

[For more information](#)

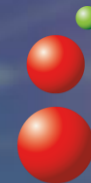


# Rebuilt Cell-Free System (PUREfrex®); As a new platform for Synthetic Biology

*Reconstituted cell-free protein synthesis kit*

**PUREfrex®**

**Takashi Ebihara, Ph.D.**  
**COO**  
**GeneFrontier Corporation**



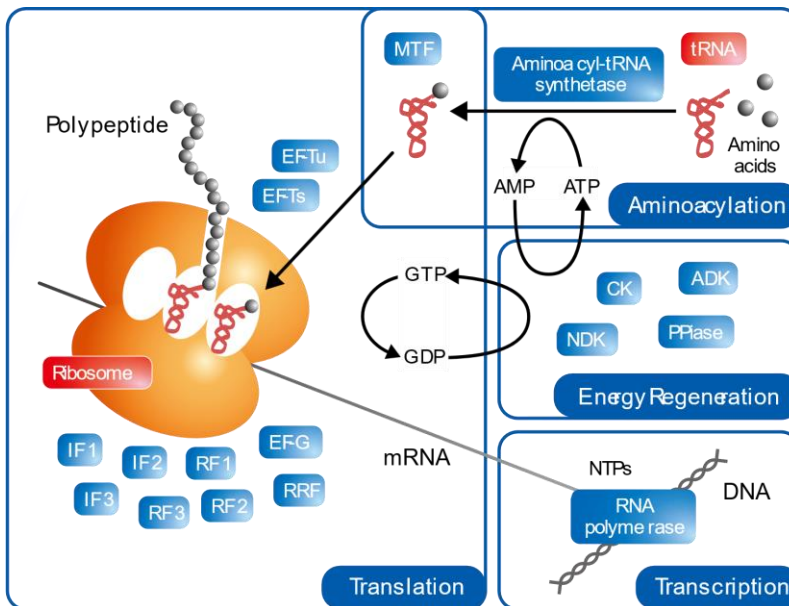
GeneFrontier

**SynBioBeta**  
**7-9 May 2024**

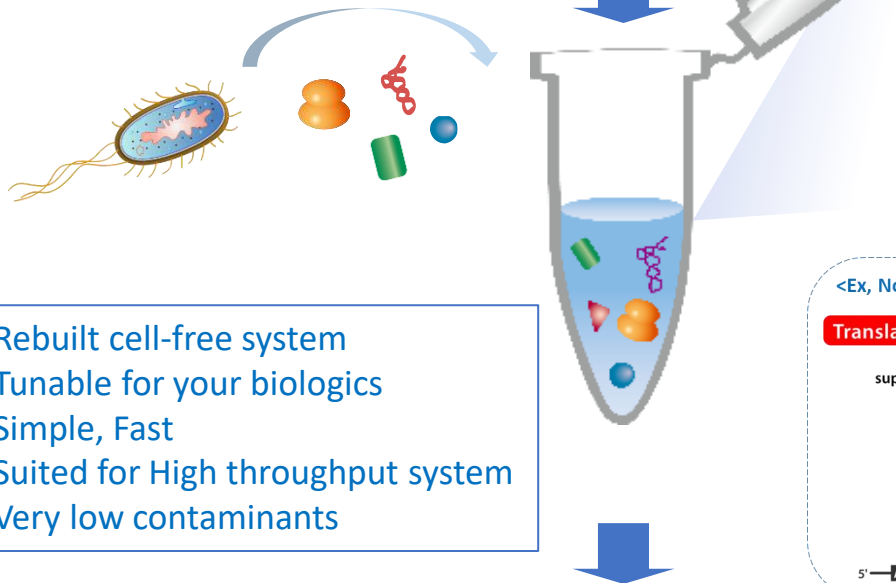
# PUREfres<sup>®</sup>

-Customize expression toolbox for your research-

## Totally constructive, molecular based system

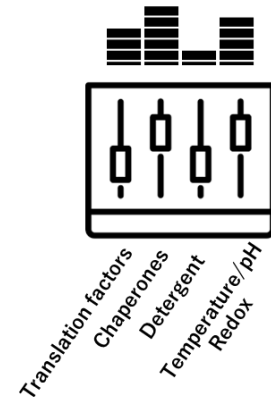
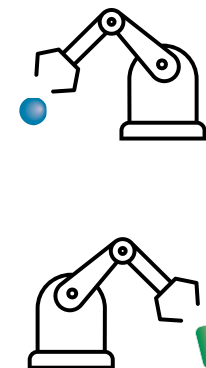


Only necessary molecules for transcription/translation



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

For more information

