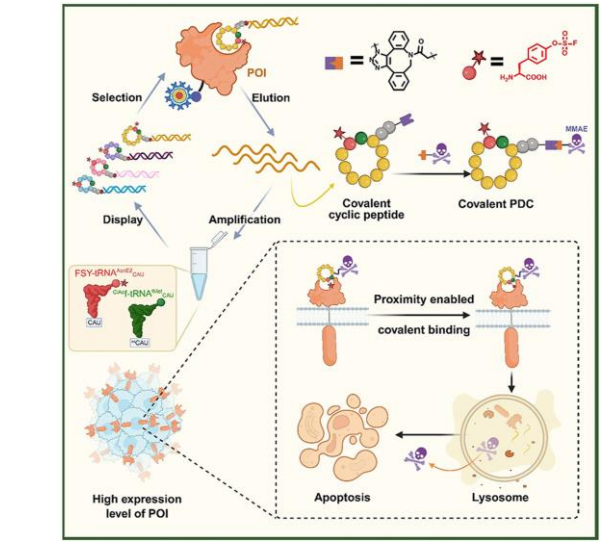
Chui Z. et al. (2024) ACS Sens, 9(6):2846-2857.



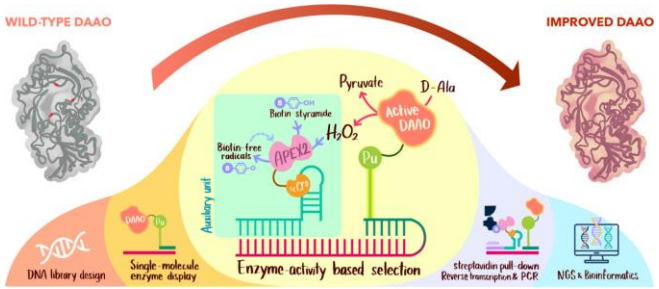
Zimmermann I. et al. (2018) eLife, 7, e34317.



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

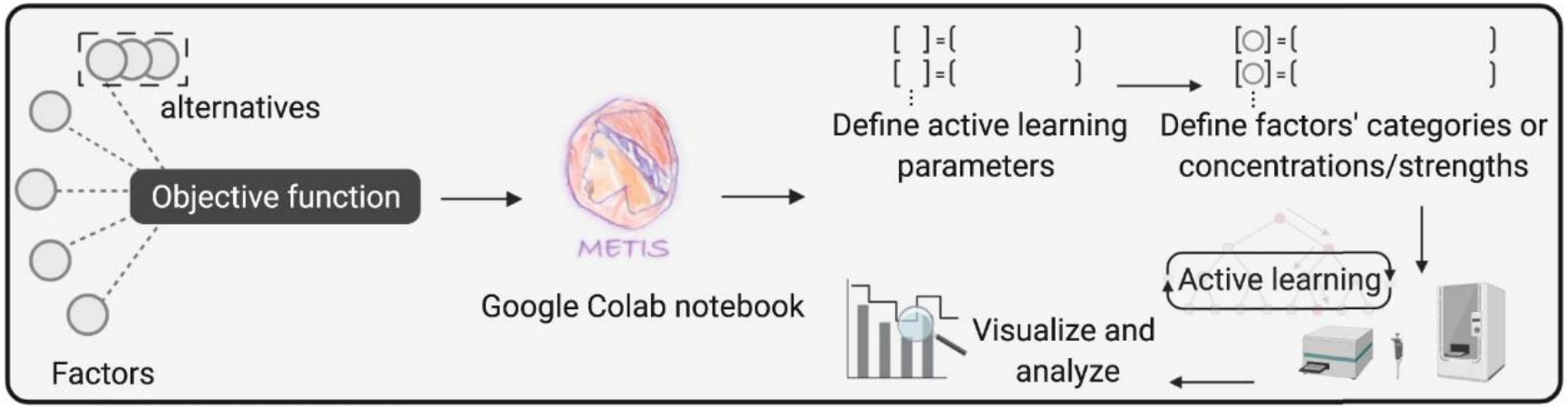


Wang et al. (2025) Acta Pharmaceutica Sinica B. vol.15(5474).

