For more information

Optimizing Cell-Free System: From Basic Research to Industrial Applications

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

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COO
GeneFrontier Corporation

GeneFrontier

PEGS Boston
13-17 May 2024

Reconstituted cell-free protein synthesis kit

PURE*frex*®

-Customize expression toolbox for your research-

For more information

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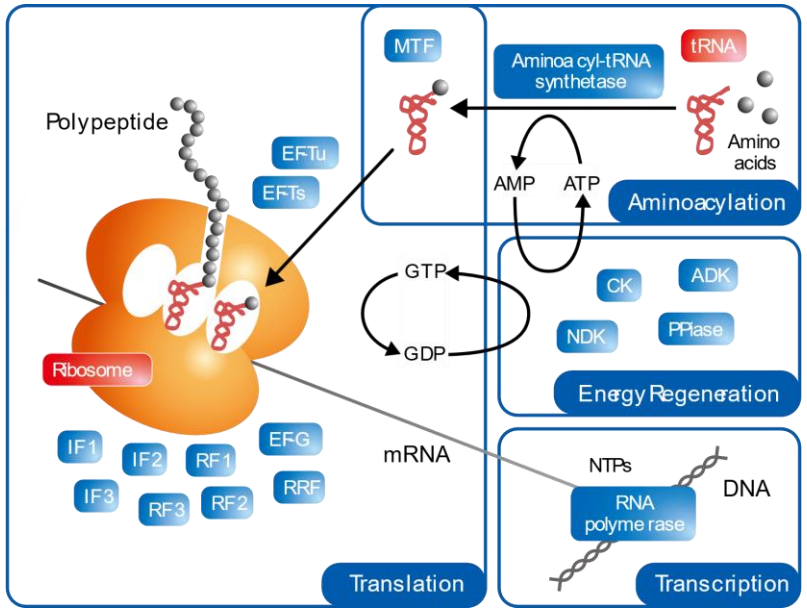
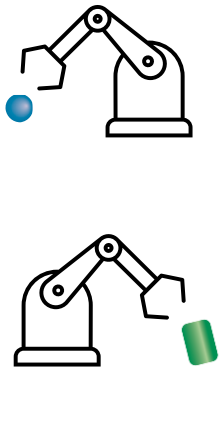
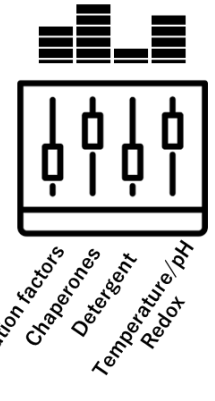
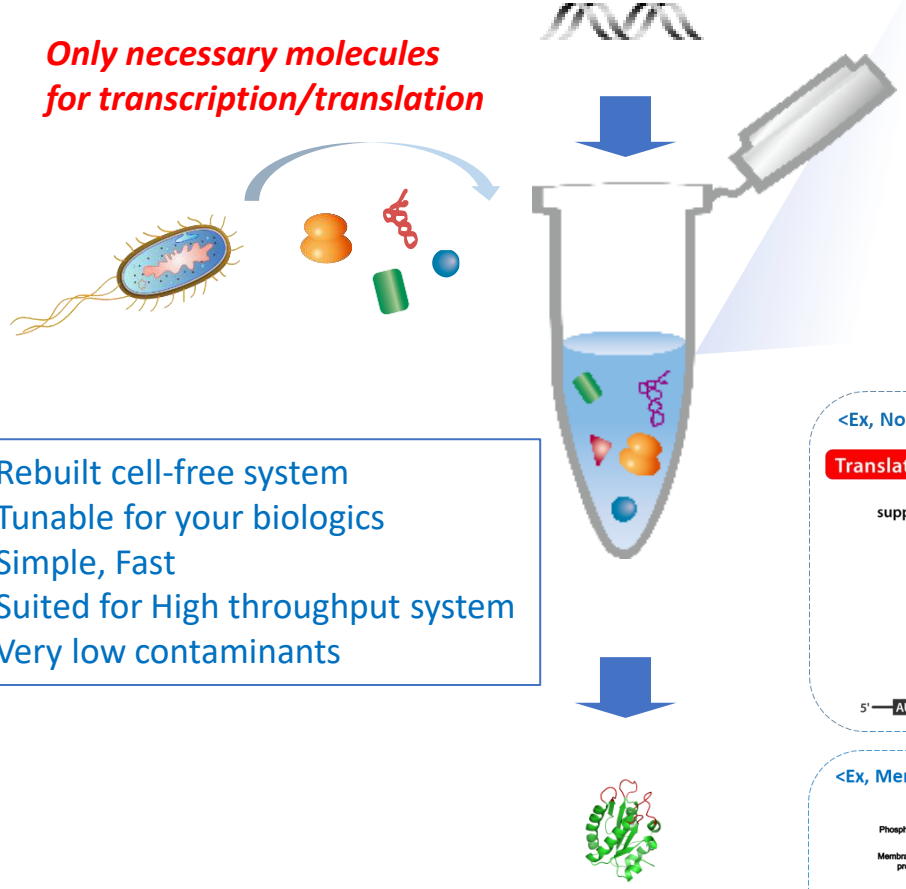
Totally constructive, molecular based system







