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DEVELOPMENT OF POLYPROTEIN 3ABC AND Fab mAb FOR FMDV DIAGNOSIS AND DIFFERENTIATION BETWEEN VACCINATED AND INFECTED ANIMALS

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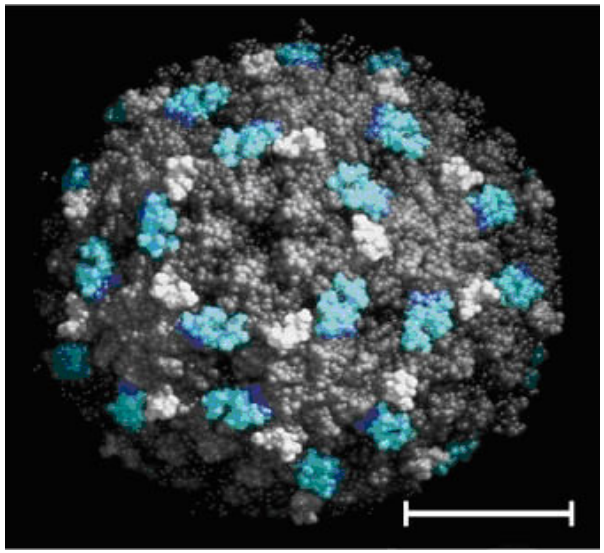
Introduction of FMDV

- Foot and mouth disease virus (FMDV)
  - The non-enveloped, plus-sense, ssRNA virus
  - Genus: *Aphthovirus*
  - Family: *Picornaviridae*



(Loeffler and Frosch, 1897)

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Introduction of FMDV

- 7 serotypes: O, A, C, Asia 1, SAT1, SAT2 and SAT3
- No cross-protection among serotypes
- 3 serotypes: O, A, and Asia1 are reported in Thailand

(Gleeson, 2002; Grubman and Baxt, 2004)

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Introduction of FMDV

- FMDV is a causative agent of foot and mouth disease (FMD)
- FMD is mainly found in cloven hoofed animals

(Loeffler and Frosch, 1898)

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Introduction of FMDV

- Clinical signs
  - Fever
  - Anorexia
  - Drooling of saliva
  - Vesicles on the membranes of mouth, tongue, feet




(Alexandersen and Mowat, 2005)

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Introduction of FMDV

- Mode of transmission
  - Respiratory route
  - Eating FMDV-contaminated food
  - Direct contact with infected animal

(Hyde et al., 1975)

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Introduction of FMDV

- The FMD causes severe economic problems
  - Reduction in the growth rate of meat animals
  - The loss of export markets through embargo of trade partner
  - The many direct and indirect costs of eradication policy

(Doel, 2003)

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Introduction of FMDV

- Disease control
  - Vaccination
  - Slaughtering of affected animals

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Introduction of FMDV

- An animal that is infected shortly after being vaccinated can be a carrier and spreads FMDV

- Differentiation between infected and vaccinated animals (DIVA) are essential to evaluate the effectiveness of control and eradication campaigns

(Sorensen et al., 1998; Lee et al., 2006)

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Introduction of FMDV

- The NSPs
  - mostly use in DIVA test
  - not serotype specific

(Rodriguez et al., 1994; Mackay et al., 1998)

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Introduction of FMDV

- The NSP 3ABC of FMDV
  - Highly immunogenicity
  - Anti-3ABC can be early detected (8-10 dpi) and detected for longer (560-742 dpi) than antibody to other NSPs

(Rodriguez et al., 1994; Silberstein et al., 1997; Sorenson et al., 1998; Malirat et al., 1998; Lu et al., 2007)

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Introduction of FMDV

- FMDV DIVA tests for detecting antigen as well as antibody rely on pAb or mAb ELISA reagents

- Recombinant Ab production using phage display technique has been popular used to produce mAbs

