

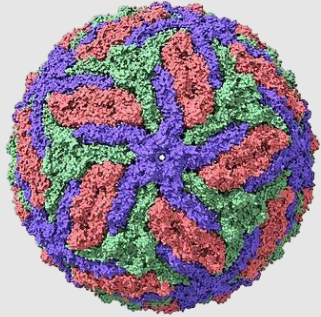
# Construction of 1B3B9 rAb in pTRIOZ expression plasmid

**Thesis title:** Construction and characterization of recombinant antibody (rAbs) against dengue virus

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Microbiology, Medicine, KKU





## Dengue virus Family Flaviviridae

- Mosquito borne disease
- 4 serotypes (DENV1-4)
- + ssRNA Envelope virus
- “Envelope protein”  
effective neutralizing target



**No specific treatment**

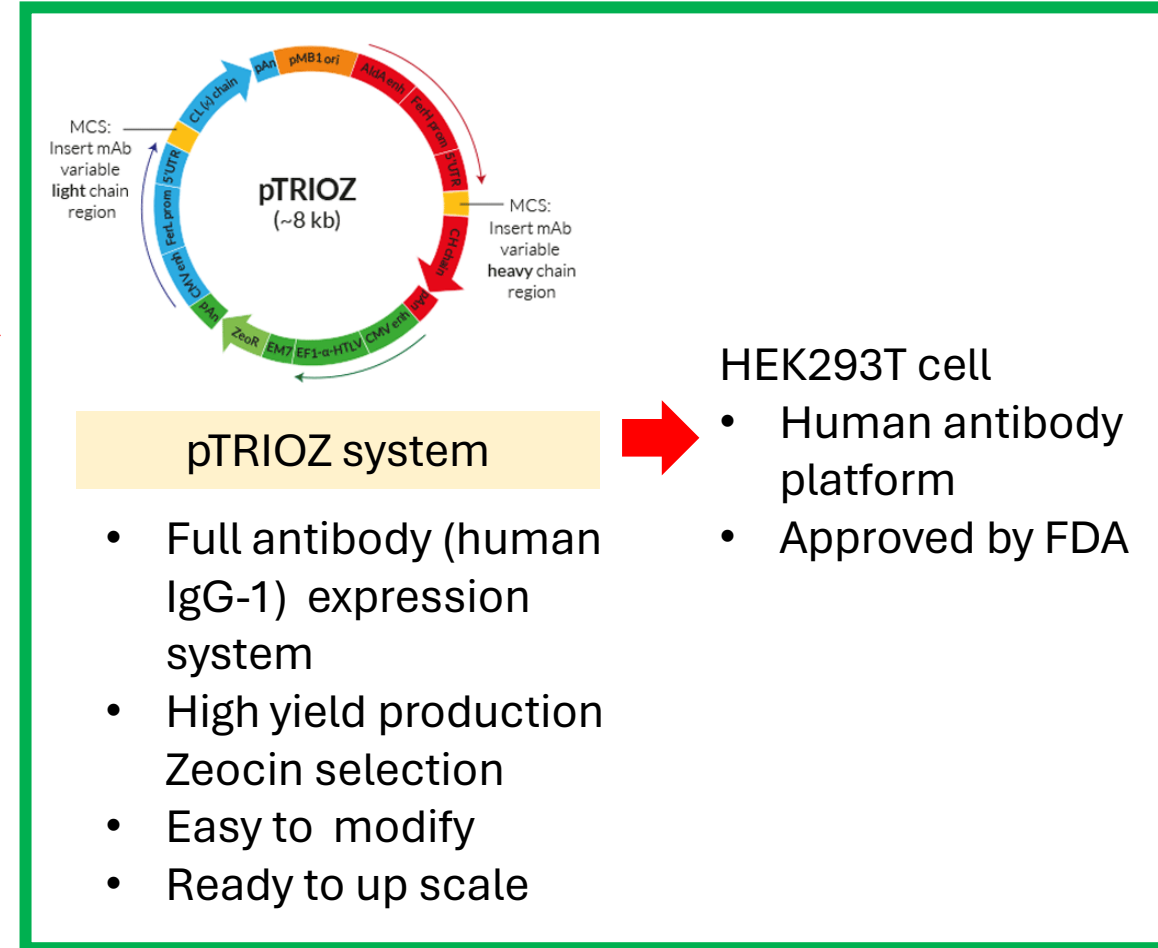
### New candidate

## 1B3B9\_V21 antibody (Screened by Machine learning)

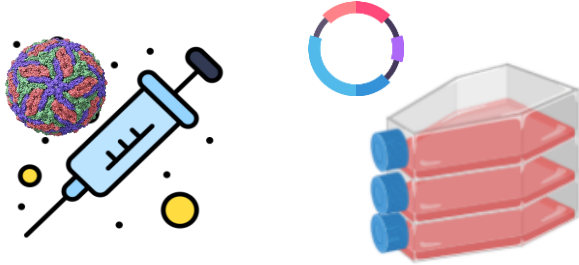


### Molecular dynamic simulation

- To accurately predict the insight interaction of 1B3B9\_V21 with 4 DENVs

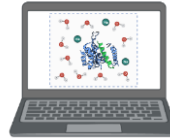


## Problem

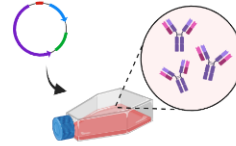


- No specific therapeutic agents
- Need simple and effective production system

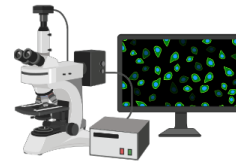
## Process



Computational analysis by Amber 22

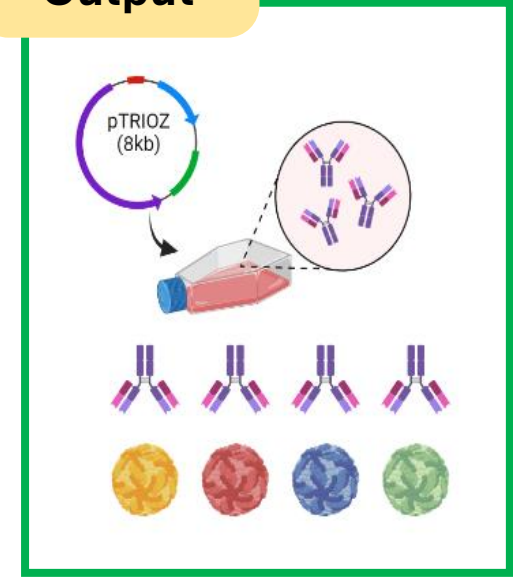


Construction and expression rAbs

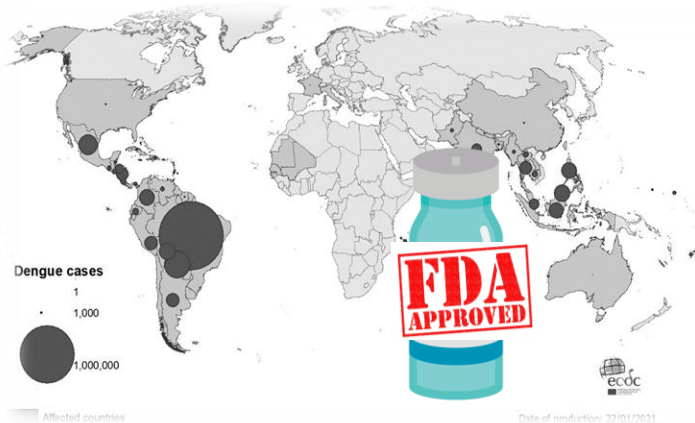


Characterization of rAbs

## Output



## Impact



## Outcome



Ready to develop as therapeutic agent



Time & Cost

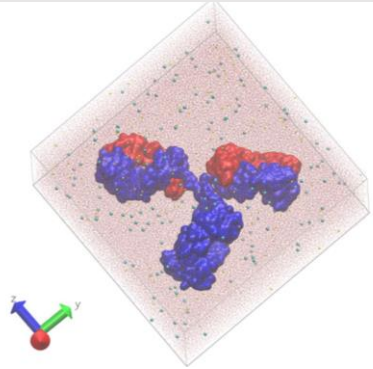


