

# Effective production of single chain variable fragment using recombinant *Escherichia coli* by DO-stat fed-batch culture

組換え大腸菌を用いたDO-stat流加培養による効率的単鎖抗体生産

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## Introduction

Recombinant protein production using *Escherichia coli* has been widely applied in the biotechnology field. However, there are some drawbacks such as formation of inclusion body and difficulty in extracellular production. In this study, we challenged to develop a method for extracellular protein production of soluble proteins by using *E. coli* with periplasmic secretion signal (pelB leader) based on DO-stat fed-batch culture for high cell density.

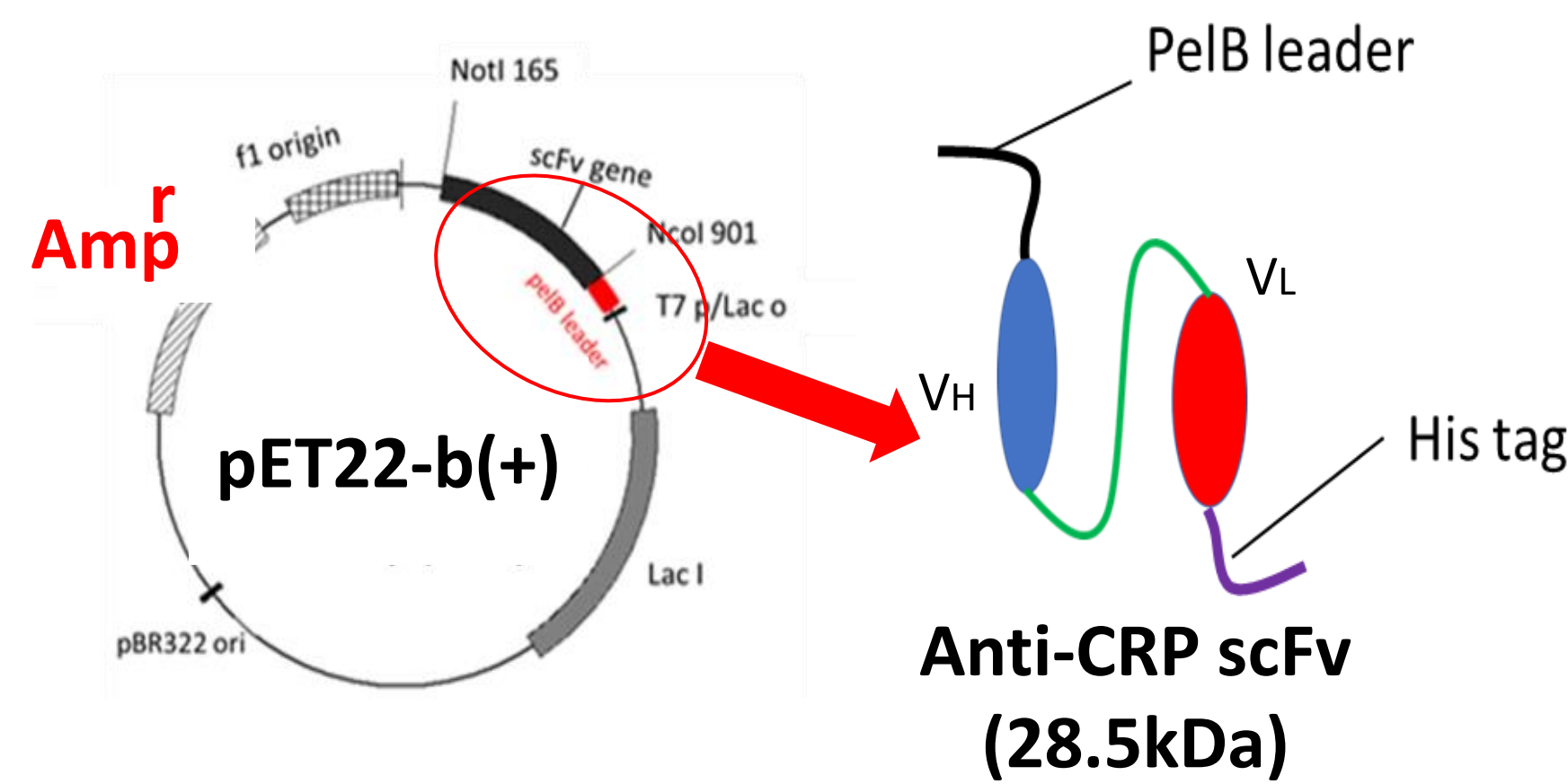
## Materials and methods

### Strains

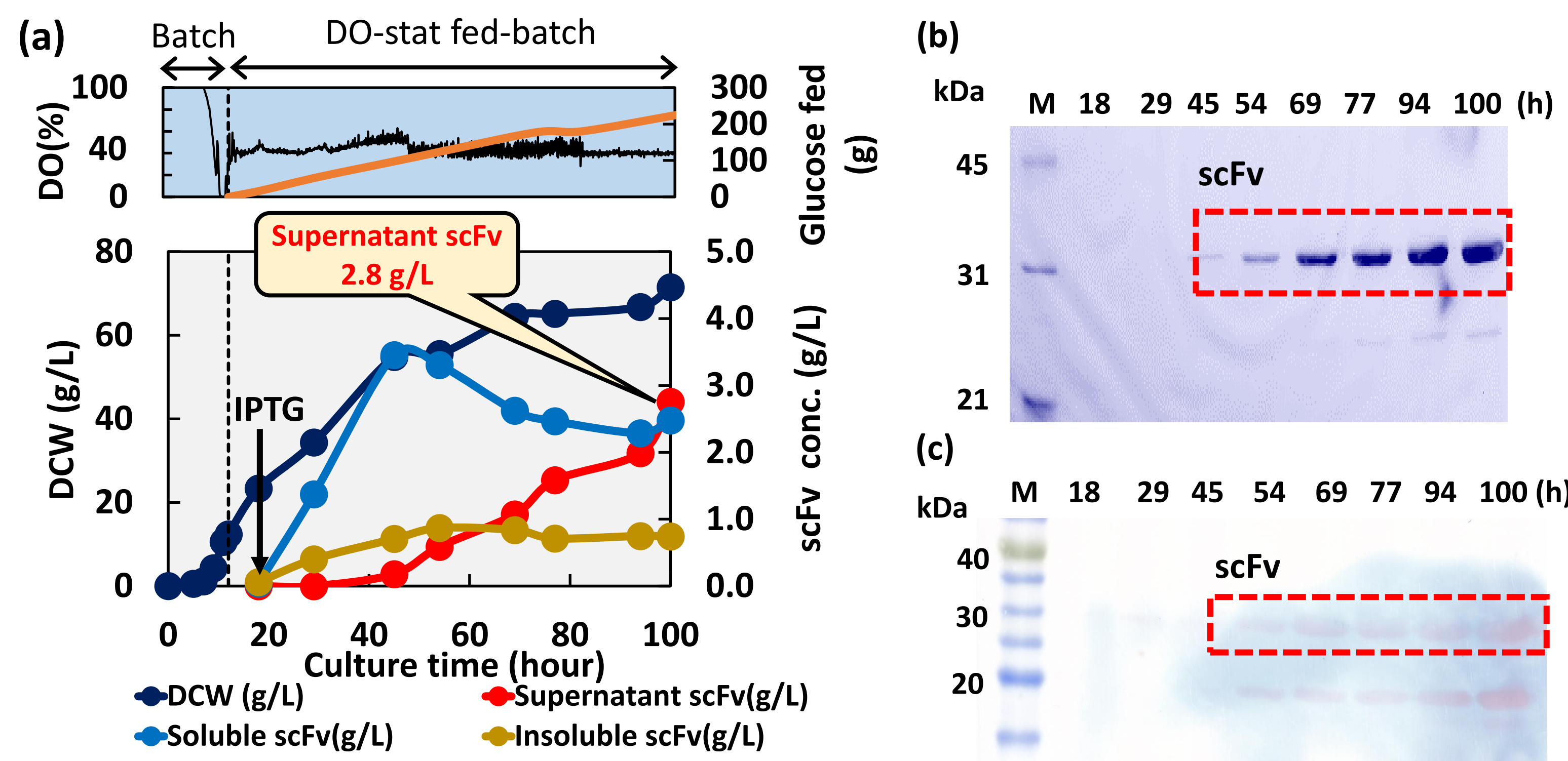
**pET expression system**  
T7 promoter  
pET22-b(+):Ampicillin resistance