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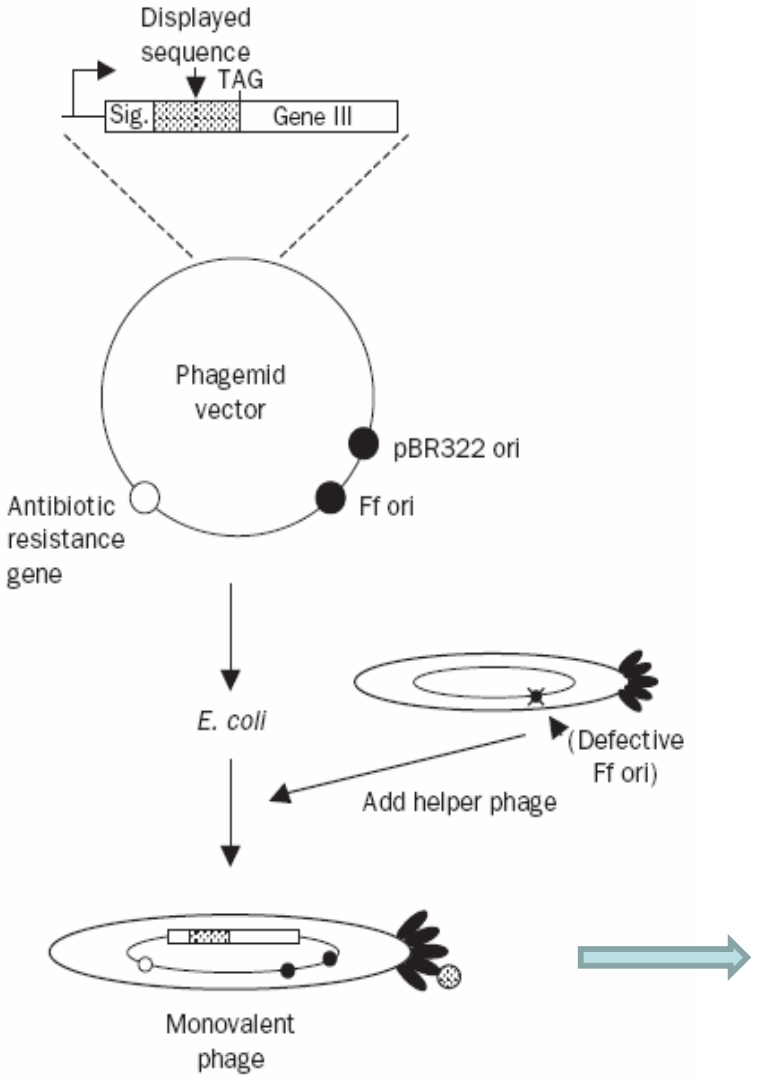
Antibody phage display technology



Select the phage libraries specific to the target protein

Clackson and Wells, 1994

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Antibody phage display technology

- Advantages of recombinant Ab
  - *In vitro* processing
  - No require the maintenance of hybridoma cells
  - Economical large scale production

Willats, 2002

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Objectives

- To produce the polyprotein 3ABC of FMDV in *E. coli* BL21
- To construct Fab mAb specific to polyprotein 3ABC by phage display technology

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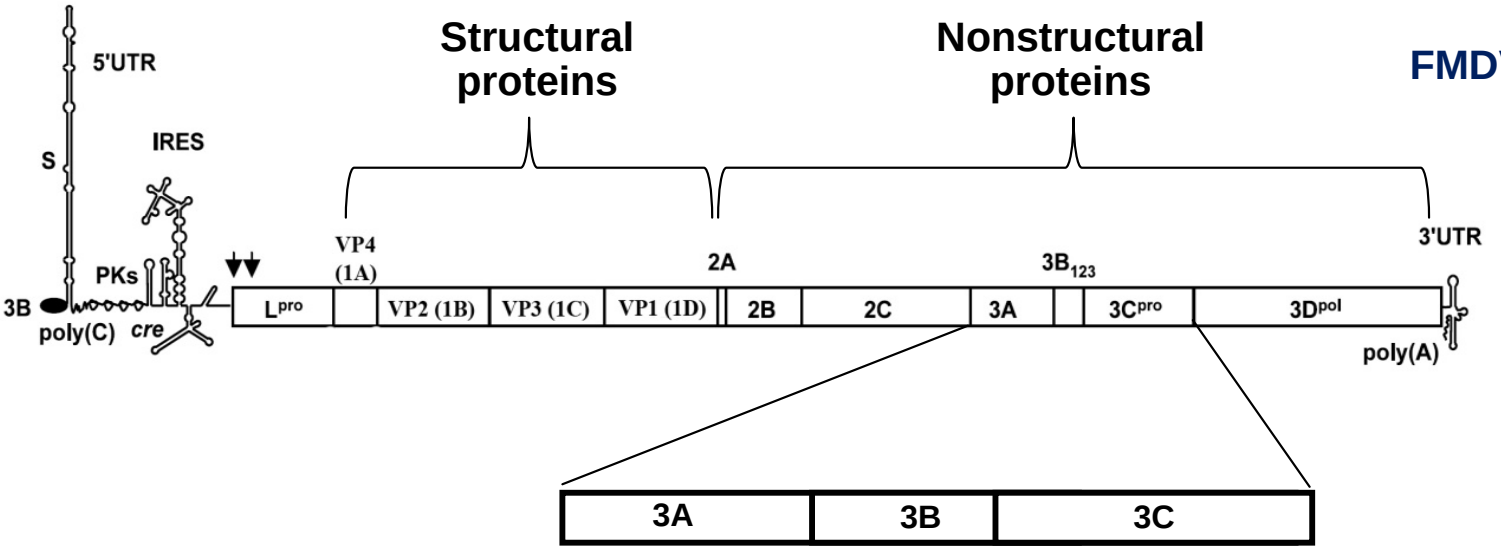
Expression of native polyprotein 3ABC



