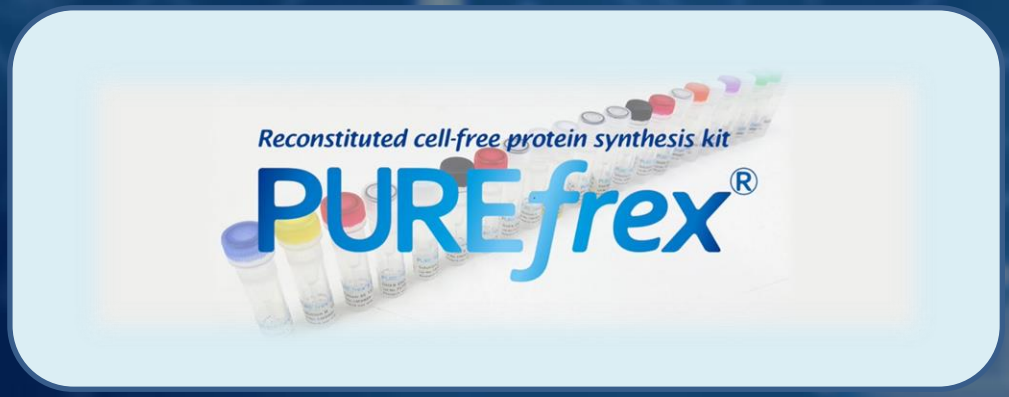
Unleashing the Power of Cell-Free:

PUREfrex® for Protein Engineering and Discovery



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

GeneFrontier

Biologics US 2025
2-3 of October, 2025

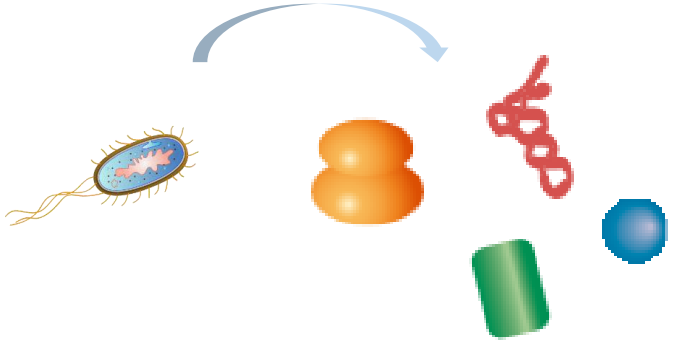
Corporate Summary

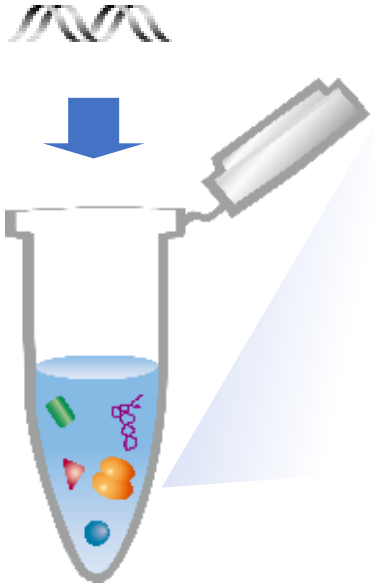
GeneFrontier

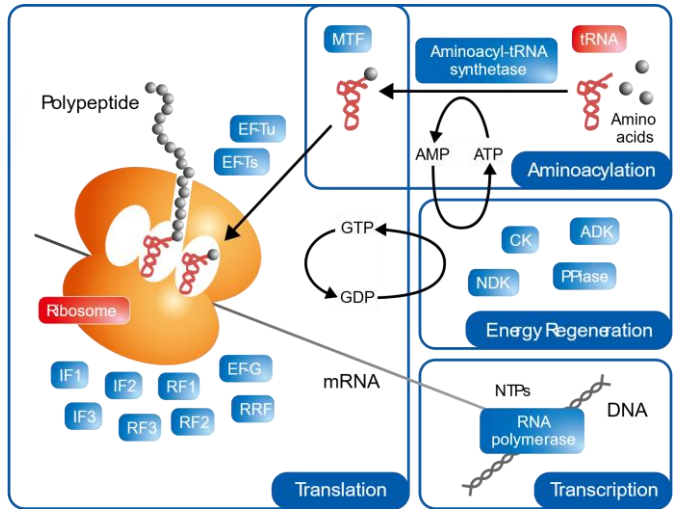
Founded :	***Oct 13th, 2010 (renewed)***
Shareholder :	***KANEKA Corporation (100%)***
People :	***17 (Ph.D. 8, MS 1)***
Place :	***Chiba, Japan***

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

Only necessary molecules
for transcription/translation





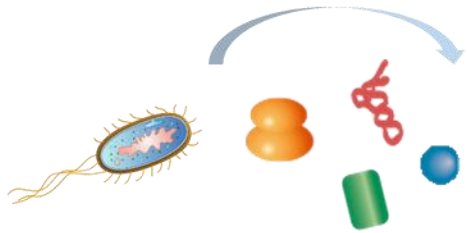


=Breaking down & Building up

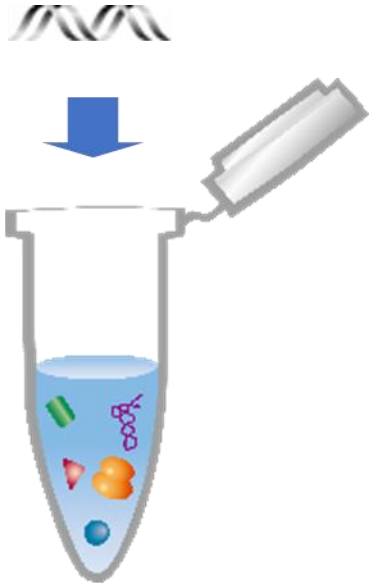
PURE system

(Protein synthesis Using Recombinant Elements)

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



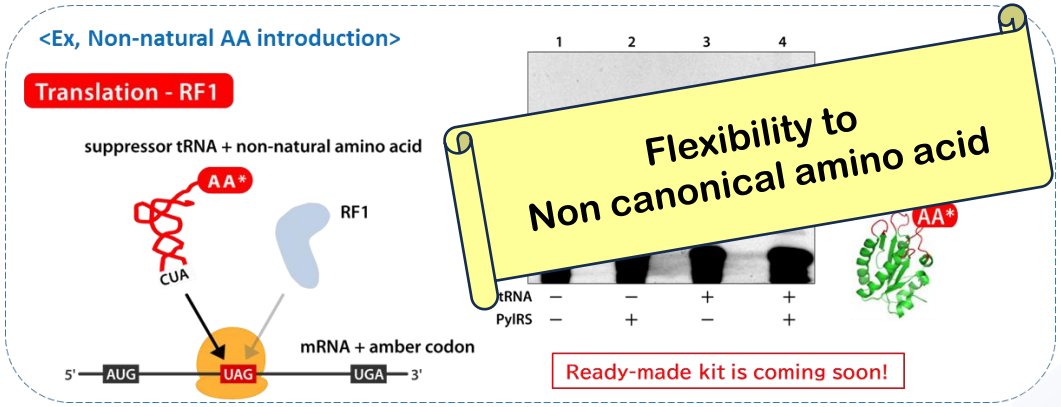
PURE system



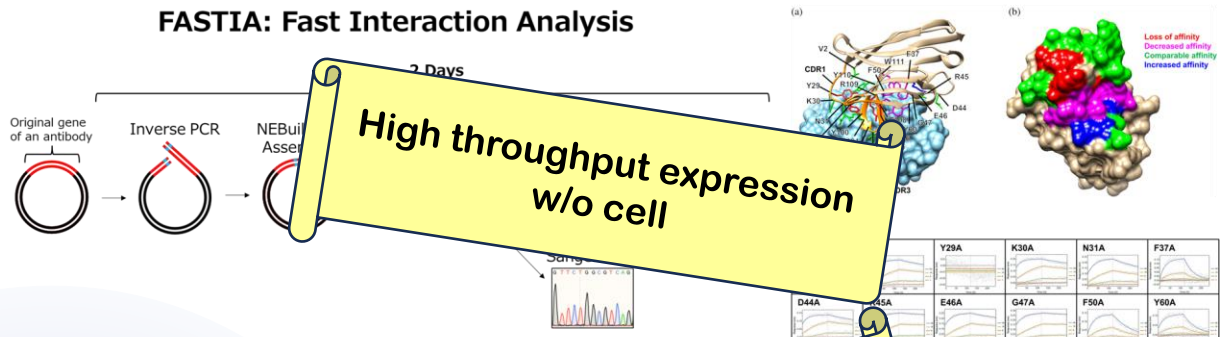
Reconstituted cell-free protein synthesis kit