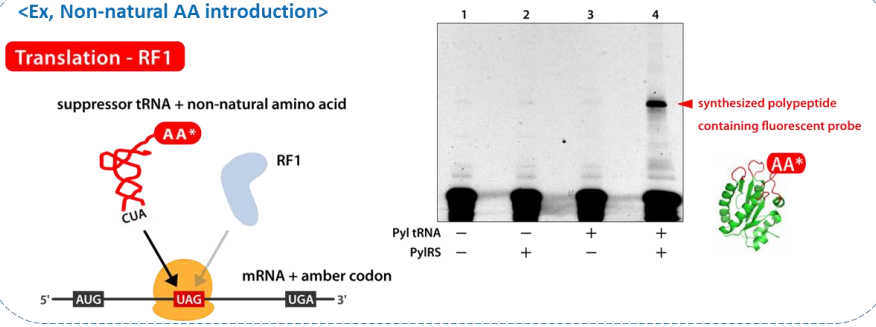
Only necessary molecules for transcription/translation



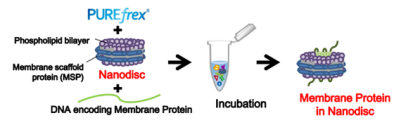
- ✓ Rebuilt cell-free system
- ✓ Tunable for your biologics
- ✓ Simple, Fast
- ✓ Suited for High throughput system
- ✓ Very low contaminants

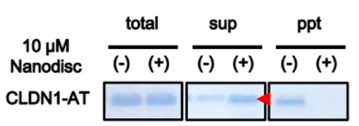
<Ex, Non-natural AA introduction>

Translation - RF1



<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.

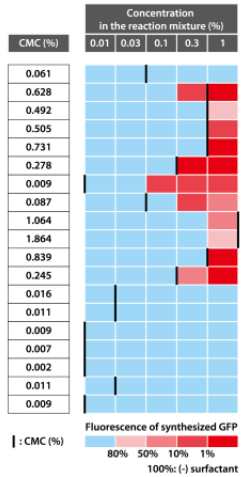
Solubilized hCLDN1 was synthesized using PURE*frex*® and Nanodisc.