Clone into expression plasmid

Express the 3ABC in *E.coli* BL21

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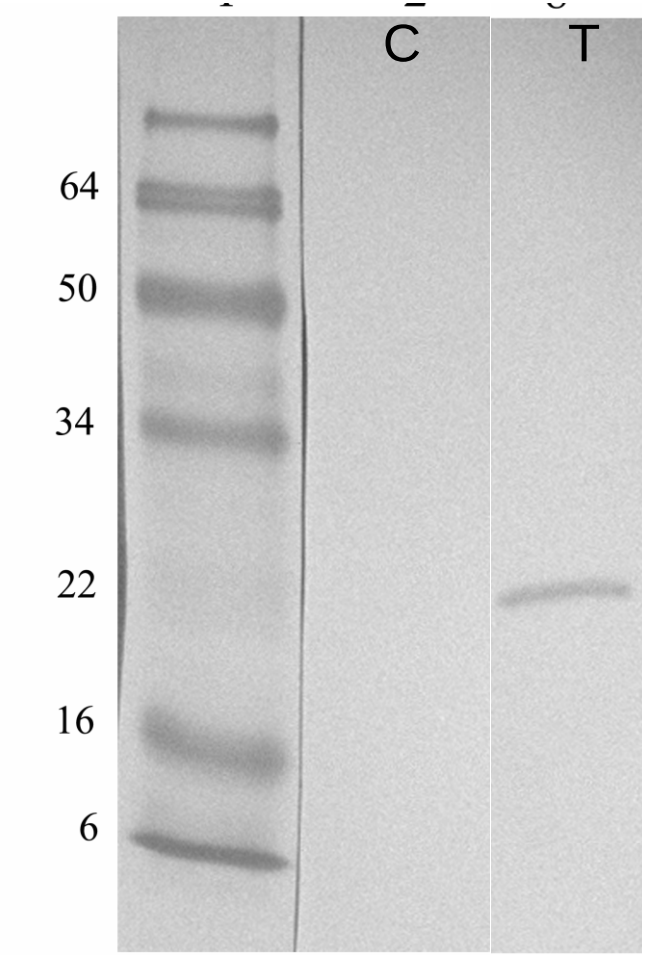
Expression of native polyprotein 3ABC



- The poly-protein 3ABC may be cleaved after expression by protease activity of 3C protein

22 kDa ⇦ expected size for 3A protein

The Western blot analysis of poly-protein 3ABC expression detecting with anti-6xHis antibody