PURE*frex*®

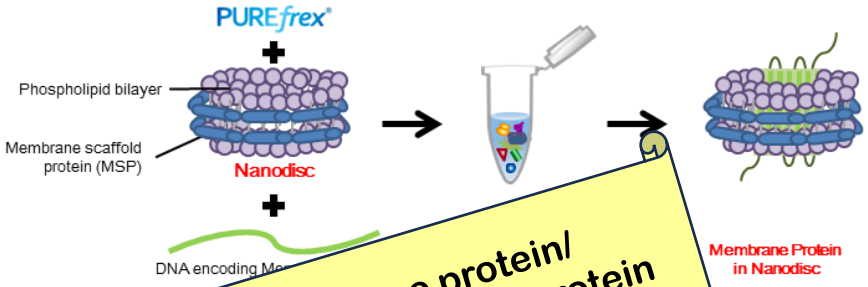
For Designing Central Dogma



Flexibility to Non canonical amino acid



High throughput expression w/o cell



Membrane protein/ Difficult-to-express protein

PURE system

Reconstituted cell-free protein synthesis kit

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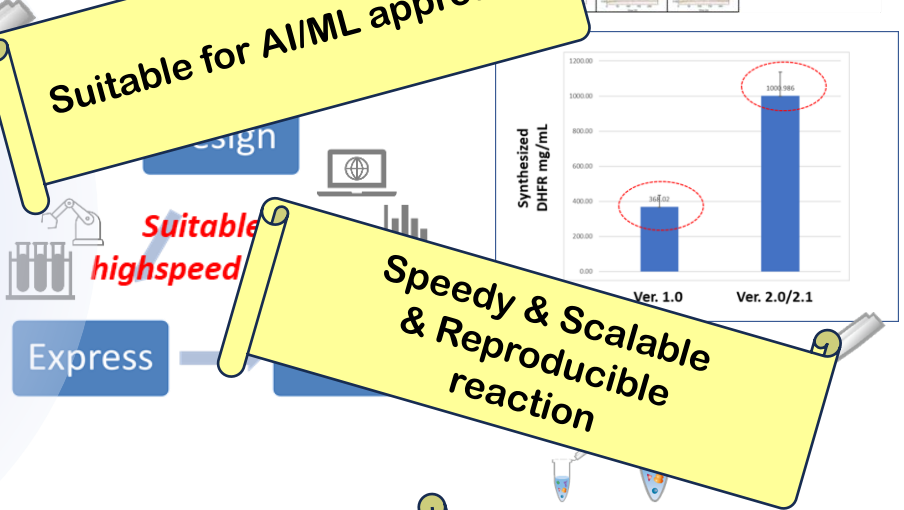
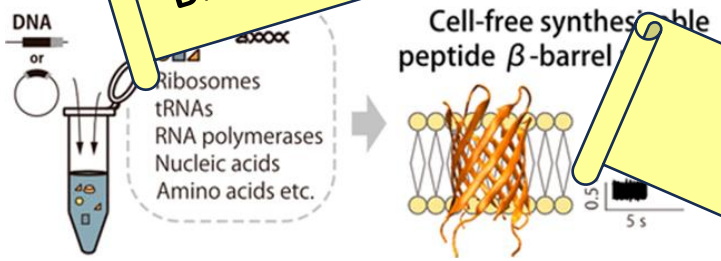
For Designing Central Dogma

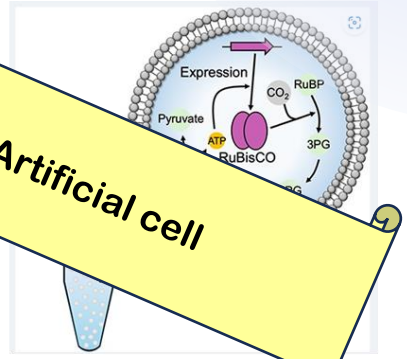


Speedy & Scalable & Reproducible reaction

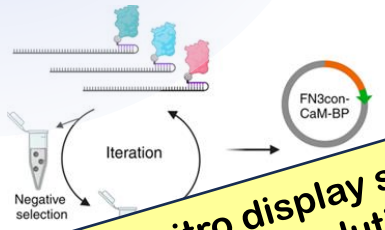


Fujita et al. (2023) ACS Nano. vol.17(4), p.3358.

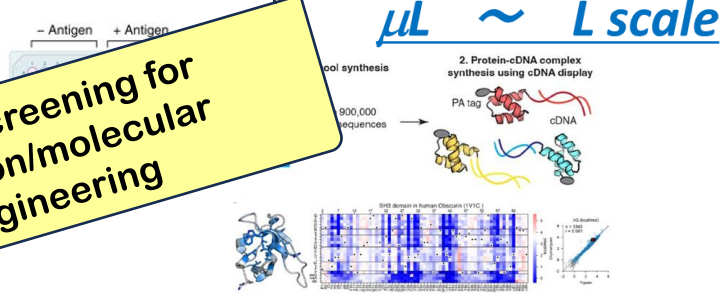
Artificial cell



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



In vitro display screening for Directed evolution/molecular evolution engineering



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

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

Reconstituted cell-free protein synthesis kit

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-Improve Validation from Weeks to Days-