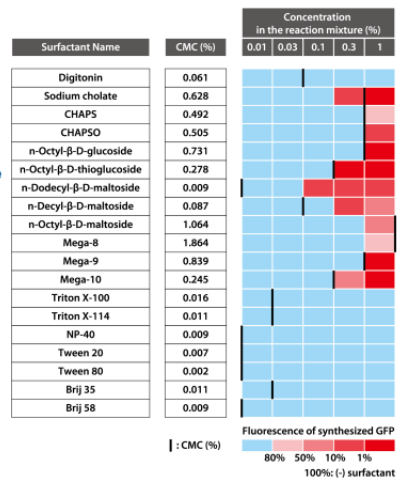


### Experimental conditions for protein synthesis

Reaction mixture	Incubation	Template DNA
PUREfres <sup>®</sup> 2.1 (4 mM GSH) + Surfactants	37°C 4 h	sfGFP PCR product (1 ng/μL)

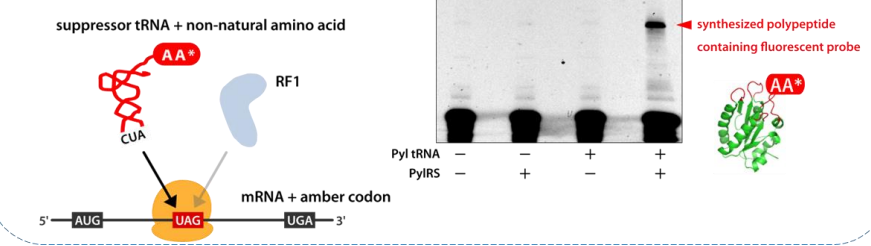
### Measurement of GFP fluorescence

- Most surfactants did not inhibit the protein synthesis reaction by PUREfres<sup>®</sup> below the CMC.
- Some surfactants such as Triton X-100 and Tween 20 could be used even above the CMC.

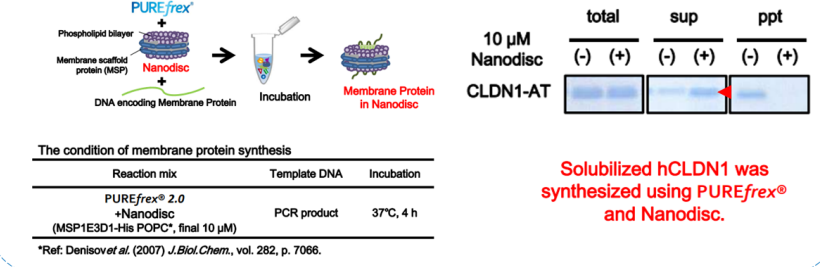


### <Ex, Non-natural AA introduction>

#### Translation - RF1



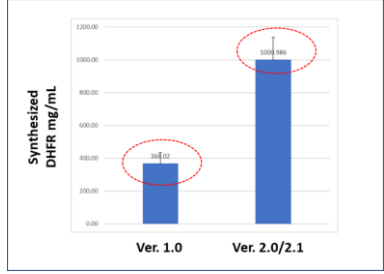
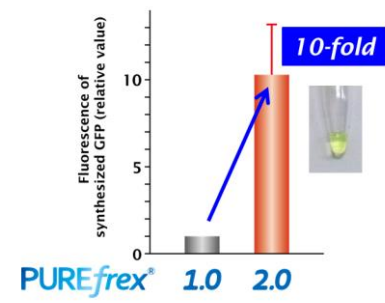
### <Ex, Membrane protein with Nanodisc; artificial membrane-like structure>