## Objective

To develop strongly neutralizing human recombinant antibody (1B3B9\_V21 rAb) against DENV-1 to DENV- 4 using pTRIOZ system.

## I. Computational analysis

MD simulations (Amber 22)



### Stabilities

- RMSD
- Atom contact
- H-bond

### Binding affinities

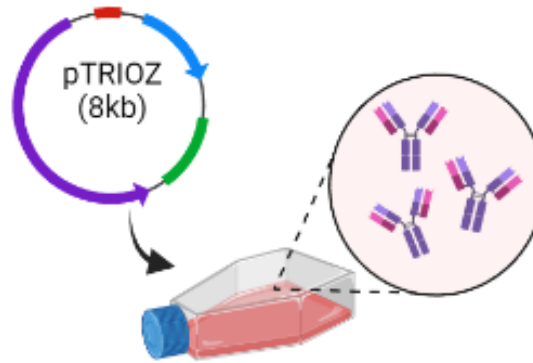
- Binding free energy

### Contact interactions

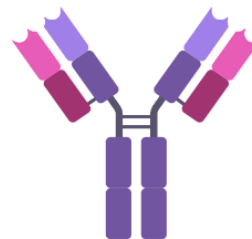
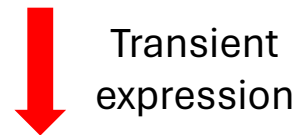
- Identification of key residues



## II. Antibody expression



HEK293T cell

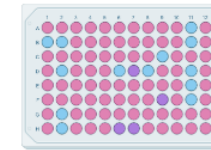


Supernatant: Full human IgG



## III. Antibody characterization

### Yields



Quantitative human IgG ELISA

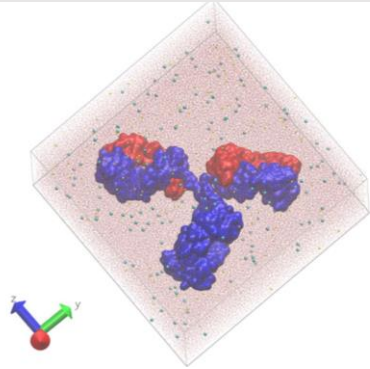
### Functions

- Cross-reactivities (IFA)
- Binding affinities by ELISA
- Neutralizing activities by FRNT
- Enhancing activities (ADE assay using K562 cell)

ELISA: enzyme link immunoassay  
IFA: immunofluorescence assay  
FRNT: Focus reduction neutralizing test  
ADE: antibody dependent enhancing

## I. Computational analysis

MD simulations (Amber 22)



### Stabilities

- RMSD
- Atom contact
- H-bond

### Binding affinities

- Binding free energy


### Contact interactions

- Identification of key residues



Antibody candidate

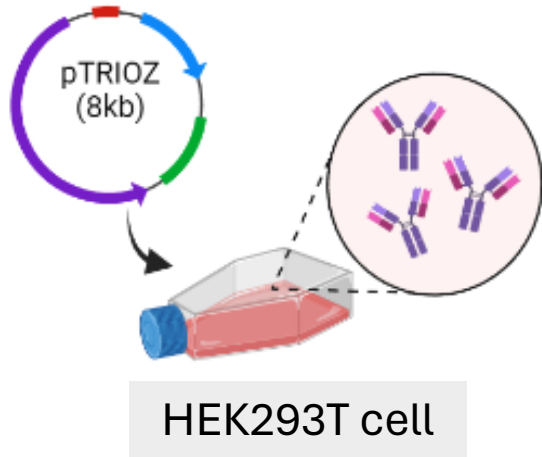
**1B3B9\_V21 Ab**

-  Binding affinity
- Stably bind with DENV 1-4
- Found key residue that impact to binding affinity