**Host**  
Roseeta2 (DE3)

**scFv gene**  
Anti-CRP scFv



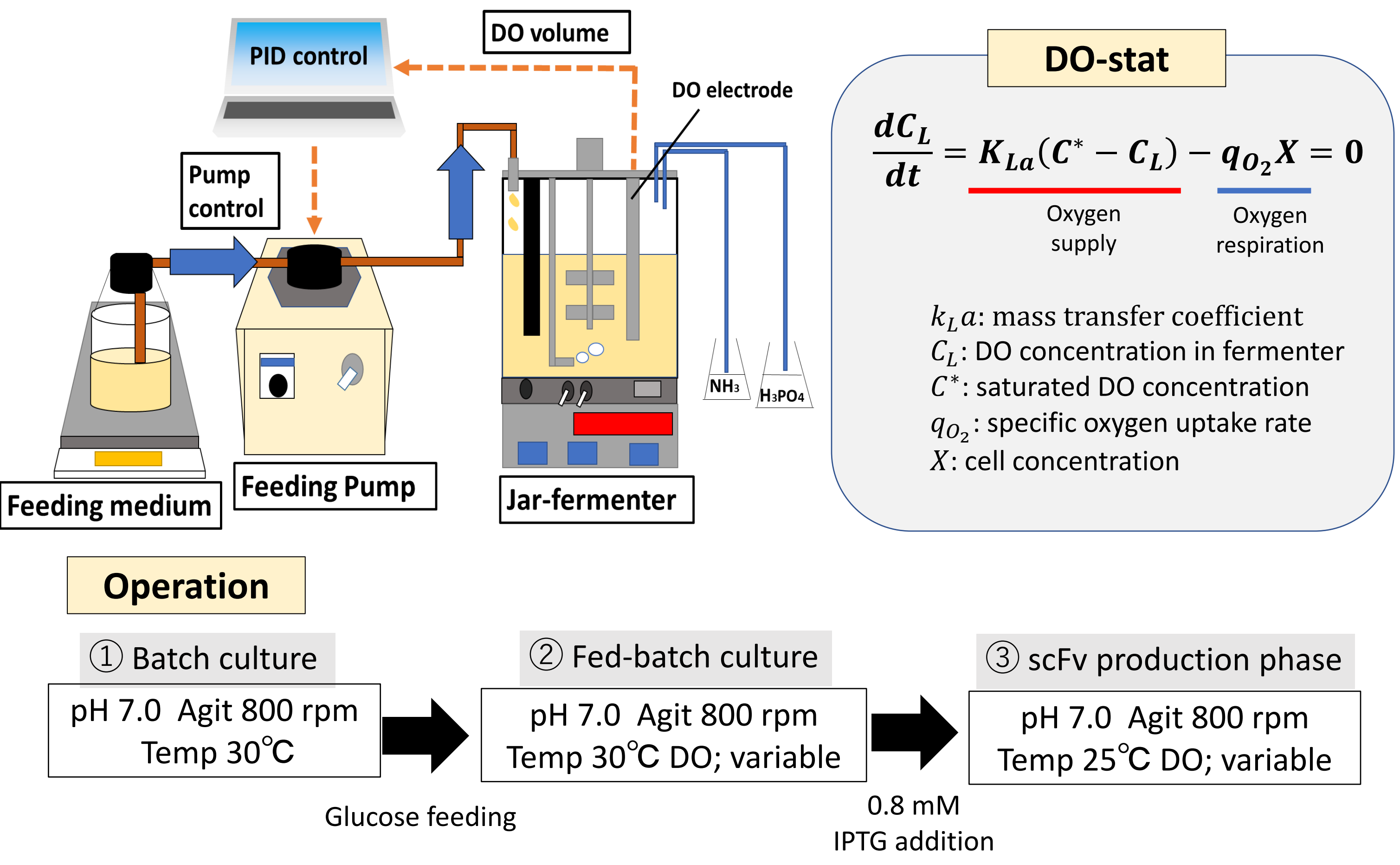
## anti-CRP scFv production by DO-stat fed-batch culture