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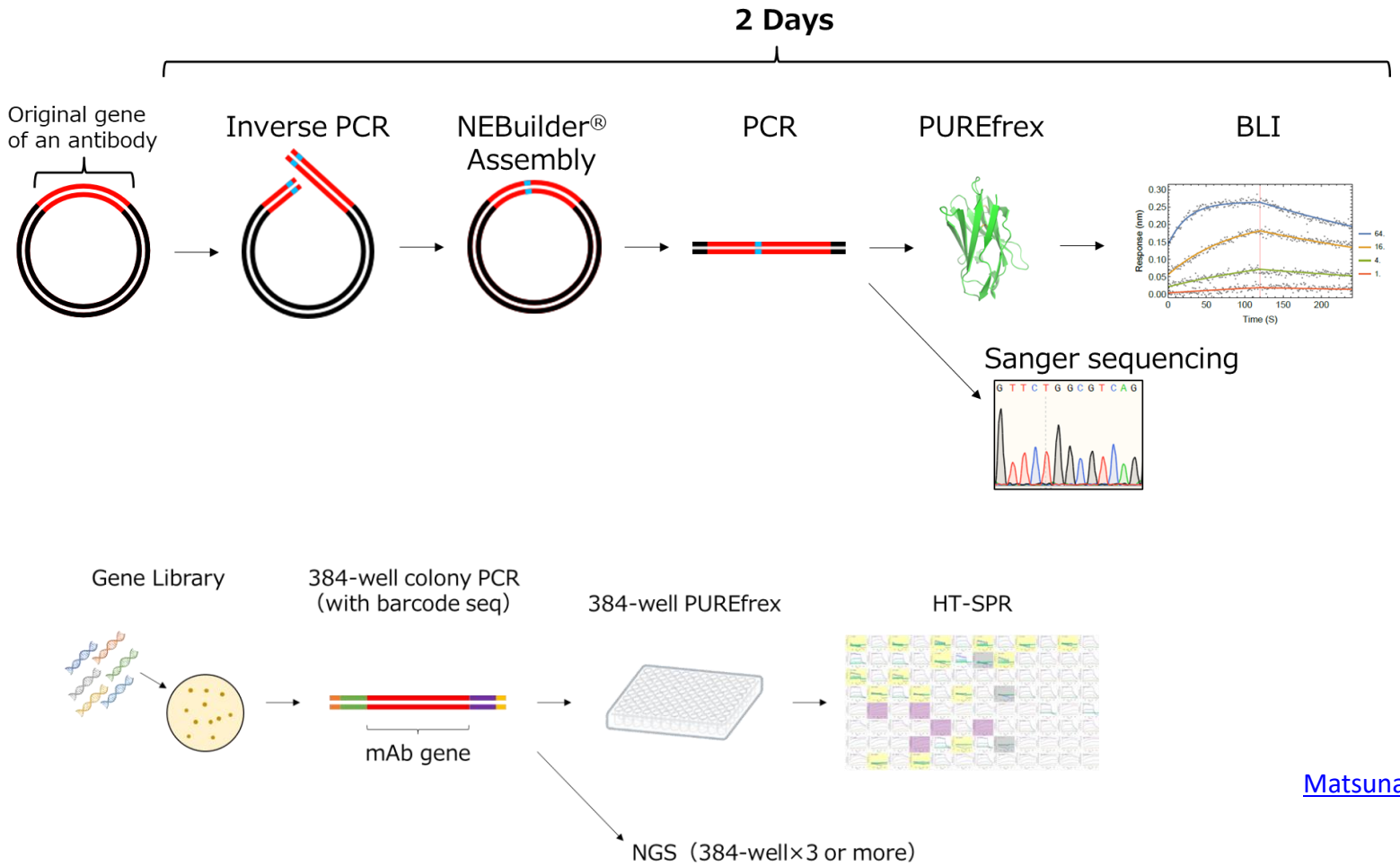
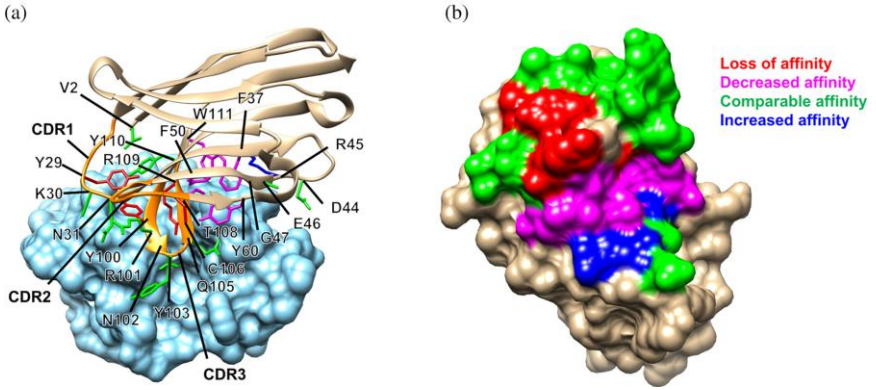
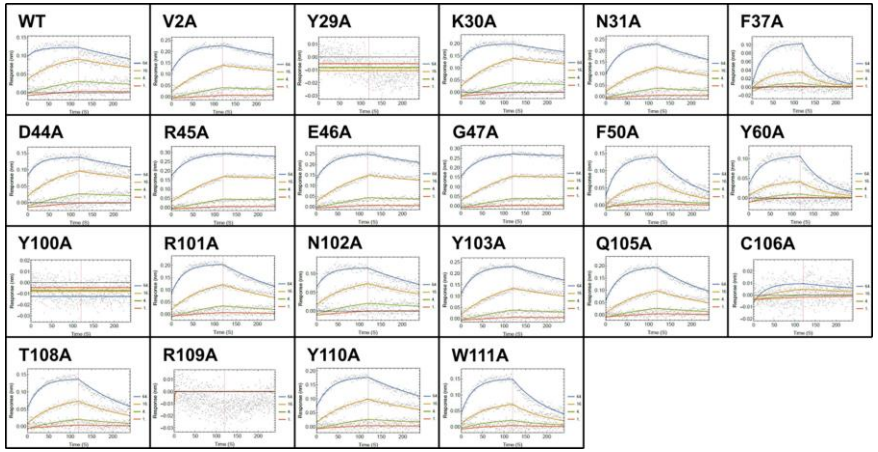
FASTIA: Fast Interaction Analysis







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

Reconstituted cell-free protein synthesis kit

PURE*frex*®

-Expression of scFv, Fab, IgG and more-

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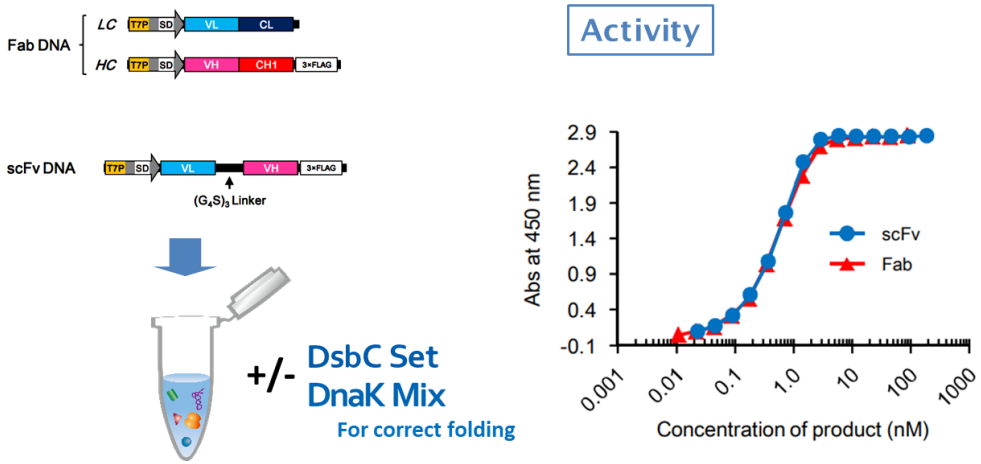
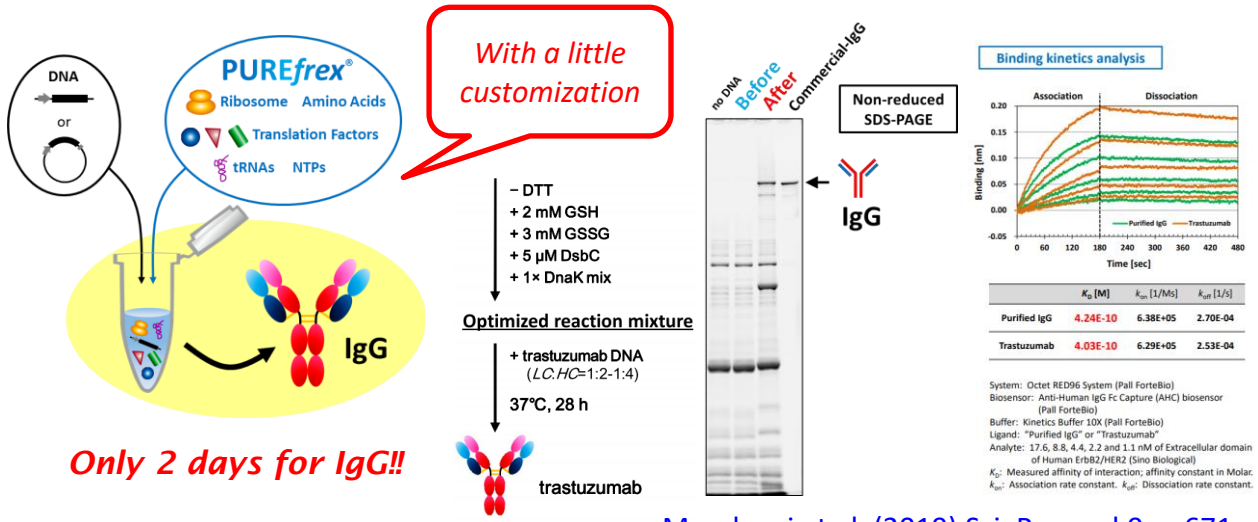
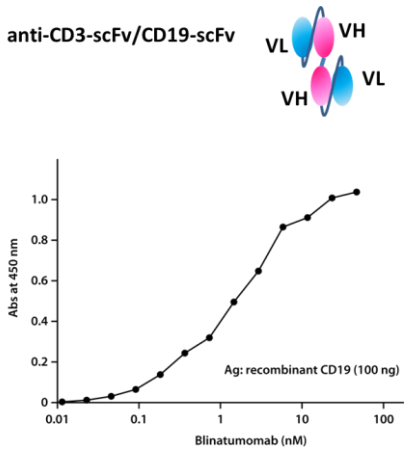