Experimental conditions for protein synthesis

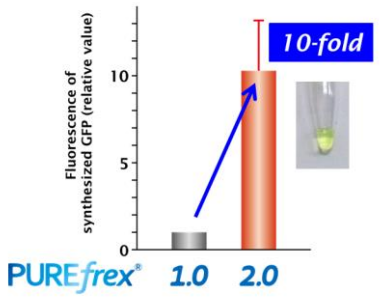
Reaction mixture	Incubation	Template DNA
PURE*frex*®2.1 (4 mM GSH) + Sufractants	37°C 4 h	sfGFP PCR product (1 ng/μL)

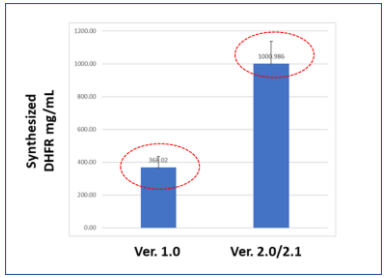
→ Measurement of GFP fluorescence

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



- 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.





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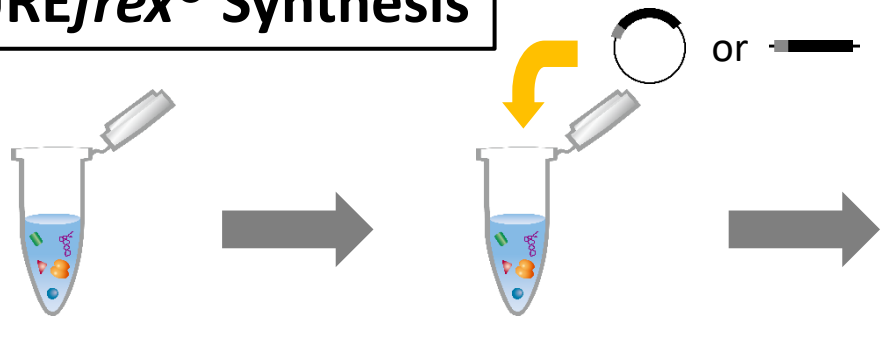
-Improve Expression from Days to Hours-

PURE*frex*® Synthesis

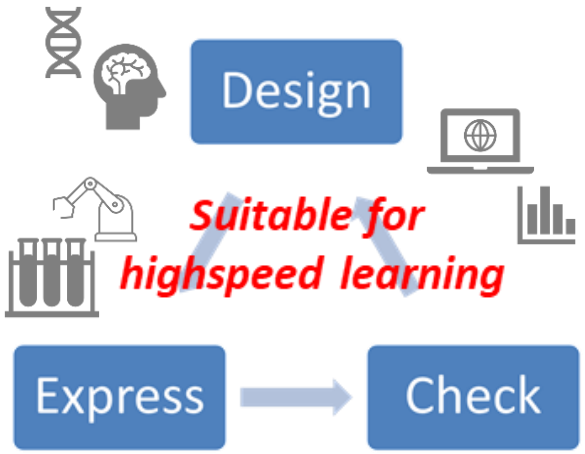


Mix solution I, II and III

Addition of template DNA

Incubation for 2-4 hours (Purification, if necessary)

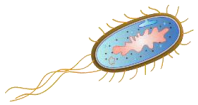
Use for application



Total: 2-4 hours

E. coli Expression

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



Total: 3-4 days

100mL package will be launched soon!

µL ~ L scale

Great Flexibility from basic research to industrial applications

Reconstituted cell-free protein synthesis kit

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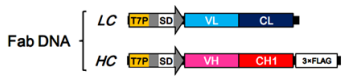
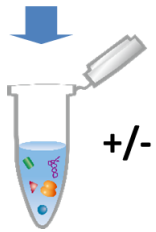
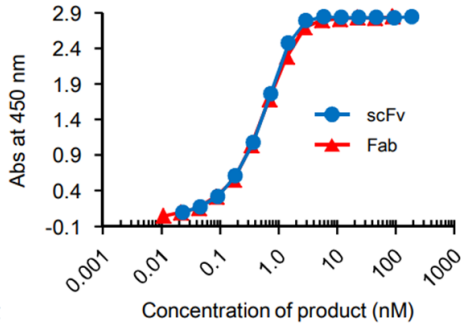
-Expression of scFv, Fab and more-





