

Rebuilding the Cell-Free System and Their Applications for R&D of Biologics

Reconstituted cell-free protein synthesis kit

PUREfrex[®]

in vitro protein selection technology

PUREfrex[®]RD

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COO
GeneFrontier Corporation

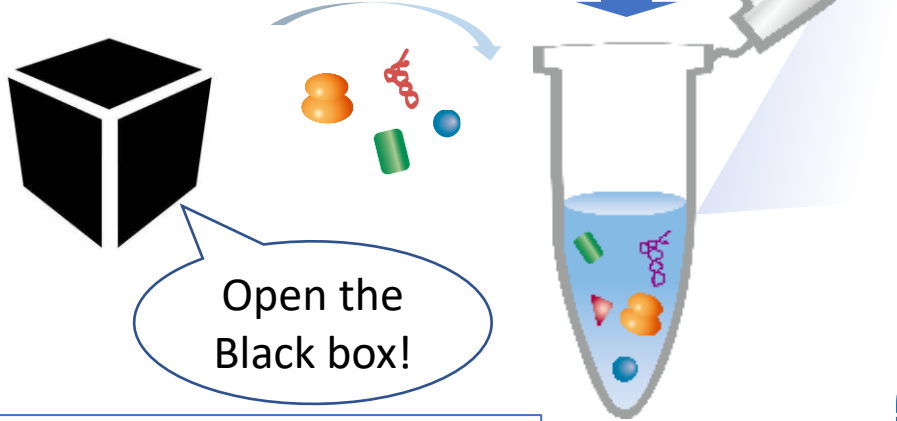


GeneFrontier

PEGS Boston
2-6 May 2022

-Customize expression toolbox for your research-

Only necessary molecules for transcription/translation

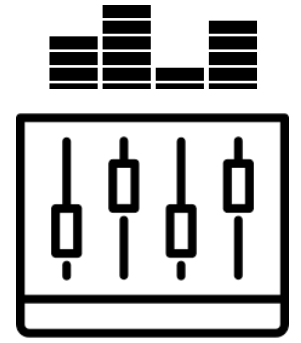
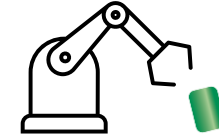
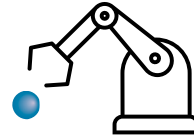
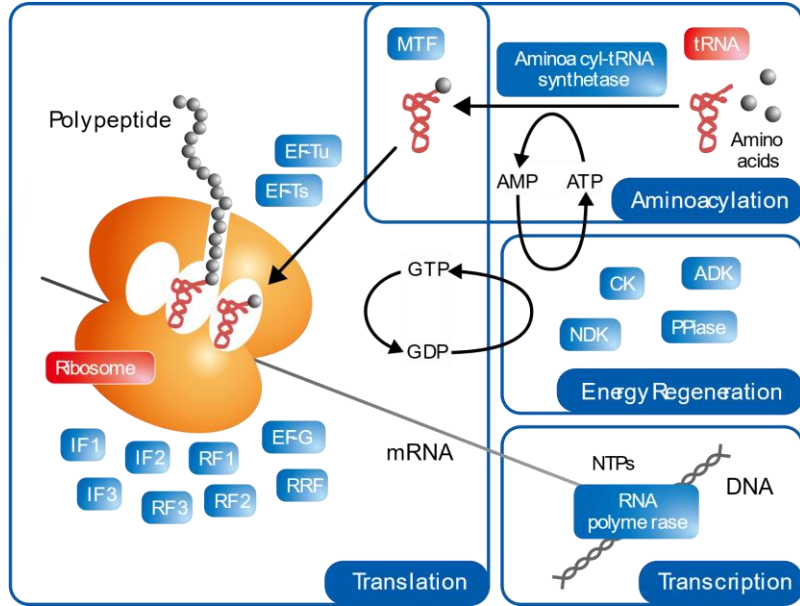


Open the Black box!

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

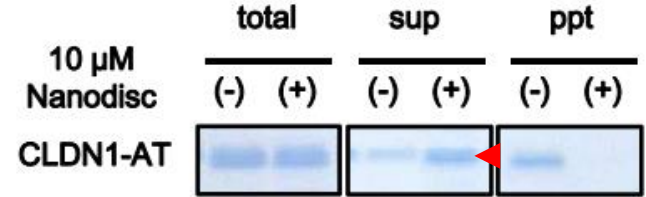
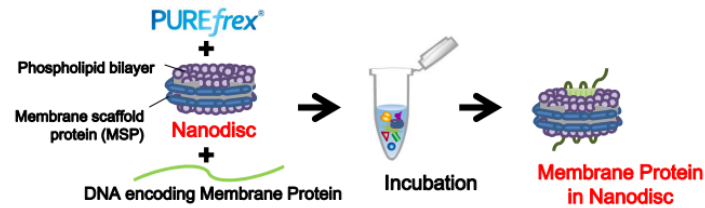


Totally constructive, molecular based system



Translation factors
Chaperones
Detergent
Temperature/pH

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



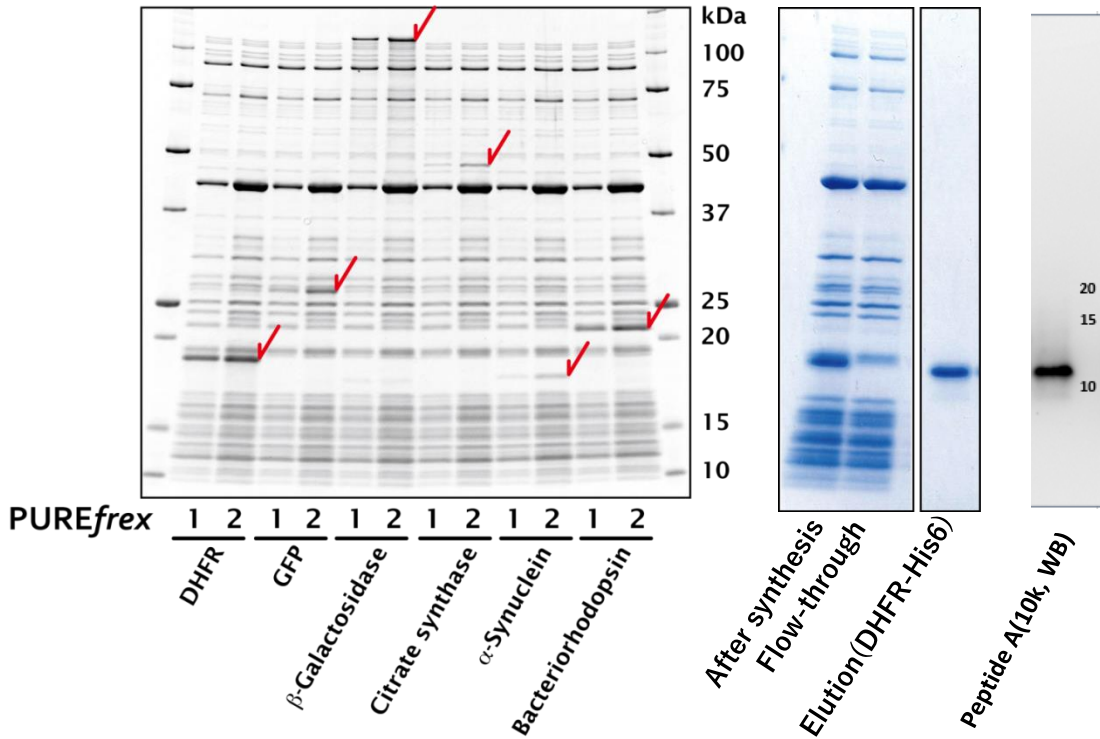
Solubilized hCLDN1 was synthesized using PUREfres[®] and Nanodisc.

