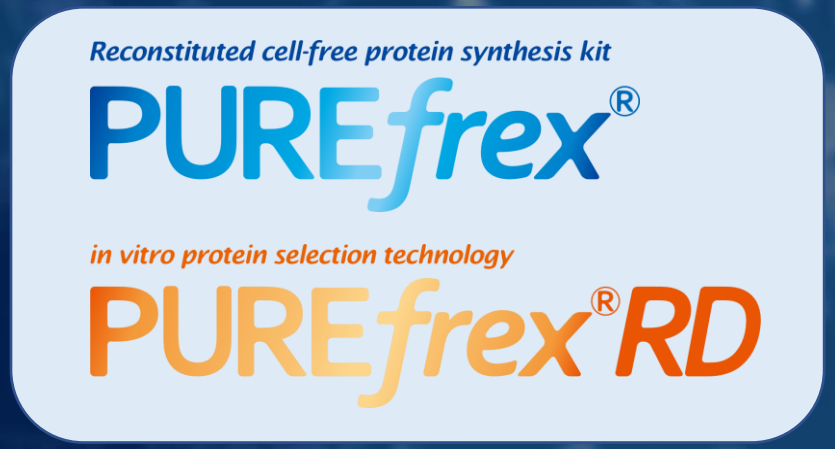
For more information

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



Takashi Ebihara, Ph.D.
COO
GeneFrontier Corporation

GeneFrontier

PEGS Europe
14-16 Nov 2022

Reconstituted cell-free protein synthesis kit

PURE*frex*®

-Customize expression toolbox for your research-

For more information

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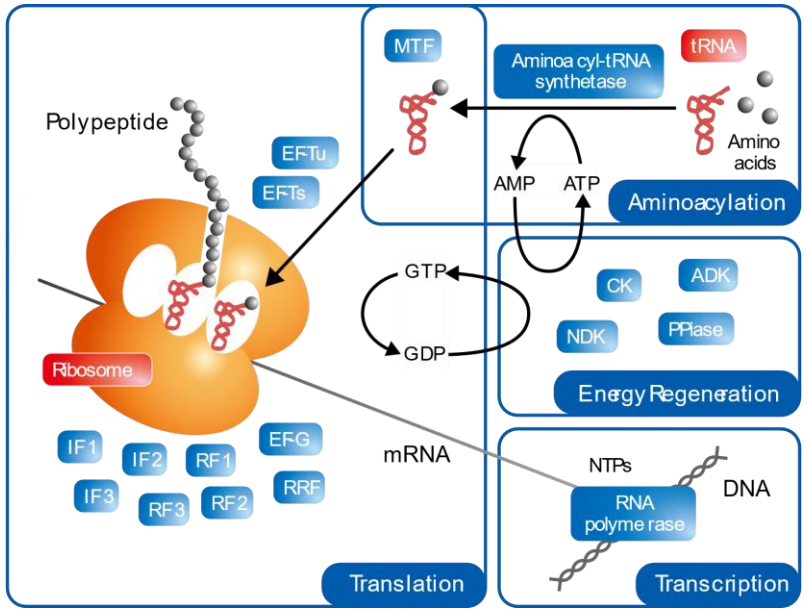
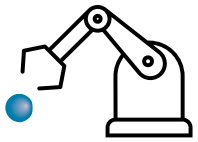
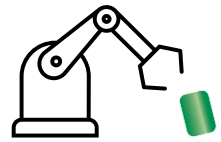
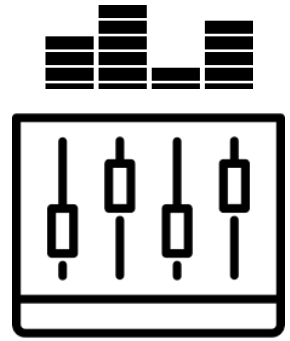
Totally constructive, molecular based system

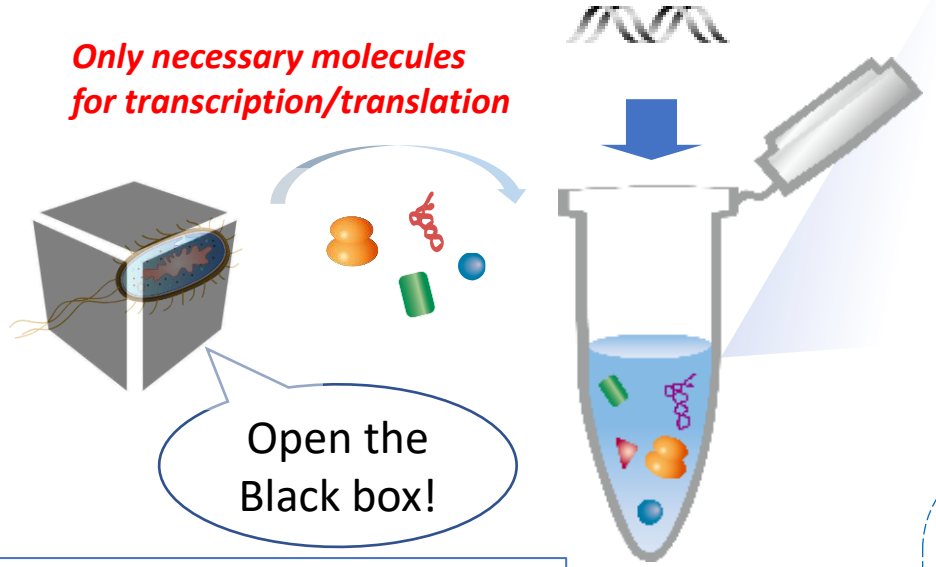








Only necessary molecules for transcription/translation



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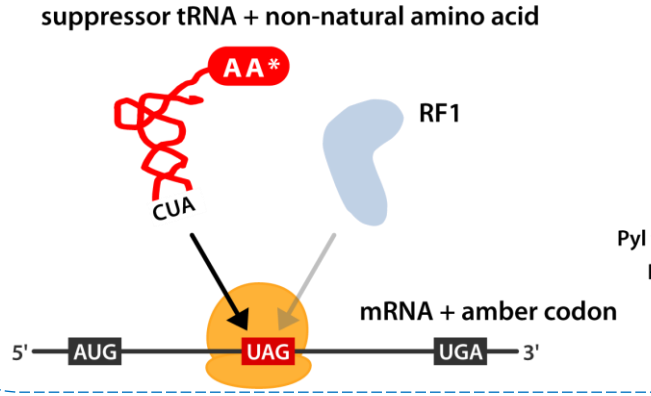
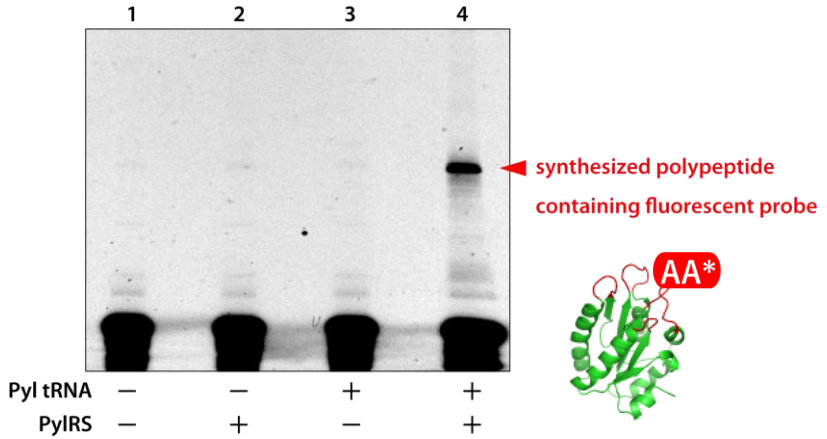
- ✓ Suited for High throughput system
- ✓ Very low contaminants

<Ex, Non-natural AA introduction>

Translation - RF1





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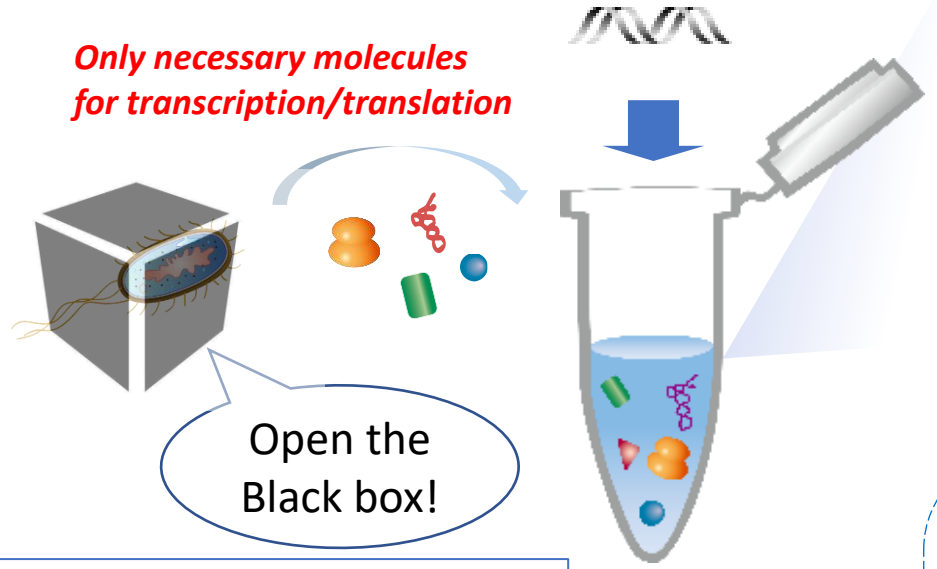
Only necessary molecules for transcription/translation



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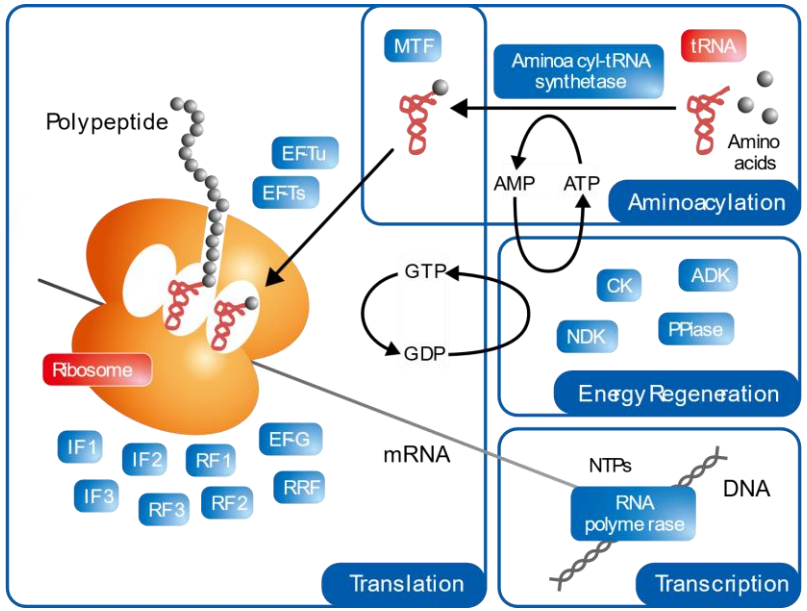
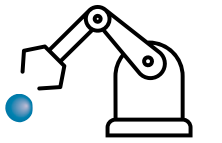
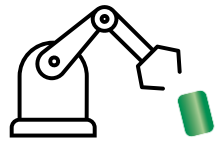
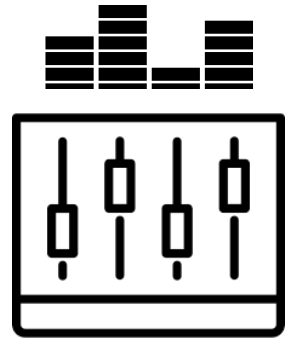
Totally constructive, molecular based system

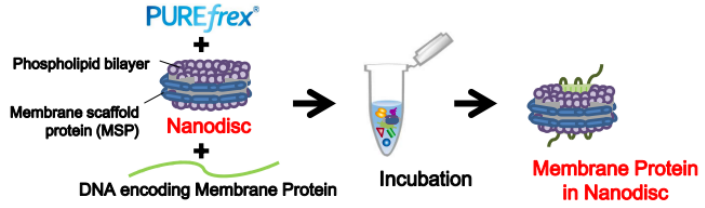


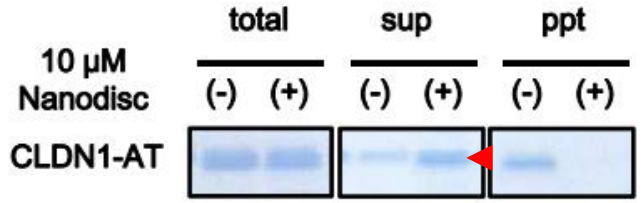






<Ex, Membrane protein with Nanodisc; artificial membrane-like structure>





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.

