

PUREfres[®] : The Re-built Protein Factory

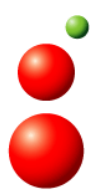


Takashi (Ebi) Ebihara, Ph.D.
COO
GeneFrontier Corporation

PEGS Boston
11-15 of May, 2026



Corporate Summary



GeneFrontier

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

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

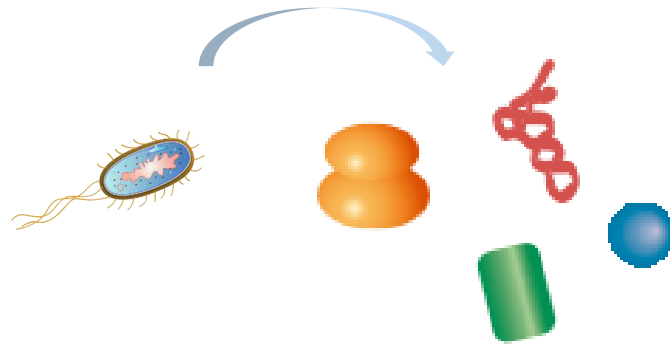
People: ***17 (Ph.D. 8, MS 1)***

Place: ***Chiba, Japan***

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



**Only necessary molecules
for transcription/translation**

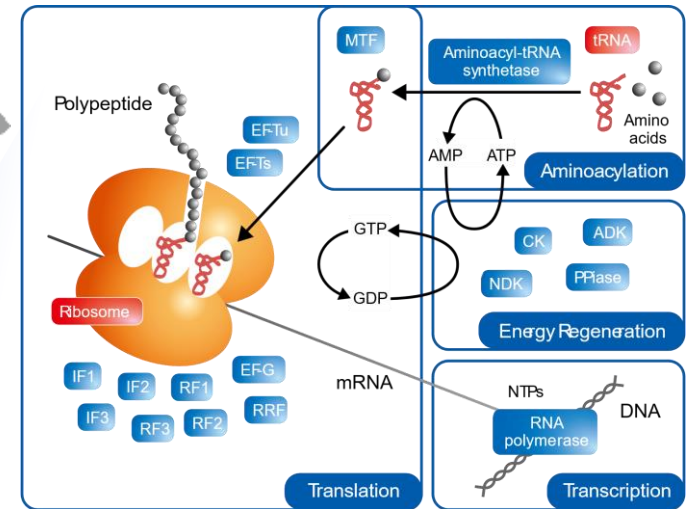
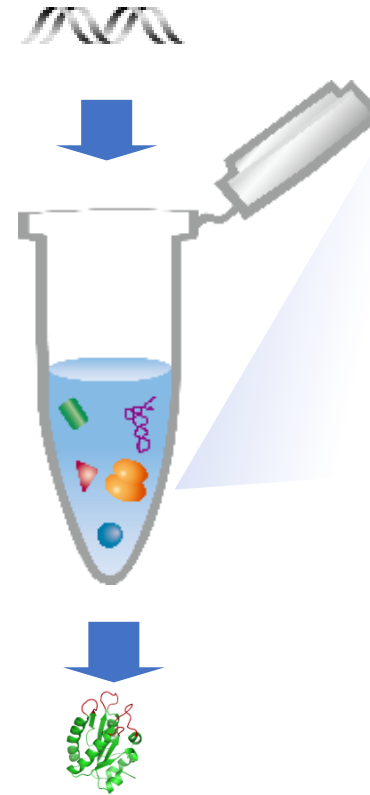


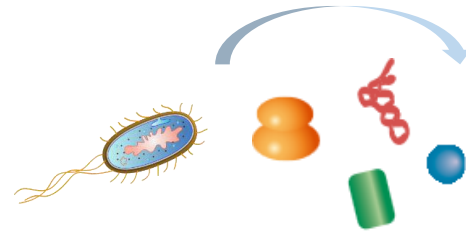
=Breaking down & Building up

PURE system

(Protein synthesis Using Recombinant Elements)