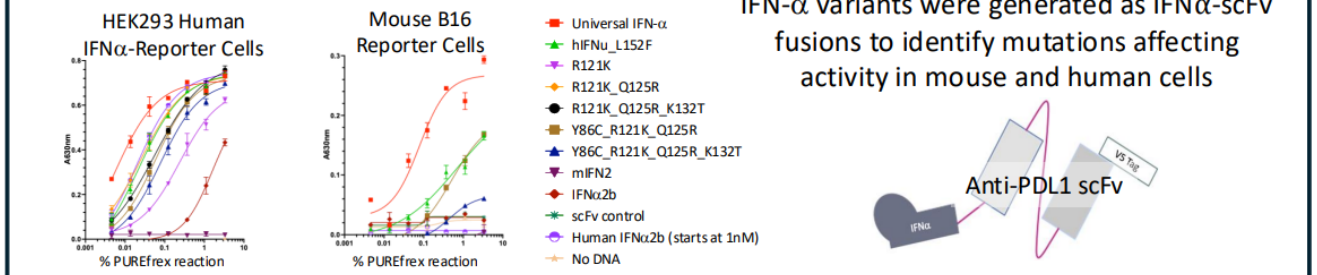


[Murakami et al. (2019) Sci. Rep. vol.9, p.671. (Supplementary Information)](#)

[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

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-Application for complex molecule-

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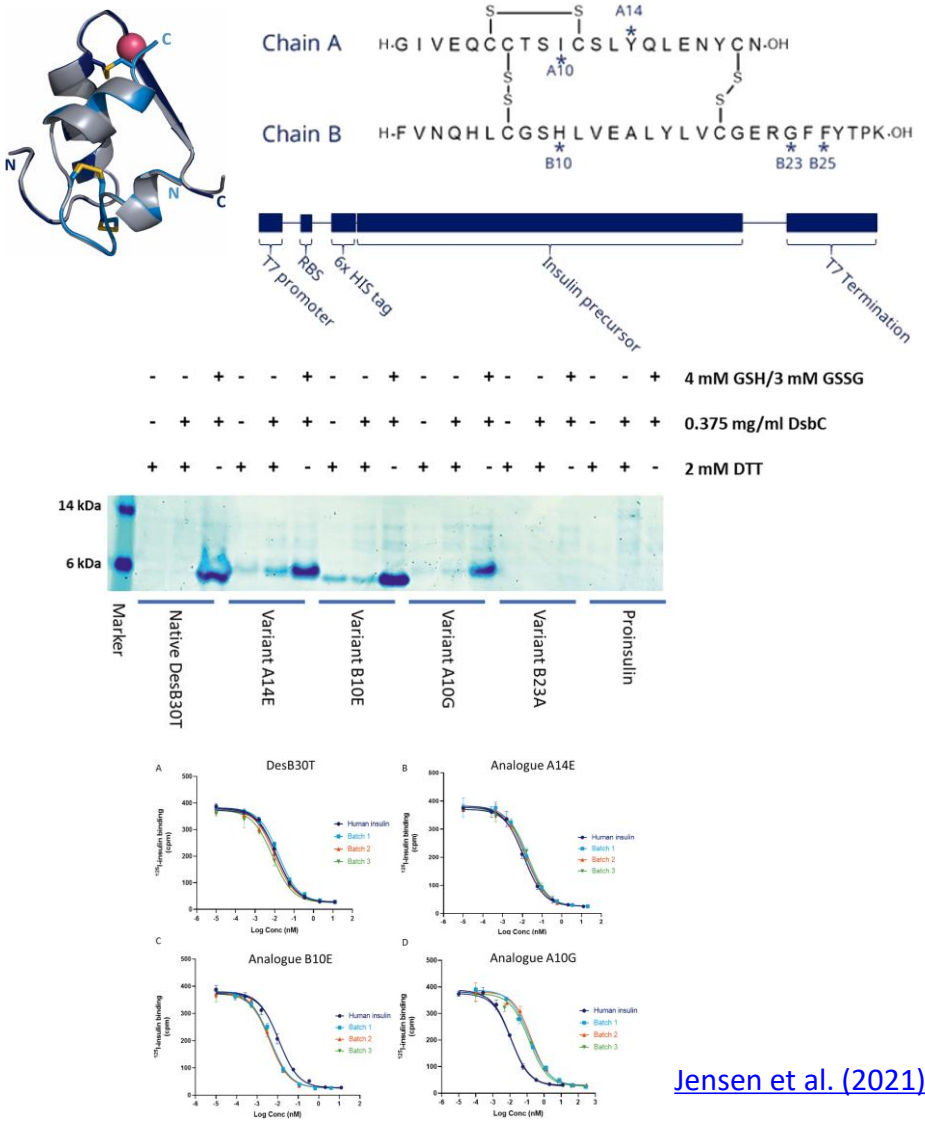

	1	2	3	4	5	6	7	8	9	10
	Proinsulin Aspart	Proinsulin Lispro	Proinsulin Glargine	Regular Proinsulin	Insulin A Chain	Insulin B Chain	Insulin A Chain Heterodimer	Insulin B Chain Heterodimer	Oxytocin	Glucagon
PURE	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Clm24	X	X	X	X	✓	X	✓	✓	✓	✓
BL21	X	X	X	X	✓	X	✓	✓	✓	X
759	✓	✓	✓	✓	✓	✓	✓	✓	✓	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

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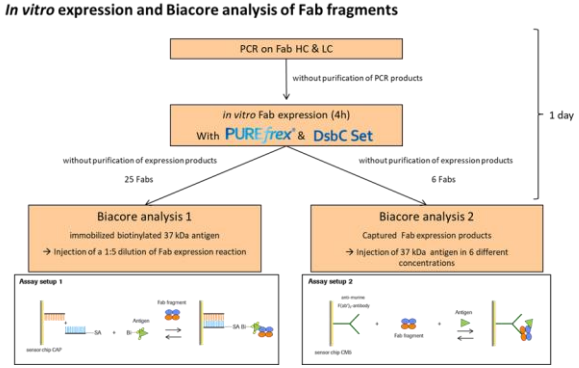
-as a platform for many companies-