Poster_MBSJ 2016

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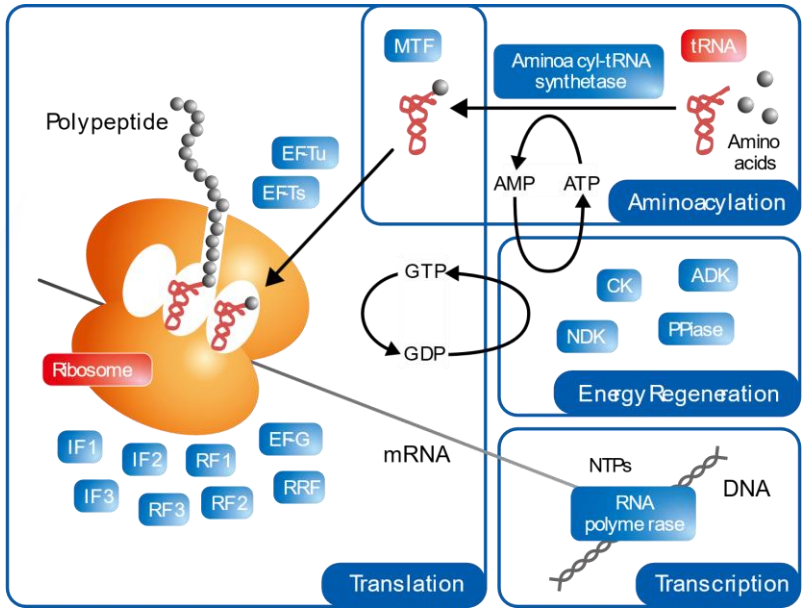
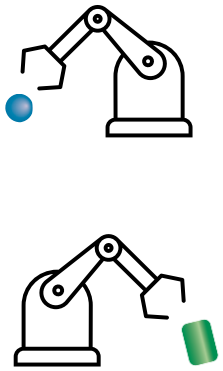
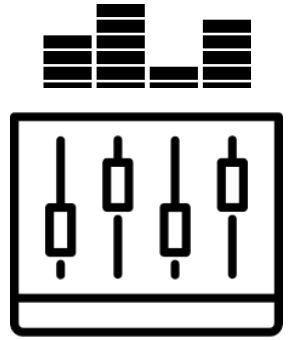
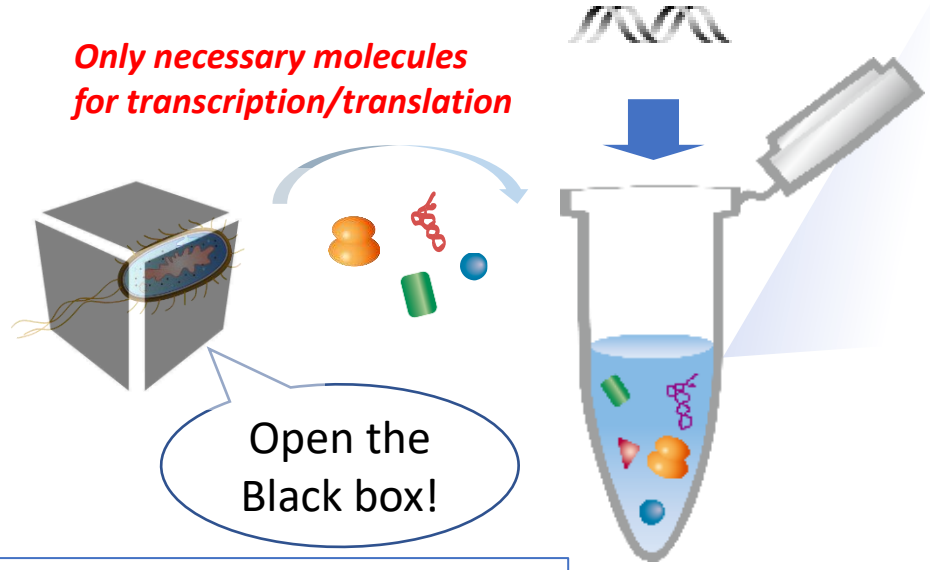
Totally constructive, molecular based system







Only necessary molecules for transcription/translation



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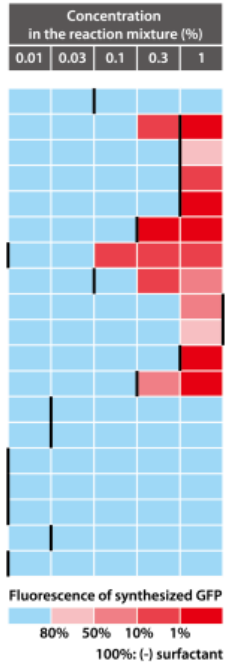
Experimental conditions for protein synthesis

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**

- 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.

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



Reconstituted cell-free protein synthesis kit

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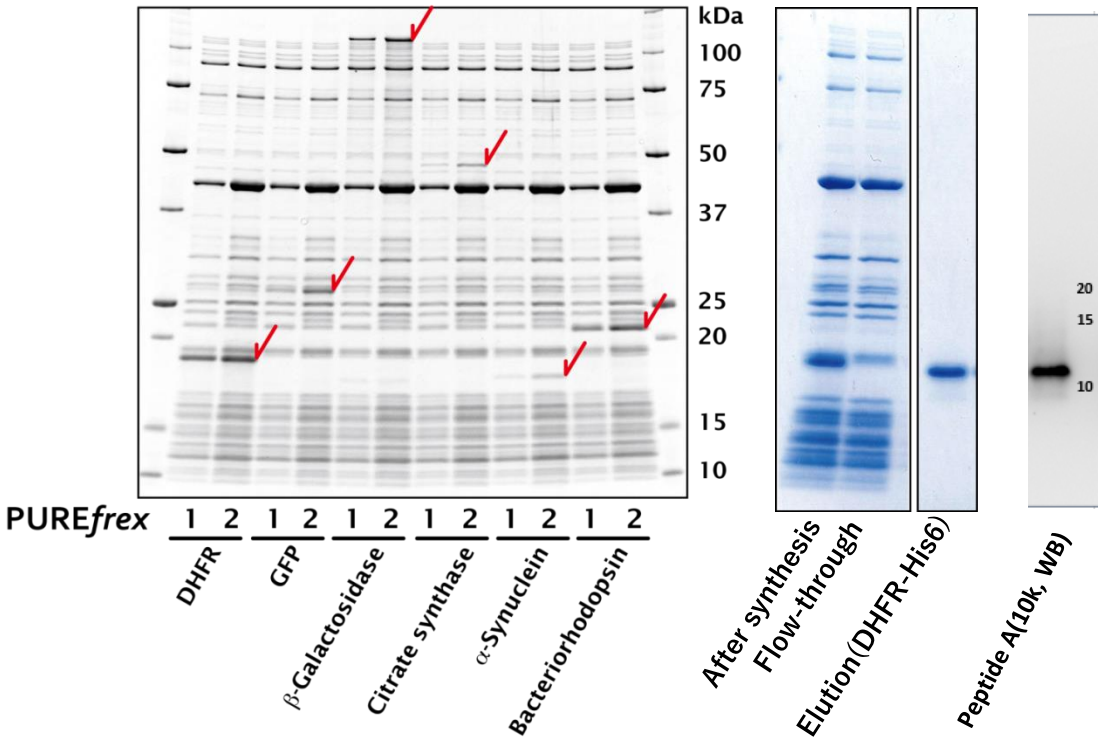
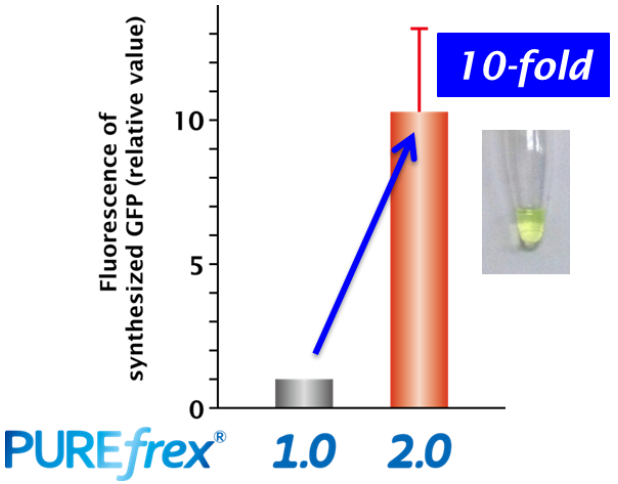
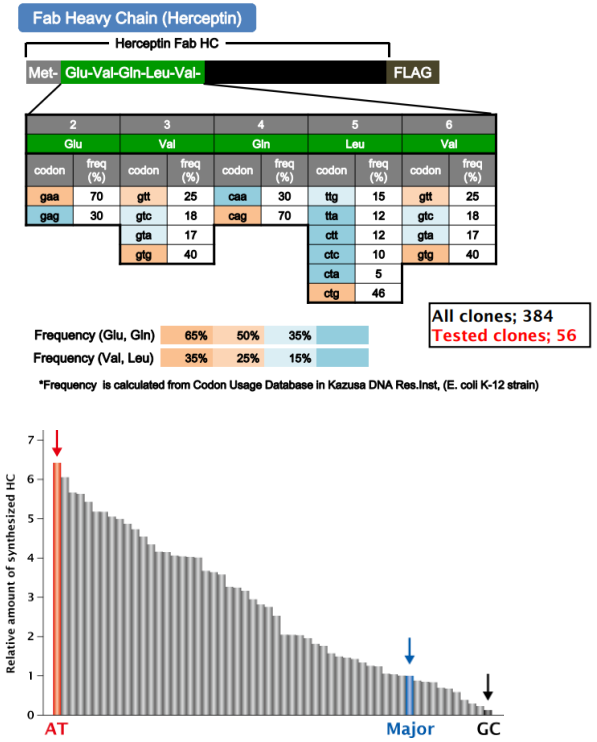
-Having good productivity-







Design of DNA template is important.