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





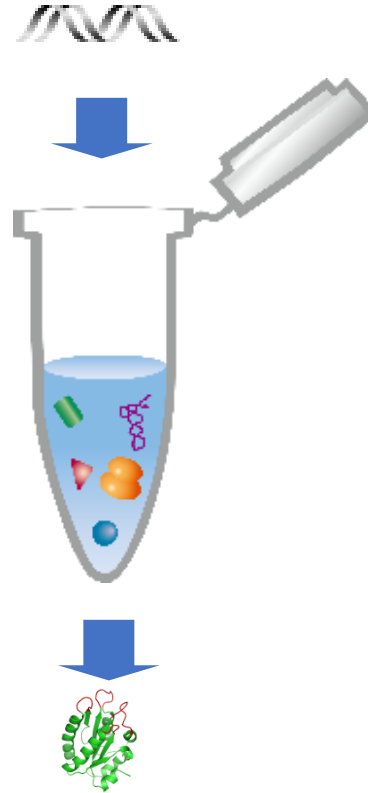
PURE system

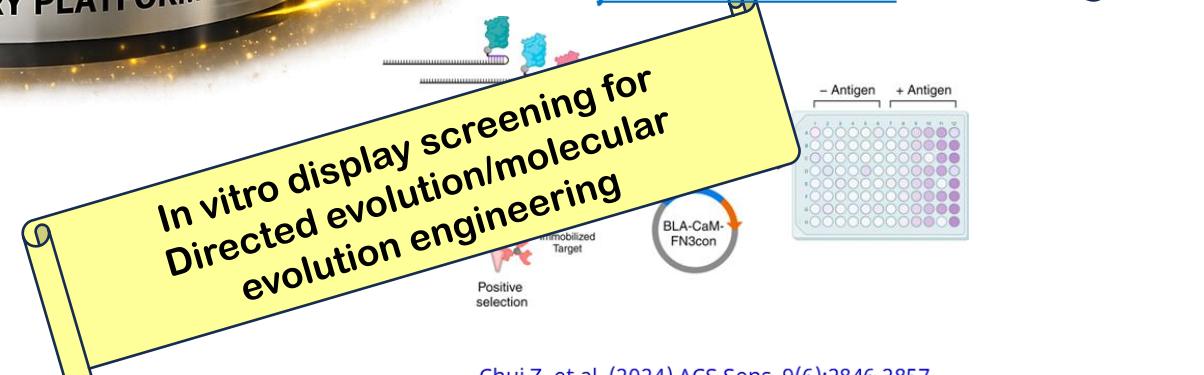
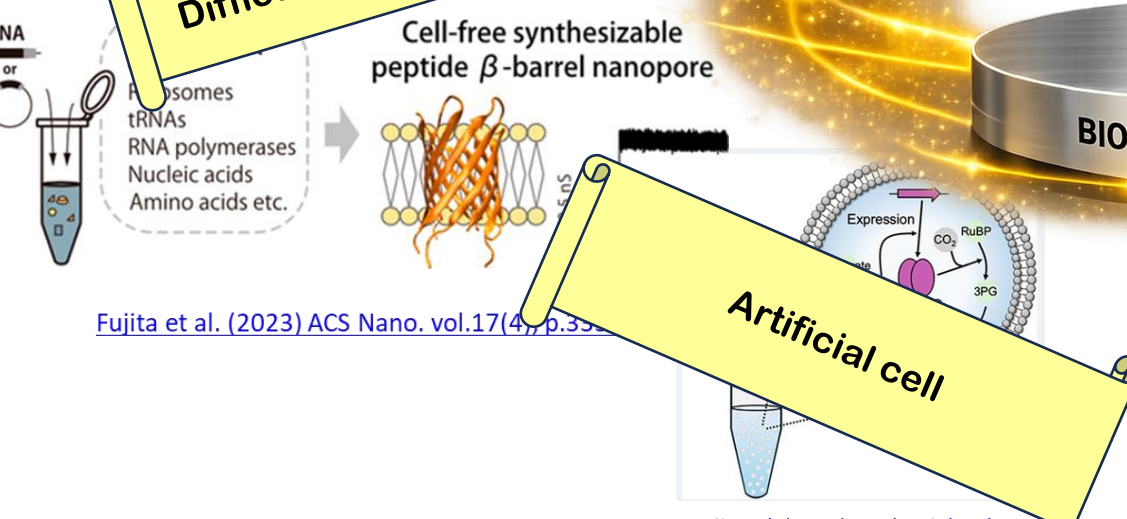
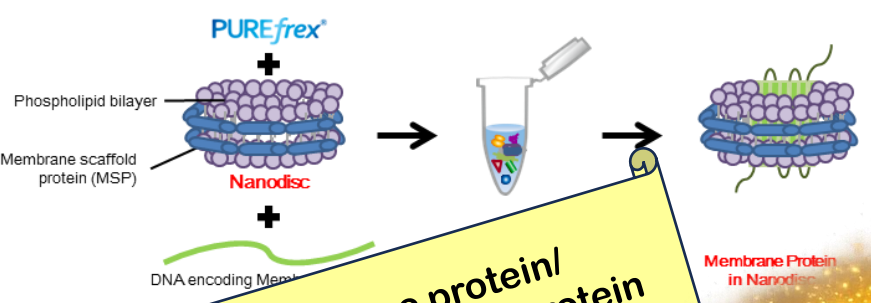
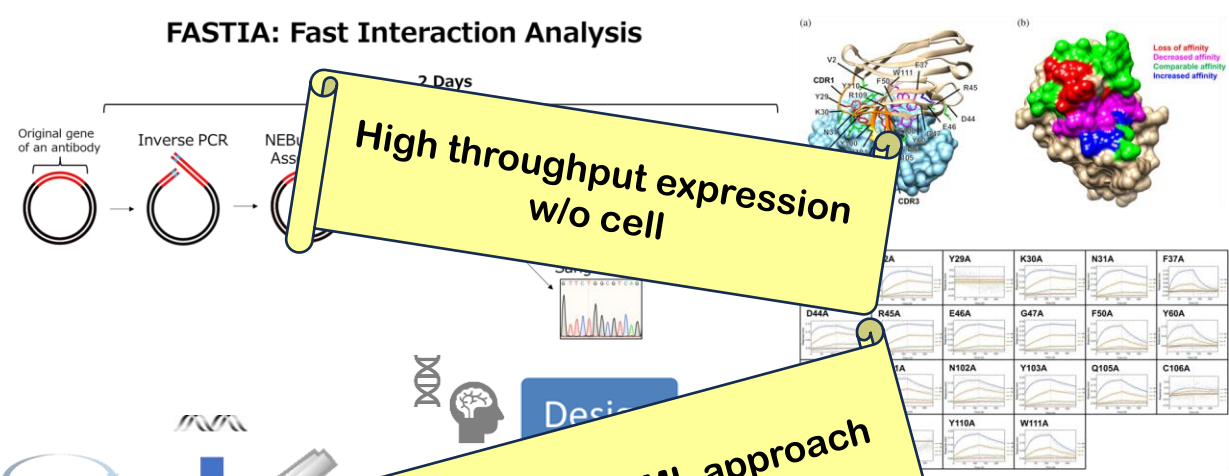
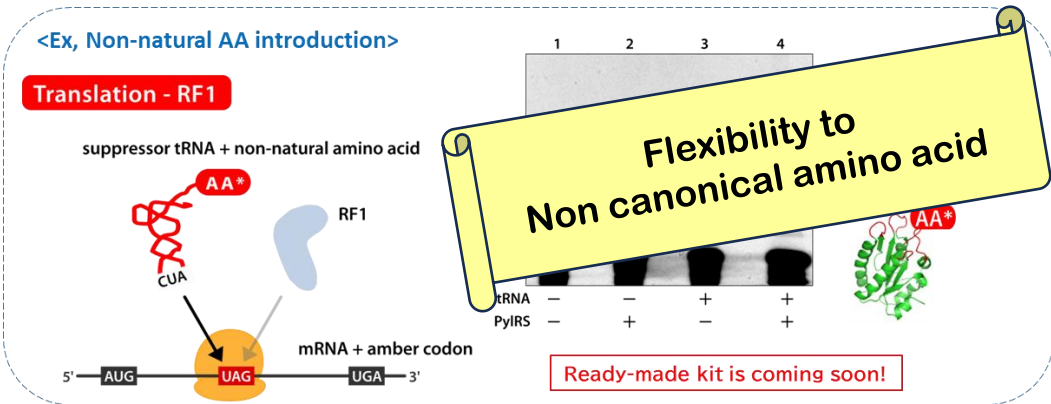


Reconstituted cell-free protein synthesis kit

PUREfrex[®]