#### The condition of membrane protein synthesis

Reaction mix	Template DNA	Incubation
PUREfres <sup>®</sup> 2.0 +Nanodisc (MSP1E3D1-His POPC*, final 10 μM)	PCR product	37°C, 4 h

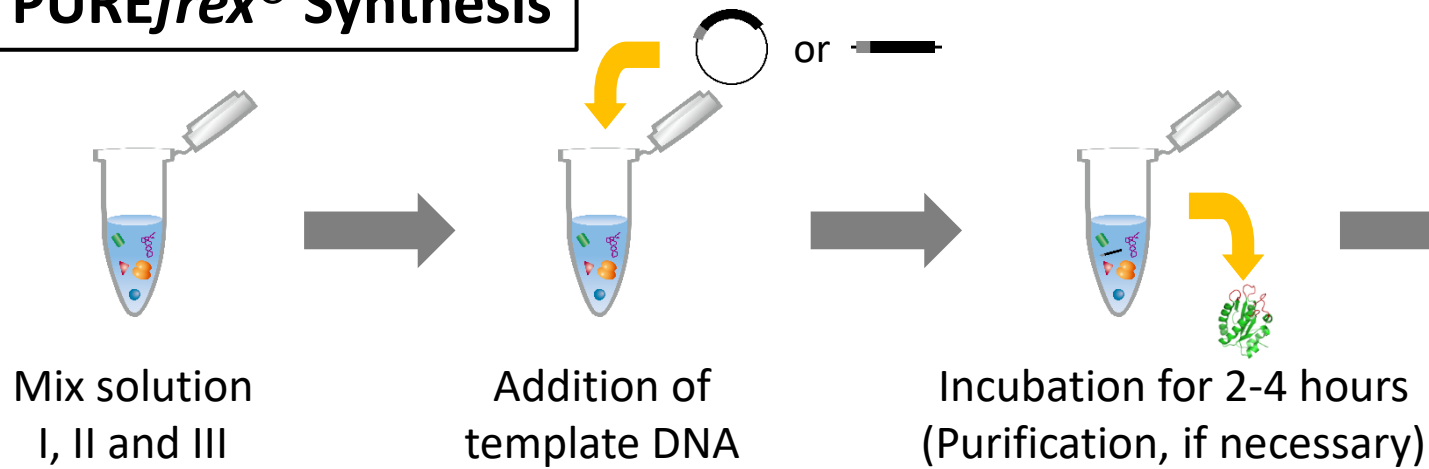
\*Ref: Denisov et al. (2007) J.Biol.Chem., vol. 282, p. 7066.





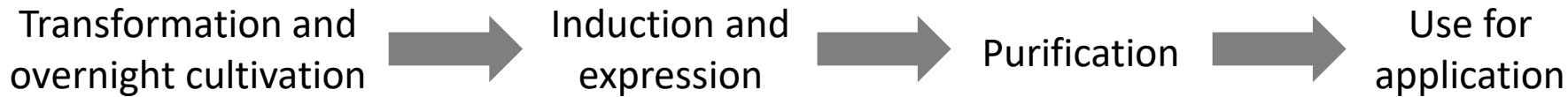
## -Improve Expression from Days to Hours-