The condition of membrane protein synthesis

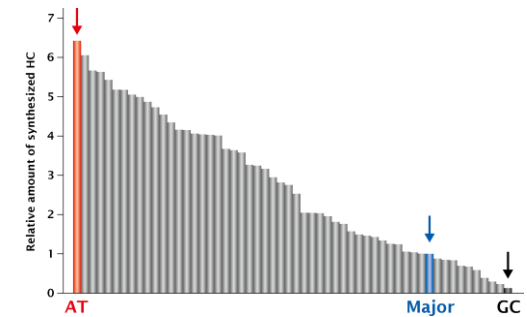
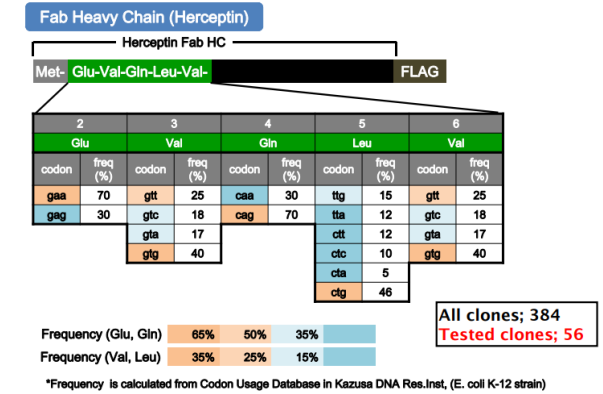
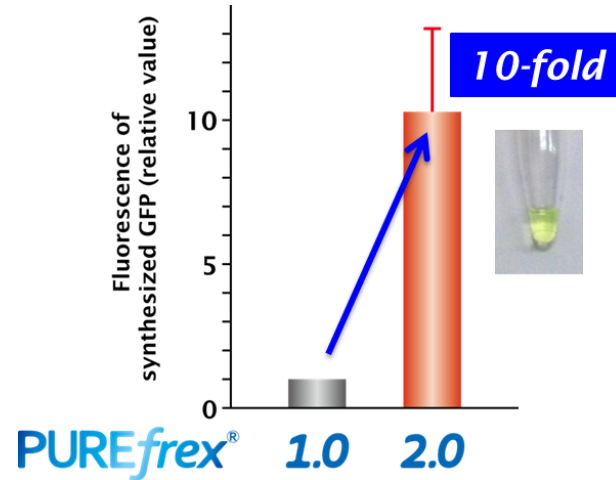
Reaction mix	Template DNA	Incubation
PUREfres [®] 2.0 + Nanodisc (MSP1E3D1-His POPC*, final 10 μM)	PCR product	37°C, 4 h

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

-Having good productivity-



- Reaction at 37°C for 4 h
- 0.5 μ L of reaction mix/ lane
- stained with Oriole (Bio-Rad) and analyzed with an image analyzer (LAS)



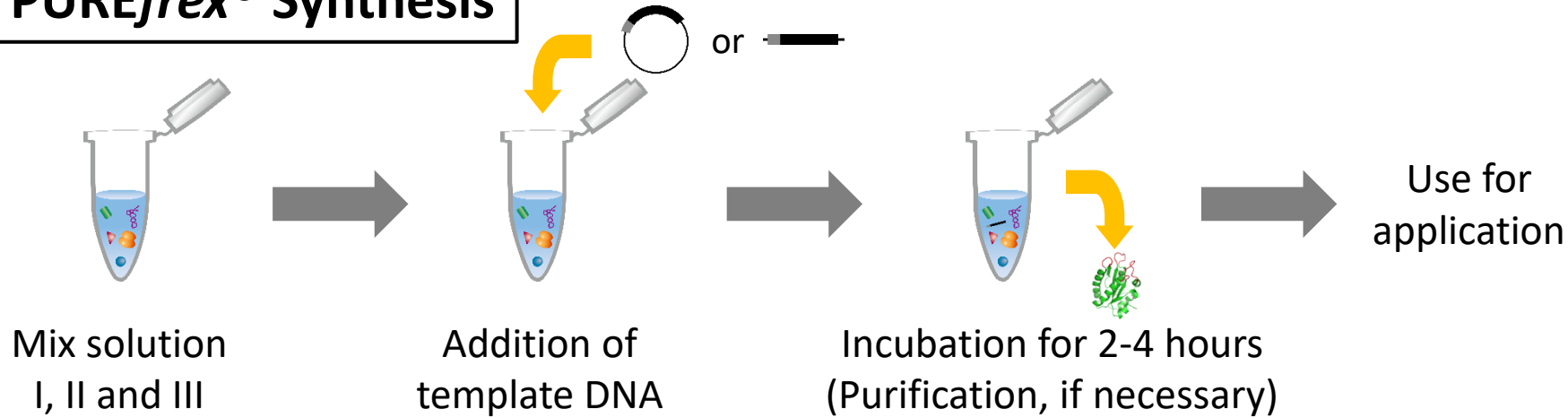
Design of DNA template is important.
Manual is Free to download from our Web site.



- ✓ Good expression for many proteins, small to large.
- ✓ Good purity with simple purification.
- ✓ Good productivity, ~g/L.

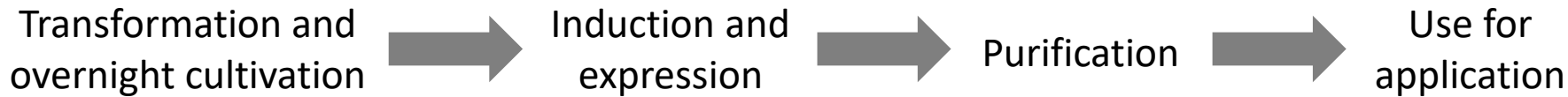
-Improve process from Days to Hours-

PUREfres[®] Synthesis

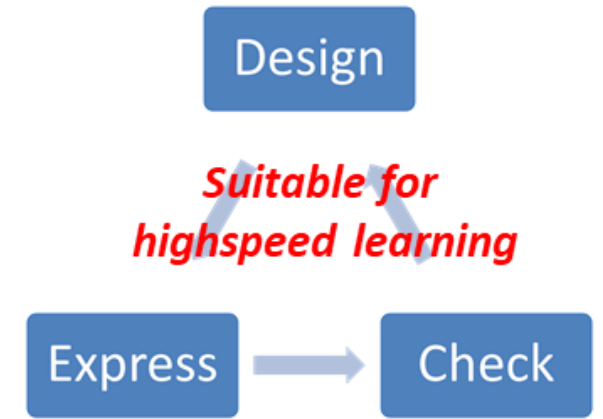


Total: 2-4 hours

E. coli Expression

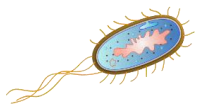


Total: 3-4 days



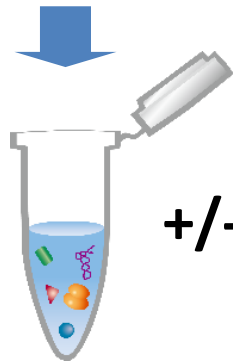
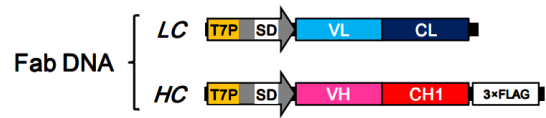
*Suitable for
highspeed learning*