Manual is Free to download from our Web site.

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

✓ Good expression for many proteins, small to large.

✓ Good purity with simple purification.

✓ Good productivity, ~g/L.

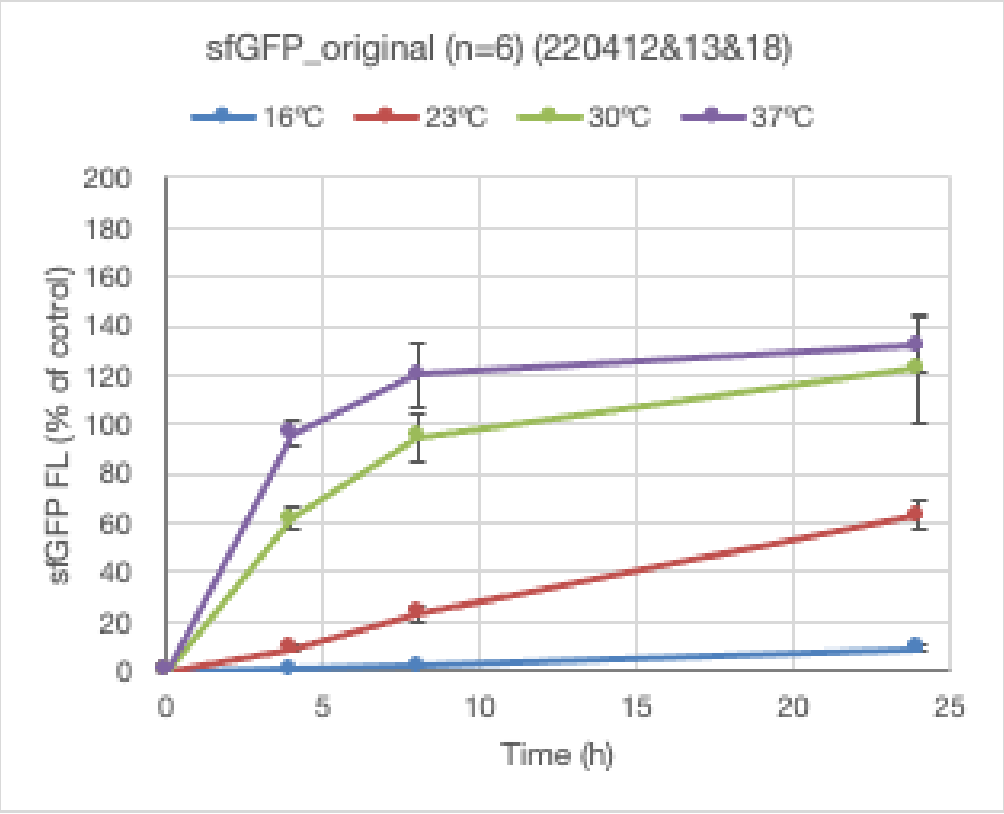
-Template, Temparature, Time-

ver. 2 (4 mM GSH), 1 ng/μL DNA
reaction at 16/23/30/37ºC for 4/8/24 hours

sfGFP

M S K G E E L F T G

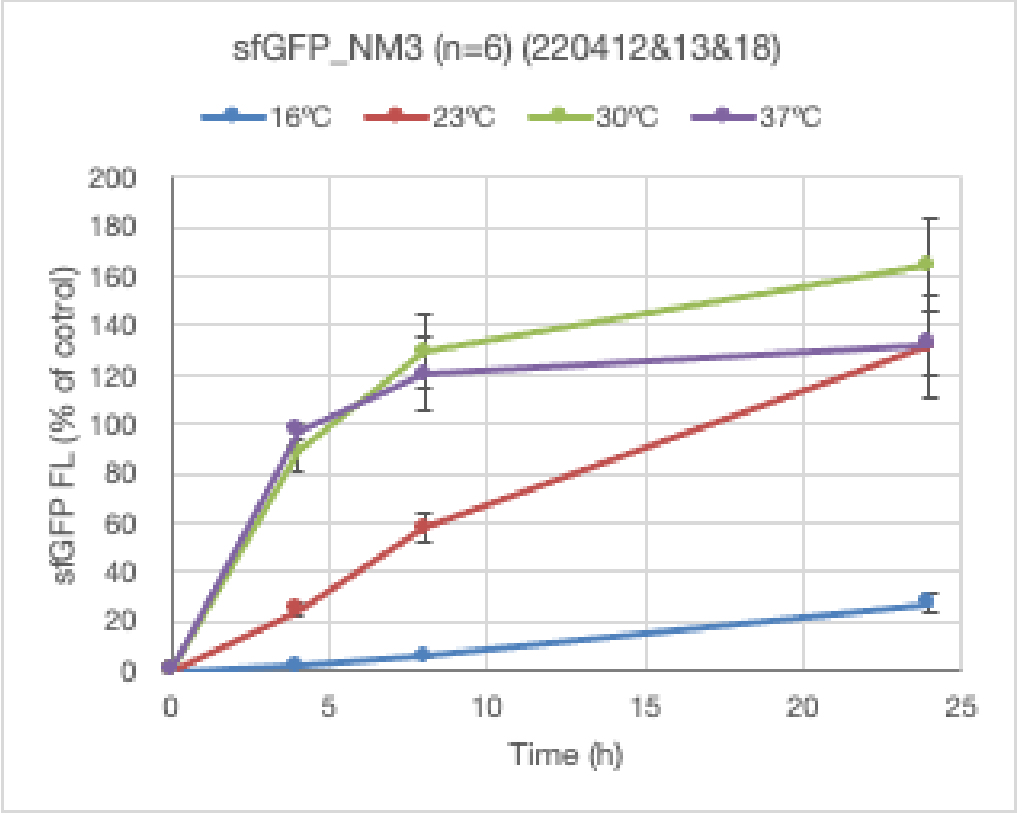
ATGAGTAAAGGAGAAGAACTTTTCACTGGA



sfGFP (ST) (NM3)

M S K S T E L F T G

ATGAGTAAATCTACTGAACTTTTCACTGGA



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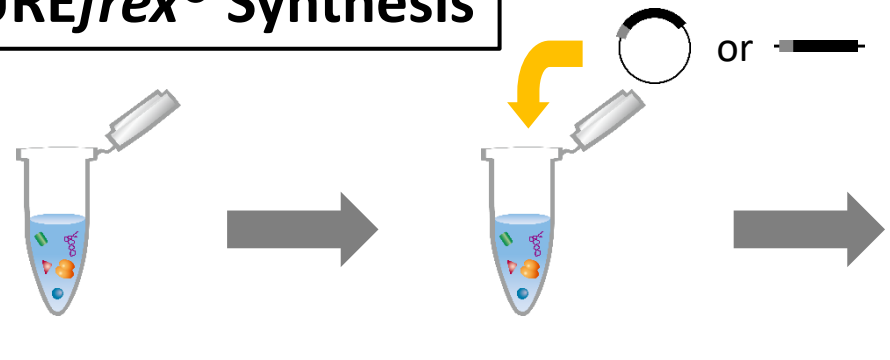
-Improve process from Days to Hours-

PURE*frex*® Synthesis

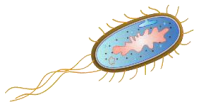


Mix solution I, II and III → Addition of template DNA → Incubation for 2-4 hours (Purification, if necessary) → Use for application