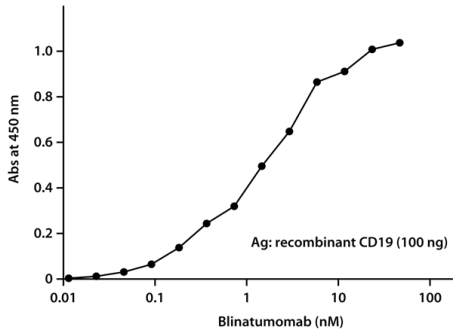
+/- DsbC Set
DnaK Mix
For correct folding

Activity



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

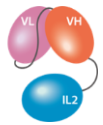
anti-CD3-scFv/CD19-scFv

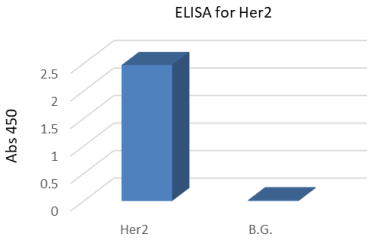


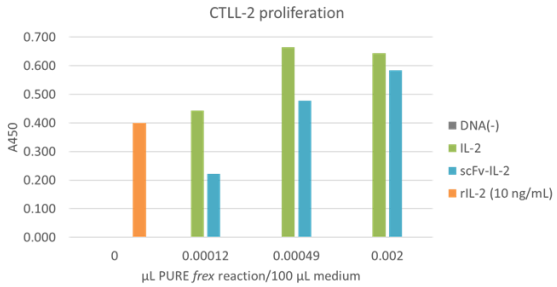
	1	2	3	4	5	6	7	8	9	10
	Proinsulin Aspart	Proinsulin Lispro	Proinsulin Glargine	Regular Proinsulin	Insulin A Chain	Insulin B Chain	Insulin A Chain Heterodimer	Insulin B Chain Heterodimer	Oxytocin	Glucagon
PURE	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Clm24	X	X	X	X	✓	X	✓	✓	✓	✓
BL21	X	X	X	X	✓	X	✓	✓	✓	X
759	✓	✓	✓	✓	✓	✓	✓	✓	✓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

scFv-IL-2







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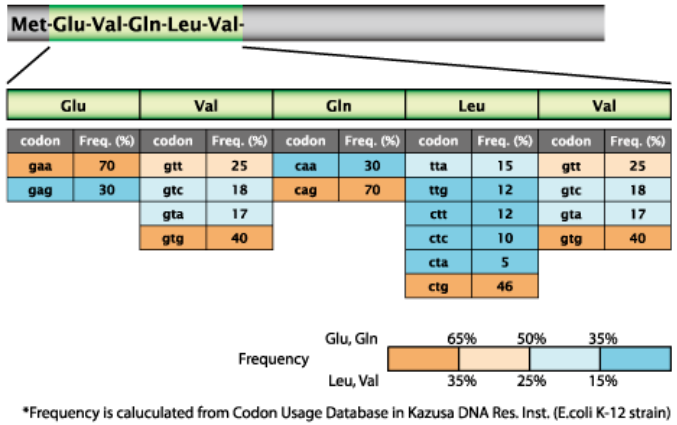
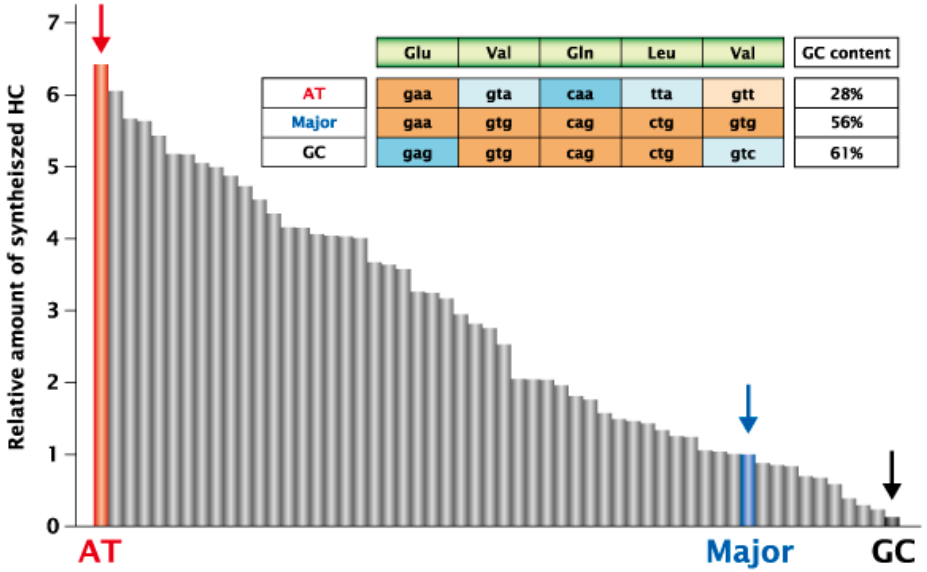
-To harness the potential of translation-