Original antibody

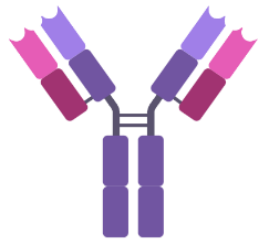
> 1B3B9 Ab

## II. Antibody expression



HEK293T cell

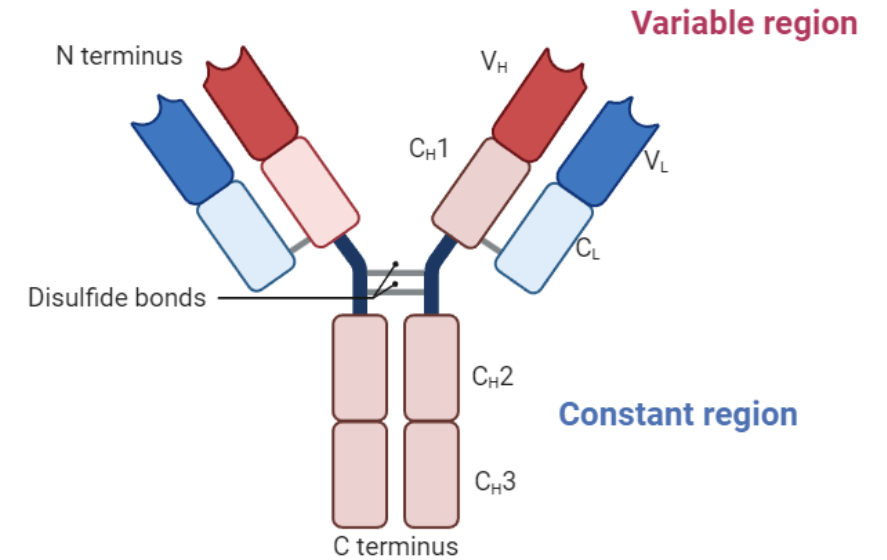
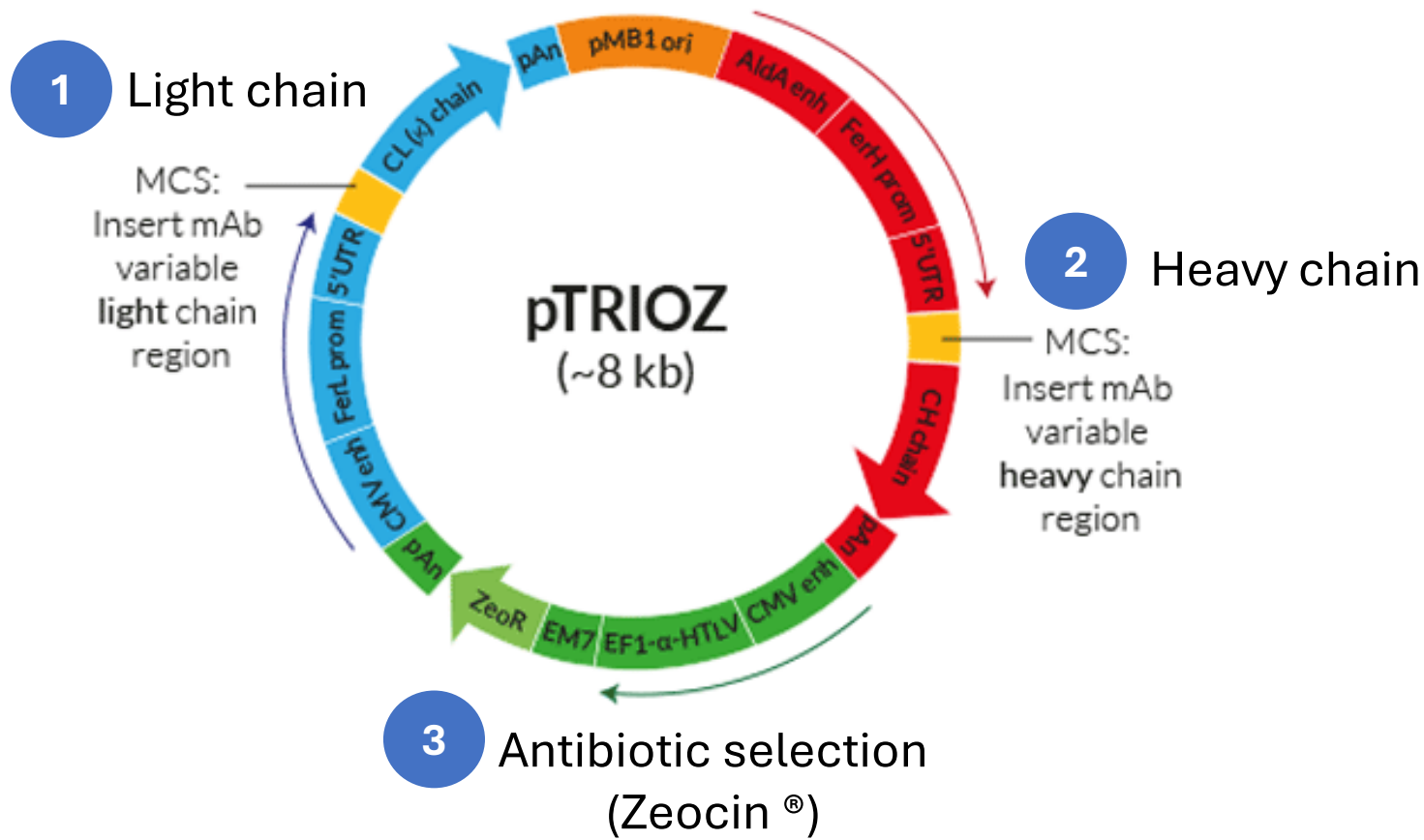
Transient expression



Supernatant: Full human IgG

### Objective

To construct antibody-expressing plasmid (pTRIOZ) and express in HEK293T cell

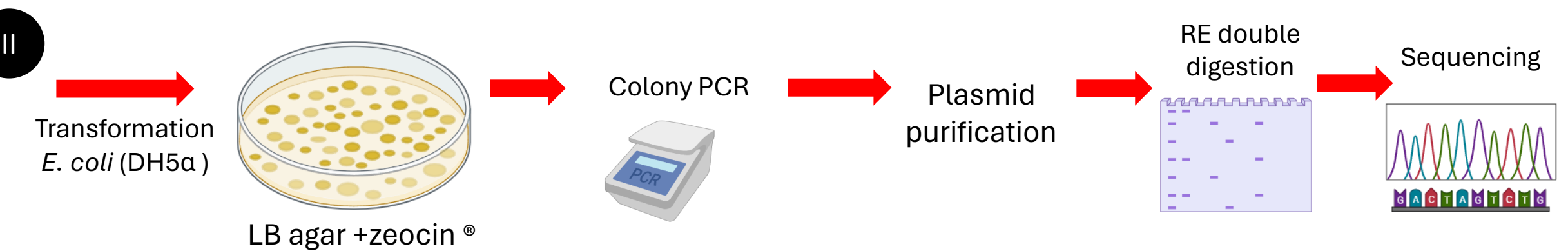
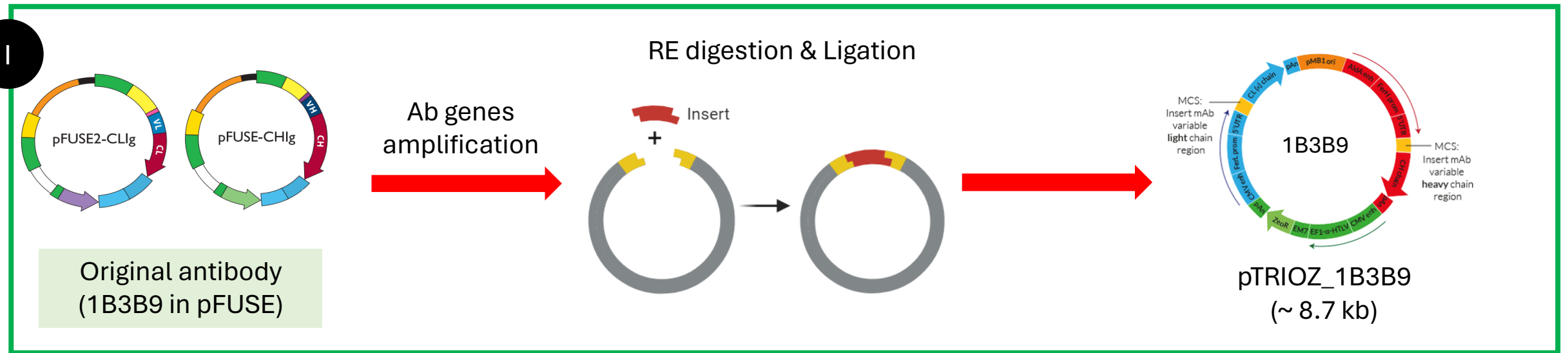