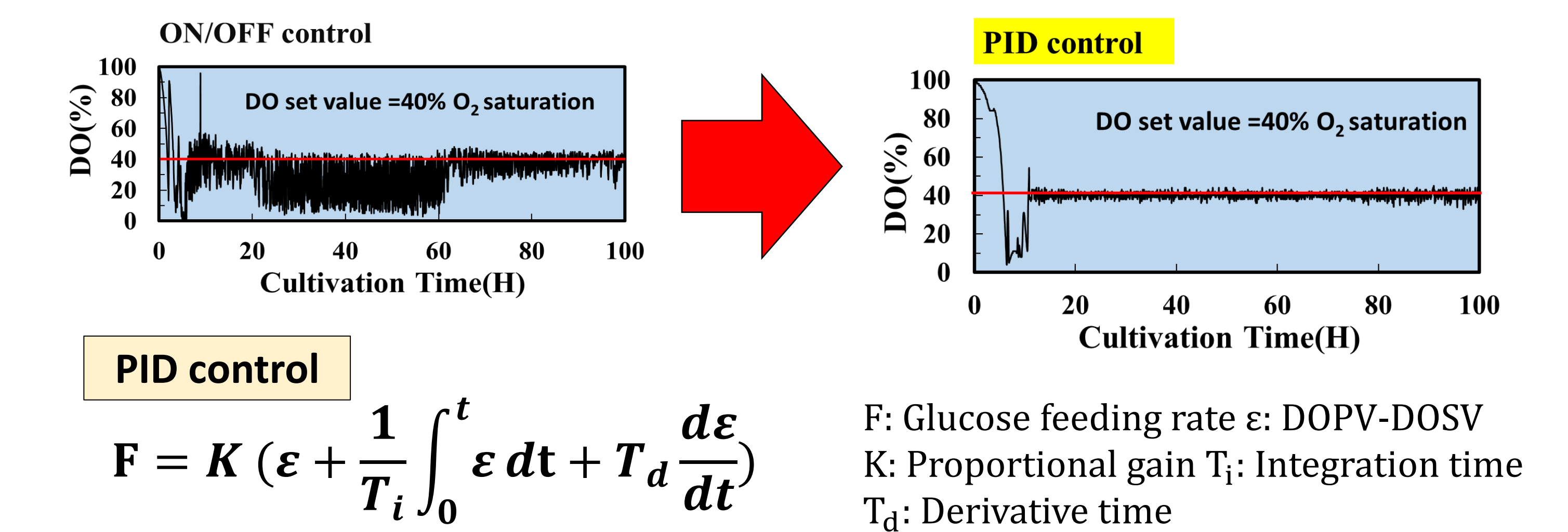
Cell Concentration: DCW=71.5 g/L  
Supernatant scFv=2.8 g/L Total scFv = 6.0 g/L

Fig. 2 (a) Time courses of DO-stat fed-batch culture for scFv production (b) SDS-PAGE after purification Supernatant (c) Western blotting after purification Supernatant

## Experimental set up for DO-stat fed-batch culture



## Introduction of PID control into DO-stat strategy



## Results and discussion

### scFv production characteristics by flask culture

Table. 1 scFv production by flask culture

Parameter	Value
DCW (g/L)	8.6 ± 0.5
Supernatant scFv (mg/L)	76 ± 5
Soluble scFv (mg/L)	35 ± 2
Insoluble scFv (mg/L)	260 ± 20
Total scFv (mg/L)	370 ± 10
Solubility (%)	30 ± 2