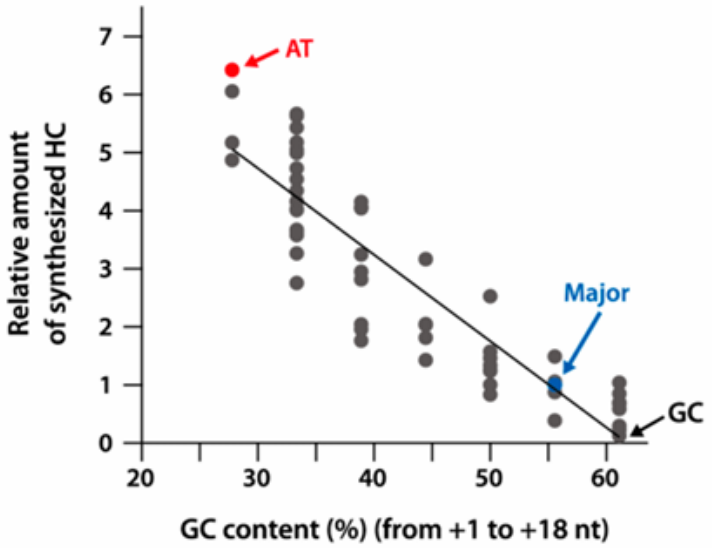
Glu		Val		Gln		Leu		Val	
codon	Freq. (%)	codon	Freq. (%)	codon	Freq. (%)	codon	Freq. (%)	codon	Freq. (%)
gaa	70	gtt	25	caa	30	tta	15	gtt	25
gag	30	gtc	18	cag	70	ttg	12	gtc	18
		gta	17			ctt	12	gta	17
		gtg	40			ctc	10	gtg	40
						cta	5		
						ctg	46		

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

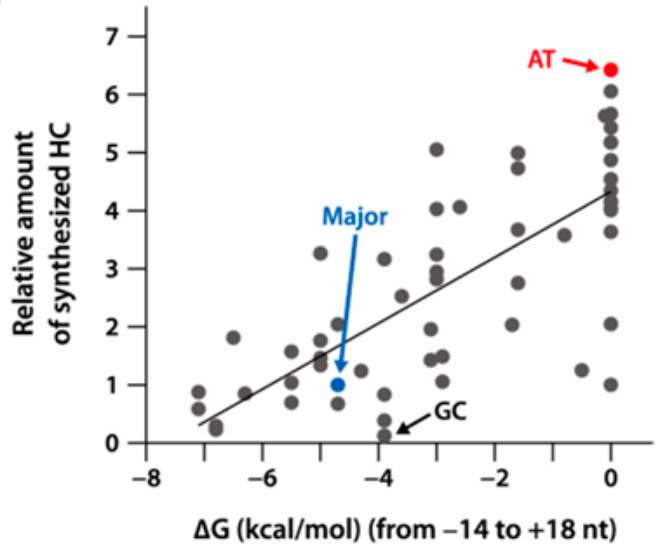


	Glu	Val	Gln	Leu	Val	GC content
AT	gaa	gta	caa	tta	gtt	28%
Major	gaa	gtg	cag	ctg	gtg	56%
GC	gag	gtg	cag	ctg	gtc	61%