- **Full IgG** expression with **one plasmid**
- **High yield** antibody production
- **Easy to scale up**

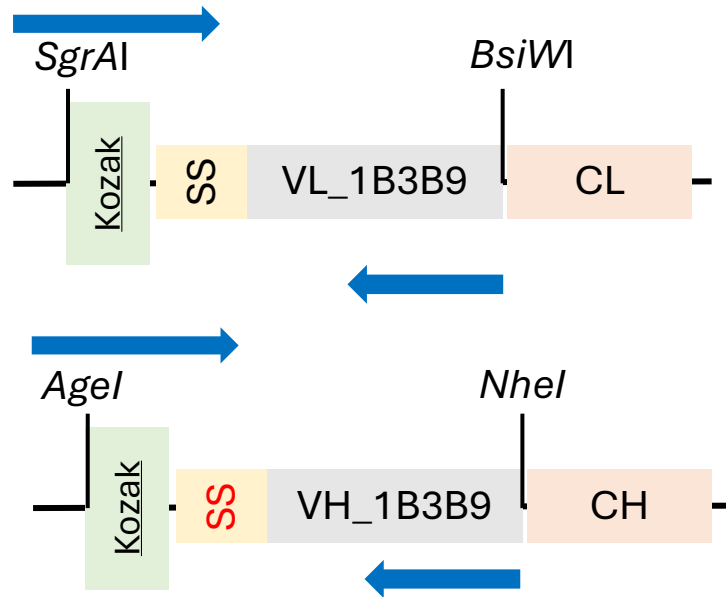


# Construction of 1B3B9 Ab in pTRIOZ plasmid

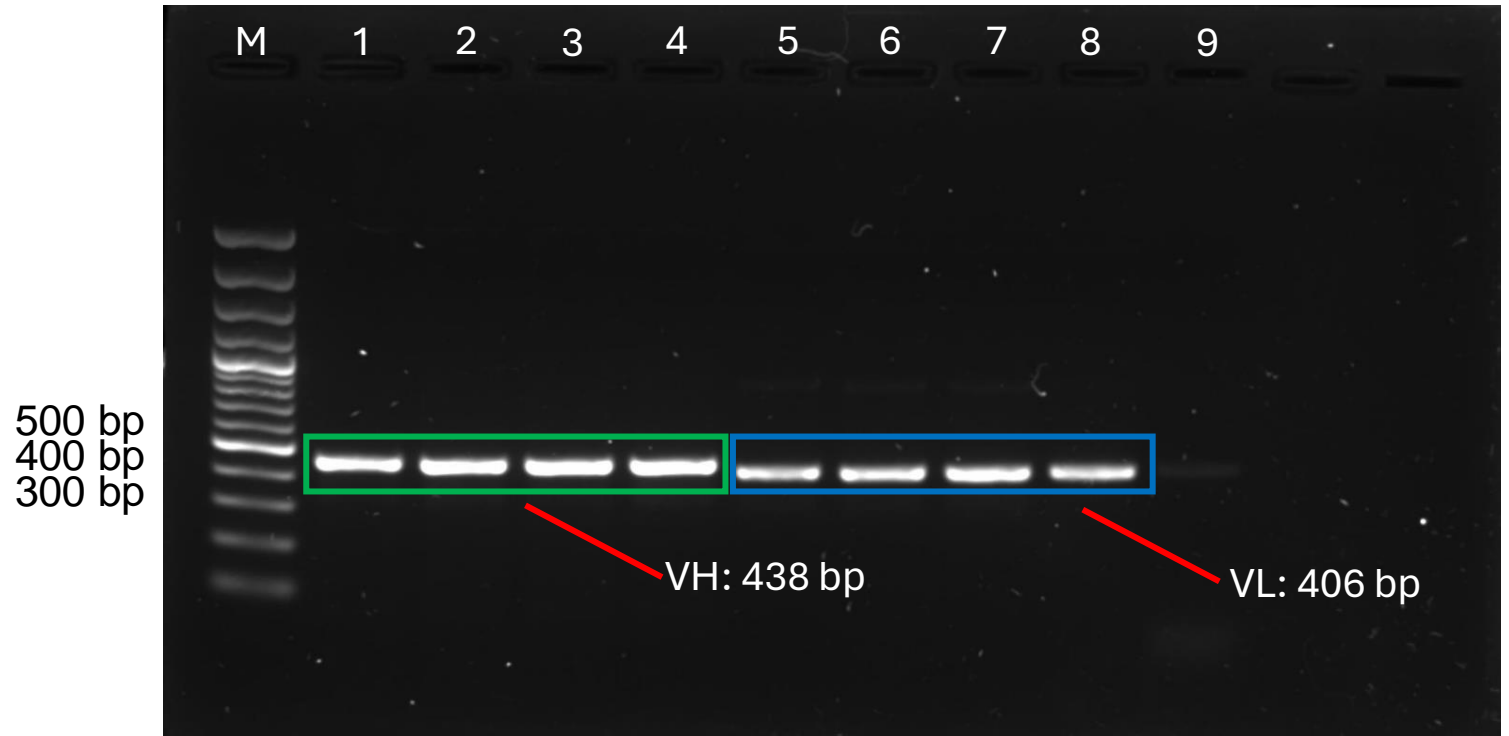
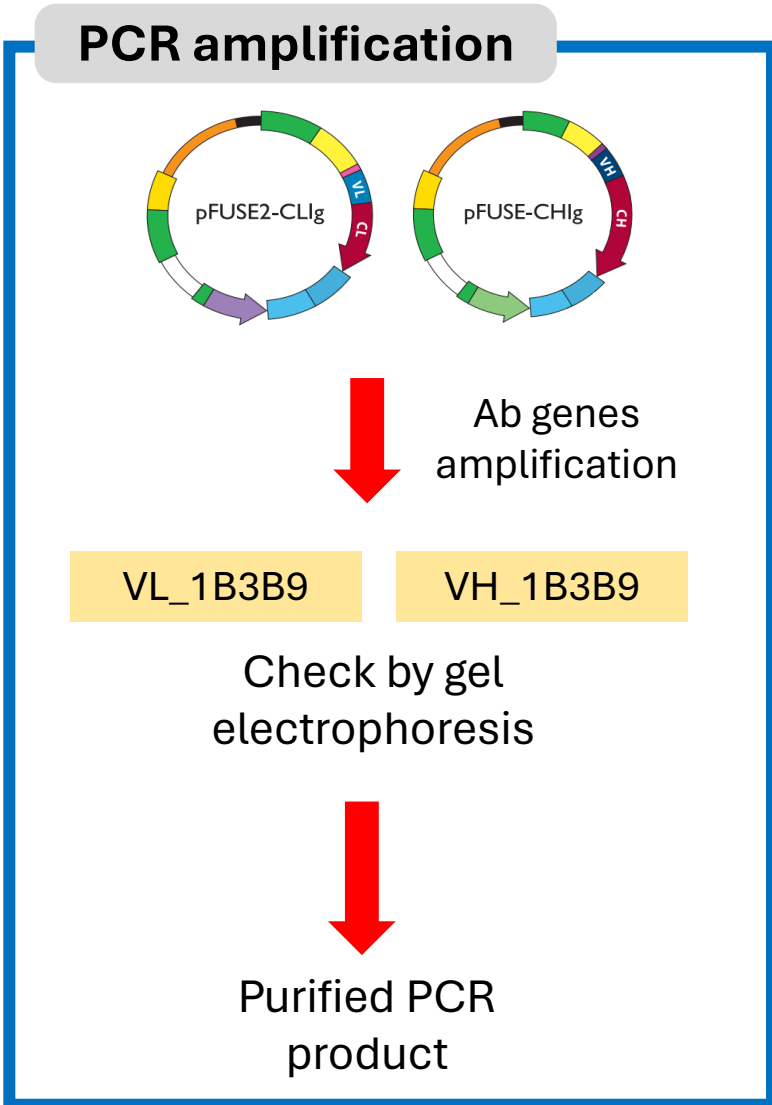
**Objective** To construct antibody-expressing plasmid (pTRIOZ) and express in HEK293T cell