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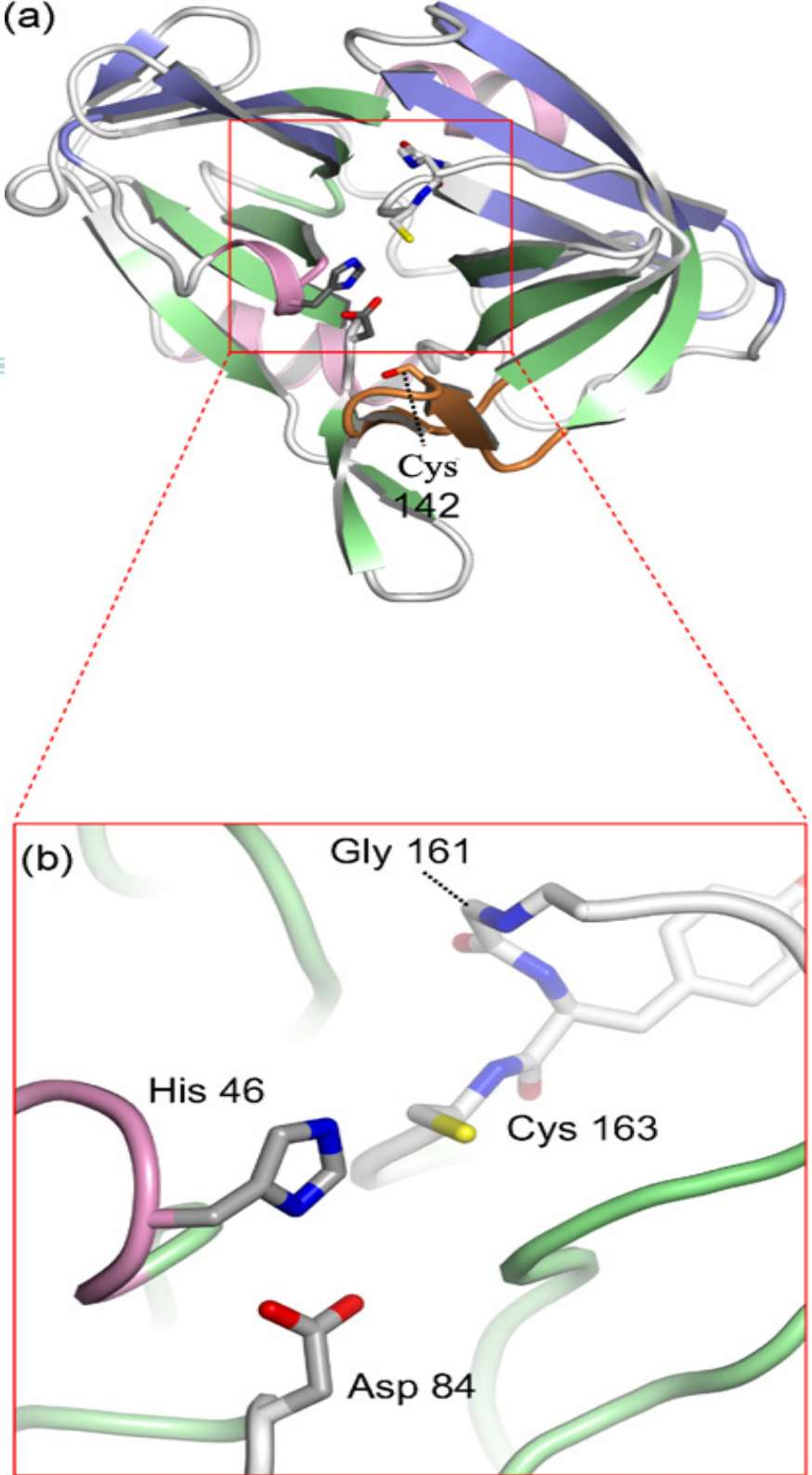
3C^{pro}

- chymotrypsin-like cystein protease enzyme
- has a Cys_{163}- His_{46}-Asp/Glu_{84} catalytic triad in the active site



(Grubman et al., 1995; Capozzo et al., 2002 ; Birtley et al., 2005; Curry et al., 2007)