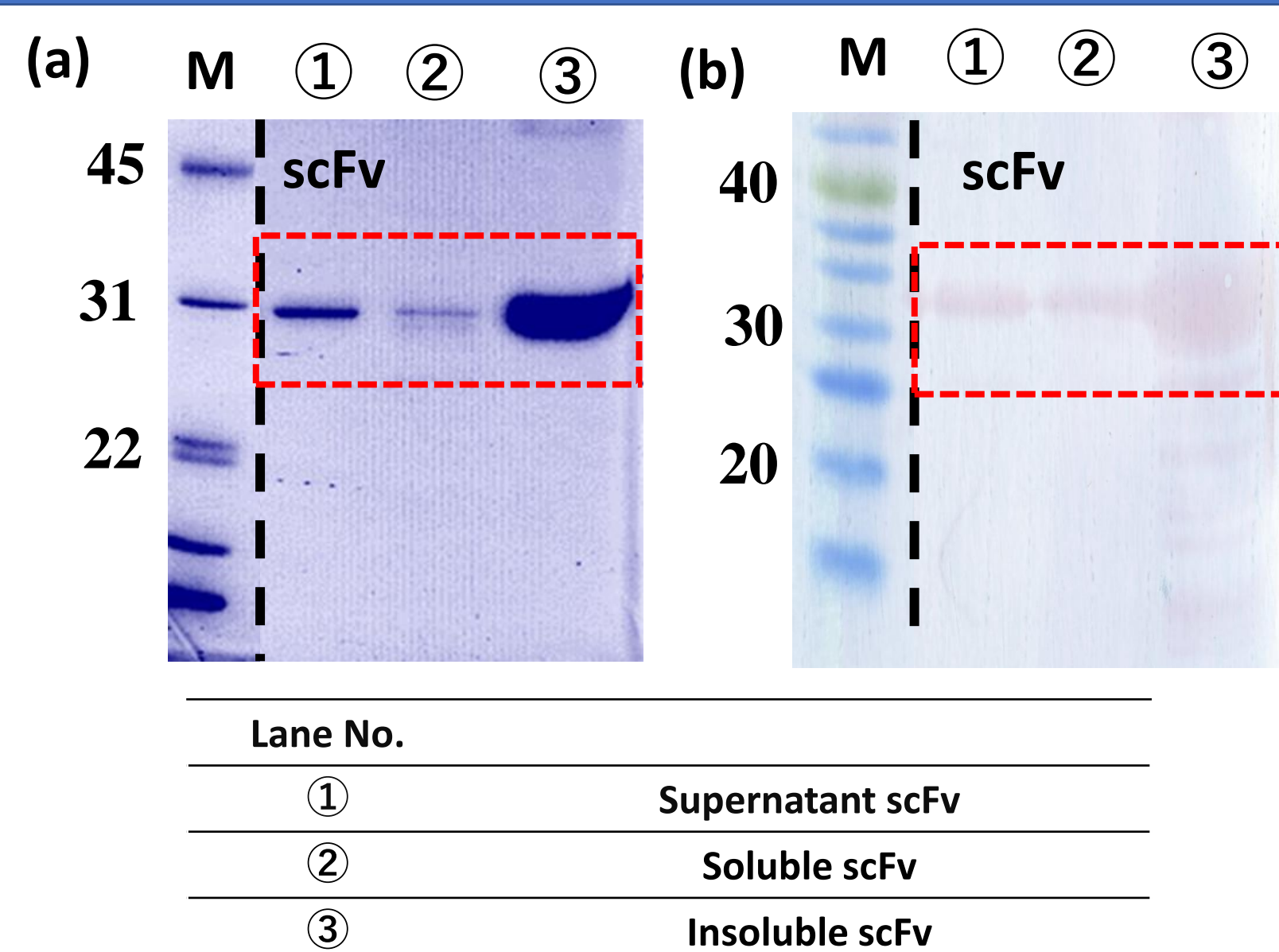


Fig. 1 (a) SDS-PAGE and (b) Western blotting after purification

- ScFvs were successfully produced.
- ScFvs were mainly accumulated as inclusion body.

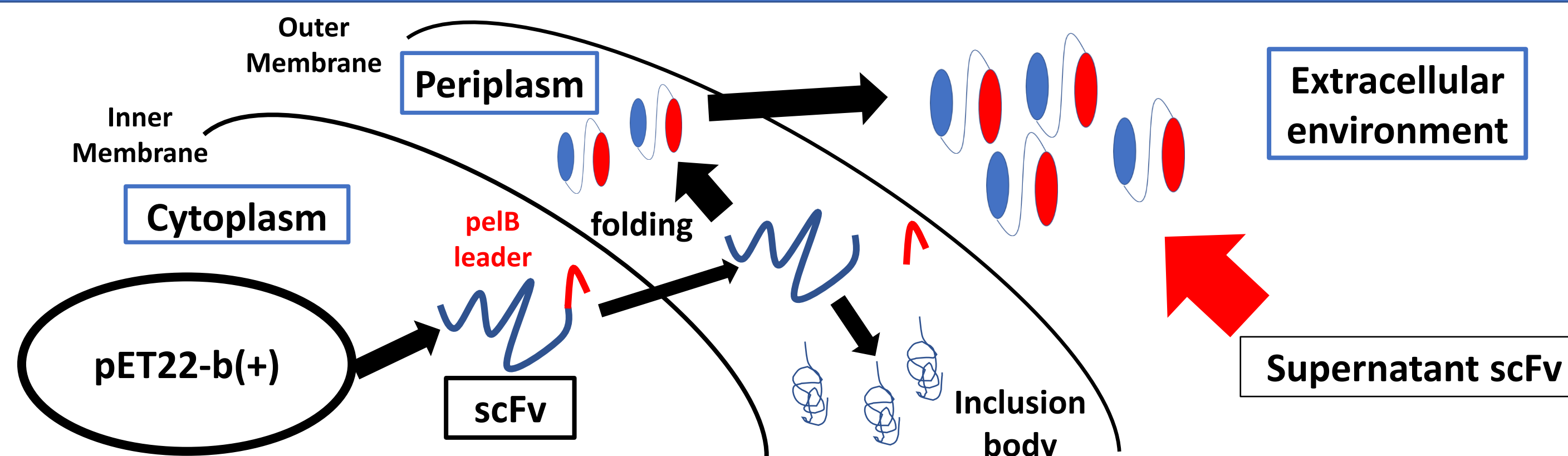
## Effects of DO on anti-CRP scFv production

Table. 2 Influence of DO on scFv production

DO (%)	Glucose feeding rate (g/h)	DCW (g/L)	Supernatant scFv (g/L)	Soluble scFv (g/L)	Insoluble scFv (g/L)	Total scFv (g/L)	Solubility (%)
40	2.4	71.5	2.8	2.5	0.7	6.0	88
50	1.9	43.3	2.4	1.6	0.7	4.7	85
60	1.5	34.1	2.9	3.2	1.0	7.2	86

- DO level has effect on glucose feeding rate and cell growth.
- Extracellular production of scFv reached to 2.4~2.9 g/L regardless of DO level.