### PUREfres<sup>®</sup> Synthesis

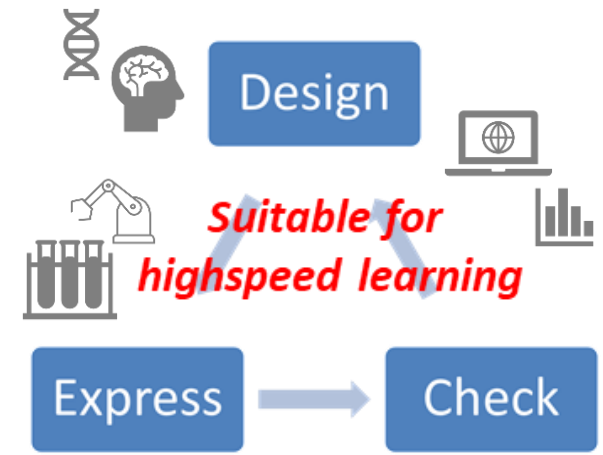


**Total: 2-4 hours**

### *E. coli* Expression



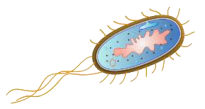
**Total: 3-4 days**



100mL package will be launched soon!

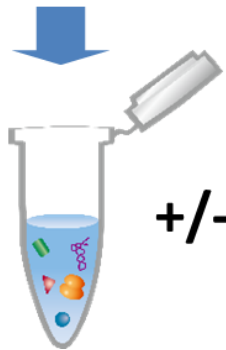
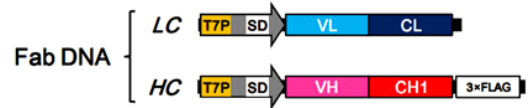


***Great Flexibility from basic research to industrial applications***



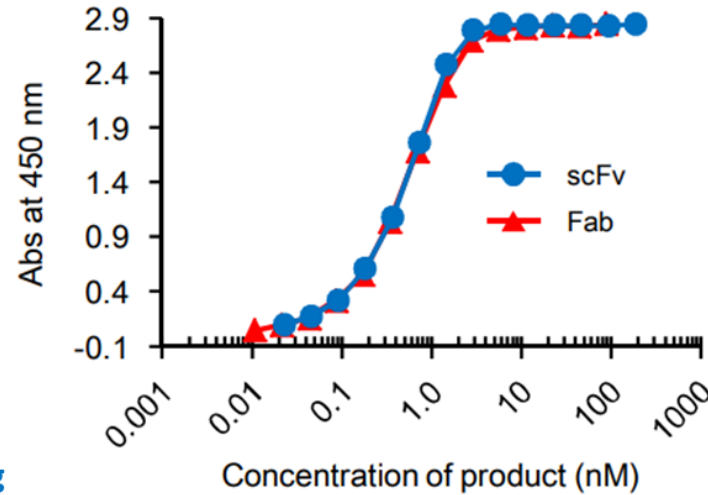


## -Expression of scFv, Fab and more-

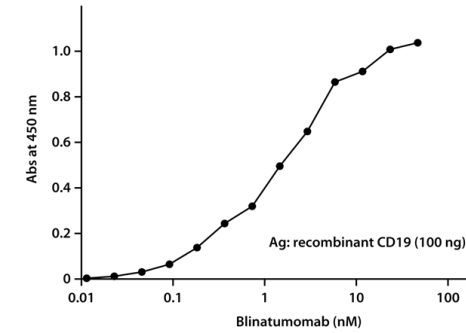


+/- DsbC Set  
DnaK Mix  
For correct folding

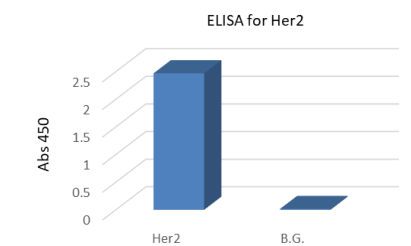
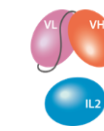
### Activity