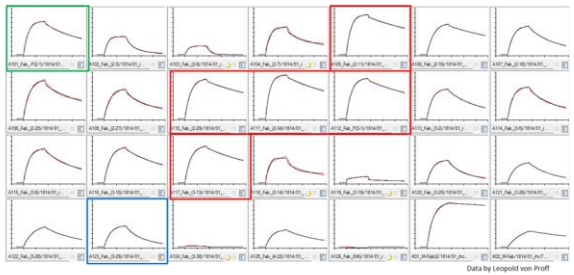
GeneFrontier

Roche

In vitro expression and Biacore analysis of Fab fragments



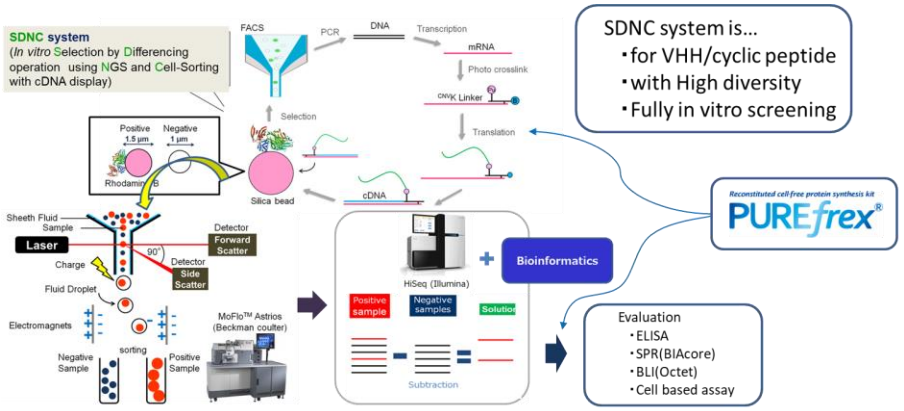
Kinetic analysis of 25 Fab binders



→ Selection of Fabs for further kinetic analysis

Epsilon Molecular Engineering

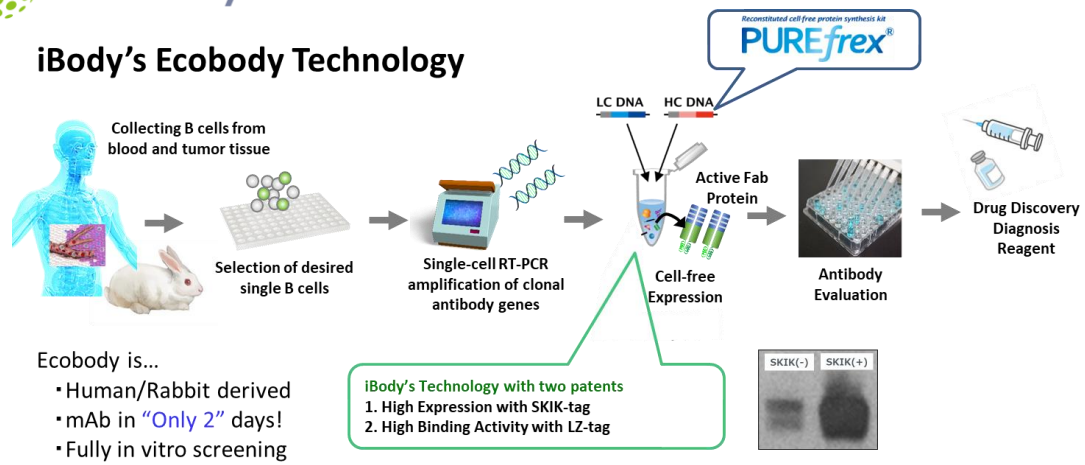
Molecular Design for Human Life



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/

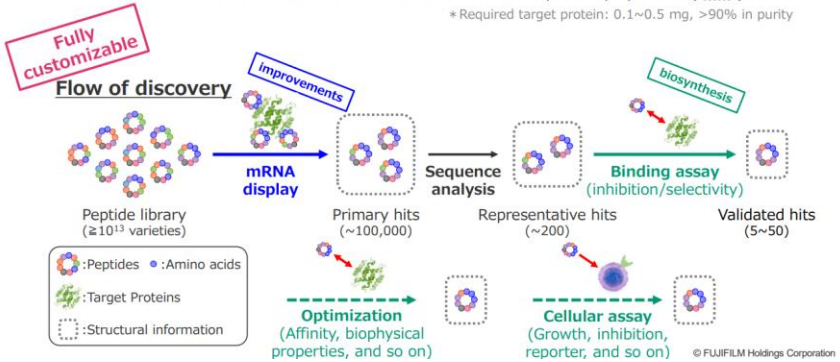
FUJIFILM peptide discovery services

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

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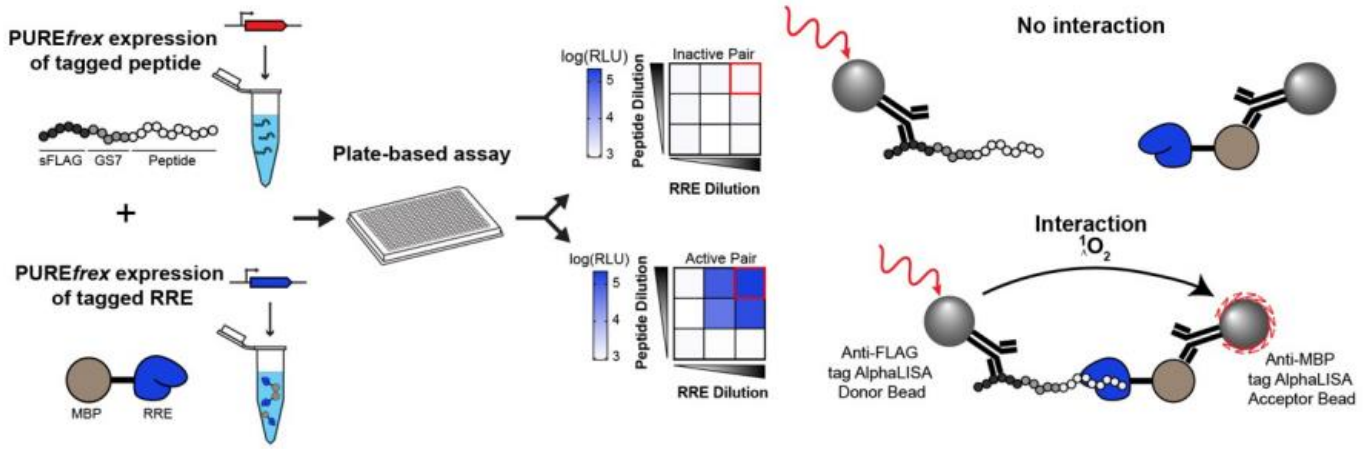
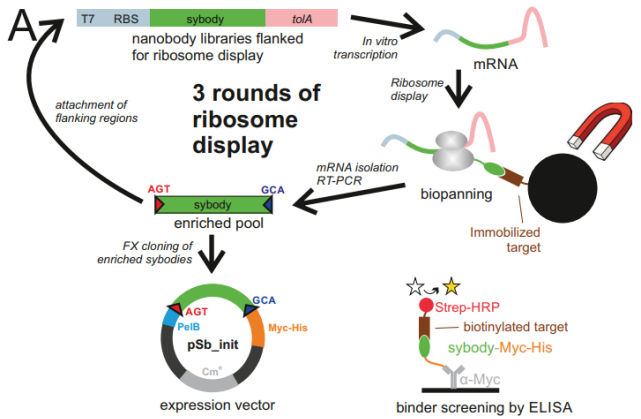
-Broad applications, yet to come!-