μ L ~
L scale

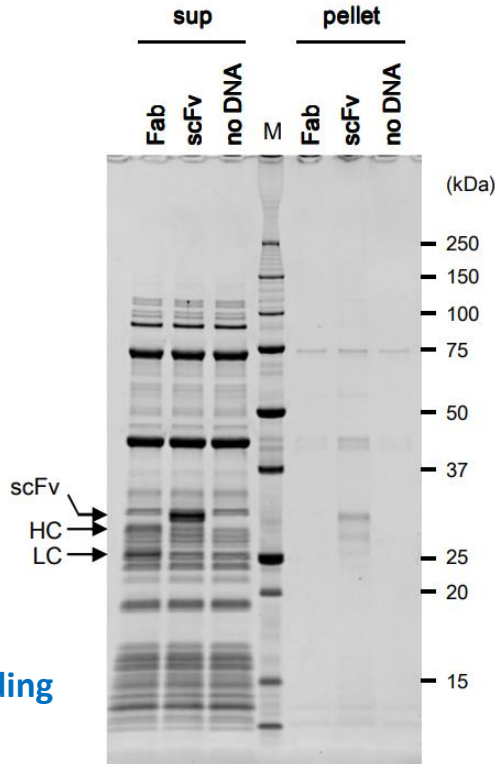


-Expression of scFv and Fab-

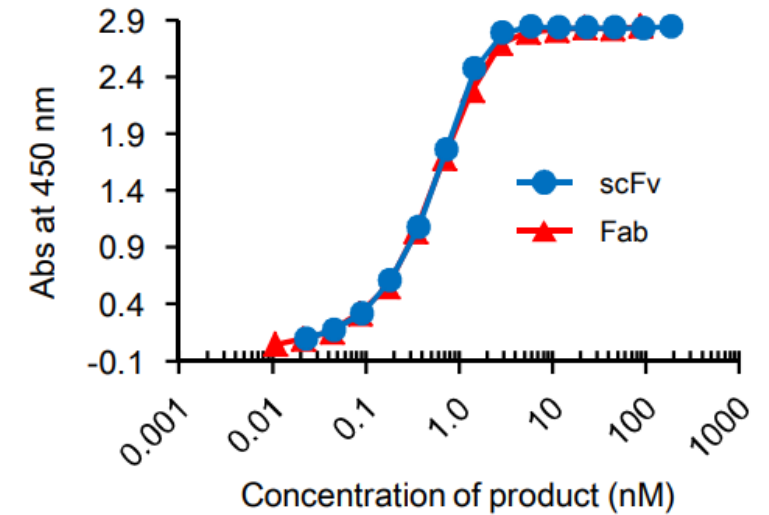
SDS-PAGE



+/- **DsbC Set**
DnaK Mix
For correct folding



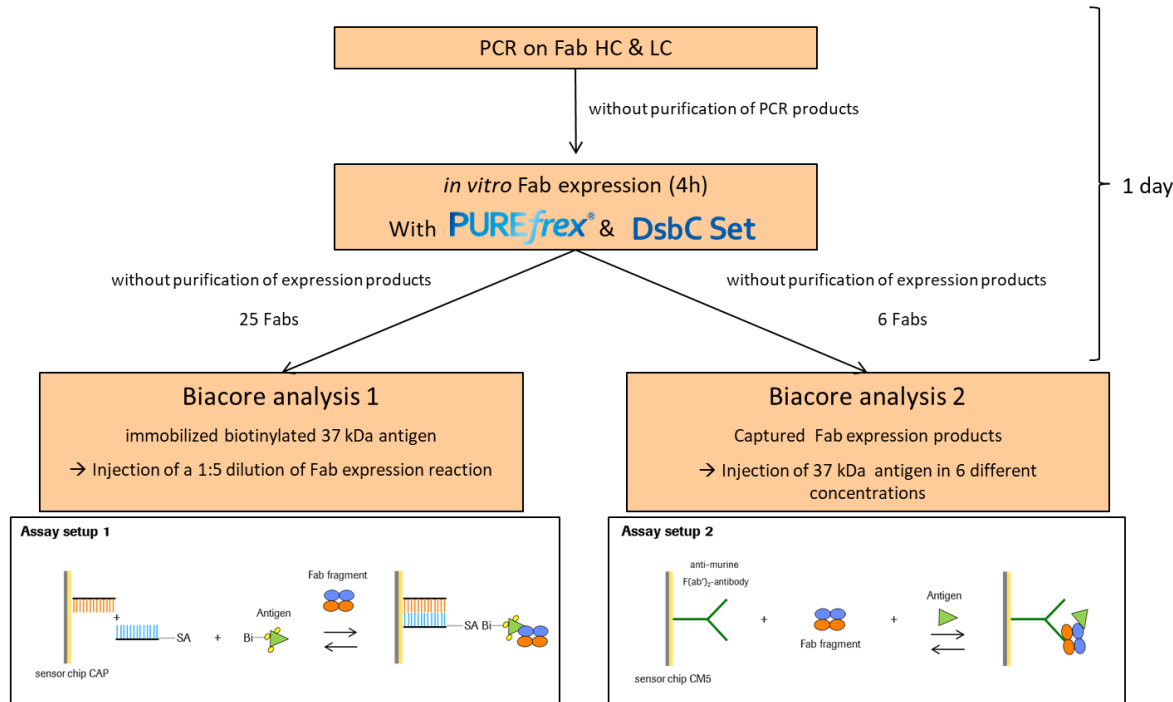
Activity



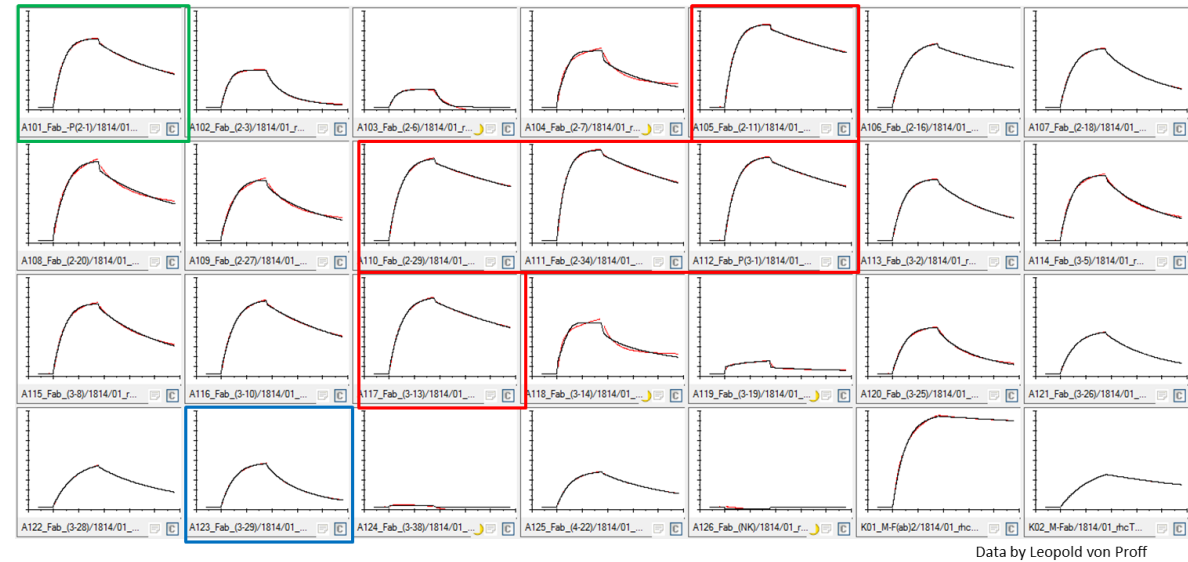
[Murakami et al. \(2019\) Sci. Rep. vol.9, p.671. \(Supplementary Information\)](#)