Total: 2-4 hours

E. coli Expression

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

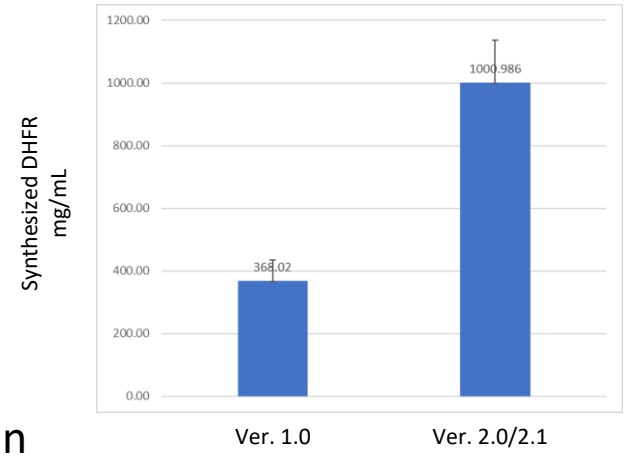


Total: 3-4 days

Design

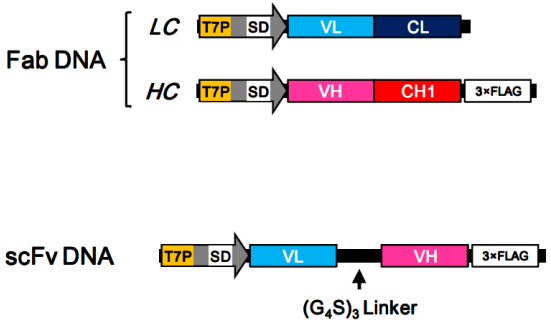
Suitable for highspeed learning

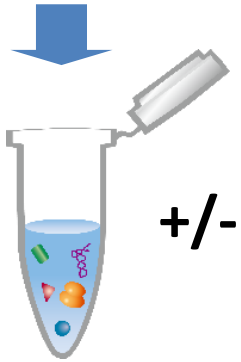
Express → Check



μL ~ L scale

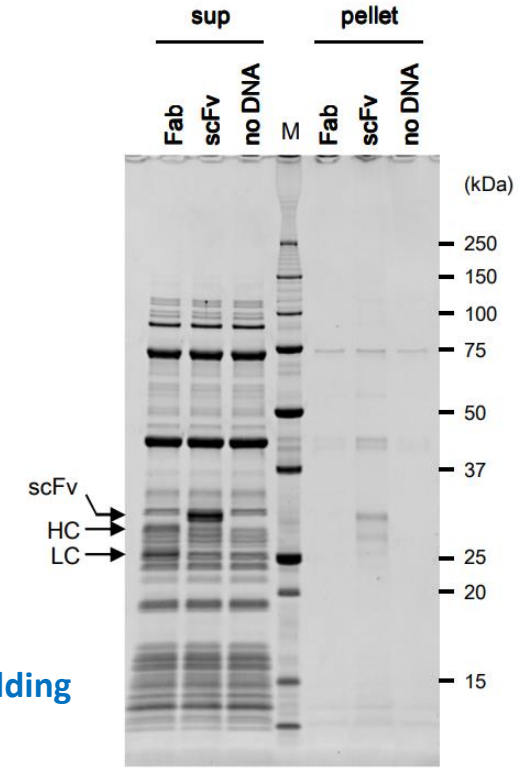
-Expression of scFv and Fab-



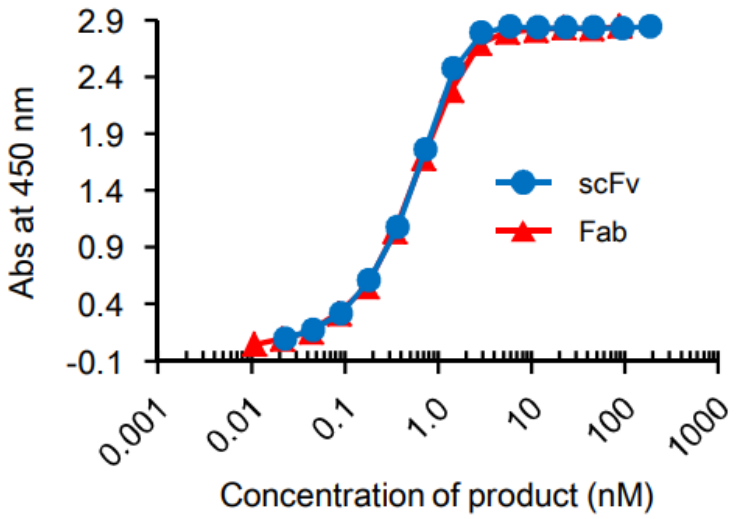


+/- **DsbC Set**
DnaK Mix
For correct folding

SDS-PAGE



Activity



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

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

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-For HTS of mAb-

Roche

For more information

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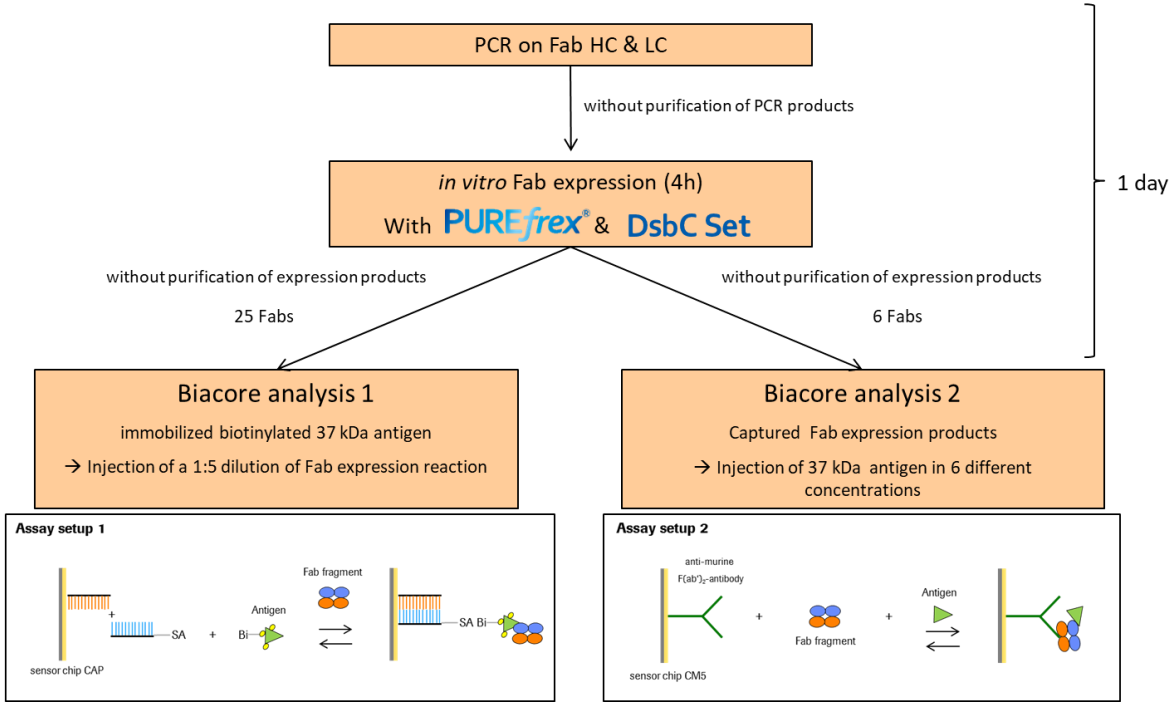
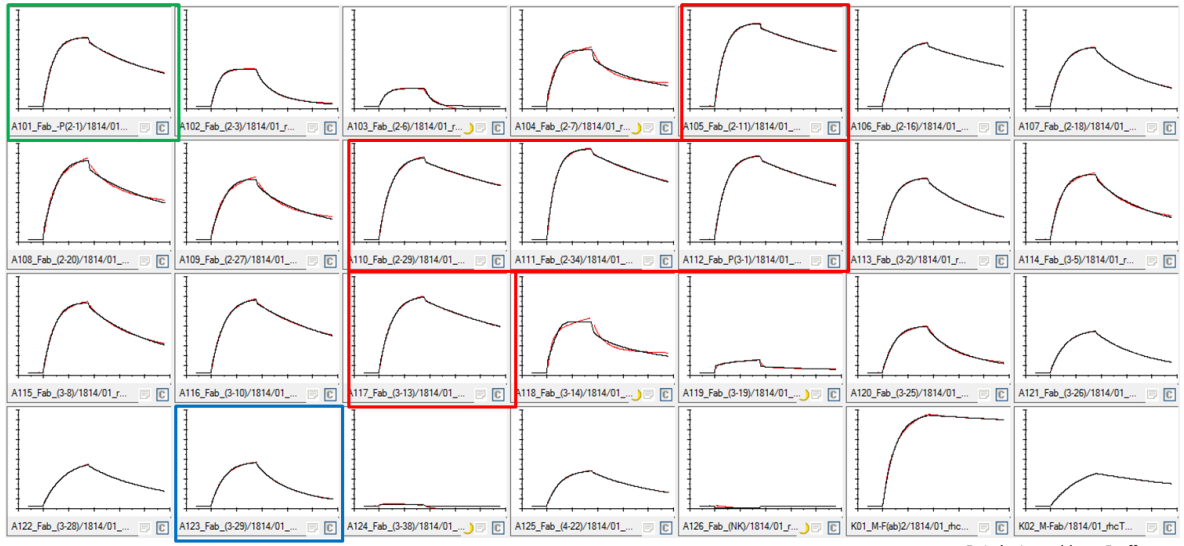
In vitro expression and Biacore analysis of Fab fragments



Kinetic analysis of 25 Fab binders

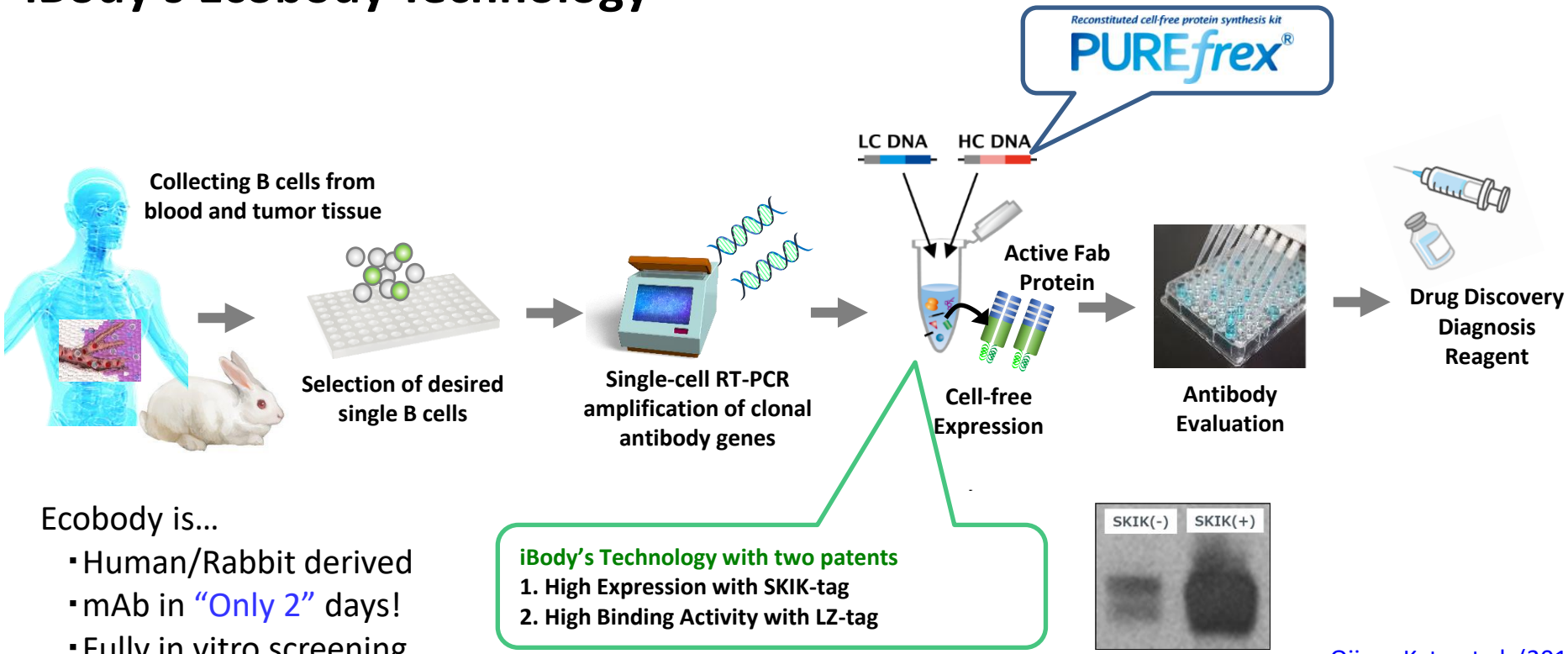


Data by Leopold von Proff

→ 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/

✓ **Active Fab is expressed/screened in HT manner.**

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-For display technology-

Epsilon Molecular Engineering
Molecular Design for Human Life

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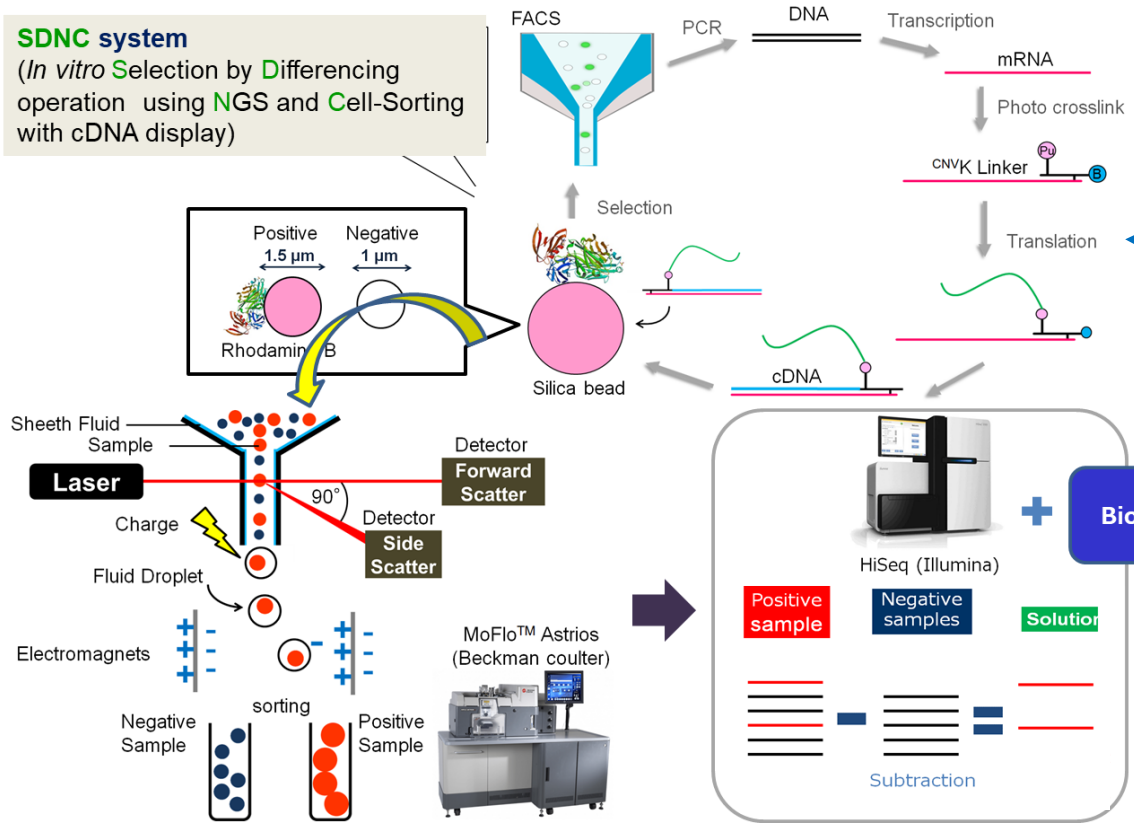
SDNC system
(*In vitro* Selection by Differencing operation using NGS and Cell-Sorting with cDNA display)