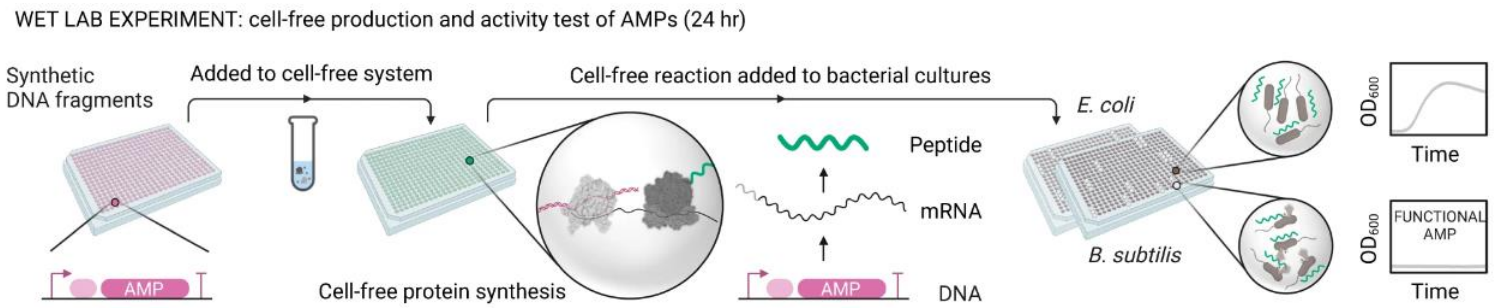
GeneFrontier



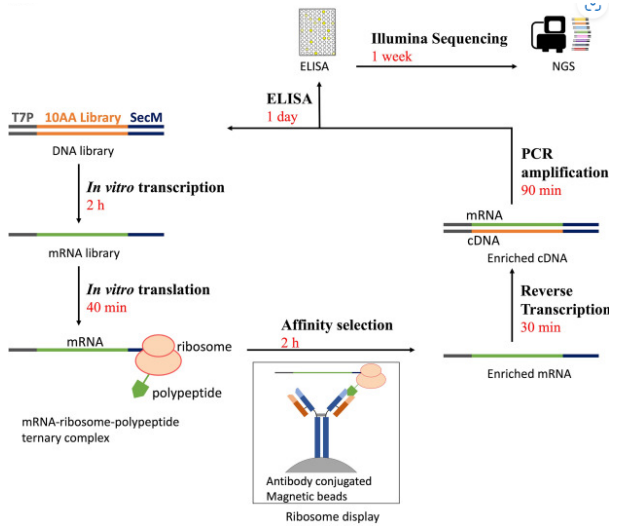
Wong et al. (2024) bioRxiv https://doi.org/10.1101/2024.03.25.586624.



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



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



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

Reconstituted cell-free protein synthesis kit

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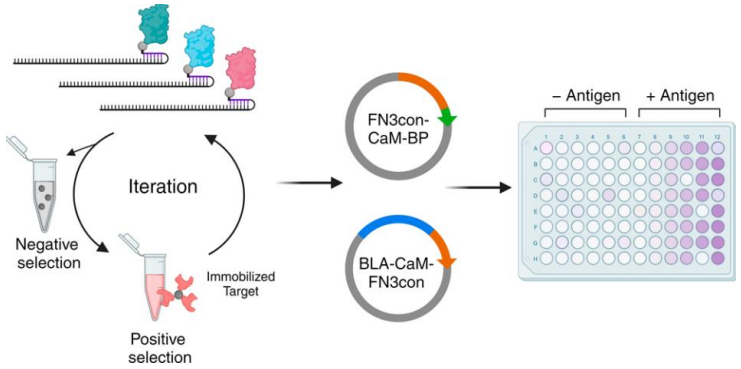
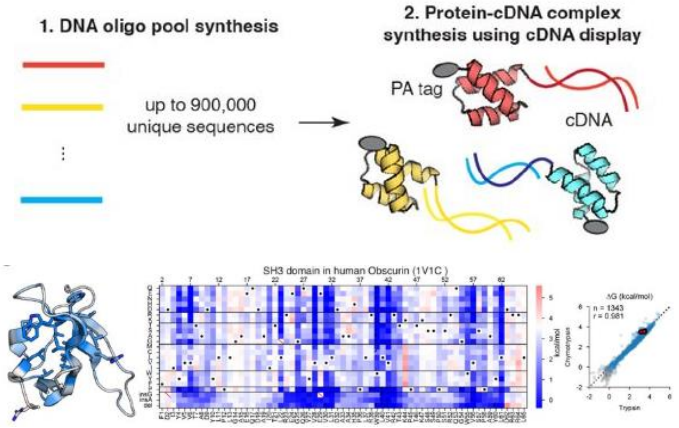
-Broad applications, yet to come!-

GeneFrontier



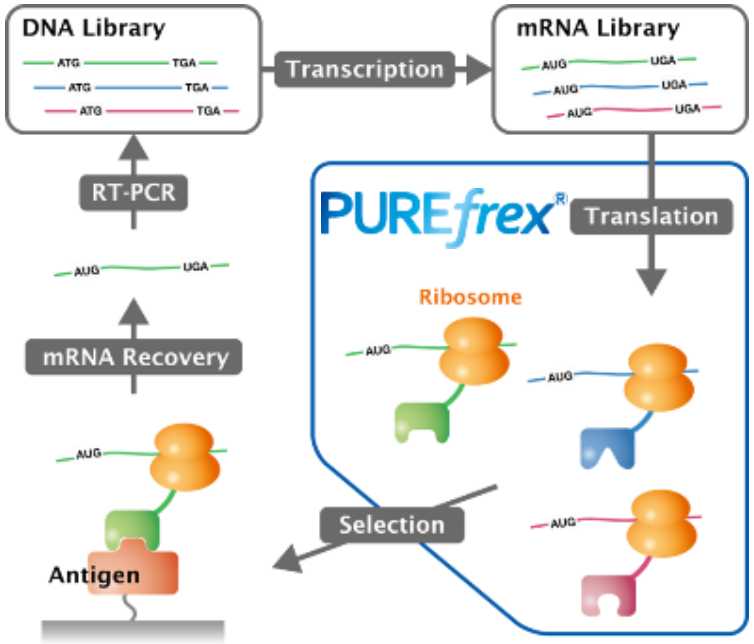
Chui Z. et al. (2024) ACS Sens, 9(6):2846-2857.



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

in vitro protein selection technology

PURE*frex*®*RD*



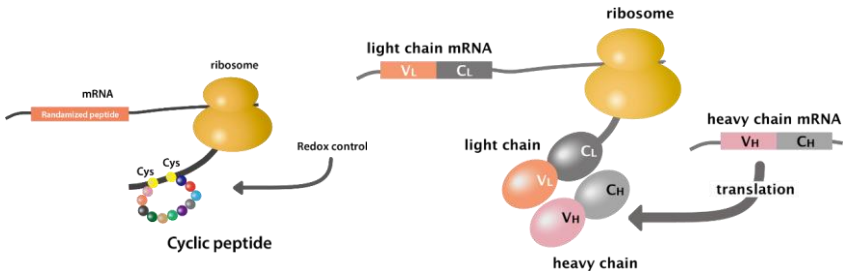
Licensed technology under JP4931135 etc.

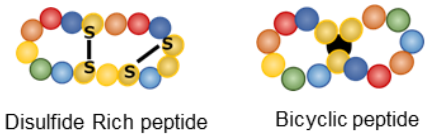
◆ **Advanced screening system for Biologics**