- Cloning 1B3B9 : antibody template



| Primer ID  | Sequence (5' -3')                      | Tm (°C) |
|------------|--|---------|
| F_1B3B9_VL | AACACCGGCGGCCACCATGGCCTGGATTCCT<br>CTC | 61      |
| R_1B3B9_VL | CGTACGACTTAGGACGGTCAGCTTAGTCC          | 61      |
| F_1B3B9_VH | ACCGGTGCCACCATGGACTGGACC TGGAGGAT      | 60      |
| R_1B3B9_VH | AAGCTAGCTGAGGAGACGGTGACCAG             | 65      |



**Fig 1:** PCR products with corrected size of VH (438 bp) and VL (406 bp) genes.

# Result-2: Colony PCR of transformed colonies with VL\_1B3B9

## Cloning procedure

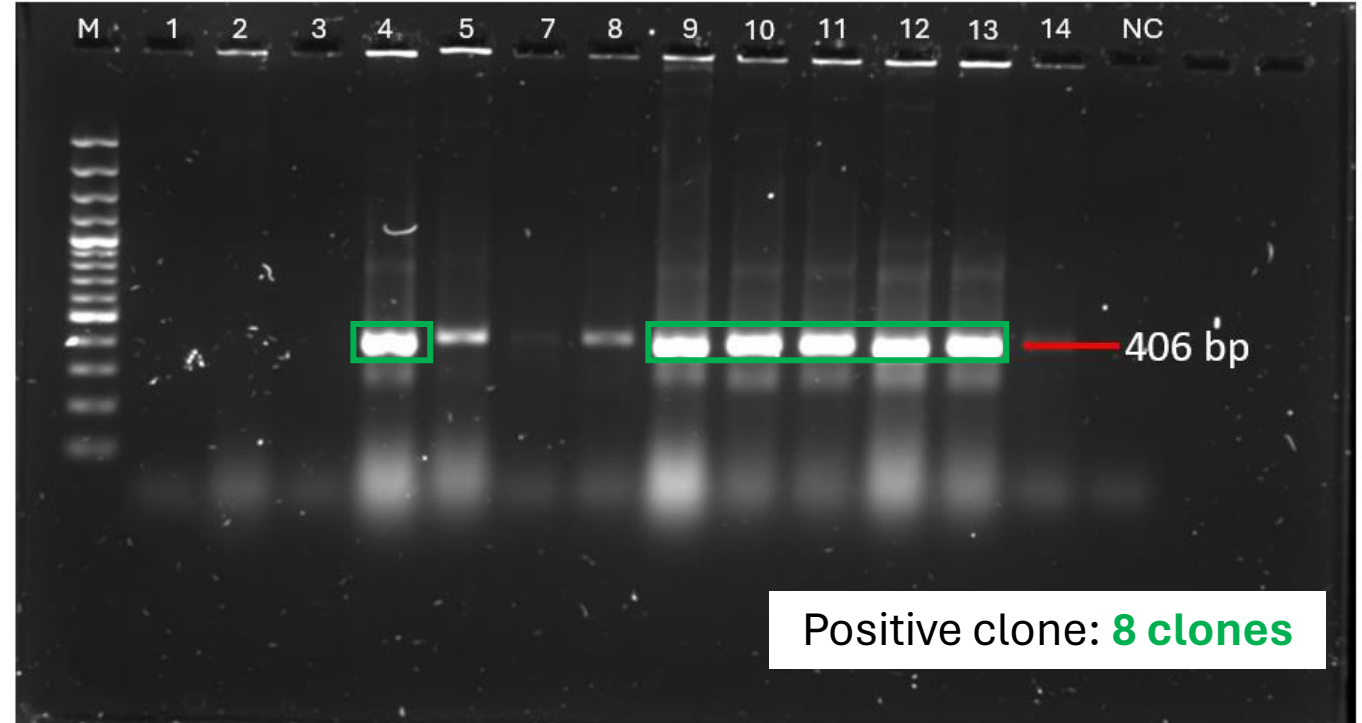
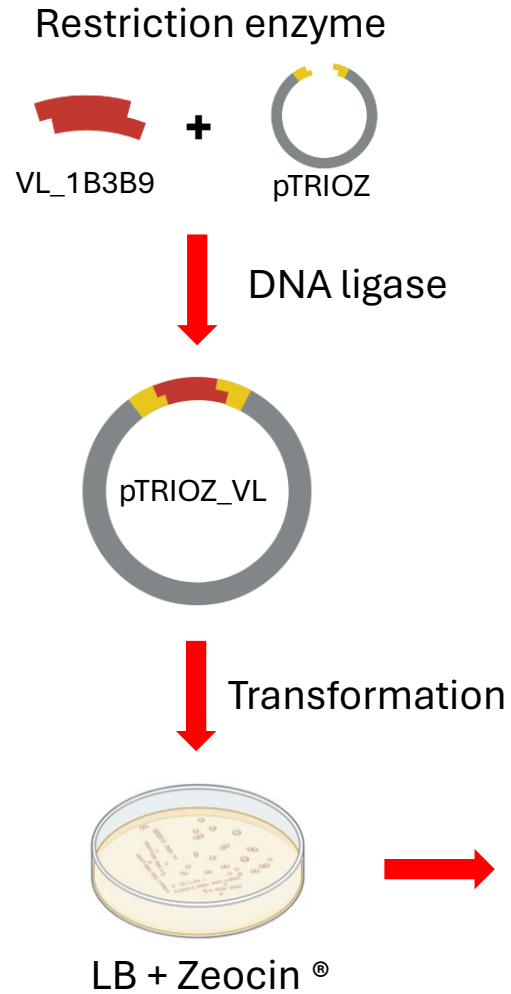
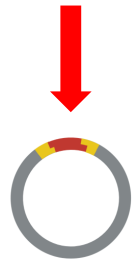