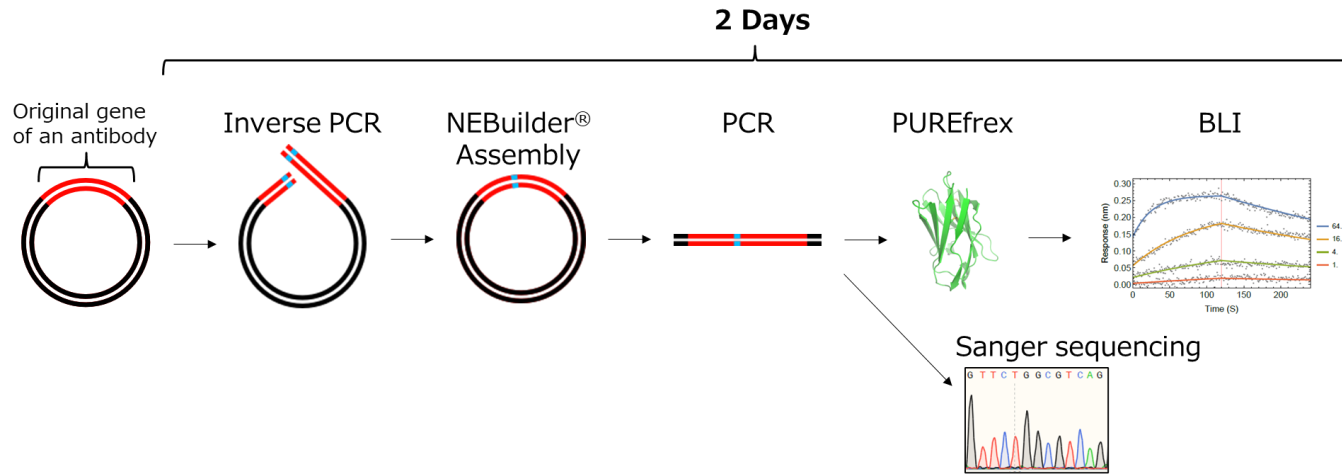
For Designing Central Dogma



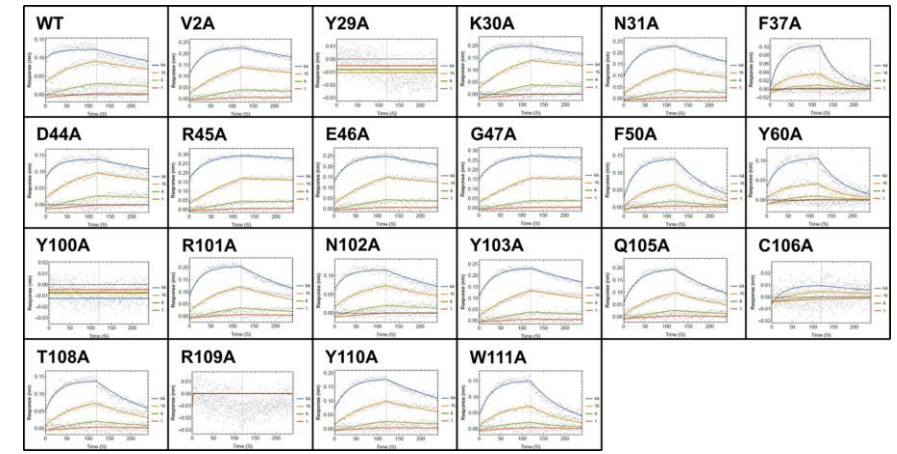
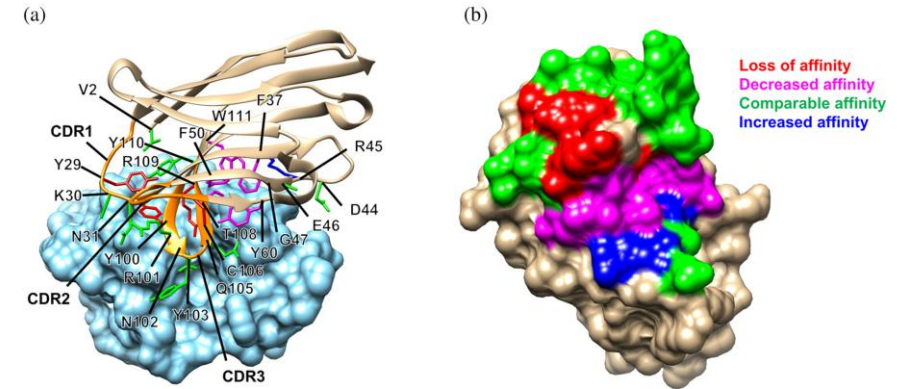
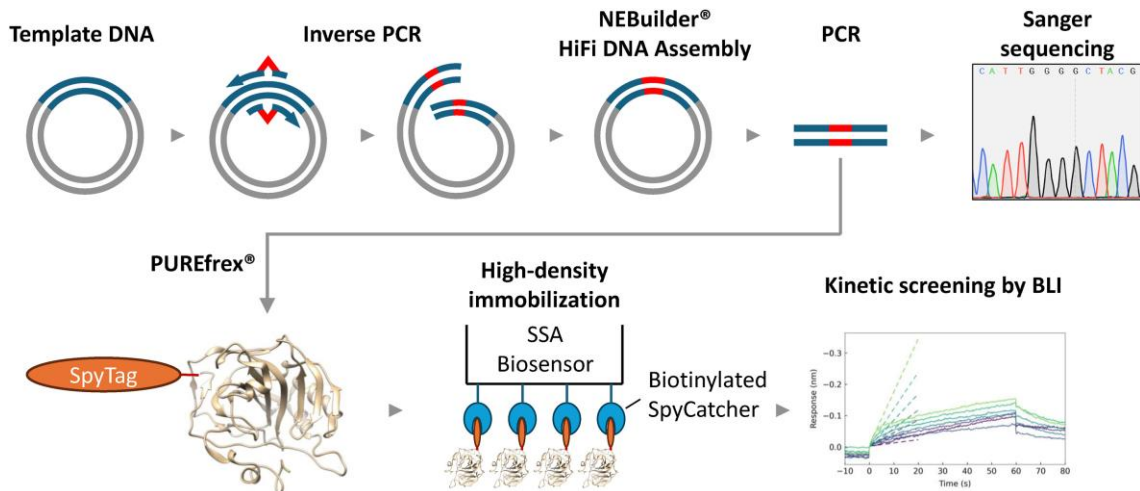


-Improve Validation from Weeks to Days-

FASTIA: Fast Interaction Analysis



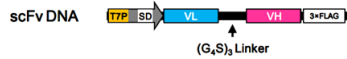
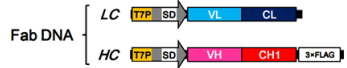
smFASTIA



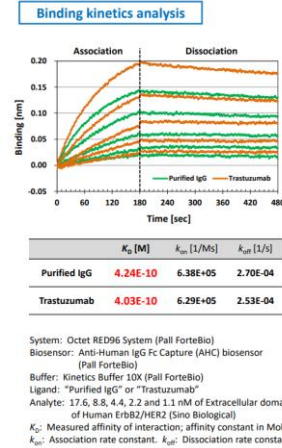
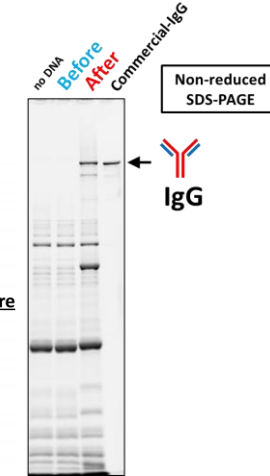
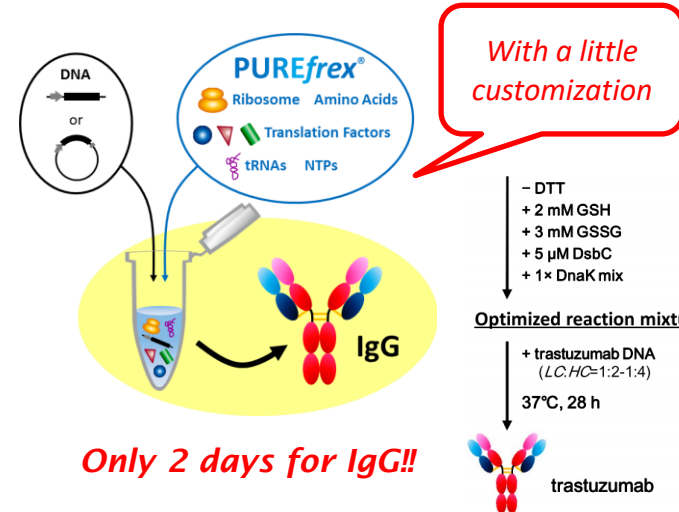
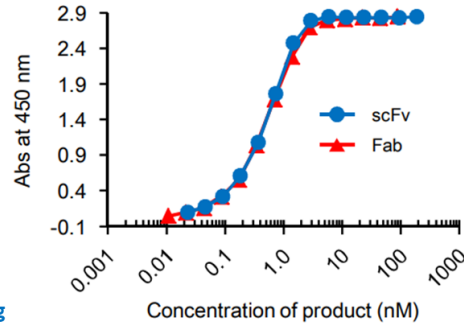
[Matsunaga et al. \(2025\) Protein Sci. Mar;34\(3\):e70065. doi: 10.1002/pro.70065.](https://doi.org/10.1002/pro.70065)