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

◆ **High Selection Efficiency**

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

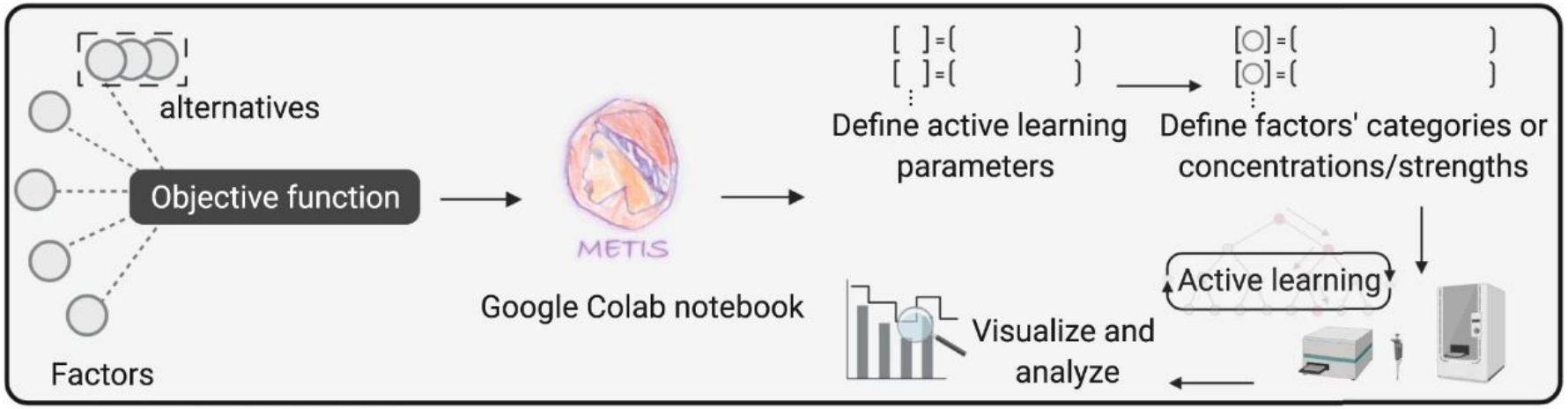




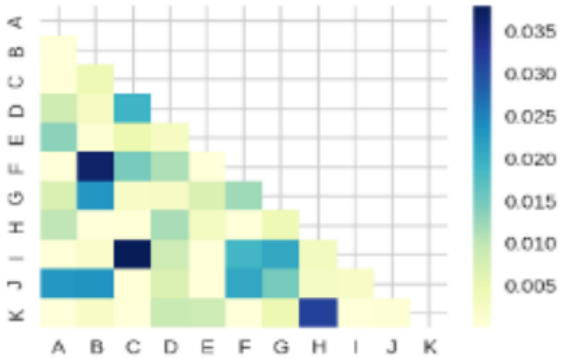
Licensed to

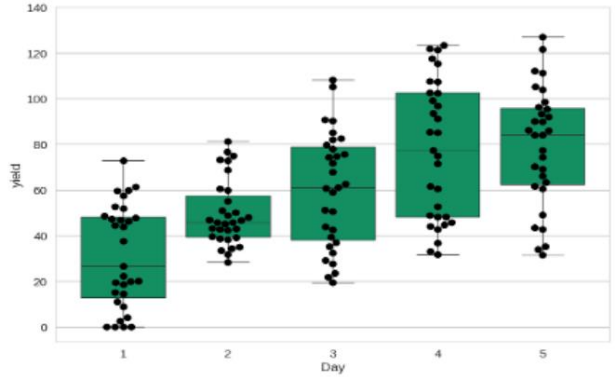
SUTRO BIOPHARMA

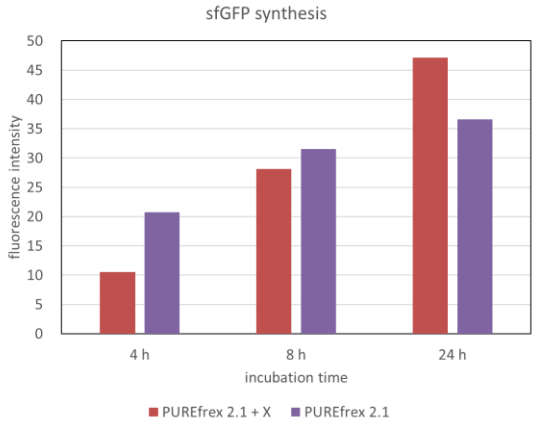
-Broad applications, yet to come!-



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.**

Reconstituted cell-free protein synthesis kit

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For more information

GeneFrontier

-KSF; AT rich codon on N-term-

Fab Heavy Chain (Herceptin)

Herceptin Fab HC

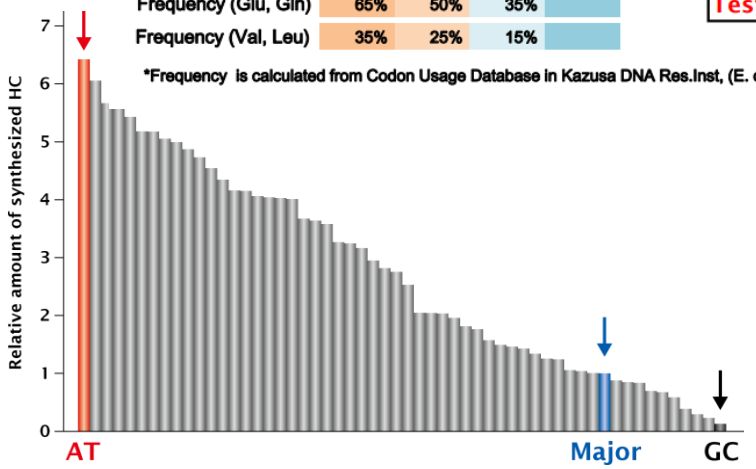
Met- Glu-Val-Gln-Leu-Val- FLAG

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)

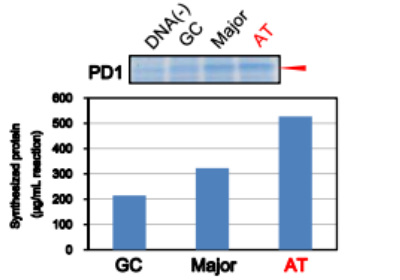


Design of DNA template is important.
Manual is Free to download from our Web site here.

PD1

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

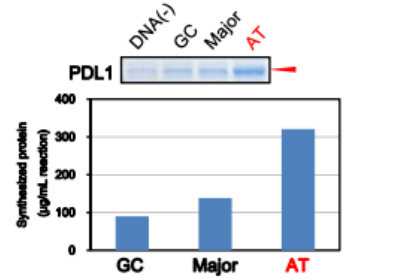
N-term type	1	2(36)	3(37)	4(38)	5(39)	6(40)	GC(%)
	Met	Thr	Phe	Ser	Pro	Ala	1-6 a.a.
GC	atg	acc	ttc	tcc	ccg	gcg	67%
Major	atg	acc	ttt	tct	ccg	gcg	56%
AT	atg	act	ttt	tca	cca	gct	39%



PDL1

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

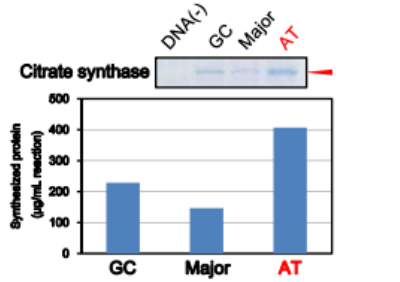
N-term type	1	2(18)	3(19)	4(20)	5(21)	6(22)	GC(%)
	Met	Ala	Phe	Thr	Val	Thr	1-6 a.a.
GC	atg	gcg	ttc	acc	gtg	acc	61%
major	atg	gcg	ttt	acc	gtg	acc	56%
AT	atg	gct	ttt	act	gta	aca	33%



Citrate Synthase

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

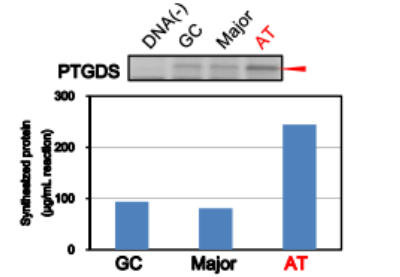
N-term type	1	2(36)	3(39)	4(40)	5(41)	6(42)	GC(%)
	Met	Ser	Ser	Ala	Ser	Glu	1-6 a.a.
GC	atg	tcc	tcc	gcg	tcc	gag	67%
major	atg	tct	tct	gcg	tct	gaa	44%
AT	atg	tca	tca	gct	tca	gaa	39%