Fig 2: PCR products of colony PCR (VL\_1B3B9) with correct size

# Result-3: Restriction enzyme analysis of positive colonies (pTRIOZ\_VL)

## DNA analysis

Culture in LB broth + Zeocin®



Plasmid purification



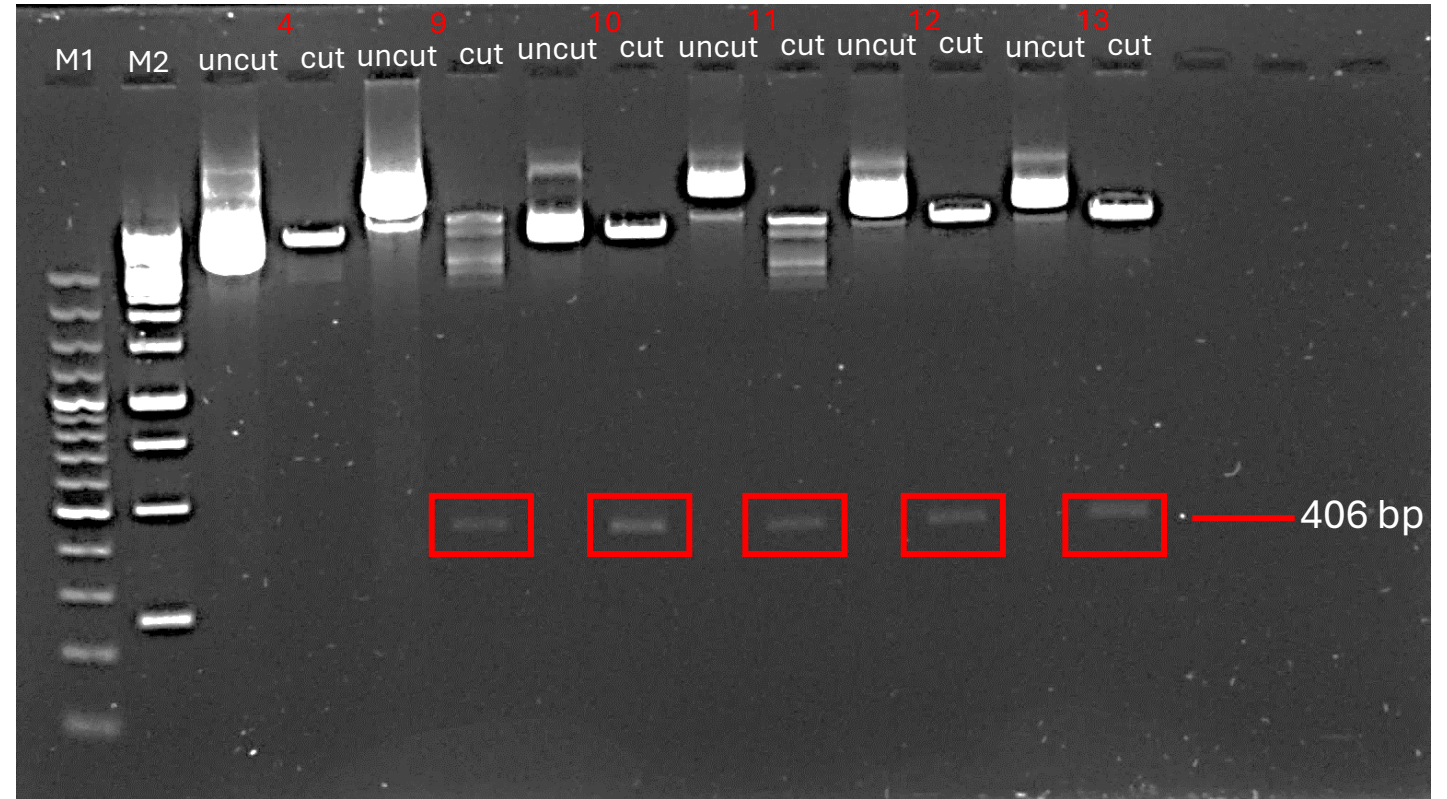
Checked by Restriction enzyme



Vector Insert



Confirmed by sequencing

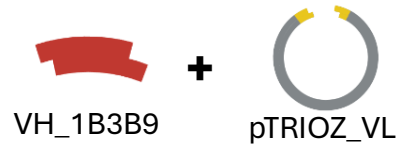


**Fig 3:** Restriction enzyme analysis. Comparing uncut and cut of positive clone from colony PCR result of pTRIOZ\_VL.

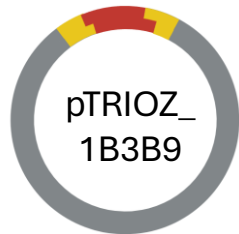
# Result-4: Colony PCR of transformed colonies with VH\_1B3B9

## Cloning procedure

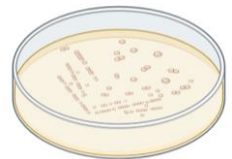
Restriction enzyme



DNA ligase



Transformation



Check by colony PCR (10 clone)