✓ **Active scFv and Fab (derived from Herceptin) can be synthesized.**

In vitro expression and Biacore analysis of Fab fragments



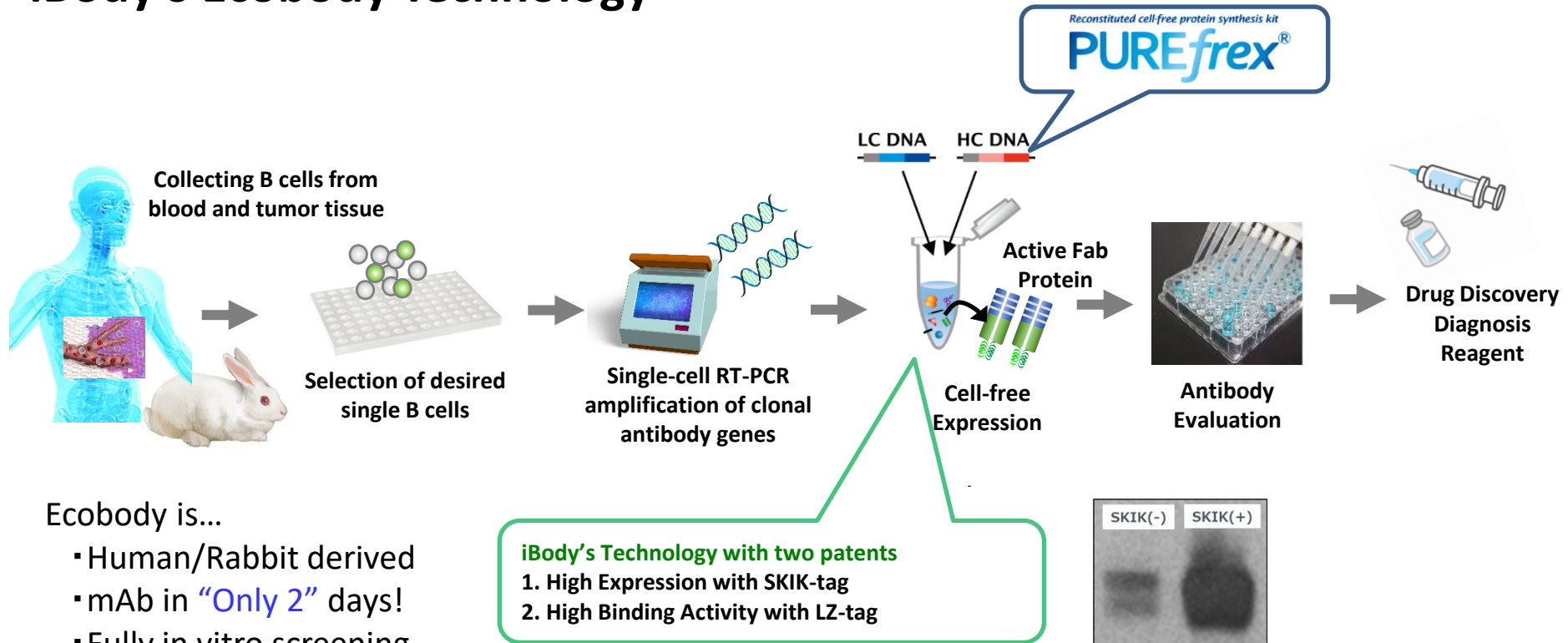
Kinetic analysis of 25 Fab binders



→ Selection of Fabs for further kinetic analysis

✓ **Active Fabs are expressed/screened in HT manner.**

iBody's Ecobody Technology



Ecobody is...

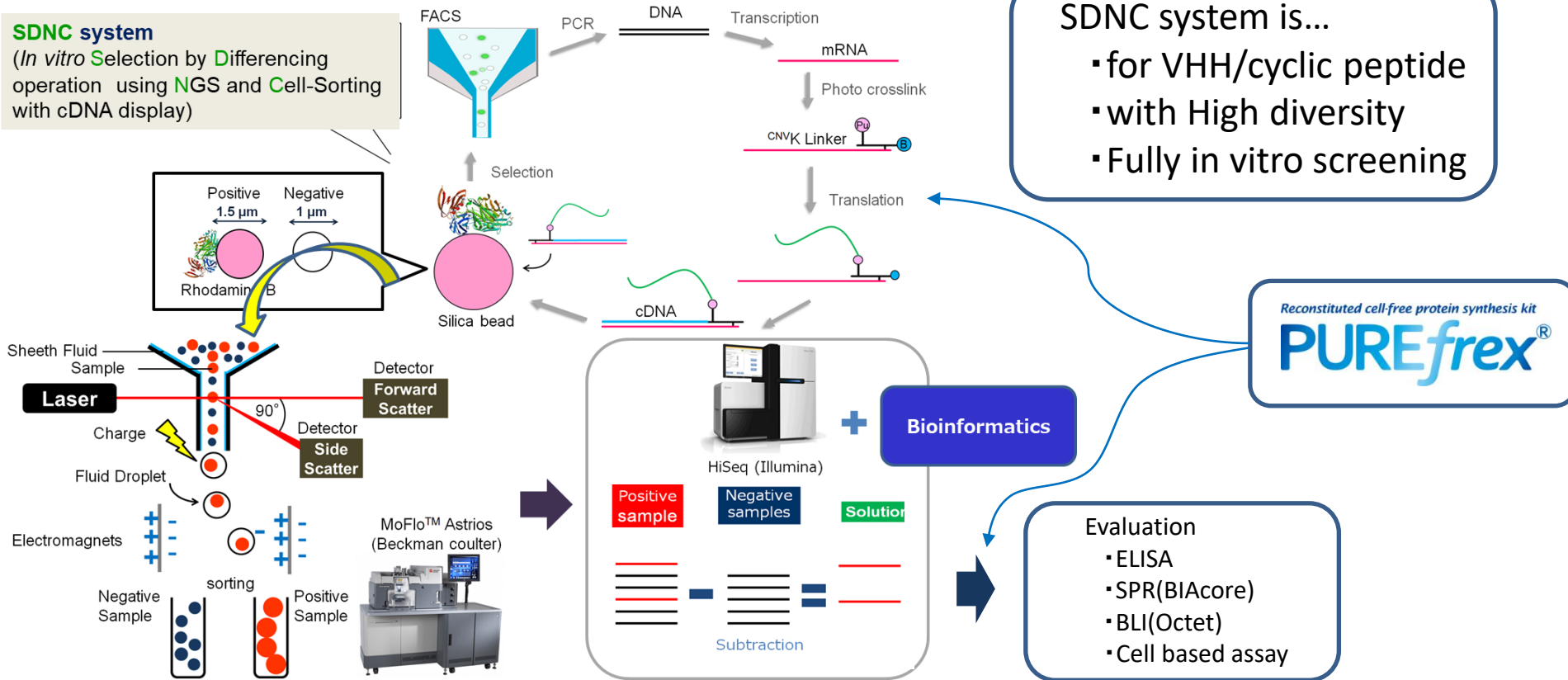
- Human/Rabbit derived
- mAb in "Only 2" days!
- Fully in vitro screening
- No culture

[Ojima-Kato et al. \(2017\) Sci. Rep., 7, 13979.](https://www.ibody.co.jp/en/)
<https://www.ibody.co.jp/en/>

✓ Active Fab is expressed/screened in HT manner.

SDNC system

(*In vitro* Selection by Differencing operation using NGS and Cell-Sorting with cDNA display)



SDNC system is...

- for VHH/cyclic peptide
- with High diversity
- Fully in vitro screening

Reconstituted cell-free protein synthesis kit
PUREfres[®]