SDNC system is…
- for VHH/cyclic peptide
- with High diversity
- Fully in vitro screening

Reconstituted cell-free protein synthesis kit
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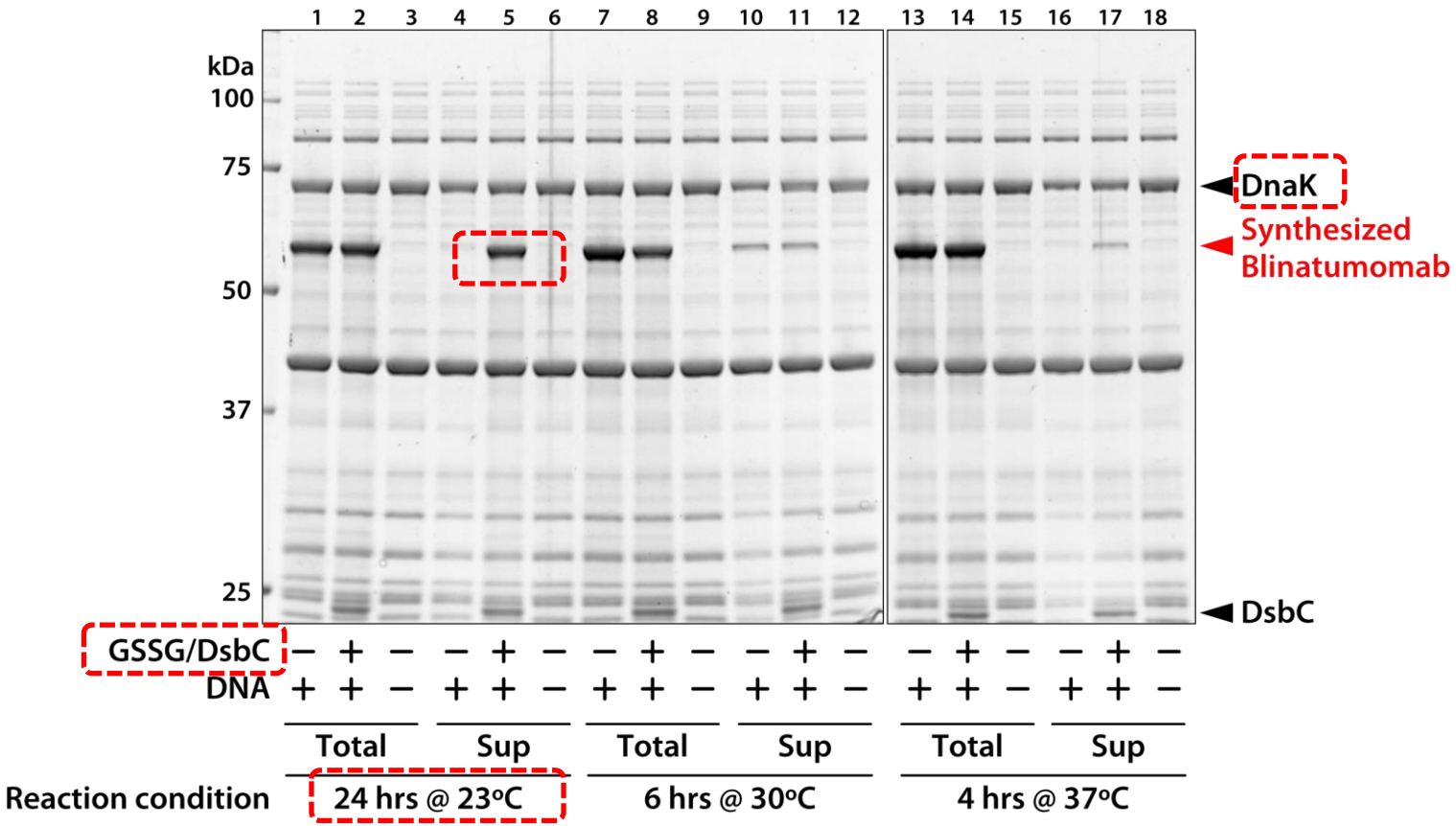
Evaluation
- ELISA
- SPR(BIAcore)
- BLI(Octet)
- Cell based assay

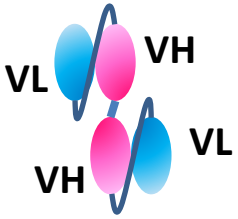
https://www.epsilon-mol.co.jp/eng/

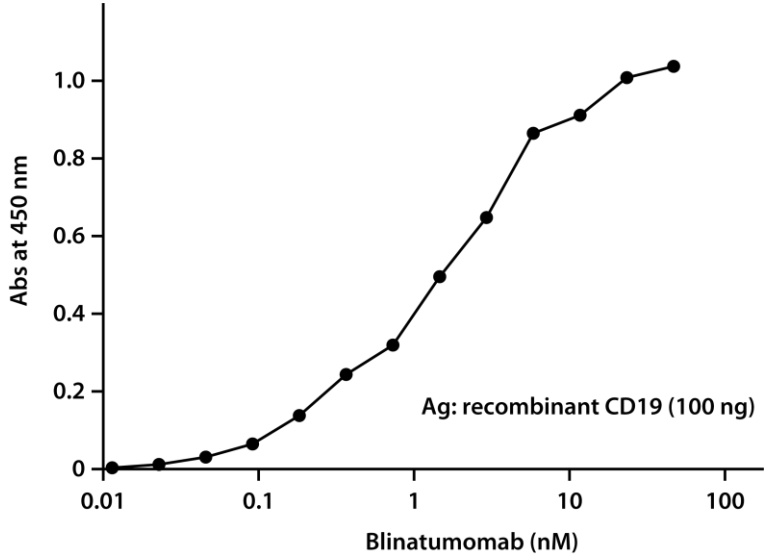
✓ PURE*frex* is applied for cDNA display based screening.

-Application for BiTE-



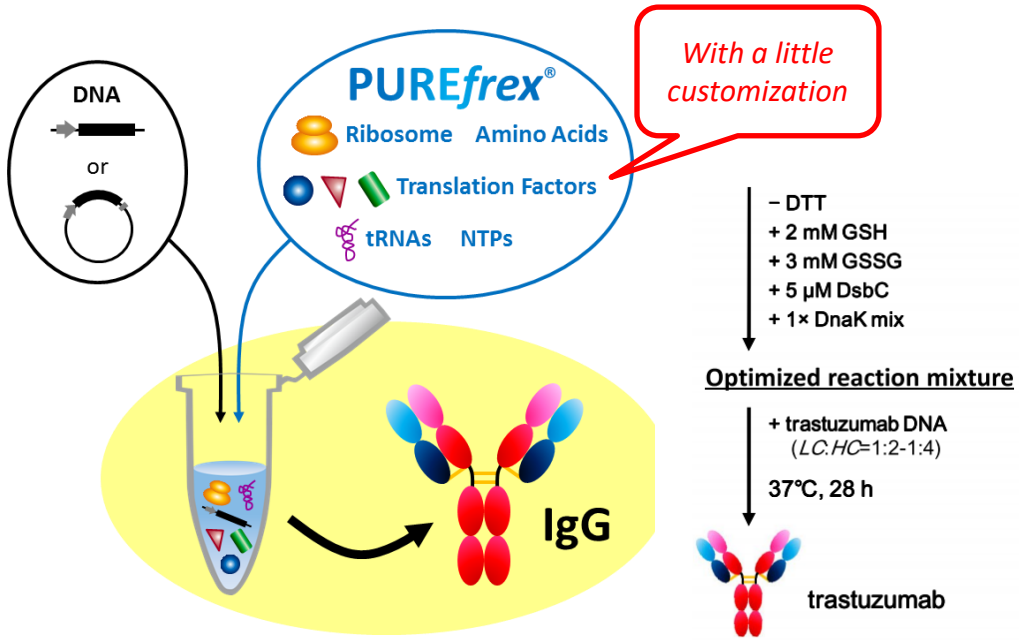
anti-CD3-scFv/CD19-scFv

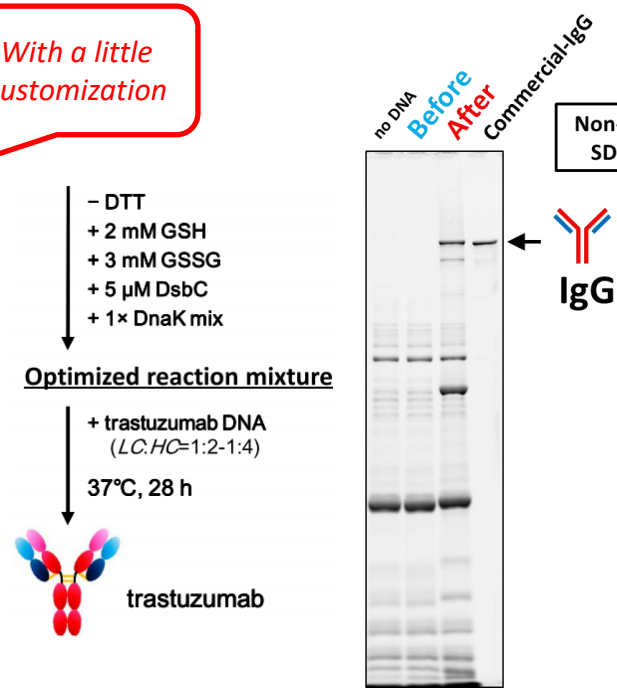


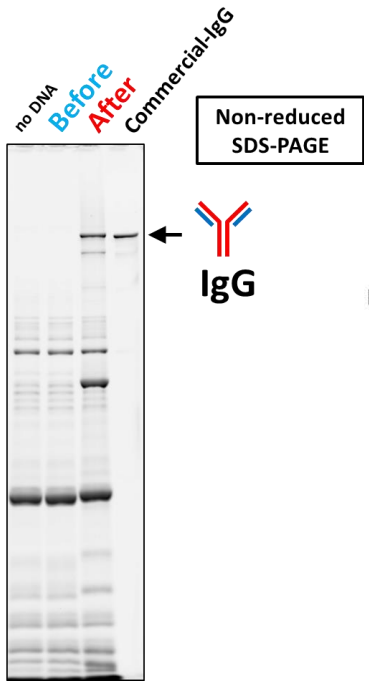


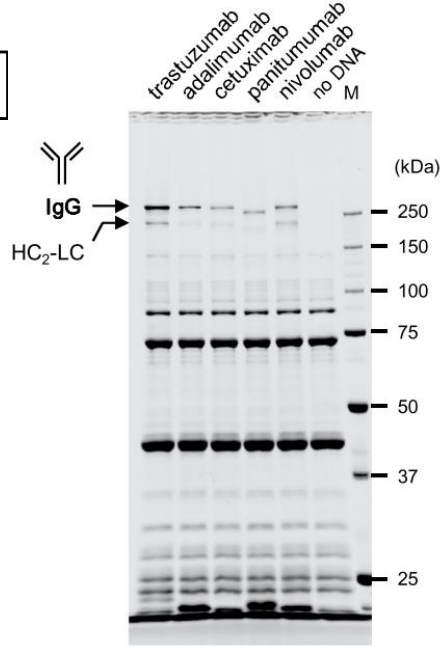
✓ **0.6mg/mL active BiTE was produced under optimized condition.**