PTGDS

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

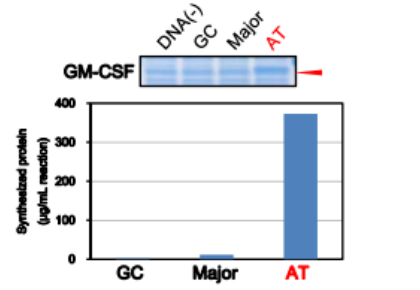
N-term type	1	2(23)	3(24)	4(25)	5(26)	6(27)	GC(%)
	Met	Ala	Pro	Glu	Ala	Gln	1-6 a.a.
GC	atg	gca	ccg	gaa	gca	cag	61%
major	atg	gcg	ccg	gaa	gcg	cag	72%
AT	atg	gca	cct	gaa	gct	caa	50%



GM-CSF

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

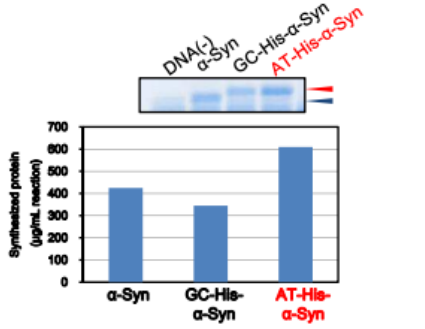
N-term type	1	2(18)	3(19)	4(20)	5(21)	6(22)	GC(%)
	Met	Ala	Pro	Ala	Arg	Ser	1-6 a.a.
GC	atg	gcg	ccg	gcg	cgc	tcc	83%
major	atg	gcg	ccg	gcg	cgc	tct	78%
AT	atg	gca	cct	gct	aga	tca	50%



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	2	3	4	5	6	7	8	9	GC(%)
	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%



Reconstituted cell-free protein synthesis kit

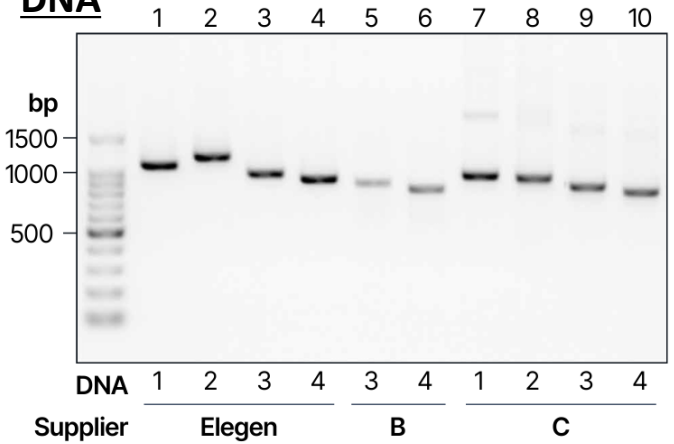
PURE*frex*®

-KSF; Quality of DNA-

GeneFrontier

#	Construct	Size (bp)	Elegen's ENFINIA DNA	Supplier B	Supplier C
			Format	Format	Format
1	HisTEV-sfGFP(G4Y)-PPG-FLAG	978	Linear dsDNA	N/A	Linear dsDNA
2	HisTEV-PPG-sfGFP(G4Y)-FLAG	978	Linear dsDNA	N/A	Linear dsDNA
3	HisTEV-sfGFP(G4Y)-FLAG	888	Linear dsDNA	Linear dsDNA	Linear dsDNA
4	sfGFP(G4Y)-FLAG	840	Linear dsDNA	Linear dsDNA	Linear dsDNA

DNA



RNA

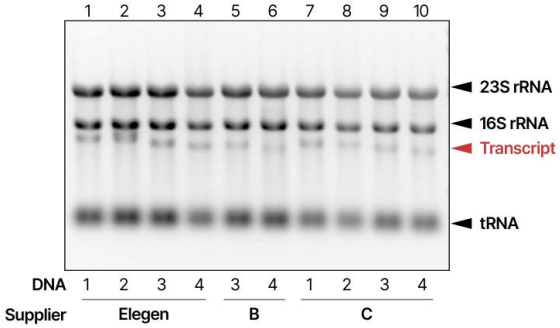
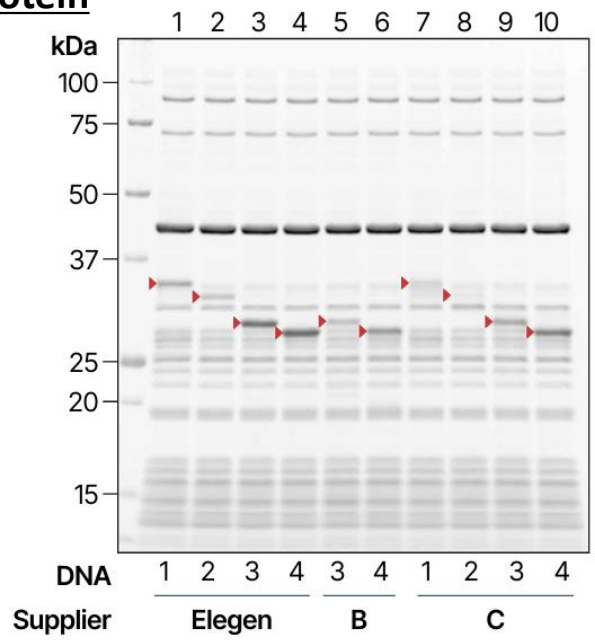
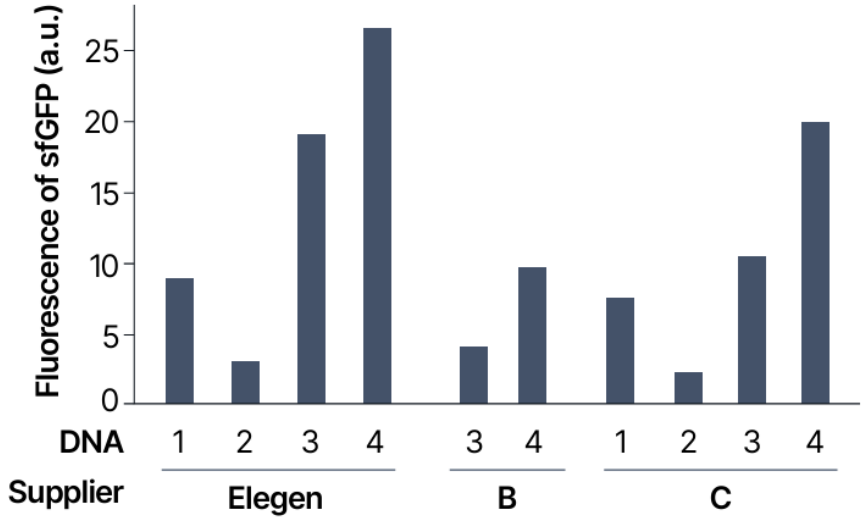


Figure 1. Analysis of DNA synthesized by three vendors. DNA synthesized by Elegen (Supplier A), Supplier B, and Supplier C quantified using a Qubit Fluorometer (Thermo Fisher Scientific) and subjected to agarose gel electrophoresis.

Protein





ELEGEN

To learn more about ENFINIA DNA, visit elegenbio.com
or contact us at info@elegenbio.com

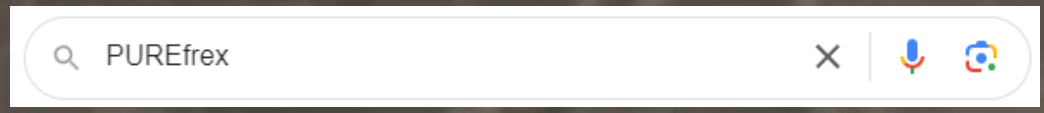
Contact information

Reconstituted cell-free protein synthesis kit

PURE*frex*®

For reagent use for expression / screening of biologics /designing central dogma

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

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- Isoelectric points between **pI 5** and **9**
- Diverse **hydrophobicity** properties

8 only **Months**

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- High-cell density fed-batch
- Control of critical fermentation parameters
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- GMP QC package
- Additional custom QC development available
- QP release