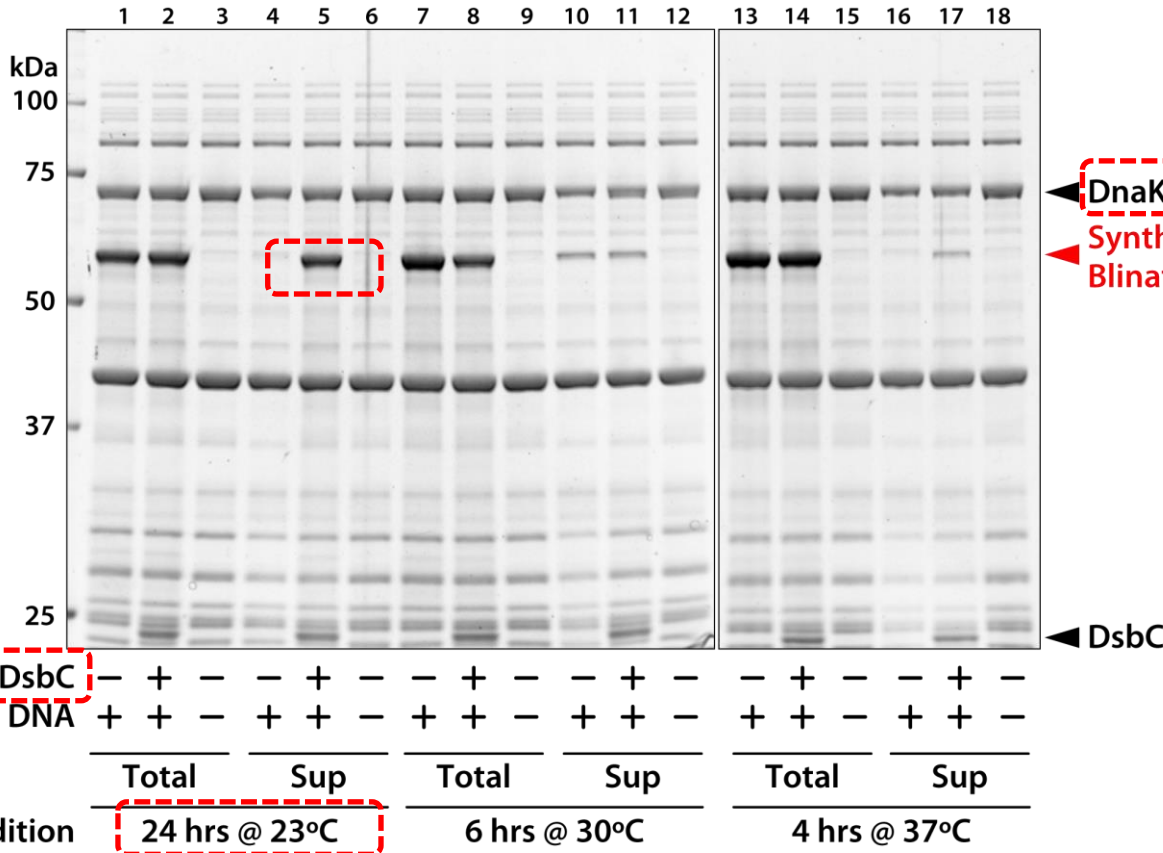
Evaluation

- ELISA
- SPR(BIAcore)
- BLI(Octet)
- Cell based assay

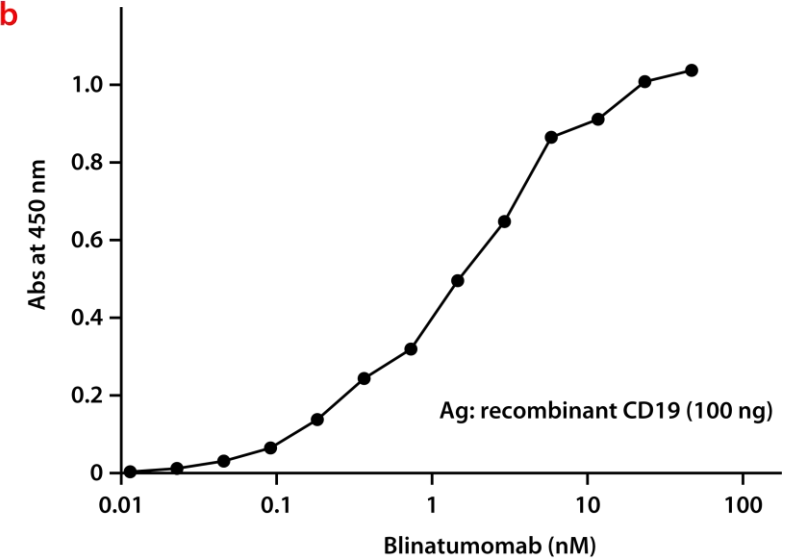
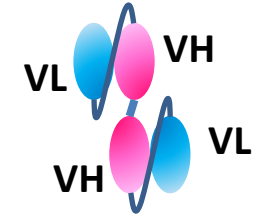
<https://www.epsilon-mol.co.jp/eng/>

✓ PUREfres is applied for cDNA display based screening.

-Application for BiTE-

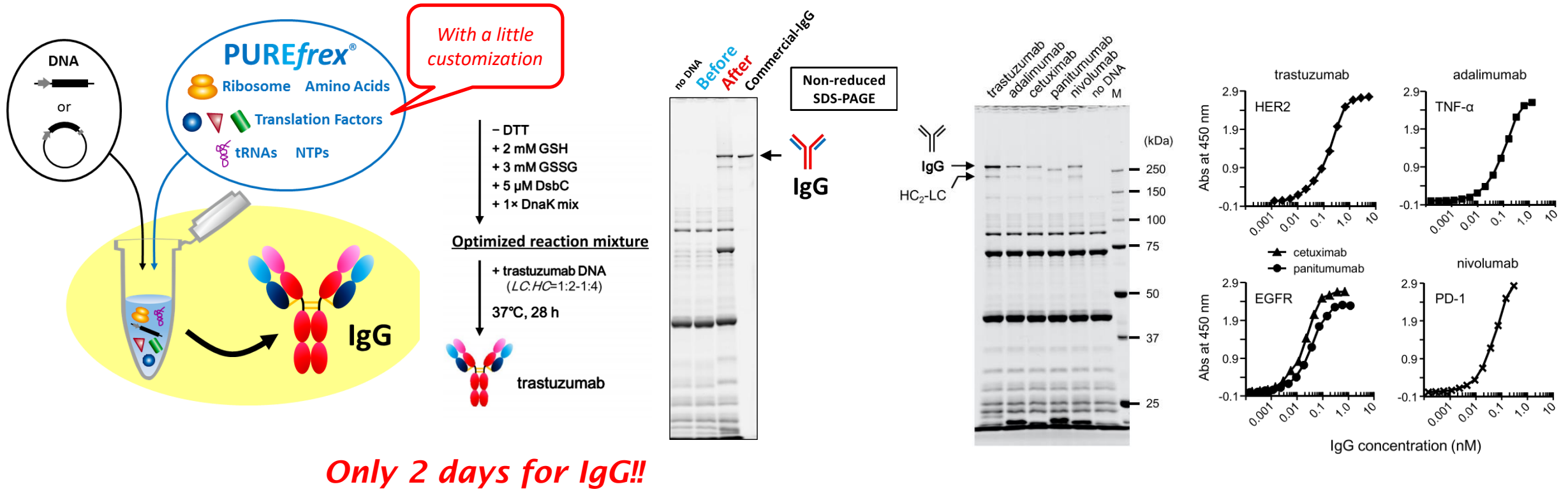


anti-CD3-scFv/CD19-scFv



✓ 0.6mg/mL active BiTE was produced under optimized condition.

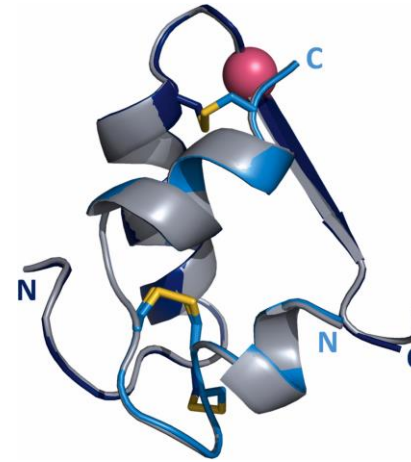
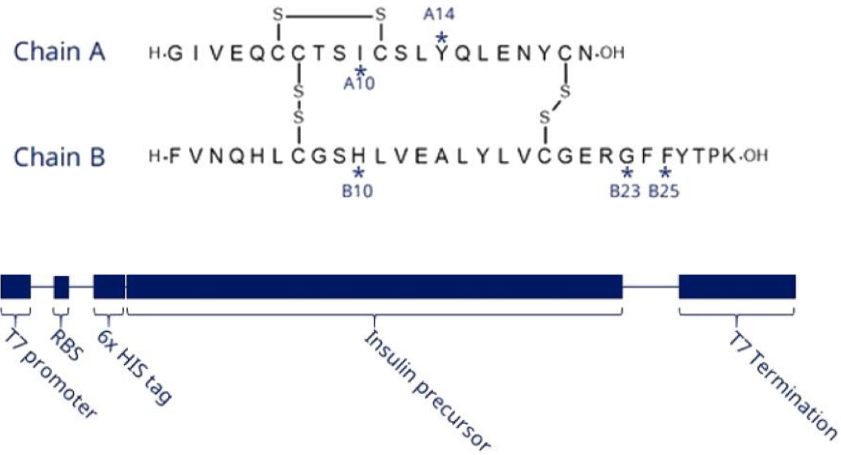
-Application for IgG-