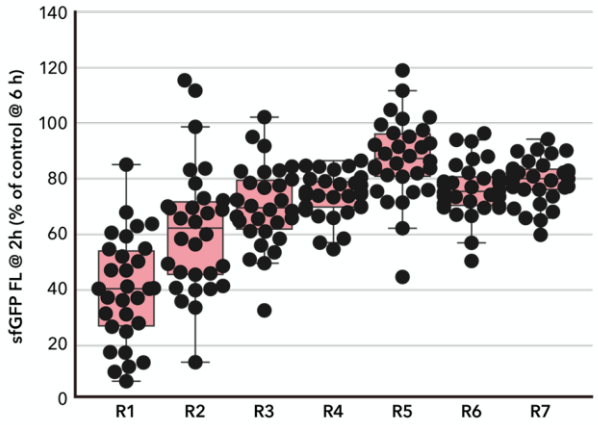


Munaweera et al. (2026) ACS Synth Biol. Feb 23. doi: 10.1021/acssynbio.5c00968.

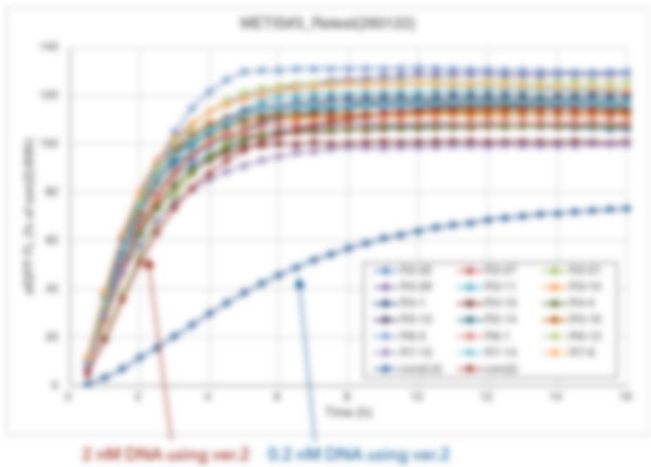
-Broad applications, yet to come!-



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.**

Reconstituted cell-free protein synthesis kit

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-KSF; AT rich codon on N-term-

GeneFrontier

Fab Heavy Chain (Herceptin)

Herceptin Fab HC

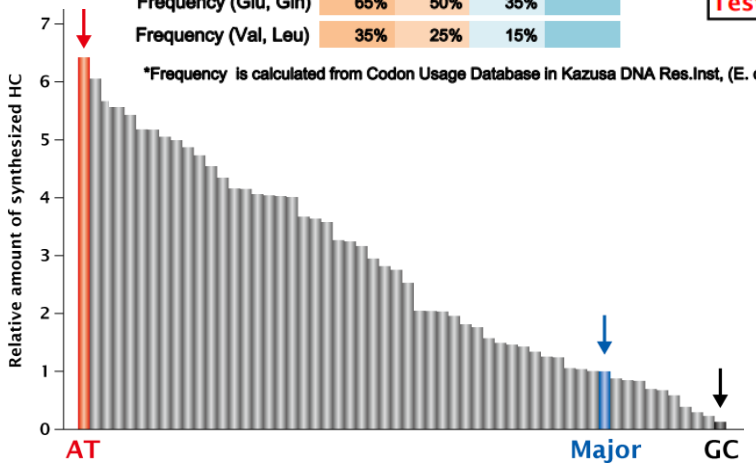
Met- Glu-Val-Gln-Leu-Val- FLAG

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)

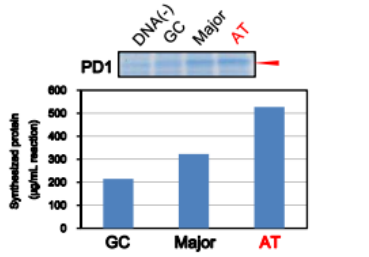


[Design of DNA template is important.](#)
[Manual is Free to download from our Web site here.](#)