A



B

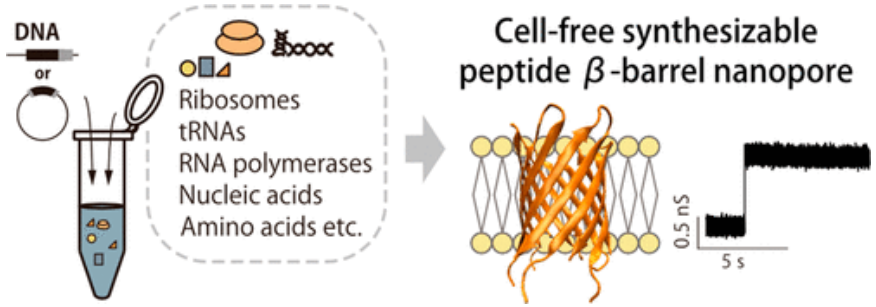


C

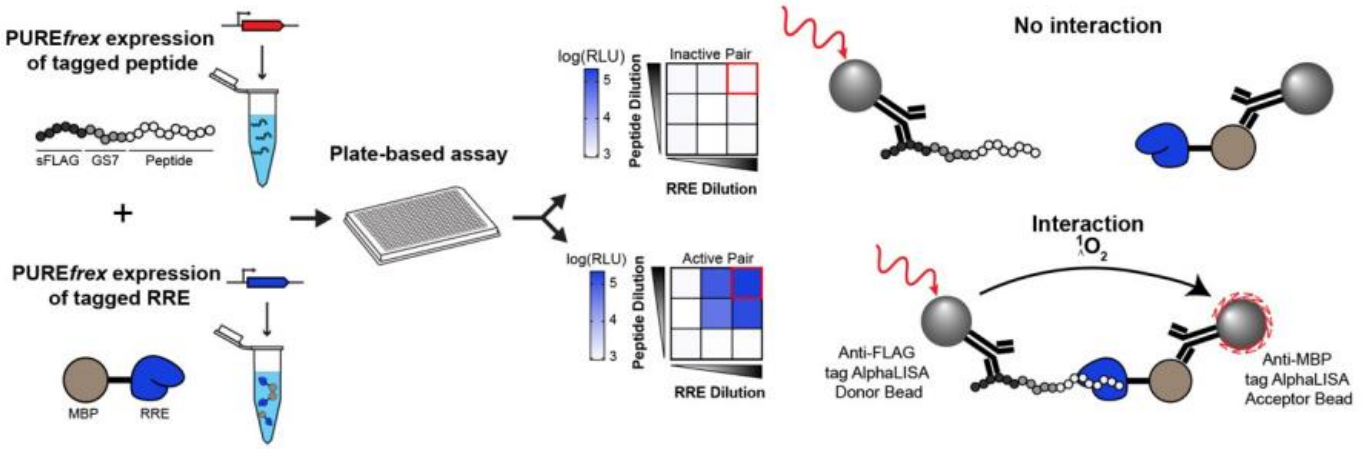
	1	2	3	4	5	6		
Name	Met	Glu	Val	Gln	Leu	Val	GC (%)	ΔG (kcal/mol)
AT	atg	gaa	gta	caa	tta	gtt	28	0.0
Major	atg	gaa	gtg	cag	ctg	gtg	56	−4.7
GC	atg	gag	gtg	cag	ctg	gtc	61	−3.9