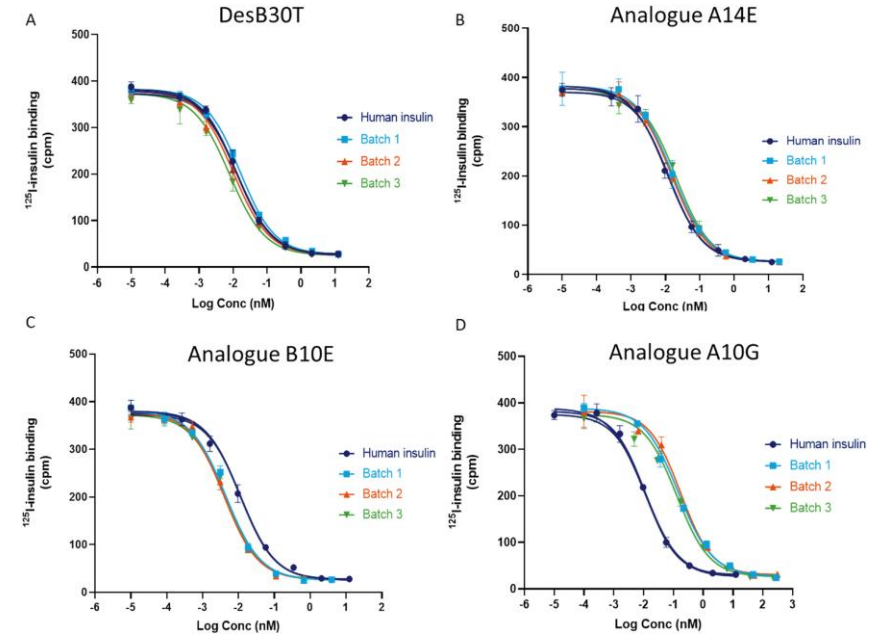
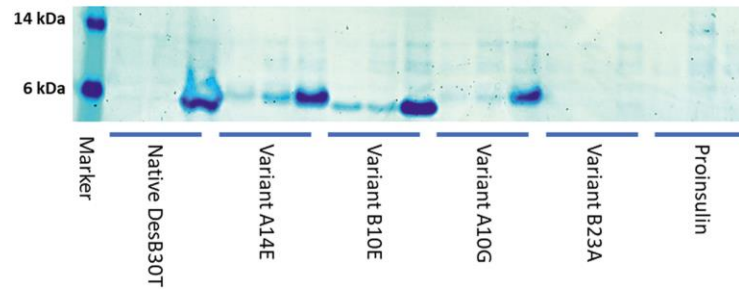
[Murakami et al. \(2019\) Sci. Rep. vol.9, p.671.](#)

✓ Full size IgG can be synthesized.

-Application for complex molecule-



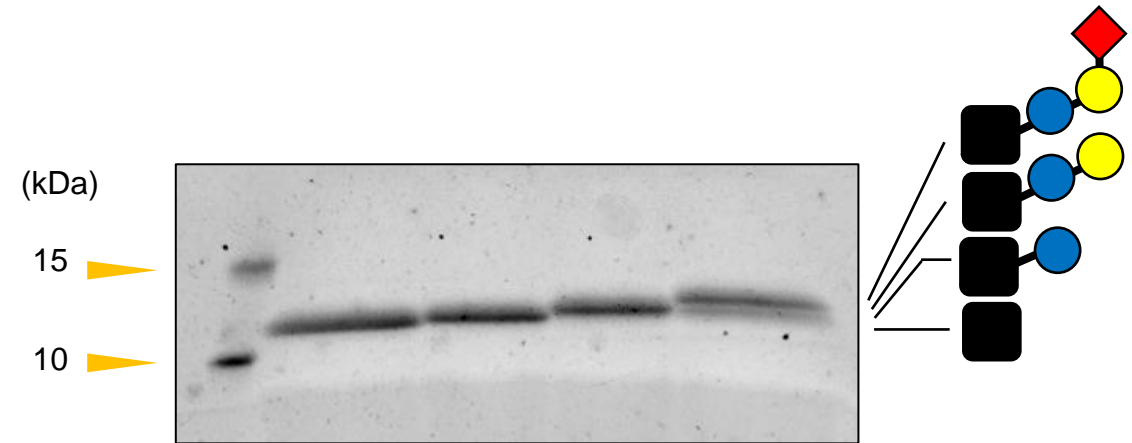
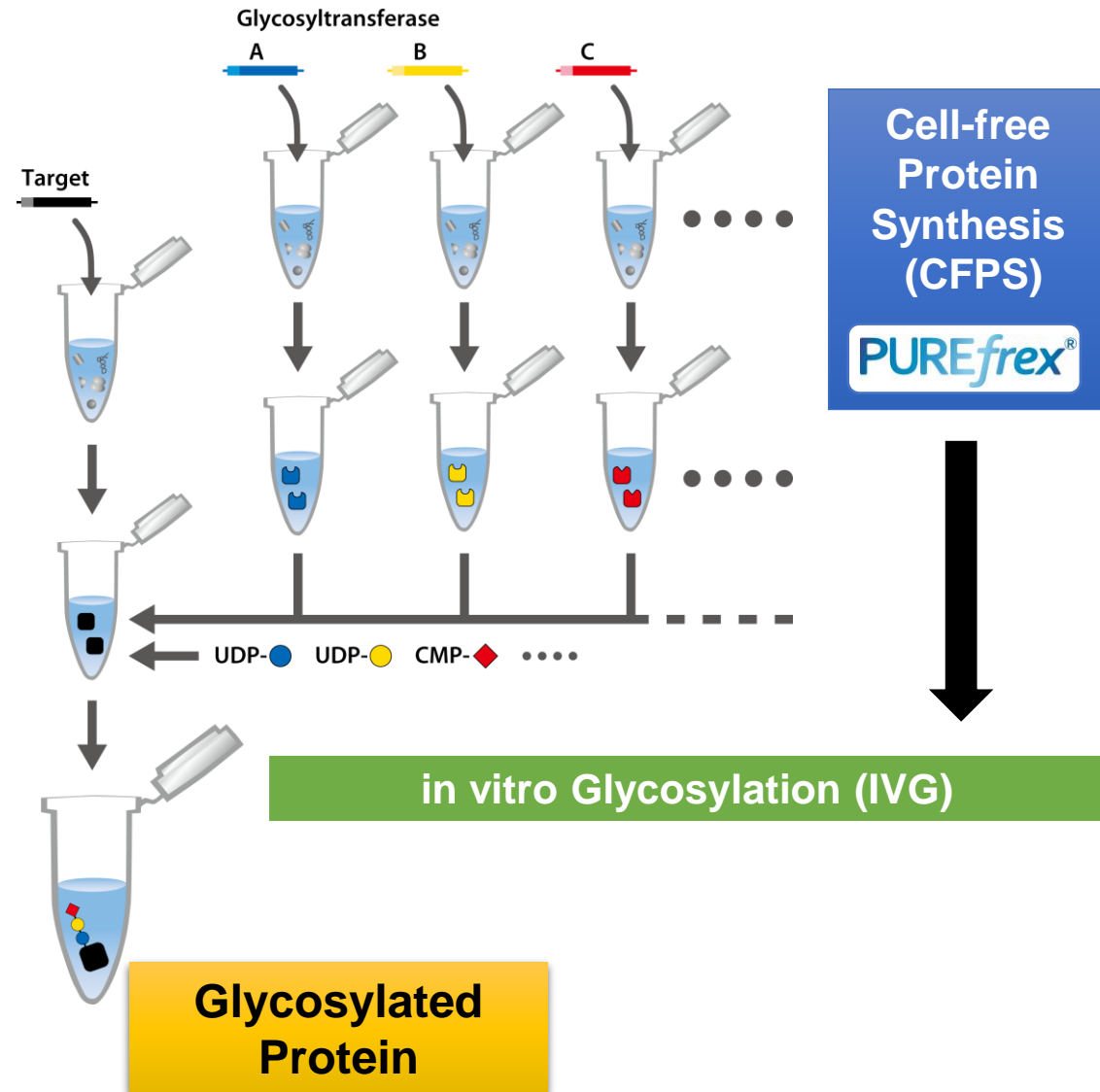
-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	4 mM GSH/3 mM GSSG
-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	0.375 mg/ml DsbC
+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	2 mM DTT