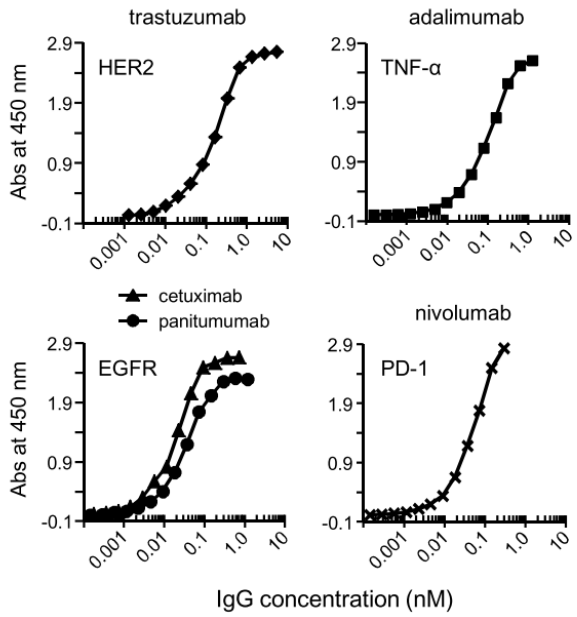
-Application for IgG-









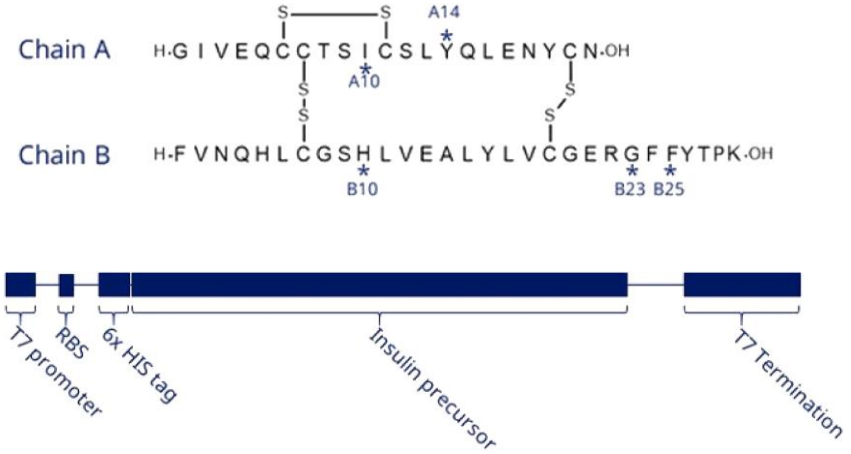


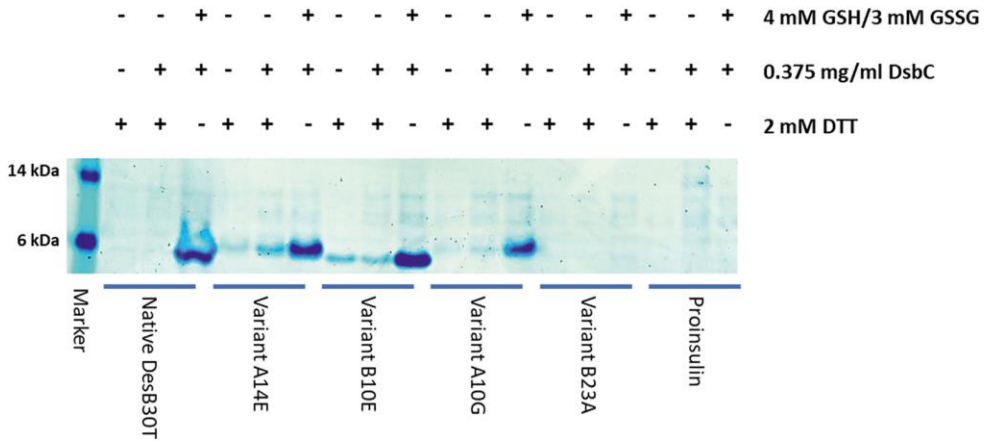
Only 2 days for IgG!!

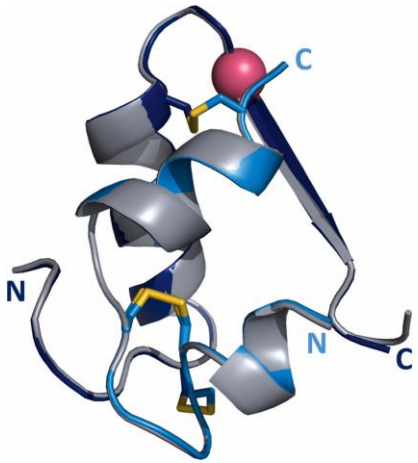
Murakami et al. (2019) Sci. Rep. vol.9, p.671.

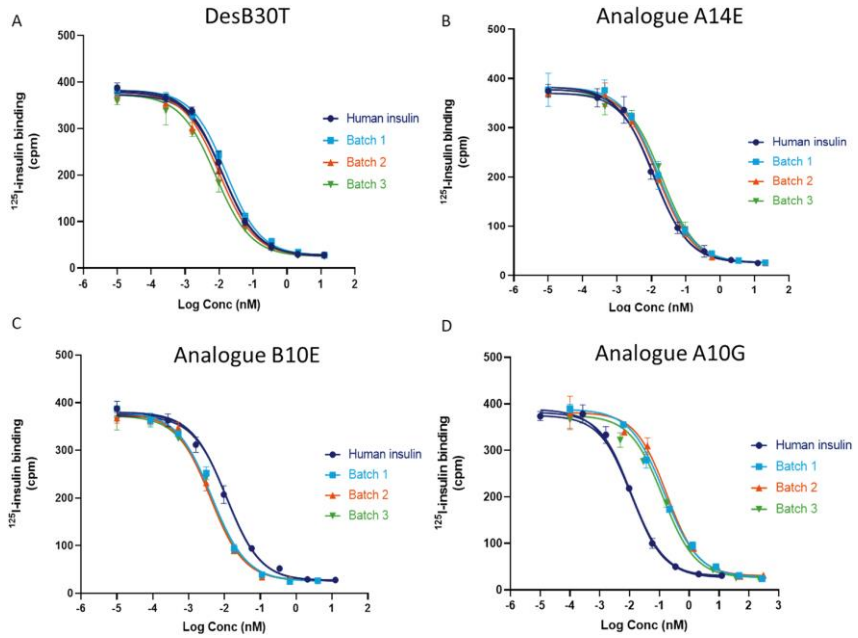
✓ **Full size IgG can be synthesized.**

-Application for complex molecule-









Jensen et al. (2021) Protein Expr. Purif., 186, 105910.

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

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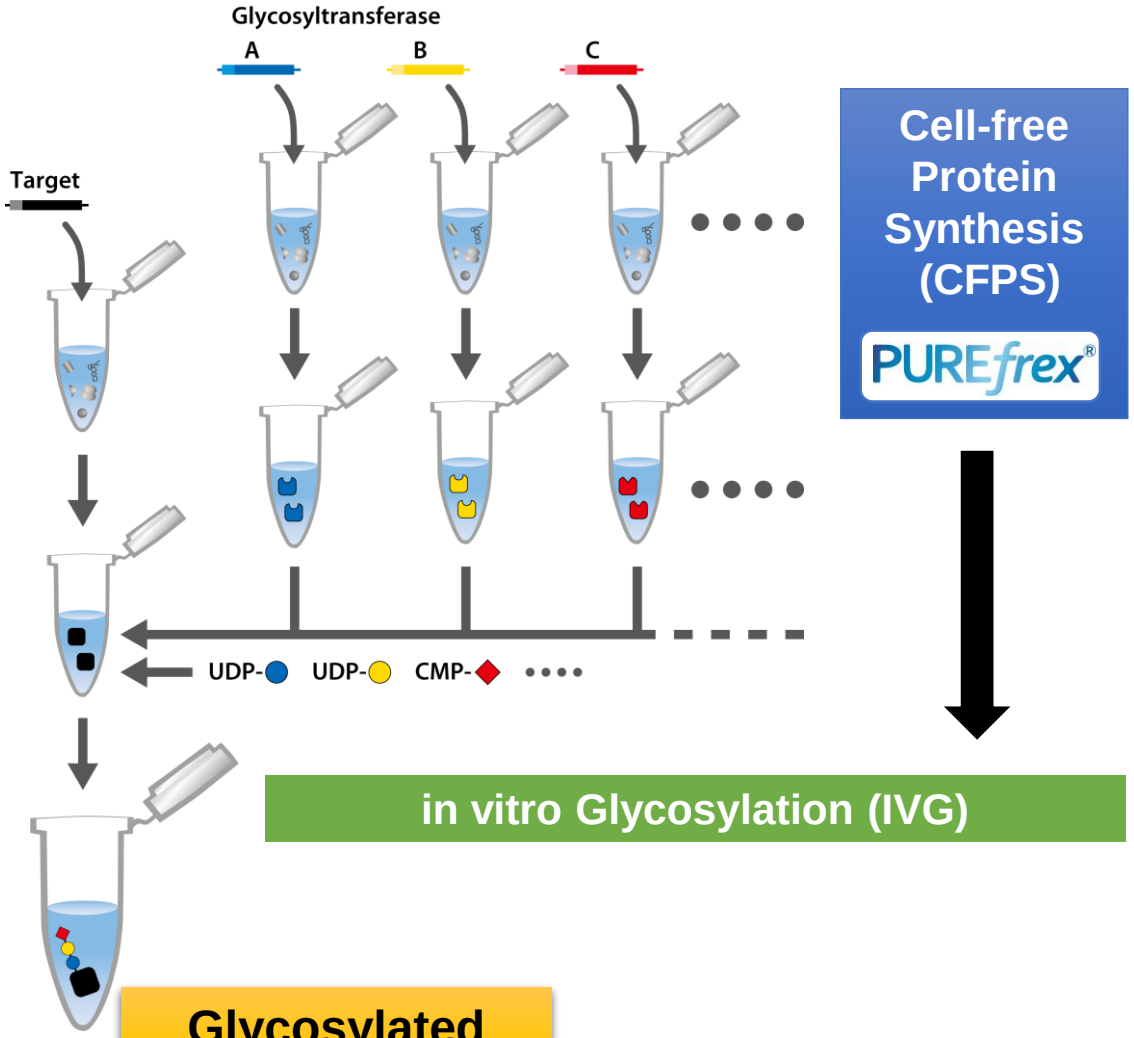
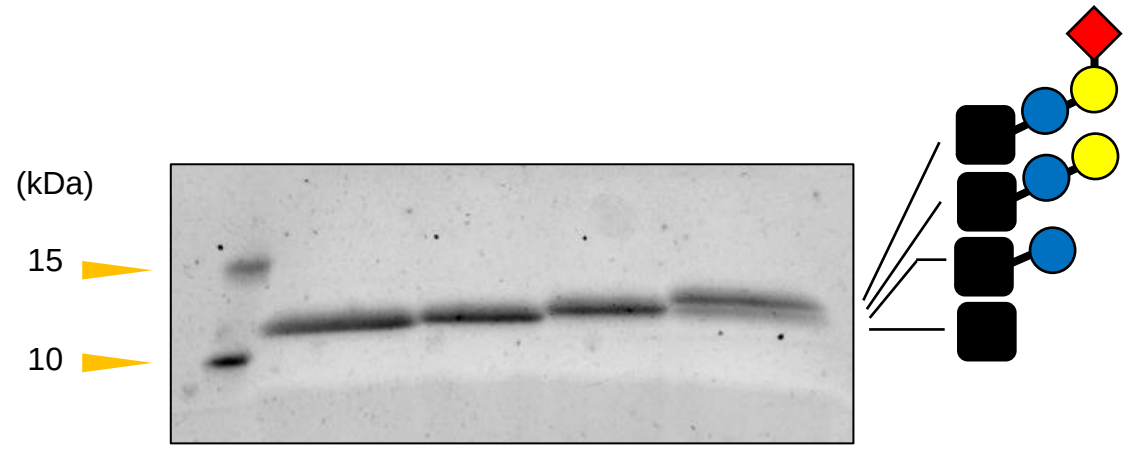
For more information