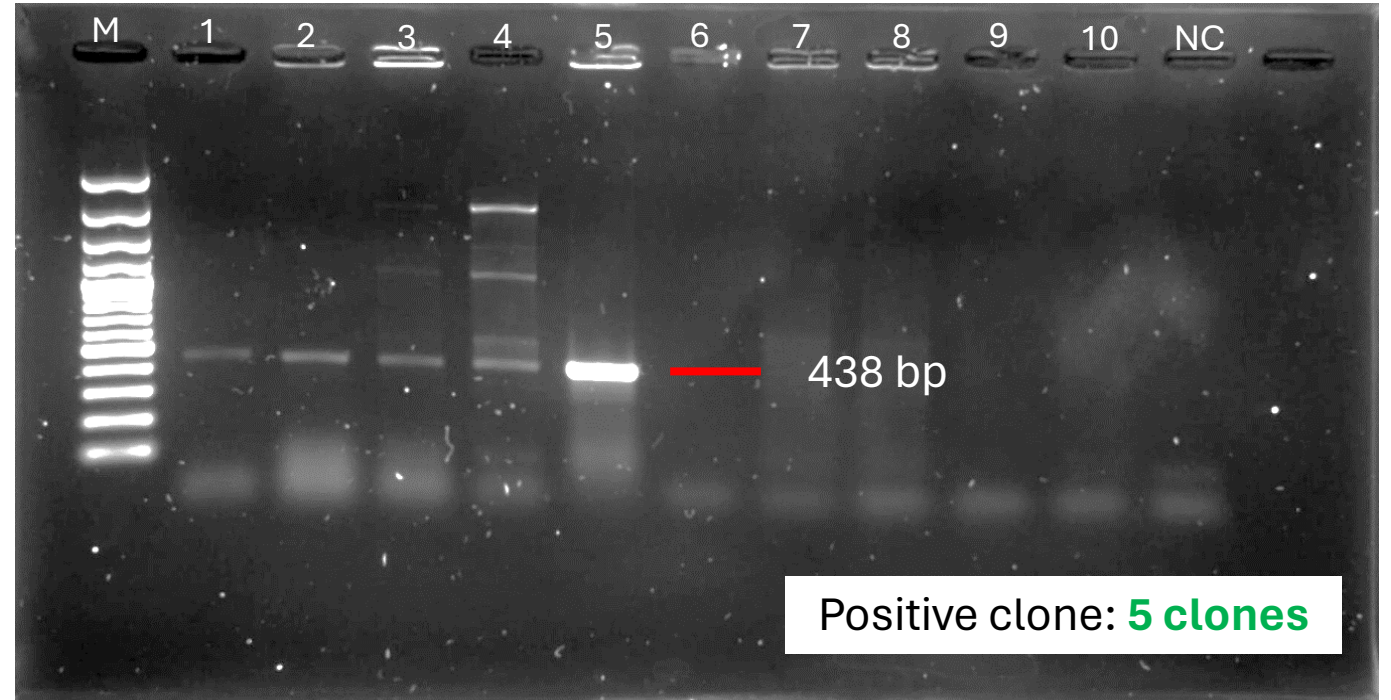
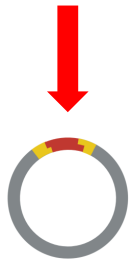


Fig 4: PCR products of colony PCR (VH\_1B3B9) with correct size

# Result-5: Restriction enzyme analysis of positive colonies (pTRIOZ\_1B3B9)

## DNA analysis

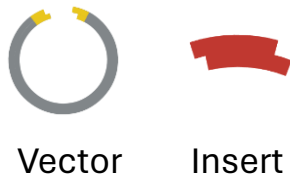
Culture in LB broth + Zeocin<sup>®</sup>