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

✓ **Functional insulin analogues can be synthesized.**

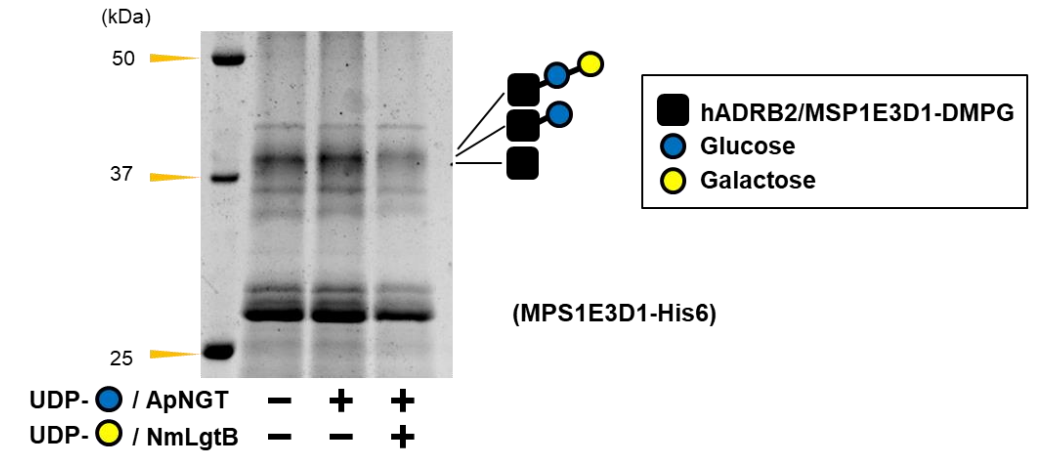
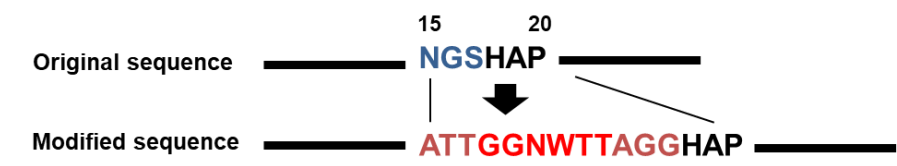
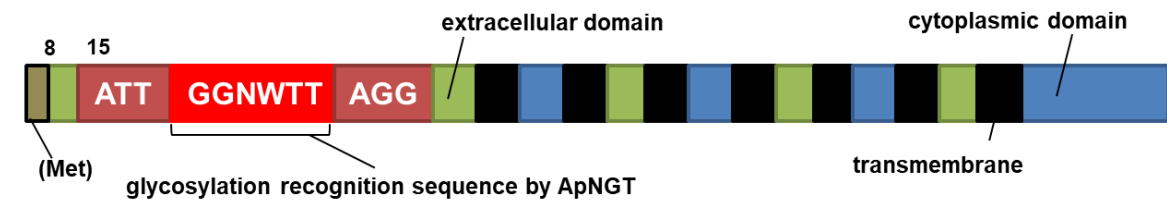
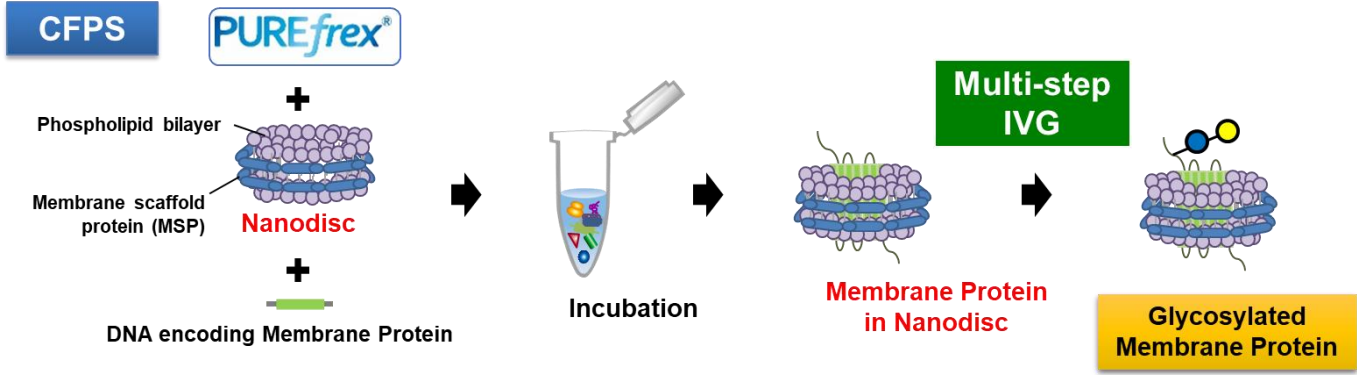
-Application for glycosylation; CFPS-IVG-



UDP-● / Enzyme A	-	+	+	+
UDP-● / Enzyme B	-	-	+	+
CMP-◆ / Enzyme C	-	-	-	+

**Applicable to glycosylation of target proteins.
Various reaction system can be designed.**

-Application for glycosylation; CFPS-IVG-



Solubilized membrane protein, hADRB2, are also glycosylated by multi-step IVG

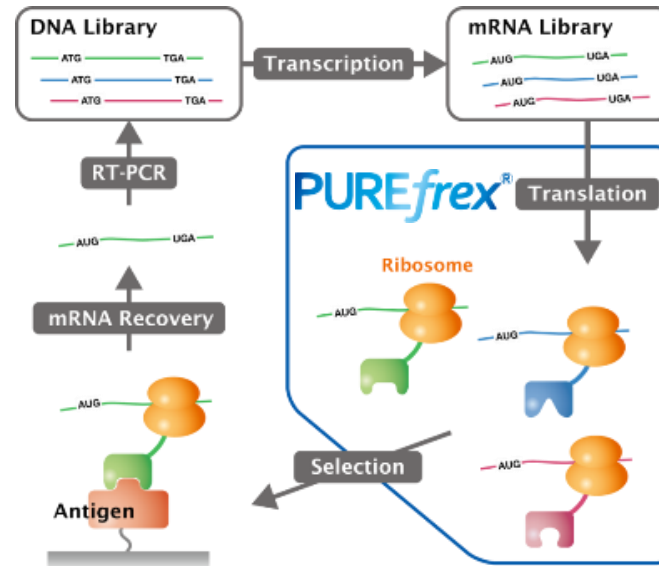
Ribosome display system using PUREfreflex

◆ Advanced screening system for Biologics

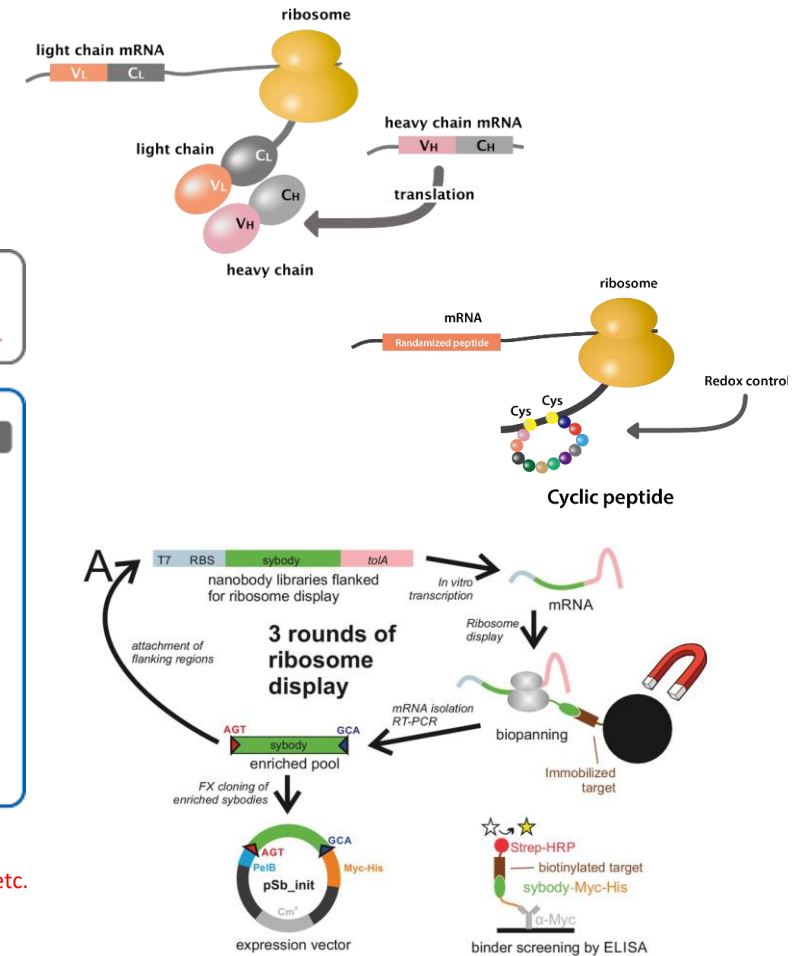
- mAb (scFv / Fab)
- VHH
- Cyclic peptide