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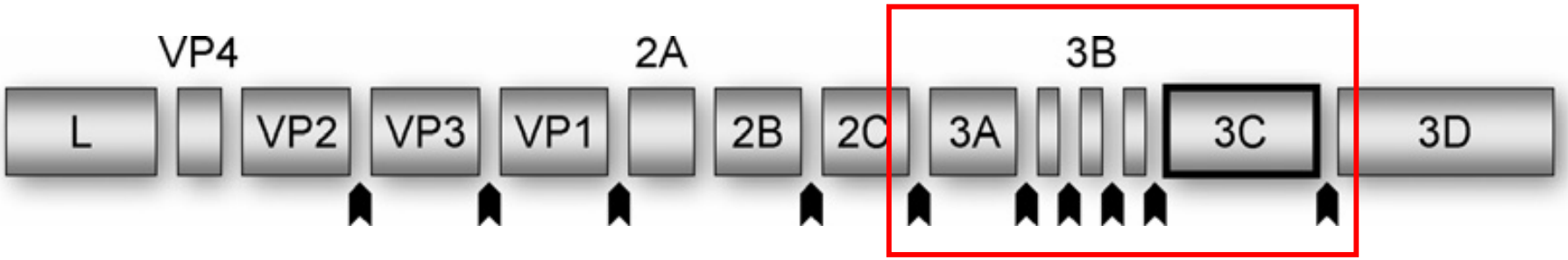
3C^{pro}

Polyprotein of FMDV



(Curry et al., 2007)

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Expression of mutated polyprotein 3ABC

- The 3ABC gene was mutated by PCR site-directed mutagenesis at 3C portions
  - Cys (TGC)-163 to Gly (GGC)
  - Cys (TGC)-142 to Ser (AGC)

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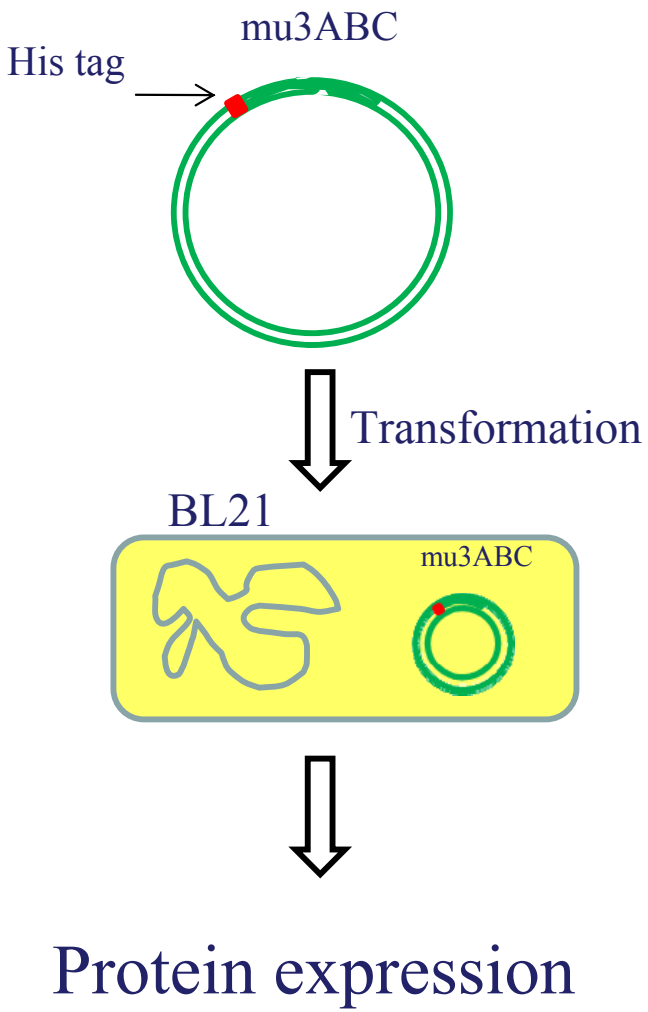
Expression of mutated polyprotein 3ABC