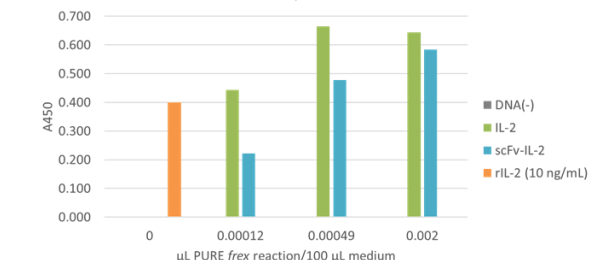
anti-CD3-scFv/CD19-scFv



scFv-IL-2



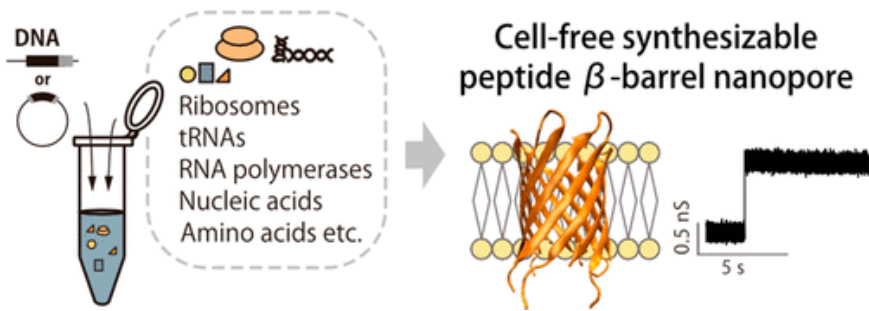
CTLL-2 proliferation



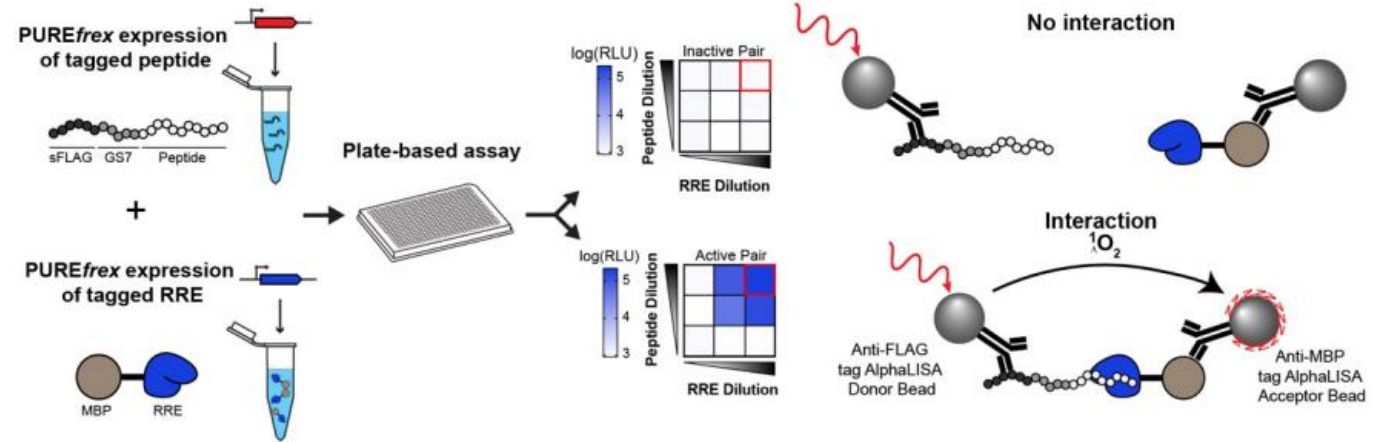
[Murakami et al. \(2019\) Sci. Rep. vol.9, p.671. \(Supplementary Information\)](#)