[Tokunaga et al. \(2026\) BBRC. Feb 19:801:153300. doi: 10.1016/j.bbrc.2026.153300.](https://doi.org/10.1016/j.bbrc.2026.153300)

-Expression of scFv, Fab, IgG and more-

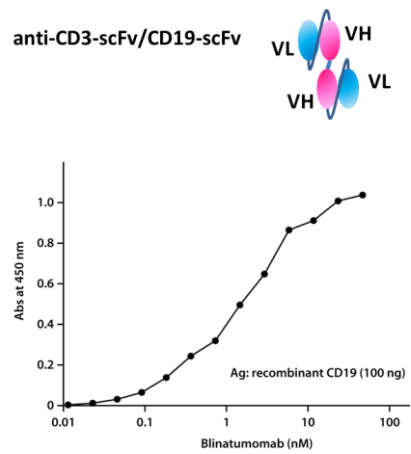


Activity



[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

HEK293 Human IFN α -Reporter Cells

Mouse B16 Reporter Cells

- Universal IFN- α
- hIFN α _L152F
- R121K
- R121K_Q125R
- R121K_Q125R_K132T
- Y86C_R121K_Q125R
- Y86C_R121K_Q125R_K132T
- mIFN2
- IFN α 2b
- scFv control
- Human IFN α 2b (starts at 1nM)
- No DNA

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

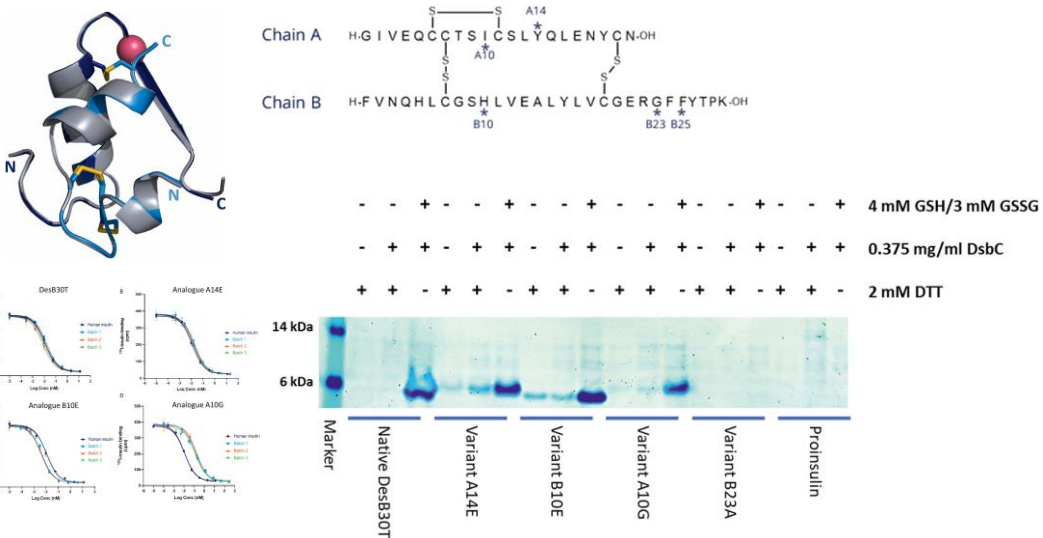
Anti-PDL1 scFv

IFN α VS Tag

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

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

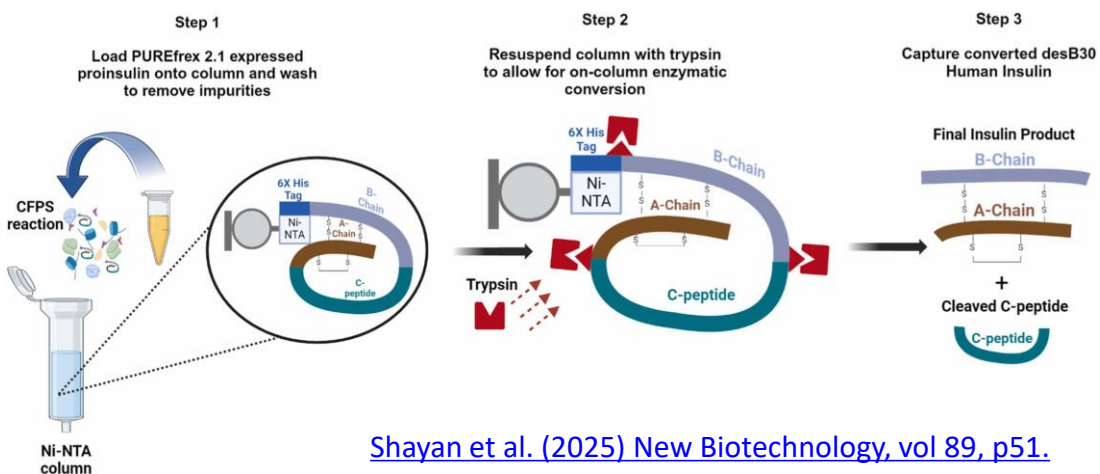
-Application for complex molecule-



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

	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	✗	✗	✗	✗	✓	✗	✓	✓	✓	✓
BL21	✗	✗	✗	✗	✓	✗	✓	✓	✓	✗
759	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗
	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	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓
BL21	✗	✗	✗	✗	✗	✗	✓	✓	✓	✓
759	✓	✓	✗	✓	✓	✓	✓	✗	✓	✓

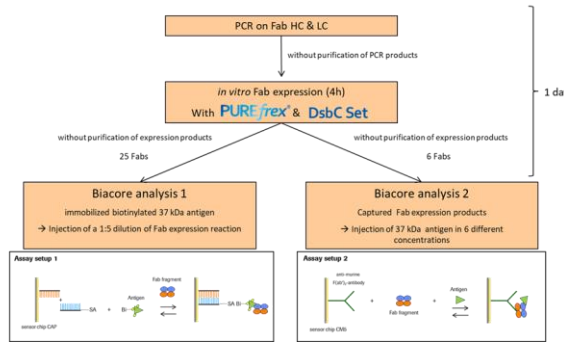
[DeWinter et al. \(2023\) ACS Synth. Biol. vol.12, 4, p1216. \(Supplementary Information\)](#)