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

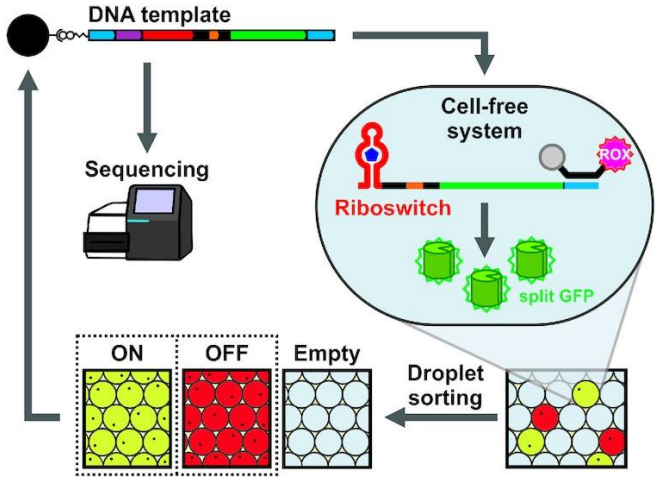
-Broad applications, yet to come!-



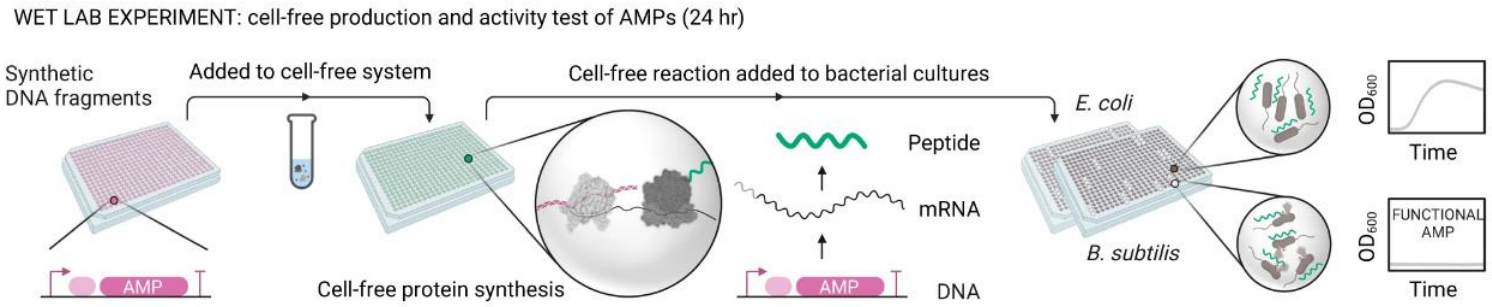
Fujita et al. (2023) ACS Nano. vol.17(4), p.3358.



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



Tabuchi et al. (2022) Nucleic Acids Res
https://doi.org/10.1093/nar/gkac152



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

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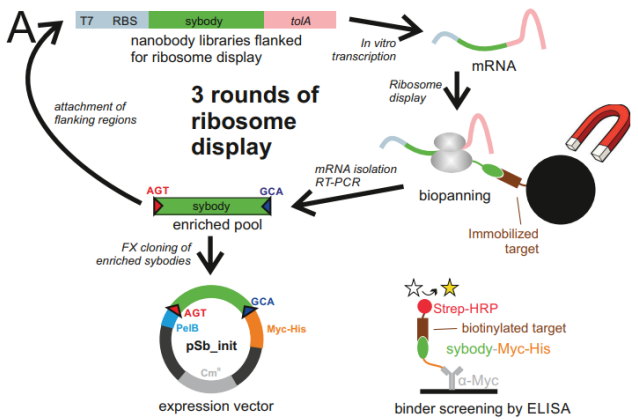
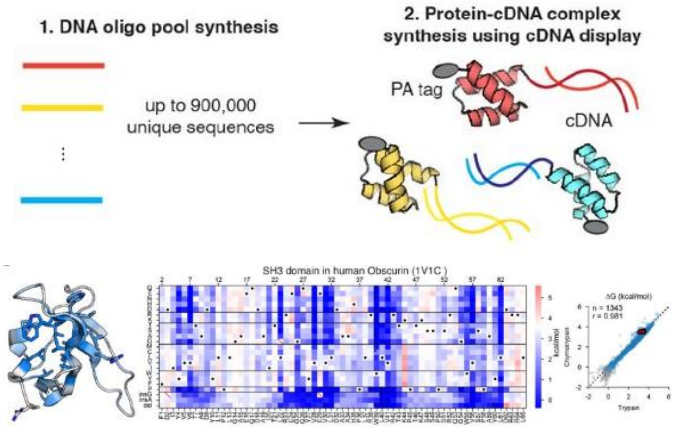
-Broad applications, yet to come!-