GeneFrontier

-Application for glycosylation; CFPS-IVG-



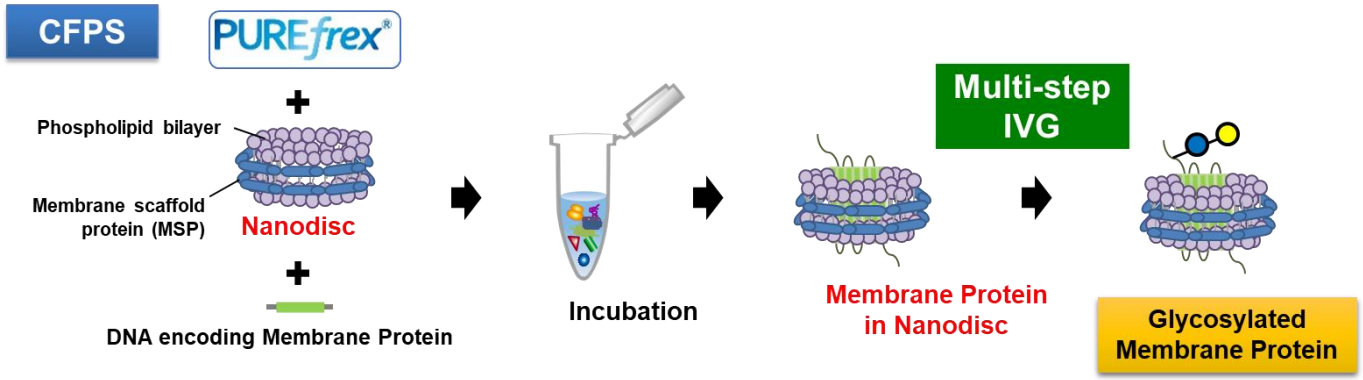


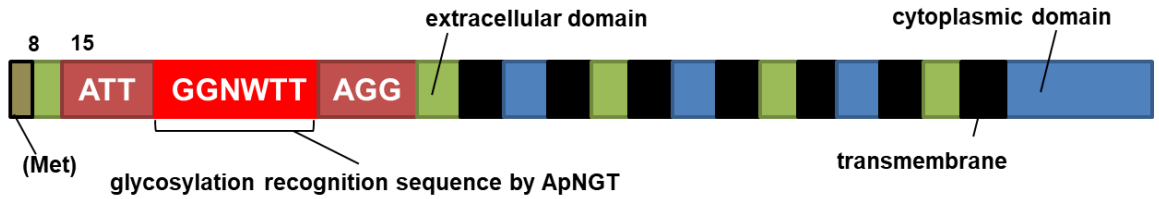
UDP-●/ Enzyme A	-	+	+	+
UDP-●/ Enzyme B	-	-	+	+
CMP-◆/ Enzyme C	-	-	-	+

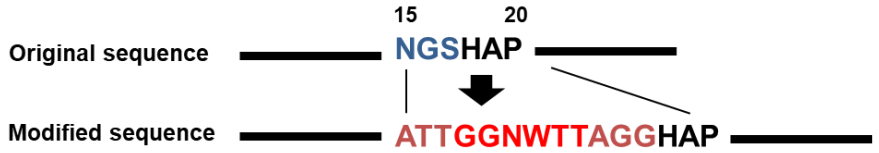
Applicable to glycosylation of target proteins.
Various reaction system can be designed.

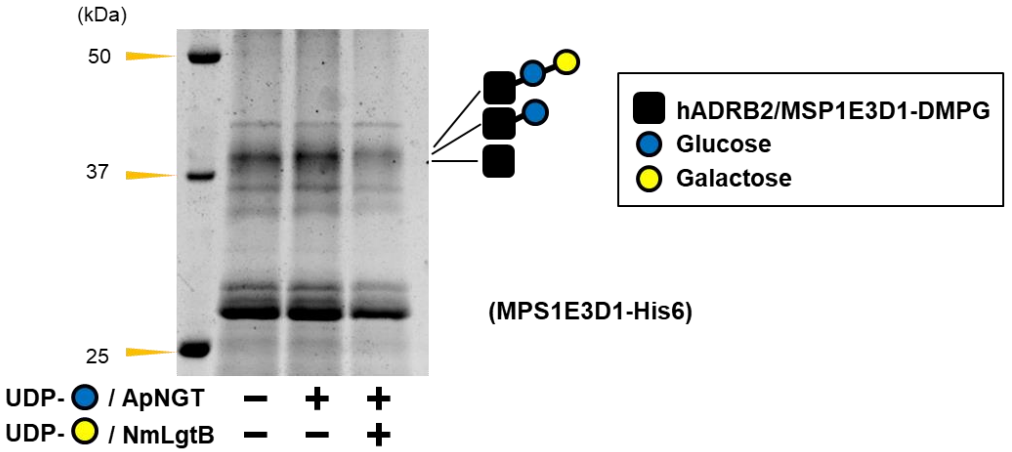
Poster_MBSJ 2021

-Application for glycosylation; CFPS-IVG-









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

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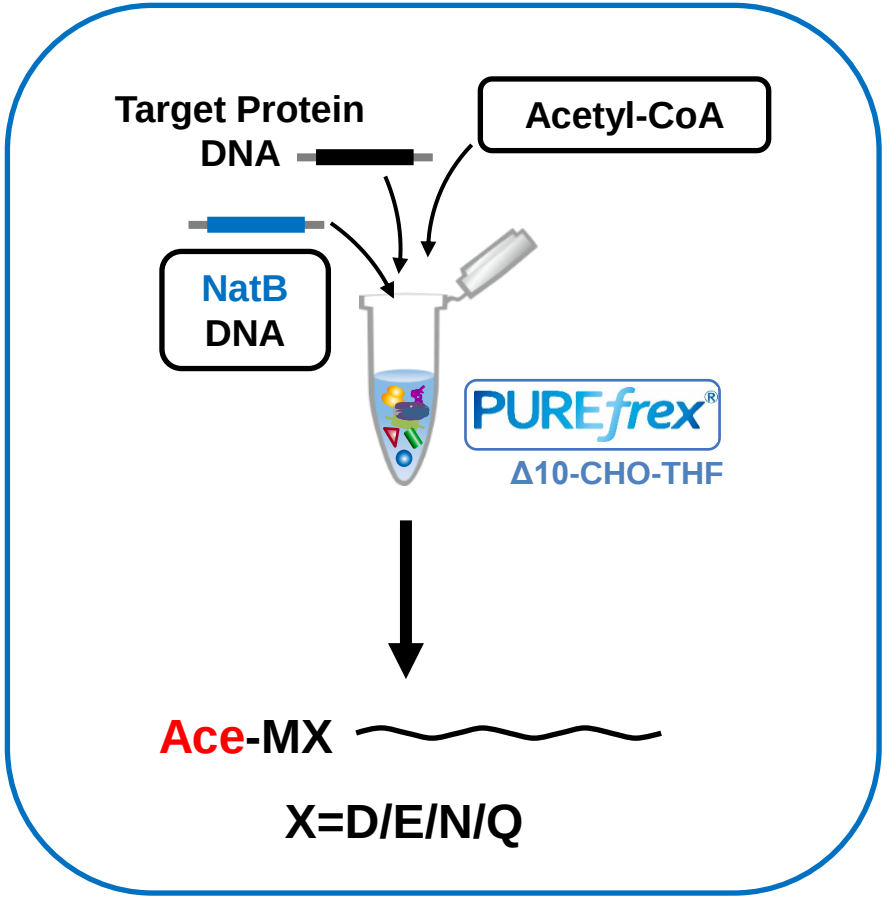
-Application for acetylation-

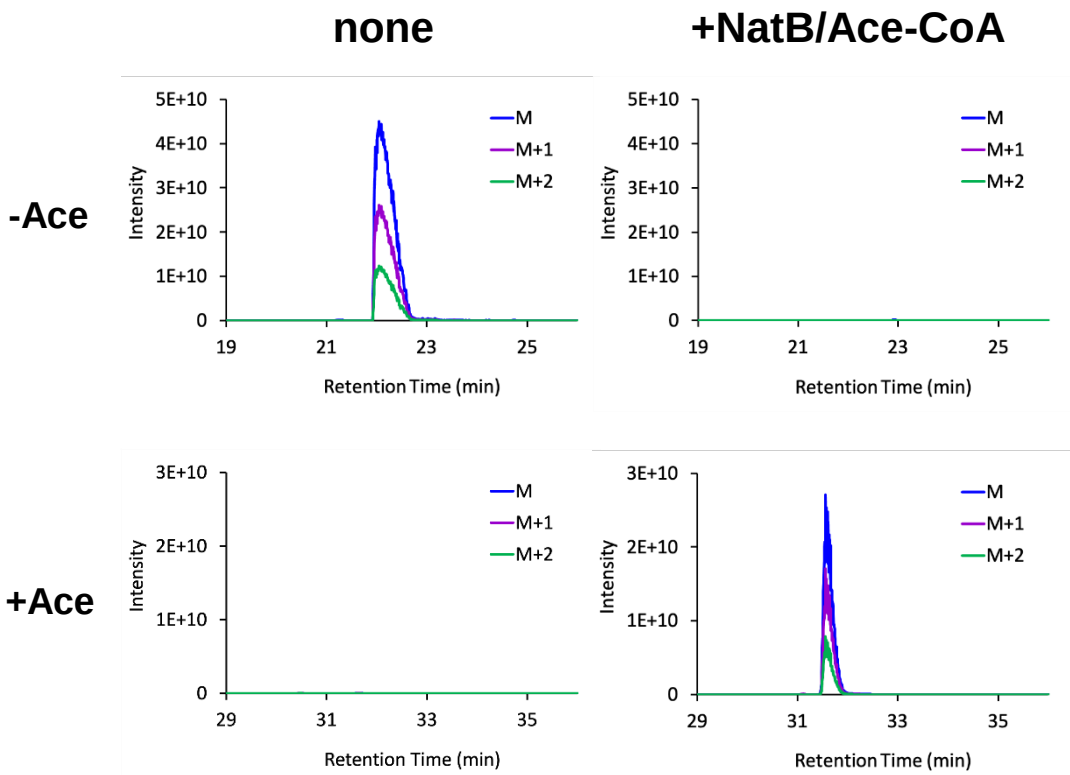
Acetylation (by NatB)



target: α-synuclein (K6A)