◆ High Selection Efficiency

- Completely molecular based system
- >10¹² diversity

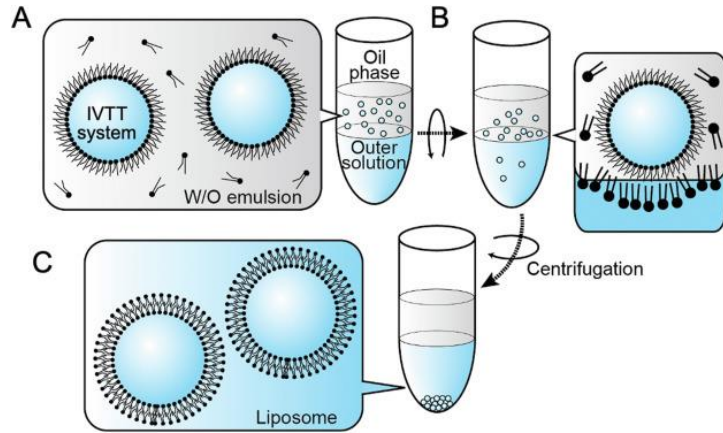


Licensed technology under JP4931135 etc.

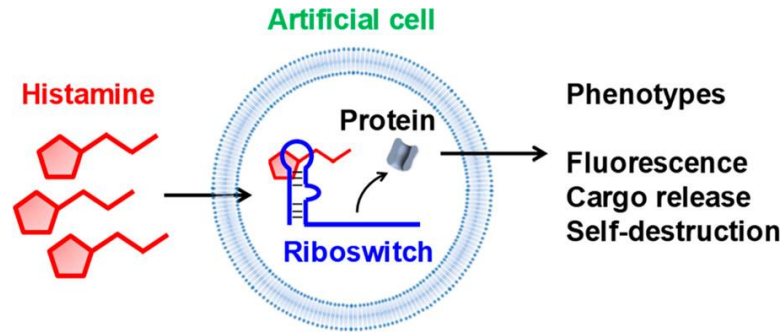


Zimmermann I. et al. (2018) eLife, 7, e34317.

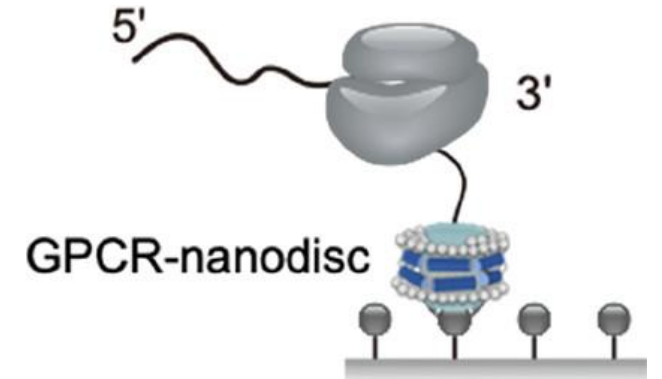
-Broad applications, yet to come!-



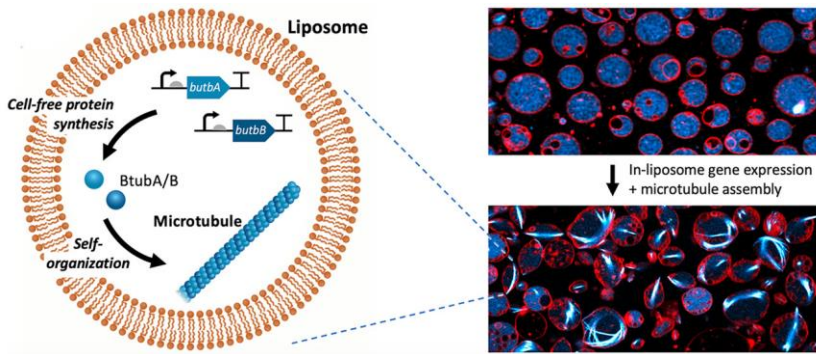
[Uyeda et al. \(2022\) J. Biosci & Bioeng. vol.133, p181.](#)



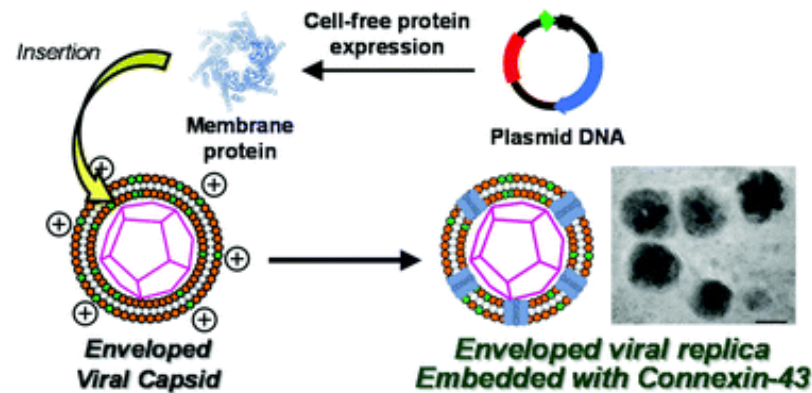
[Dwidar et al. \(2019\) J. Am. Chem. Soc. vol.141, p.11103.](#)



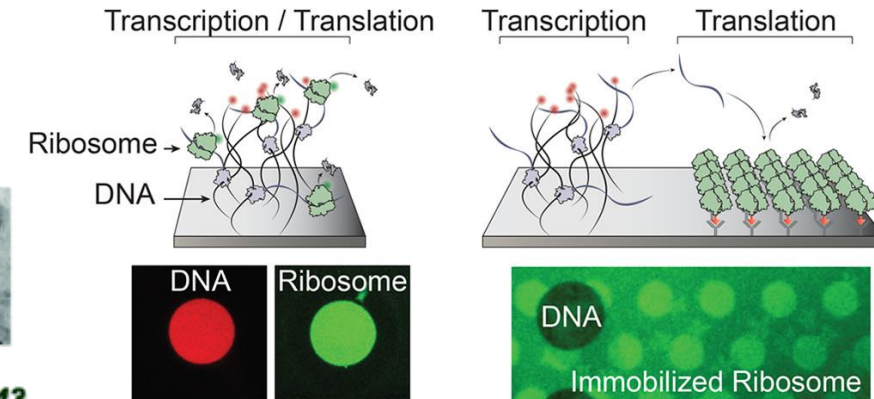
[Nakai et al. \(2022\) Anal. Chem. vol.94, p.3831.](#)



[Kattan et al. \(2021\) ACS. Synth. Biol. vol.10, p.2447.](#)



[Furukawa et al. \(2022\) RSC. Chem. Biol. vol.3, p.231.](#)



[Levy et al. \(2021\) ACS. Synth. Biol. vol.10, p.609.](#)

Contact information

Booth# 1218



Reconstituted cell-free protein synthesis kit

PUREfrex[®]

For reagent use for expression and screening of biologics

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



in vitro protein selection technology

PUREfrex[®]RD

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

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