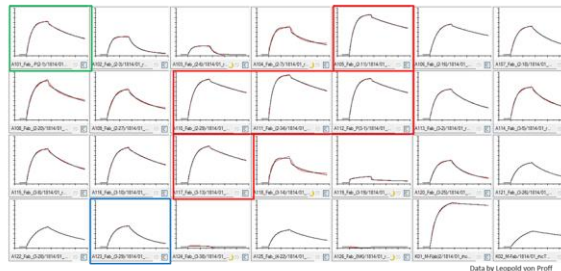
[Shayan et al. \(2025\) New Biotechnology, vol 89, p51.](#)



In vitro expression and Biacore analysis of Fab fragments



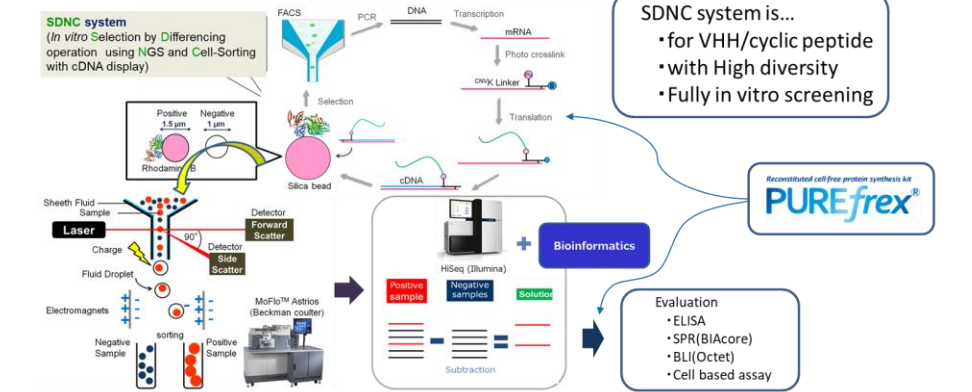
Kinetic analysis of 25 Fab binders



→ Selection of Fabs for further kinetic analysis

EME Epsilon Molecular Engineering

Molecular Design for Human Life



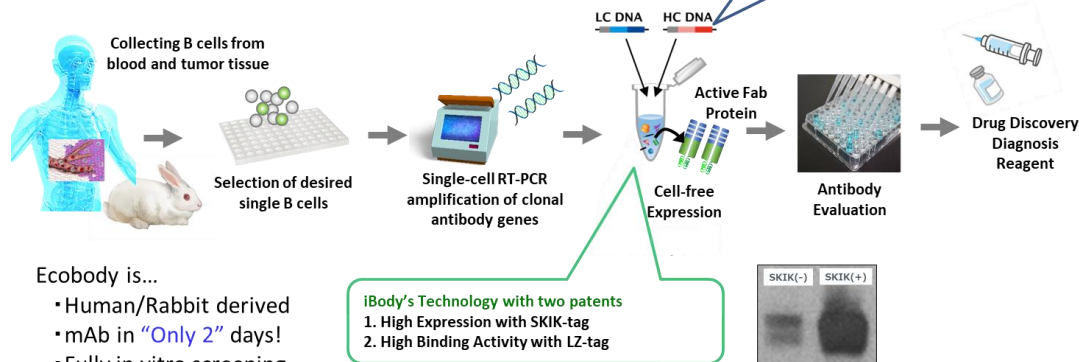
SDNC system is...
 • for VHH/cyclic peptide
 • with High diversity
 • Fully in vitro screening



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

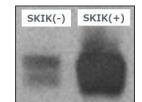


iBody's Ecobody Technology



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

iBody's Technology with two patents
 1. High Expression with SKIK-tag
 2. High Binding Activity with LZ-tag