PD1

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

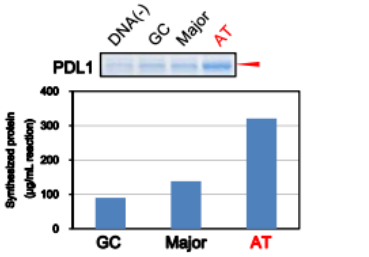
N-term type	1 Met	2(36) Thr	3(37) Phe	4(38) Ser	5(39) Pro	6(40) Ala	GC(%) 1-6 a.a.
GC	atg	acc	ttc	tcc	ccg	gcg	67%
Major	atg	acc	ttt	tct	ccg	gcg	56%
AT	atg	act	ttt	tca	cca	gct	39%



PDL1

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

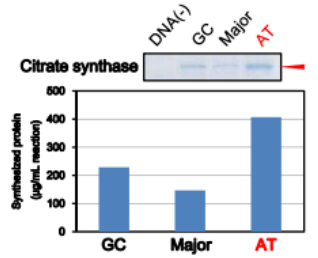
N-term type	1 Met	2(18) Ala	3(19) Phe	4(20) Thr	5(21) Val	6(22) Thr	GC(%) 1-6 a.a.
GC	atg	gcg	ttc	acc	gtg	acc	61%
major	atg	gcg	ttt	acc	gtg	acc	56%
AT	atg	gct	ttt	act	gta	aca	33%



Citrate Synthase

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

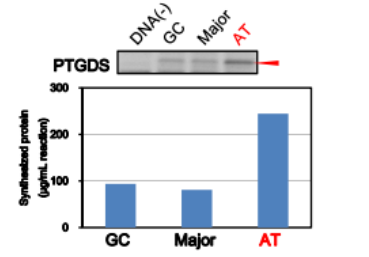
N-term type	1 Met	2(38) Ser	3(39) Ser	4(40) Ala	5(41) Ser	6(42) Glu	GC(%) 1-6 a.a.
GC	atg	tcc	tcc	gcg	tcc	gag	67%
major	atg	tct	tct	gcg	tct	gaa	44%
AT	atg	tca	tca	gct	tca	gaa	39%



PTGDS

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

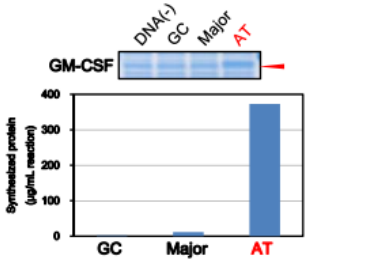
N-term type	1 Met	2(23) Ala	3(24) Pro	4(25) Glu	5(26) Ala	6(27) Gln	GC(%) 1-6 a.a.
GC	atg	gca	ccg	gaa	gca	cag	61%
major	atg	gcg	ccg	gaa	gcg	cag	72%
AT	atg	gca	cct	gaa	gct	caa	50%



GM-CSF

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

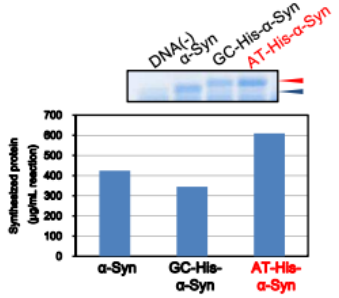
N-term type	1 Met	2(18) Ala	3(19) Pro	4(20) Ala	5(21) Arg	6(22) Ser	GC(%) 1-6 a.a.
GC	atg	gcg	ccg	gcg	cgc	tcc	83%
major	atg	gcg	ccg	gcg	cgc	tct	78%
AT	atg	gca	cct	gct	aga	tca	50%



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%



DNA design for PURExfrex

Amino acid sequence entry form

Consultation is free of charge!

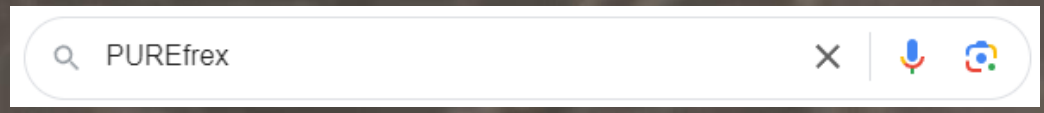
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- QP release