## Possible mechanism of extracellular production of scFv



## Effects of amino acid sequence on scFv production

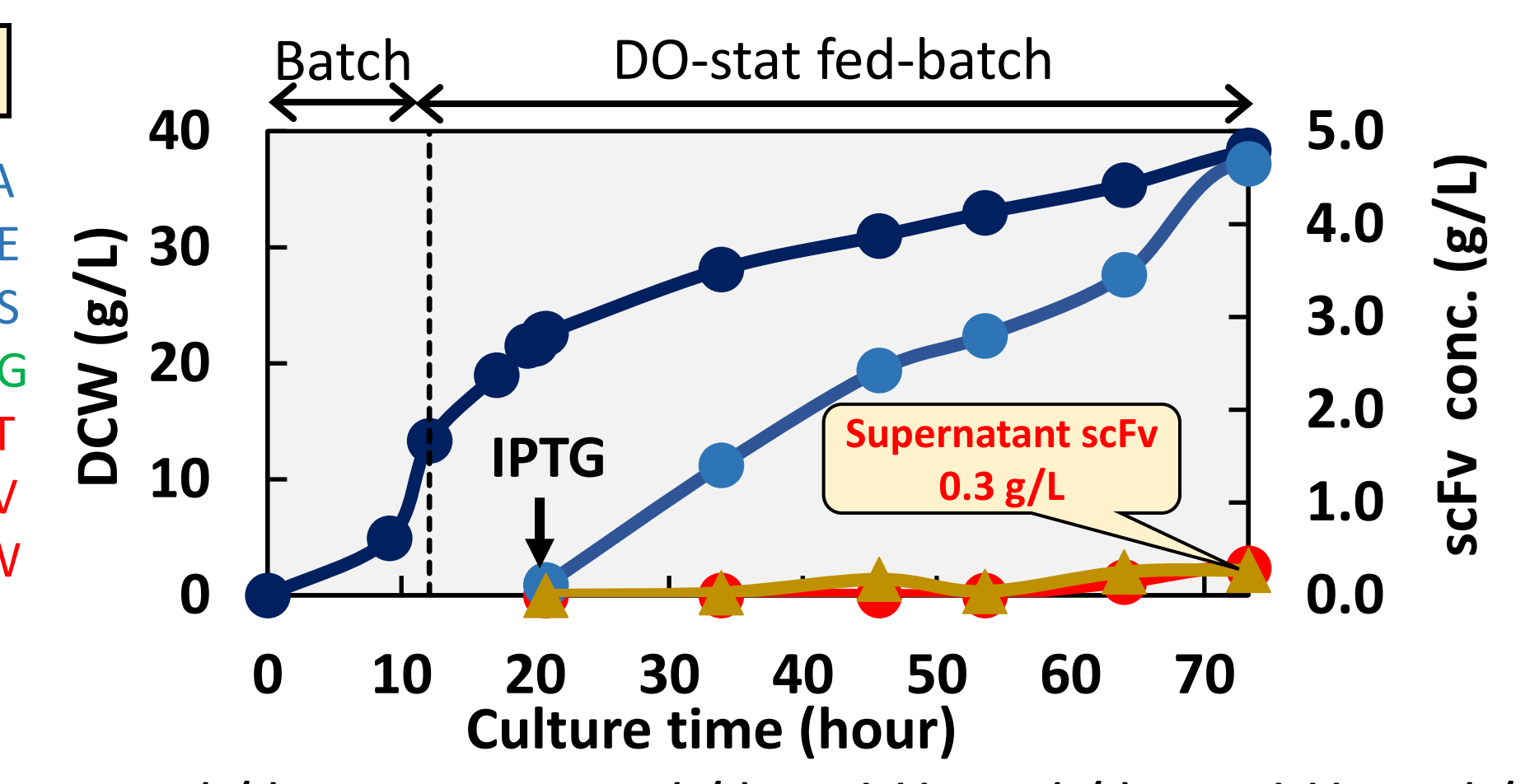
### Amino acid sequence of anti-CRP scFv\*