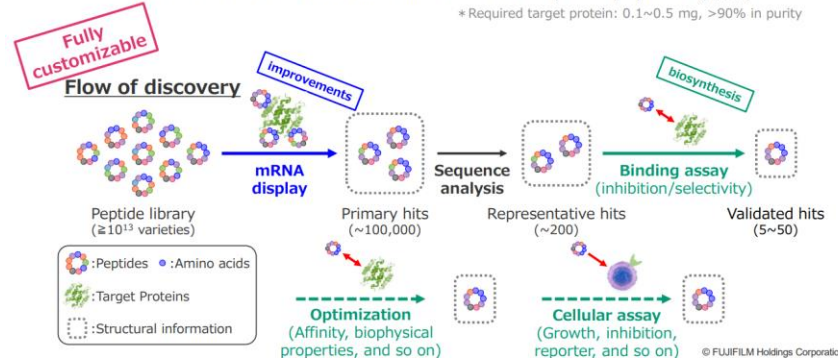
<https://www.ibody.co.jp/en/>

FUJIFILM peptide discovery services

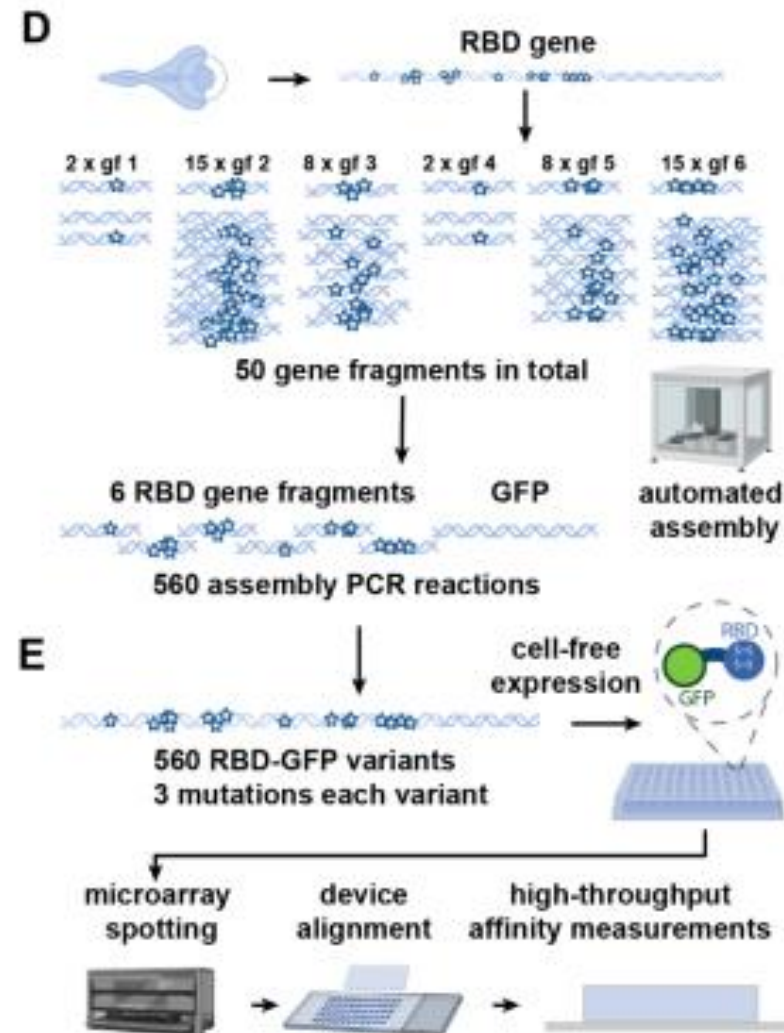
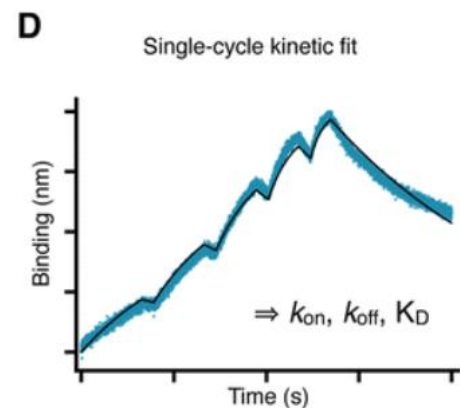
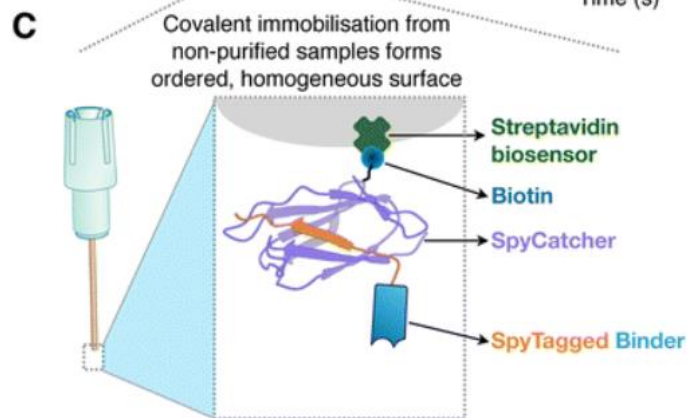
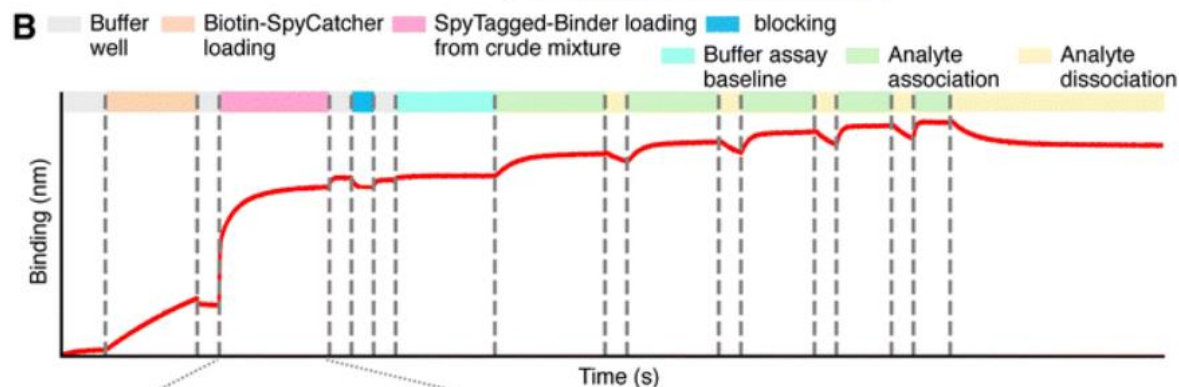
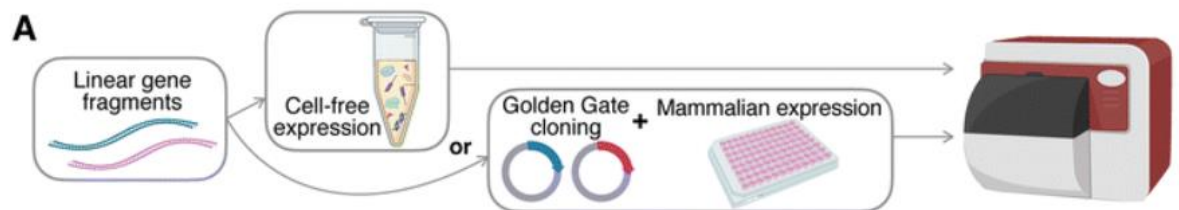
collaborated with PUREfres

- ✓ Innovative improvements in mRNA display enable screening from >10¹³ 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



-Broad applications, yet to come!-

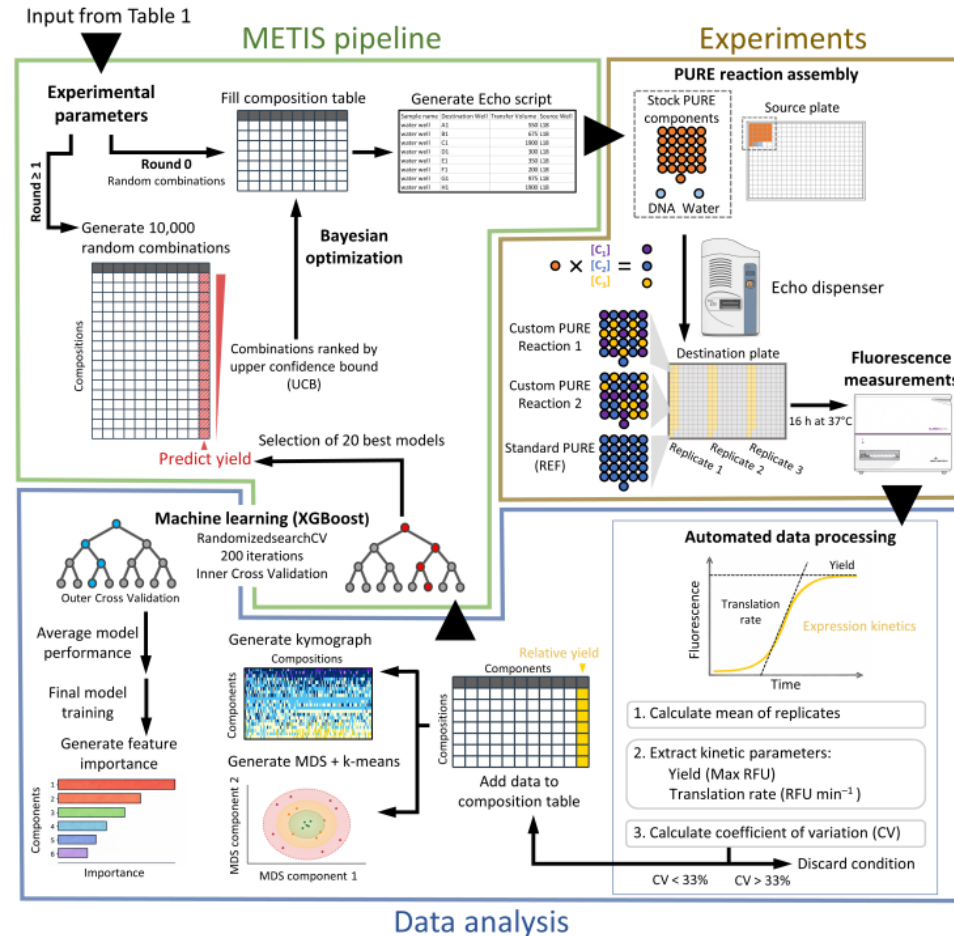


[Predenia et al. \(2025\) RSC Chem. Biol, 6\(1313\).](https://doi.org/10.1039/C5SC01313A)