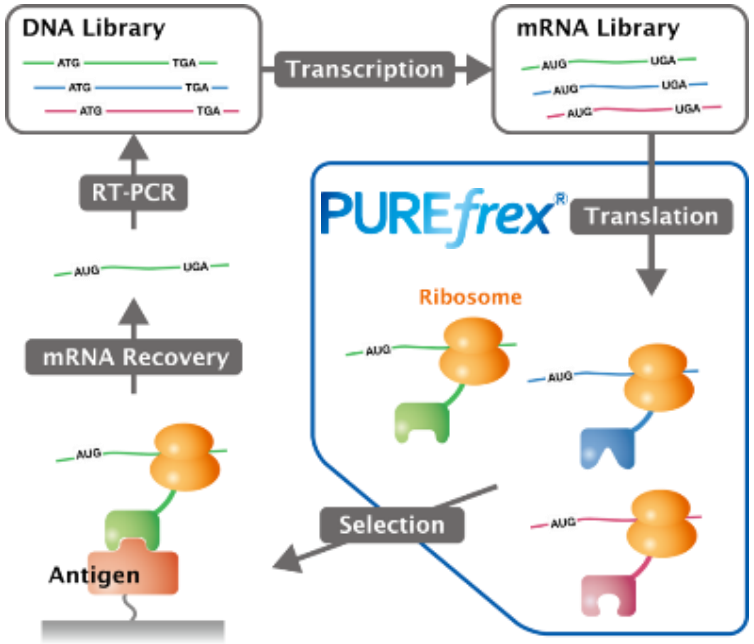
Zimmermann I. et al. (2018) eLife, 7, e34317.



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

in vitro protein selection technology

PURE*frex*® RD



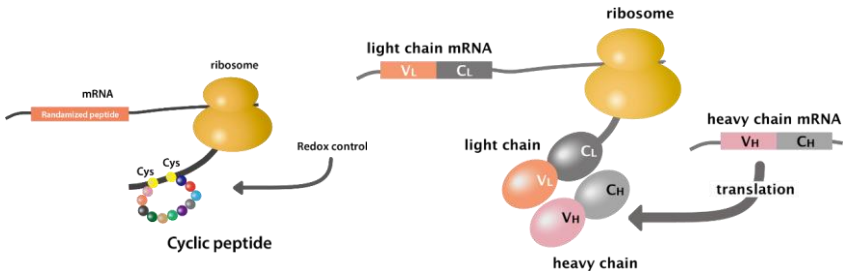
Licensed technology under JP4931135 etc.

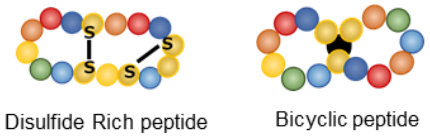
◆ **Advanced screening system for Biologics**

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

◆ **High Selection Efficiency**

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





Licensed to

SUTRO BIOPHARMA

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PURE*frex*®

For reagent use for expression / screening of biologics

https://purefrex.genefrontier.com/

in vitro protein selection technology

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

Takashi Ebihara, Ph.D., COO, GeneFrontier

E-mail: ebihara@genefrontier.com