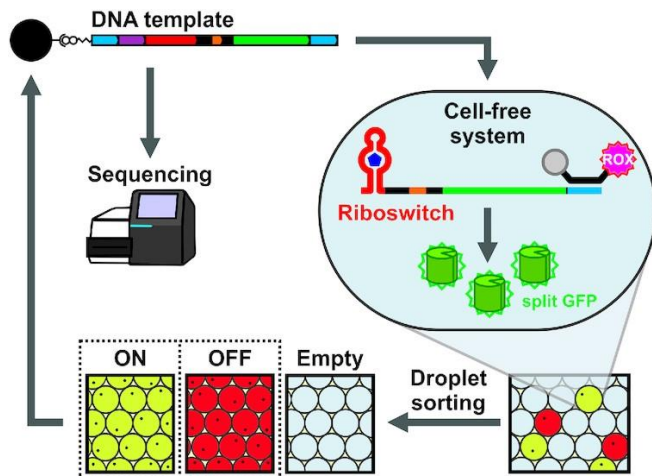
## -Broad applications, yet to come!-



[Fujita et al. \(2023\) ACS Nano. vol.17\(4\), p.3358.](#)

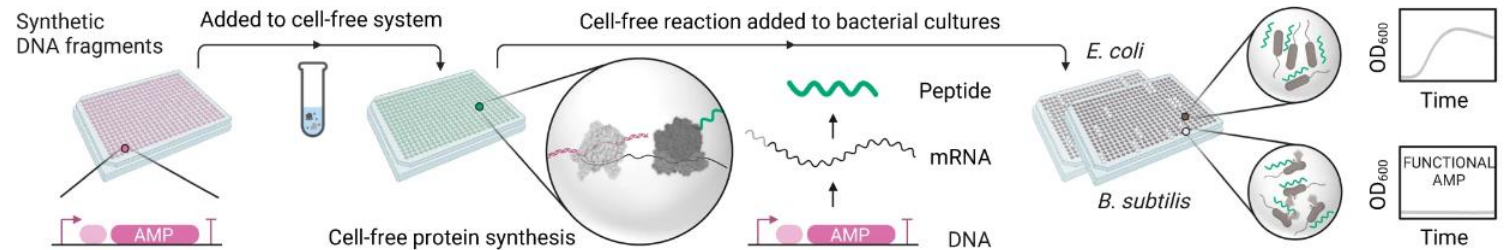


[Wong et al. \(2024\) bioRxiv <https://doi.org/10.1101/2024.03.25.586624>.](#)



[Tabuchi et al. \(2022\) Nucleic Acids Res <https://doi.org/10.1093/nar/gkac152>](#)

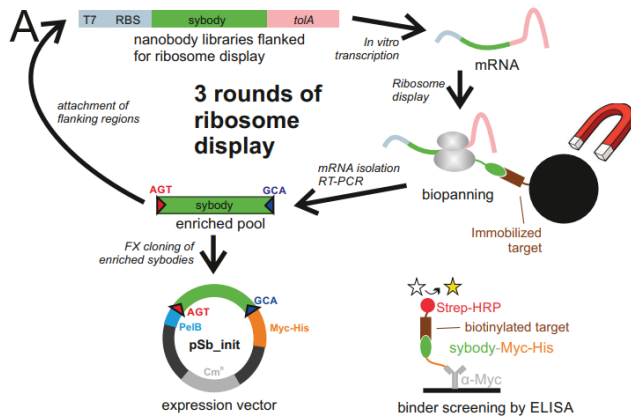
WET LAB EXPERIMENT: cell-free production and activity test of AMPs (24 hr)



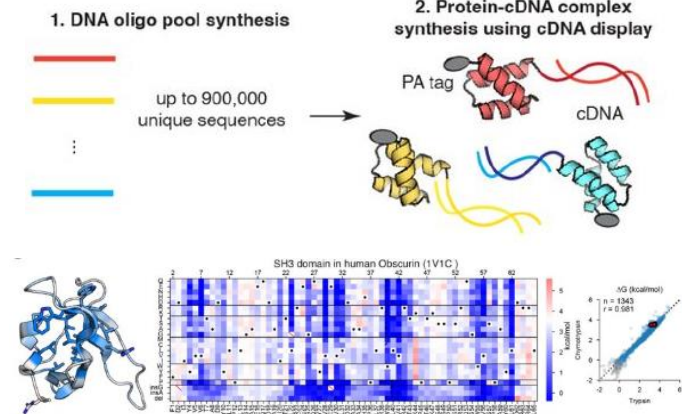
[Pandi et al. \(2023\) Nat Communications. vol.14\(7197\).](#)