MDVFMAGLSK･･･ Hisx6

Chromatogram of MS1 intensity



Perfect control of N-terminal acetylation of α-synuclein

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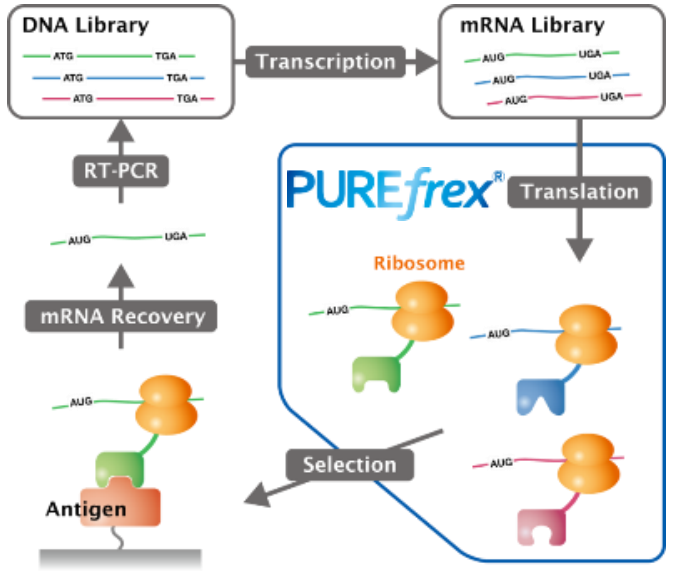
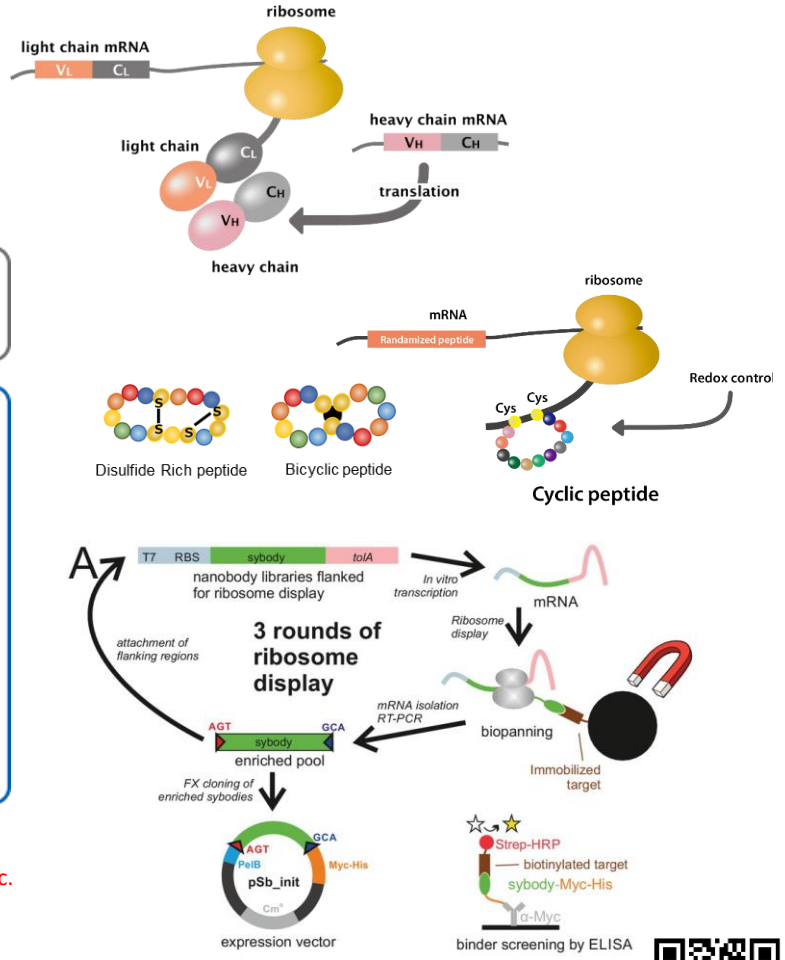
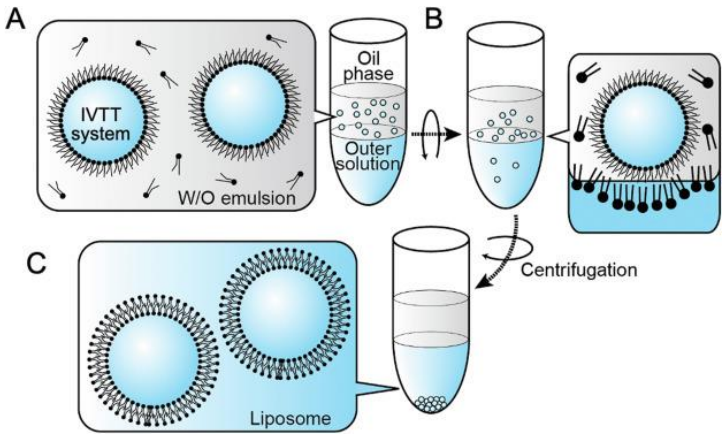


Licensed technology under JP4931135 etc.

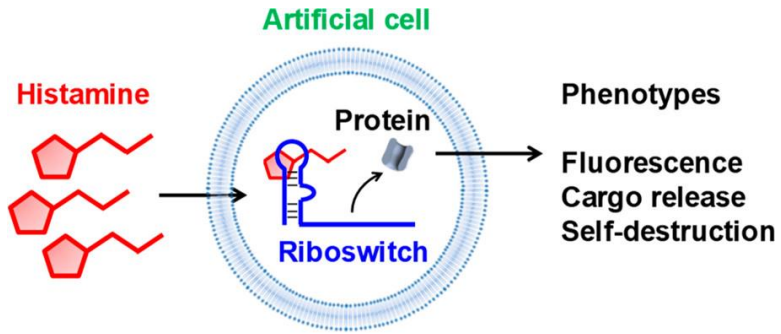


Zimmermann I. et al. (2018) eLife, 7, e34317.

-Broad applications, yet to come!-



Uyeda et al. (2022) J. Biosci & Bioeng. vol.133, p181.



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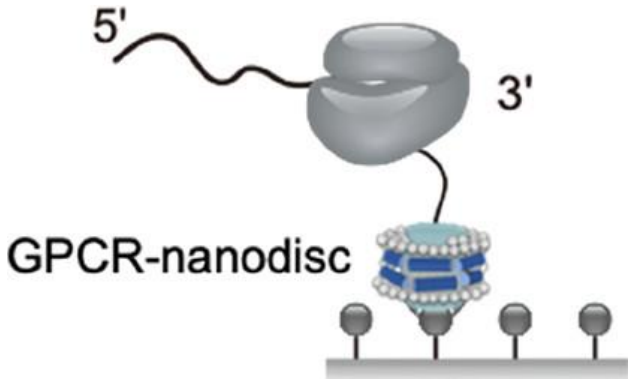

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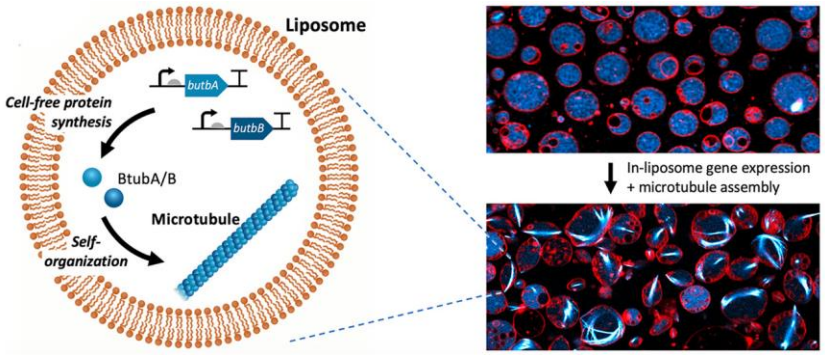

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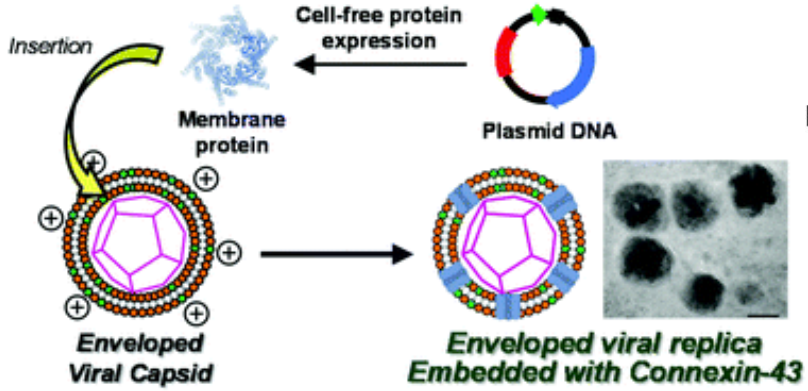

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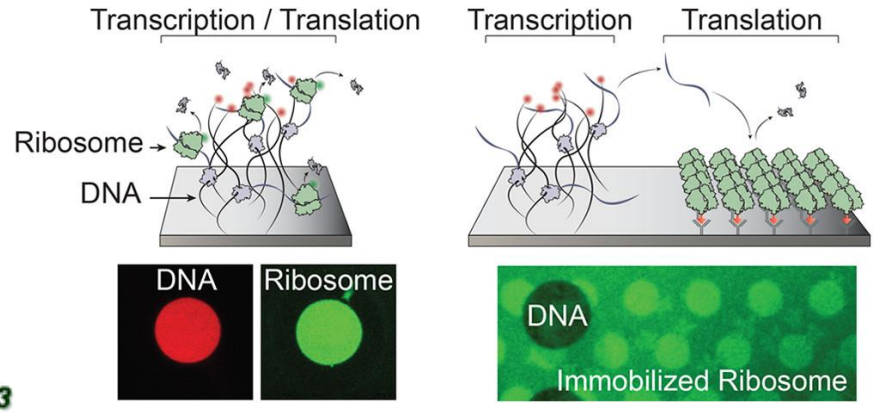

Levy et al. (2021) ACS. Synth. Biol. vol.10, p.609.

Contact information

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Takashi Ebihara, Ph.D., COO, GeneFrontier

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