MKYLPTAAAGLLLLAAQPAMA MDASQVQLQQSGA  
ELVKPGASVKLSCTASGFNIKDYMHVWVKRTEQGLE  
WIGRIDPEDGETKYAPKFGKATITADTSSNTAYLQLSS  
LTSEDTAVYYCARGYGSSEAMDYWGQGTSLTVSSGGG  
GSGGGGSGGGGSGTGSIVMTQSHKFMSTVSGDRVSIT  
CKASQDVNTAVAWYQKQKPGQSPKLLIYWASTRHTGV  
PDRFTGSGFGTDYTLTISVQAEDLALYYCQHYSTPV  
TFGGGTLKLEIKRADAAPTVAALAEHHHHHHH

\*Modified anti-CRP scFv was removed the amino acid sequences shown in yellow.

anti-CRP scFv  
M.W:28.5 kDa  
pI: 6.35

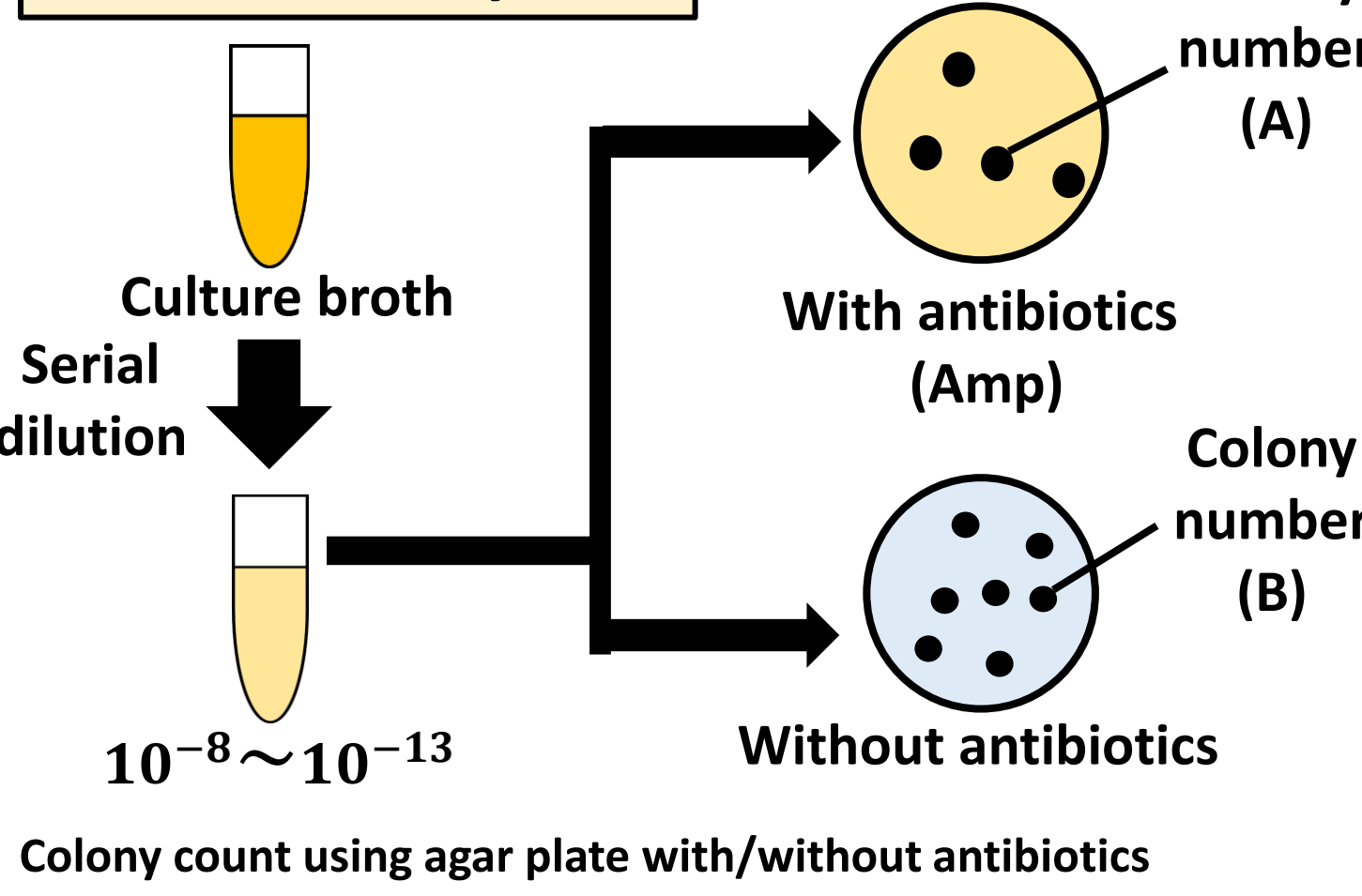
Modified anti-CRP scFv  
M.W: 27.3 kDa  
pI: 6.86