## -Broad applications, yet to come!-



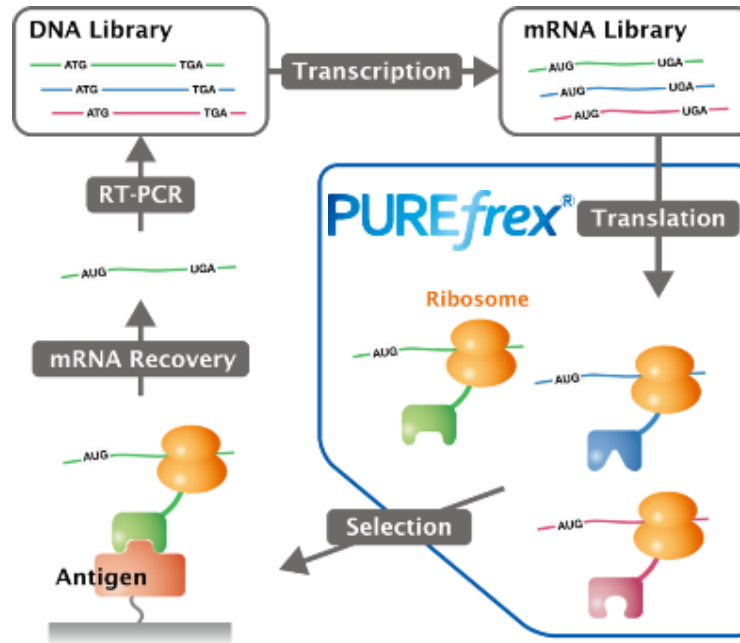
[Zimmermann I. et al. \(2018\) eLife, 7, e34317.](#)



[Tsuboyama et al. \(2023\) Nature, 620, p434.](#)

in vitro protein selection technology

# PUREfres<sup>®</sup> RD



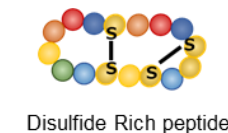
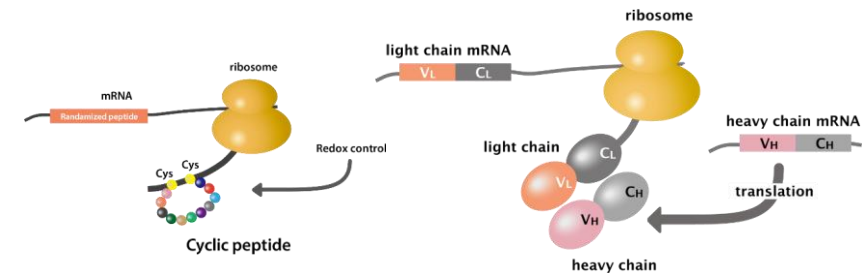
Licensed technology under JP4931135 etc.

### ◆ Advanced screening system for Biologics

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

### ◆ High Selection Efficiency

- Completely molecular based system
- >10<sup>12</sup> diversity



Licensed to  
**SUTRO**  
BIOPHARMA

## Contact information



*Reconstituted cell-free protein synthesis kit*

**PUREfrex<sup>®</sup>**

*For reagent use for expression / screening of biologics*

<https://purefrex.genefrontier.com/>



*in vitro protein selection technology*

**PUREfrex<sup>®</sup>RD**

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

Takashi Ebihara, Ph.D., COO, GeneFrontier

[E-mail: ebihara@genefrontier.com](mailto:ebihara@genefrontier.com)