Plasmid purification



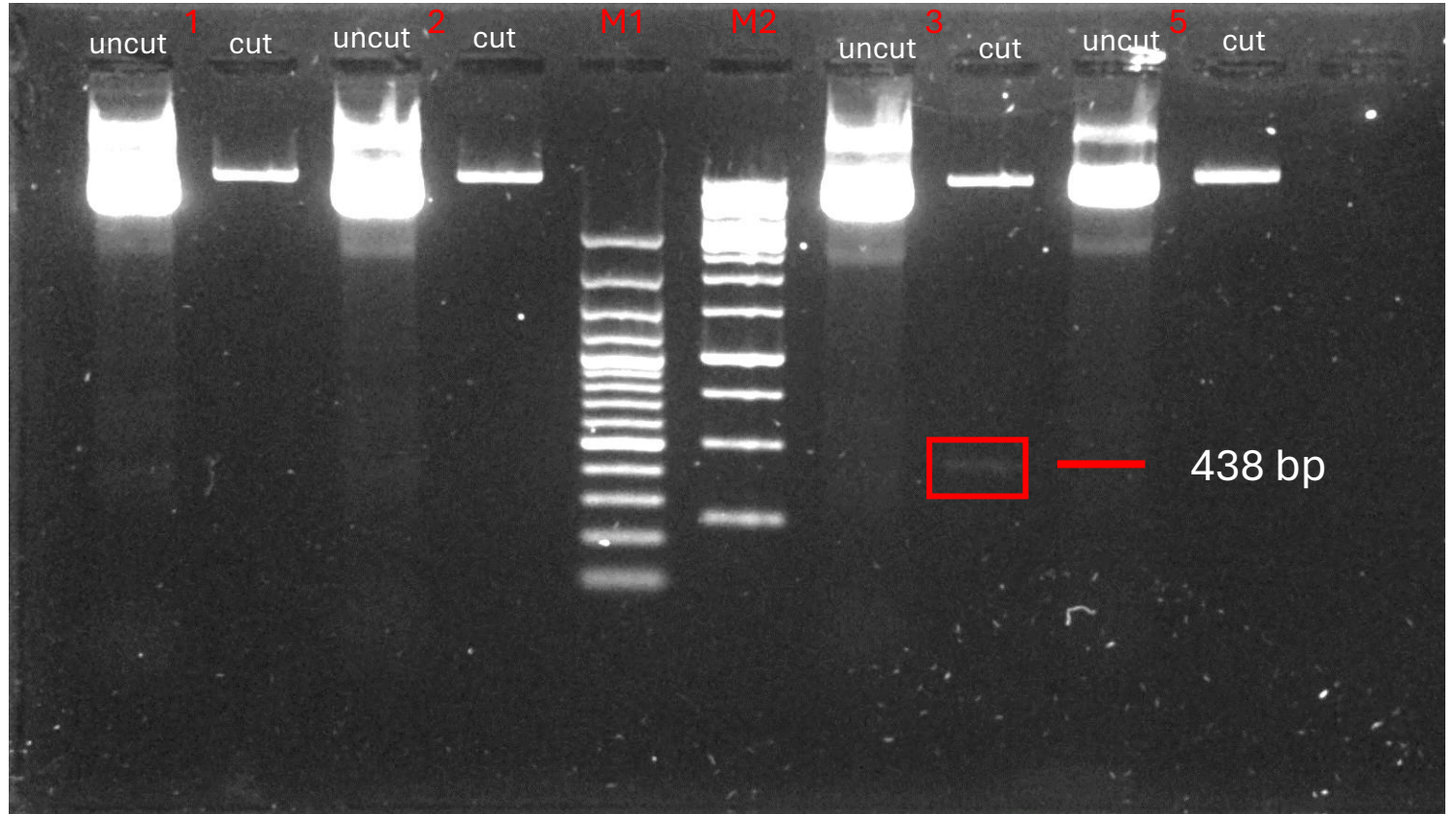
Checked by Restriction enzyme



Vector    Insert



Confirmed by sequencing



**Fig 3:** Restriction enzyme analysis. Comparing uncut and cut of positive clone from colony PCR result of pTRIOZ\_VL.

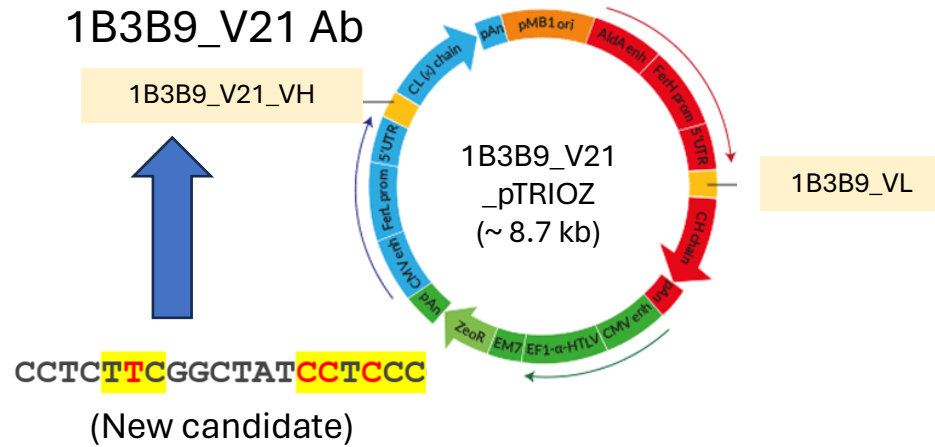
- Successfully constructed **1B3B9\_pTRIOZ expression plasmid**
- Successfully find out the **optimal conditions for cloning VL and VH genes into pTRIOZ** single plasmid (PCR amplification, double restriction enzymes digestion, ligation)
- Additionally, we **optimize the transfection parameters** for expressing rAb in mammalian HEK293T cells including cell density, transfection method, and transfection complex ratio. → plan to proceeding



## Construction of new Ab candidate (1B3B9\_V21)



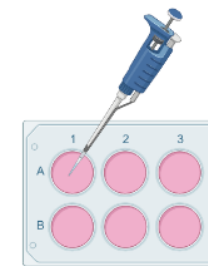
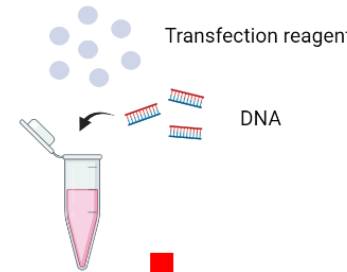
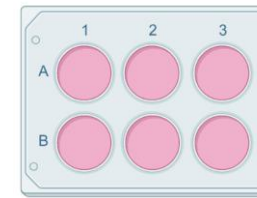
## Transient expression in HEK293T cell



Seeding HEK293T  $5.5 \times 10^5$  cell/ml



Incubate 24 h, 37 C, 5%CO<sub>2</sub>



### Optimization

- ✓ Cell density
- ✓ Transfection reagent
- ✓ Ratio of DNA: transfection reagent

Take supernatant to test

Register 8 credit

## Semester 2 /2023

### Plan

Nov    Dec    Jan    Feb    March    April    May

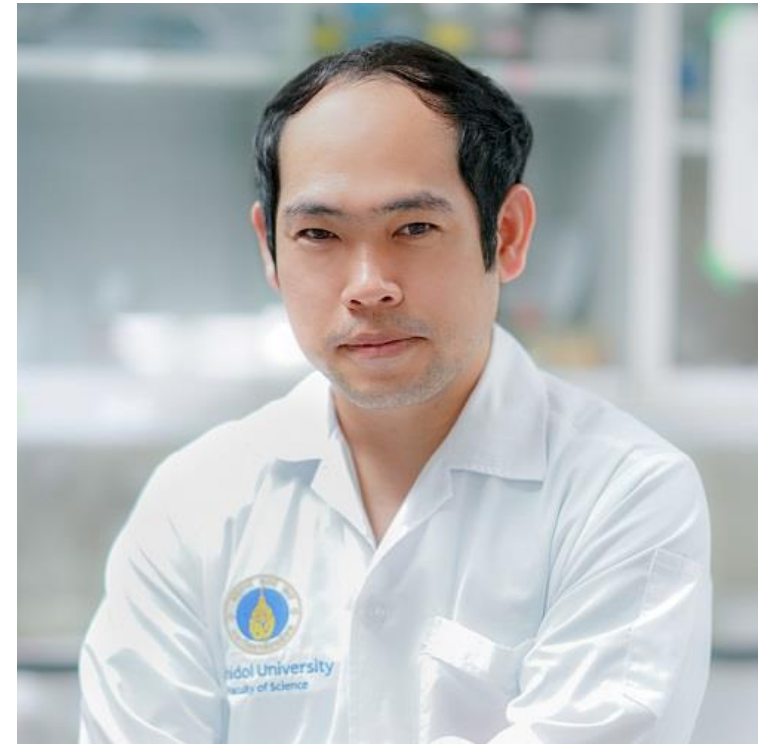
- Construction of 1B3B9 & 1B3B9\_21 rAb in pTRIOZ
- Antibody expression in HEK293T
- Characterization of rAbs
- Data analysis
- National proceeding
- Thesis examination



# Acknowledgement



**Assist .Prof. Dr. Chonlatip Pipattanaboon**  
**Advisor**



**Dr. Surachet Benjathammarak**  
**Co-advisor**



**Thank you for your kind attention .**

**Q&A**

**Suggestion**