[Grasemann et al. \(2025\) bioRxiv. https://doi.org/10.1101/2025.09.23.678000.](https://doi.org/10.1101/2025.09.23.678000)

-Broad applications, yet to come!-

Abbreviation	Components	Function	PUREfres 2.0 solution	Calibration
hepes	HEPES-KOH	Buffer and reducing agents	I	GPSA
k-glut	Potassium glutamate	Buffer and reducing agents	I	GPSA
mg-acet	Magnesium acetate	Buffer and reducing agents	I	GPSA
gsh	Glutathione	Buffer and reducing agents	I	GP
spermidine	Spermidine	Buffer and reducing agents	I	GPSB
fthf	10-formyl-tetrahydrofolate	Aminoacylation	I	GPSA
cp	Creatine phosphate	Energy regeneration	I	GP
aa18	All amino acids except tyrosine & cysteine	Aminoacylation	I	GPSA
tyr	Tyrosine	Aminoacylation	I	GPSA
cys	Cysteine	Aminoacylation	I	GP
atp-gtp	Adenosine triphosphate, guanosine triphosphate	Transcription Aminoacylation Translation	I	GPSB
ctp-utp	Cytidine triphosphate, uridine triphosphate	Transcription	I	GPSA
tRNAs	Transfer RNA mix	Aminoacylation Translation	I	GP
aaRSs-mtf	All aminoacyl-tRNA synthetases, Methionyl-tRNA formyltransferase	Aminoacylation	II	GP
IFs	Initiation factors: IF-1, IF-2, IF-3	Translation	II	GP
EFs	Elongation factors: EF-Tu, EF-Ts, EF-G	Translation	II	GP
RFs	Release factors: RF-1, RF-2, RF-3	Translation	II	GPSB
t7pol	T7 RNA polymerase	Transcription	II	GP
ndk-mk-ck-ppiase	Energy regeneration enzymes: nucleoside diphosphate kinase, myokinase, creatine kinase, pyrophosphatase	Energy regeneration	II	GPSA
factors	Other cofactors/stabilizers (proprietary)	-	II	GPSB
ribosomes	Ribosomes	Translation	III	GPSA
DNA	<i>meyfp</i> gene	-	-	GP
Water	Water	-	-	GP

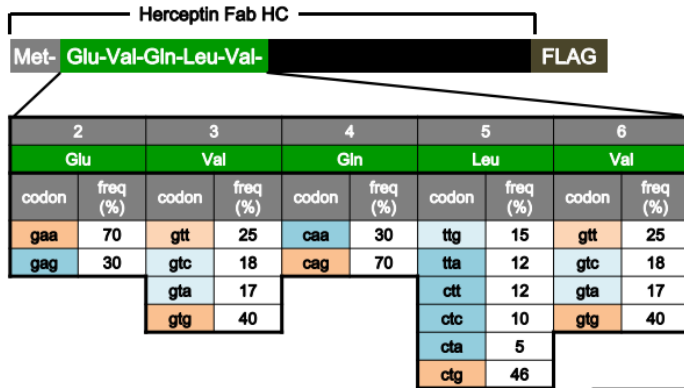


Bernard-Lapeyre Y et al. (2026) <https://doi.org/10.64898/2026.03.23.713685>.

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

-KSF; AT rich codon on N-term-

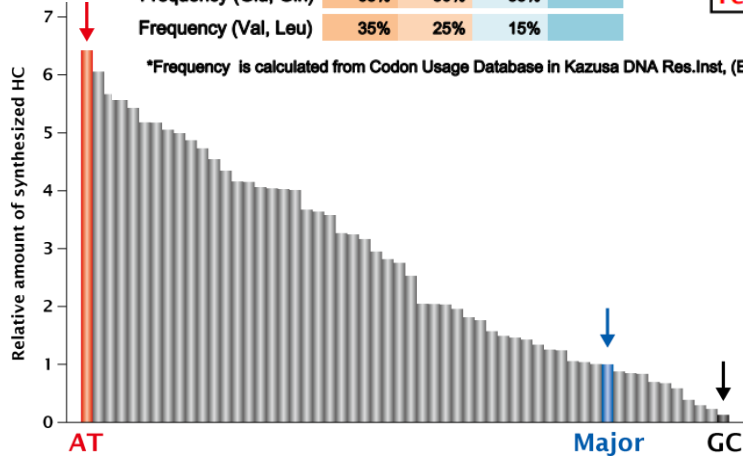
Fab Heavy Chain (Herceptin)



All clones; 384
Tested clones; 56

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

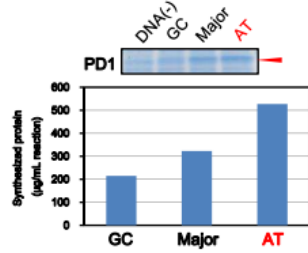
*Frequency is calculated from Codon Usage Database in Kazusa DNA Res.Inst. (E. coli K-12 strain)



PD1

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

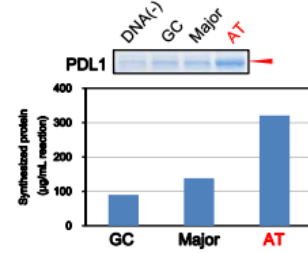
N-term type	1	2(36)	3(37)	4(38)	5(39)	6(40)	GC(%) 1-6 a.a.
Met	Thr	Phe	Ser	Pro	Ala		
GC	atg	acc	ttc	tcc	cgc	gcg	67%
Major	atg	acc	ttt	tct	cgc	gcg	56%
AT	atg	act	ttt	tca	cca	gct	39%



PDL1

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

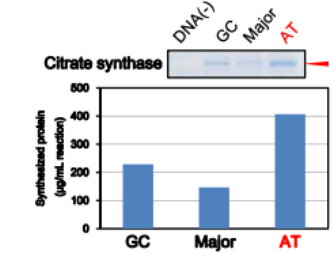
N-term type	1	2(18)	3(19)	4(20)	5(21)	6(22)	GC(%) 1-6 a.a.
Met	Ala	Phe	Thr	Val	Thr		
GC	atg	gcg	ttc	acc	gtg	acc	61%
Major	atg	gcg	ttt	acc	gtg	acc	56%
AT	atg	gct	ttt	act	gta	aca	33%



Citrate Synthase

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

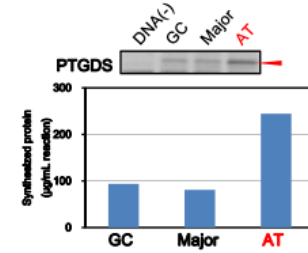
N-term type	1	2(38)	3(39)	4(40)	5(41)	6(42)	GC(%) 1-6 a.a.
Met	Ser	Ser	Ala	Ser	Glu		
GC	atg	tcc	tcc	gcg	tcc	gag	67%
Major	atg	tct	tct	gcg	tct	gaa	44%
AT	atg	tca	tca	gct	tca	gaa	39%