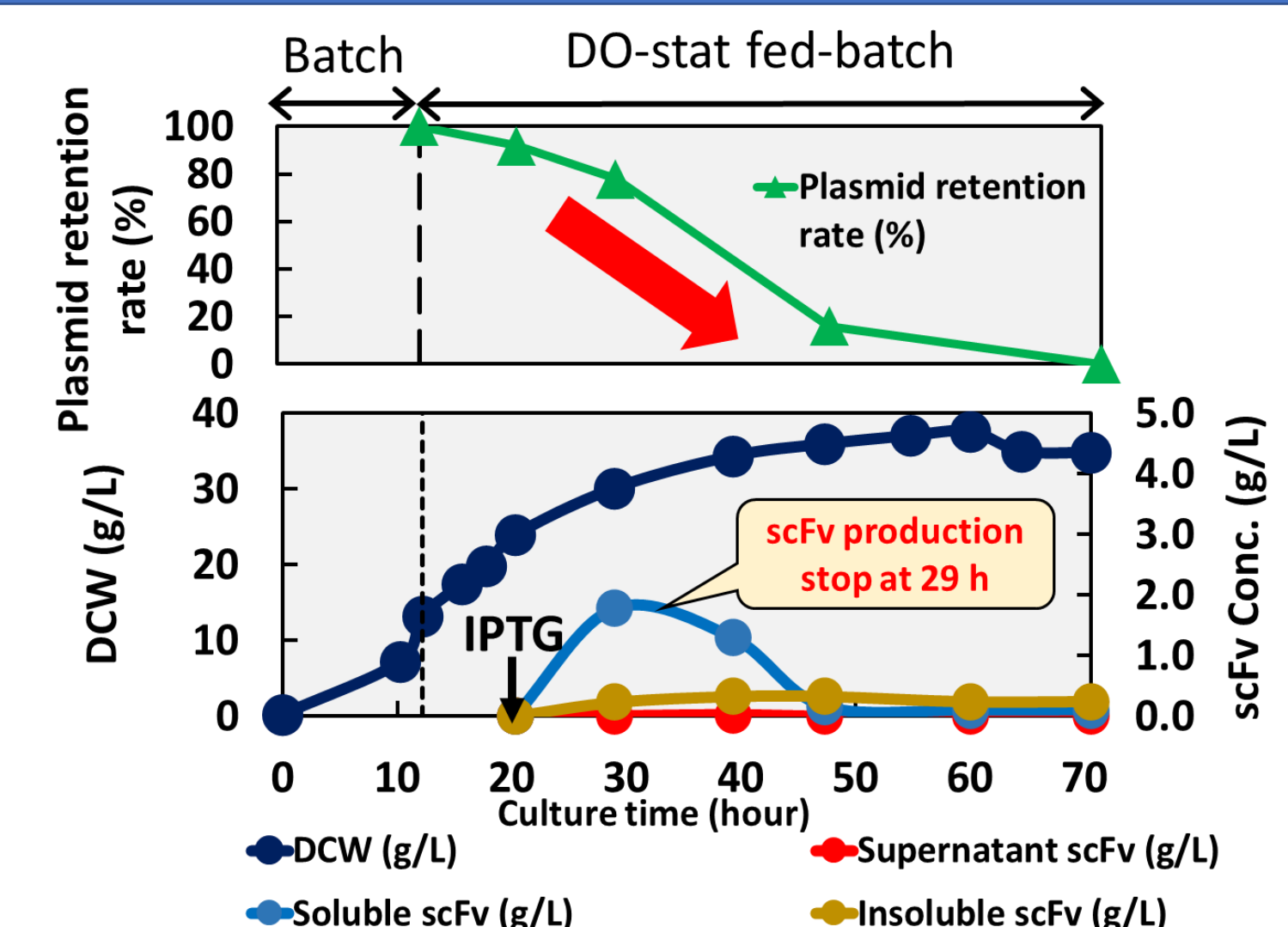
- Slight modification of amino acid sequence seriously affected the secretion of scFv.

## Effects of plasmid stability on scFv production

### Plasmid stability test



$$\text{Plasmid retention rate (\%)} = \frac{(A)}{(B)} \times 100$$



- The ratio of plasmid retention cells drastically decreased in the later phase of fed-batch culture.

## Conclusions

- The extracellular production of scFv was confirmed by fed-batch culture using *E. coli* with pelB leader.
- Slight difference in amino acid sequence of scFv and plasmid stability influenced the characteristics of extracellular scFv production.

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