PTGDS

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

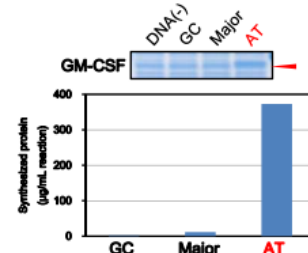
N-term type	1	2(23)	3(24)	4(25)	5(26)	6(27)	GC(%) 1-6 a.a.
Met	Ala	Pro	Glu	Ala	Gln		
GC	atg	gca	cgc	gaa	gca	cag	61%
Major	atg	gca	cgc	gaa	gca	cag	72%
AT	atg	gca	cct	gaa	gct	caa	50%



GM-CSF

Organism: *Homo sapiens*
Synthesized region: 18Ala-144Glu
Length: 128 a.a.
Molecular weight: 14,808 Da

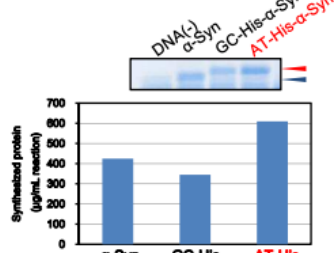
N-term type	1	2(18)	3(19)	4(20)	5(21)	6(22)	GC(%) 1-6 a.a.
Met	Ala	Pro	Ala	Arg	Ser		
GC	atg	gca	cgc	gca	cgc	tcc	83%
Major	atg	gca	cgc	gca	cgc	tct	78%
AT	atg	gca	cct	gct	aga	tca	50%



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.
Met	His	His	His	His	His	His	His	Gly	Ser	
GC	atg	cac	ccc	cac	cac	cac	cac	ggt	tct	59%
Major	atg	cac	cac	cac	cac	cac	cac	ggt	tct	59%
AT	atg	cat	cat	cat	cat	cat	cat	ggt	tct	37%



Design of DNA template is important.

Manual is Free to download from our Web site here.



DNA design for PUREfres



Amino acid sequence entry form

Consultation is free of charge!

-KSF; Quality of DNA-

#	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

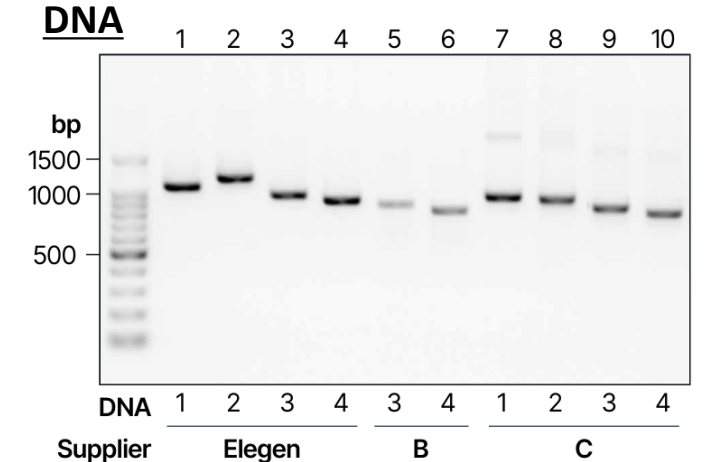
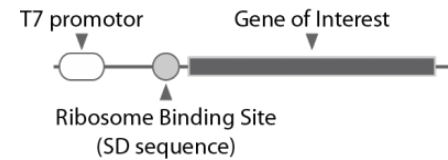
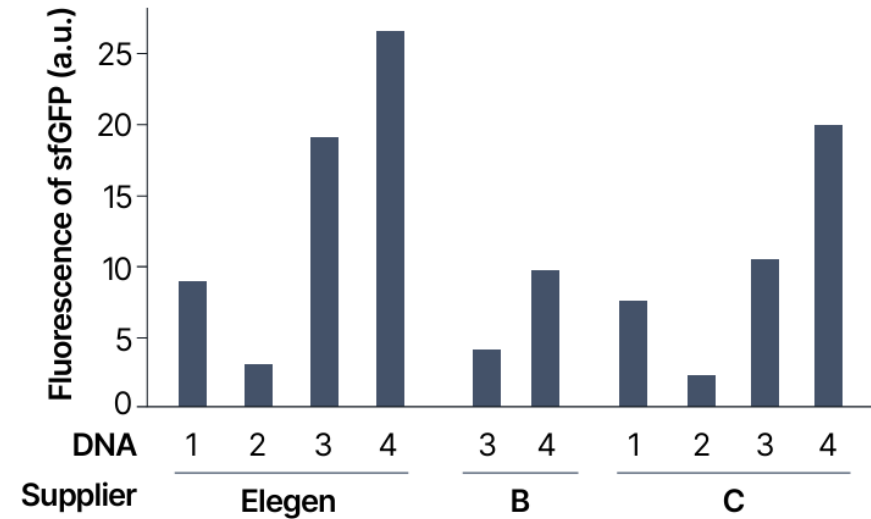
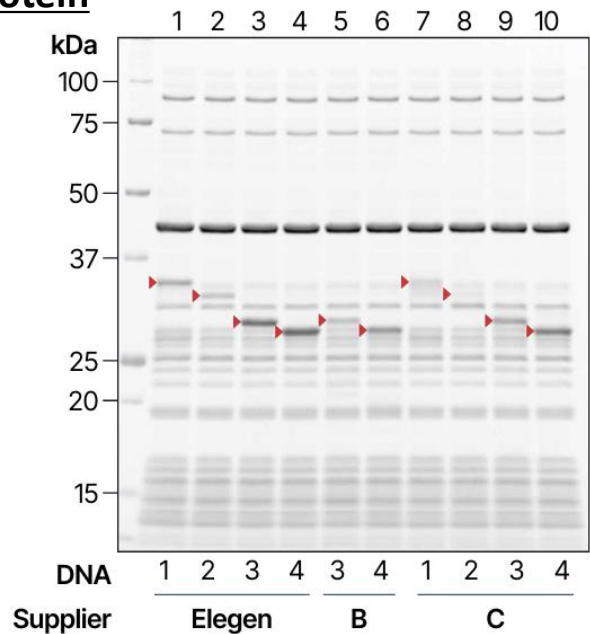


Figure 1. Analysis of DNA synthesized by three vendors. DNA synthesized by Elegen (Supplier A), Supplier B, and Supplier C was quantified using a Qubit Fluorometer (Thermo Fisher Scientific) and subjected to agarose gel electrophoresis.

Protein



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

Contact information

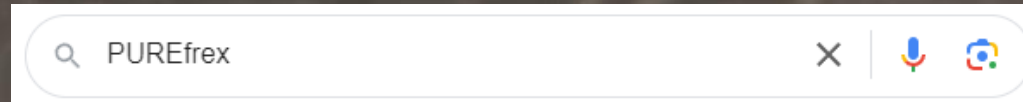


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