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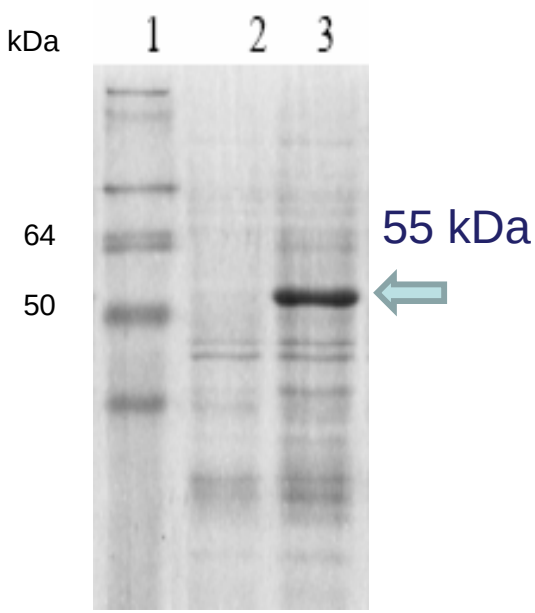
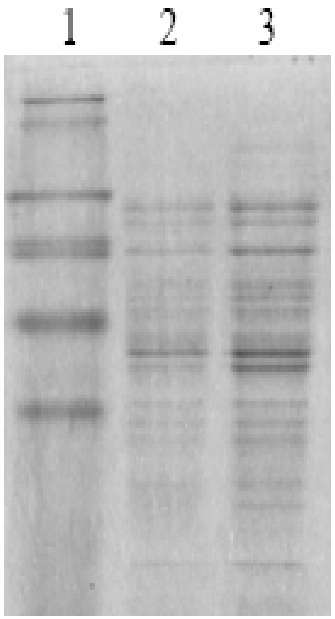
Results

Insoluble part



Soluble part



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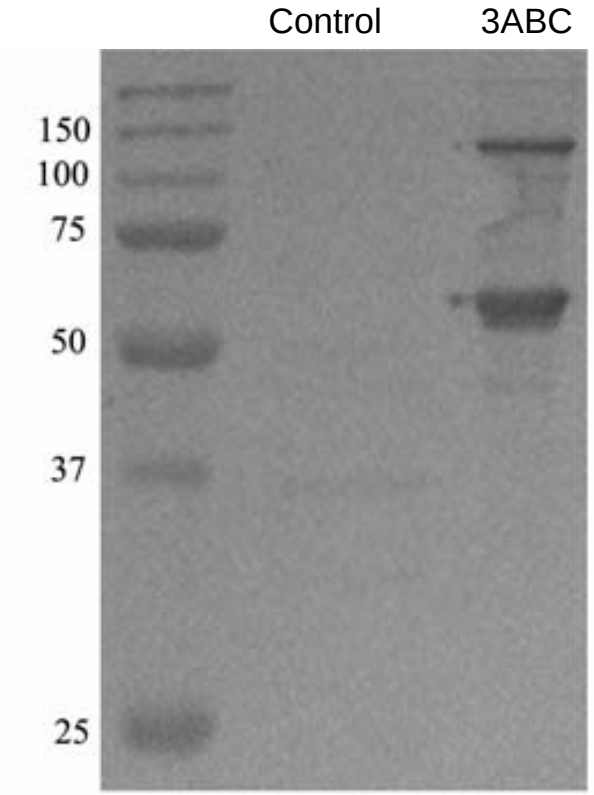
Results



Western blotting using anti-3ABC antibody from FMDV infected cattle

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Objectives

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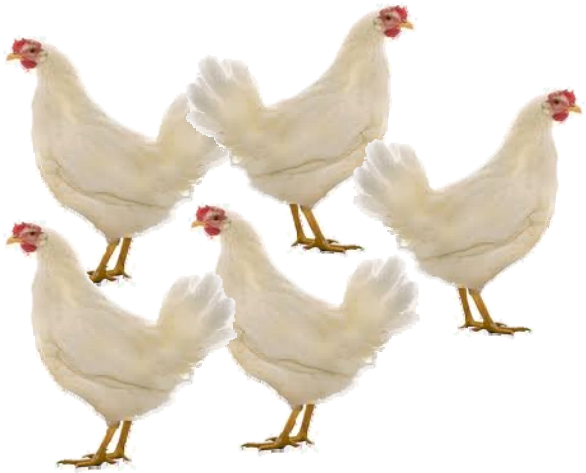
Construction of chimeric chicken/ Human Fab fragment



4 week-year-old white leghorn chickens (SPF)

Immunized with 250 µg of mu3ABC protein that mixed (1:1) with α-tocopherol (vitamin E) based adjuvant at intramuscular (at 3-4 points)

Euthanized and collected the spleens for RNA extraction

Synthesized cDNA

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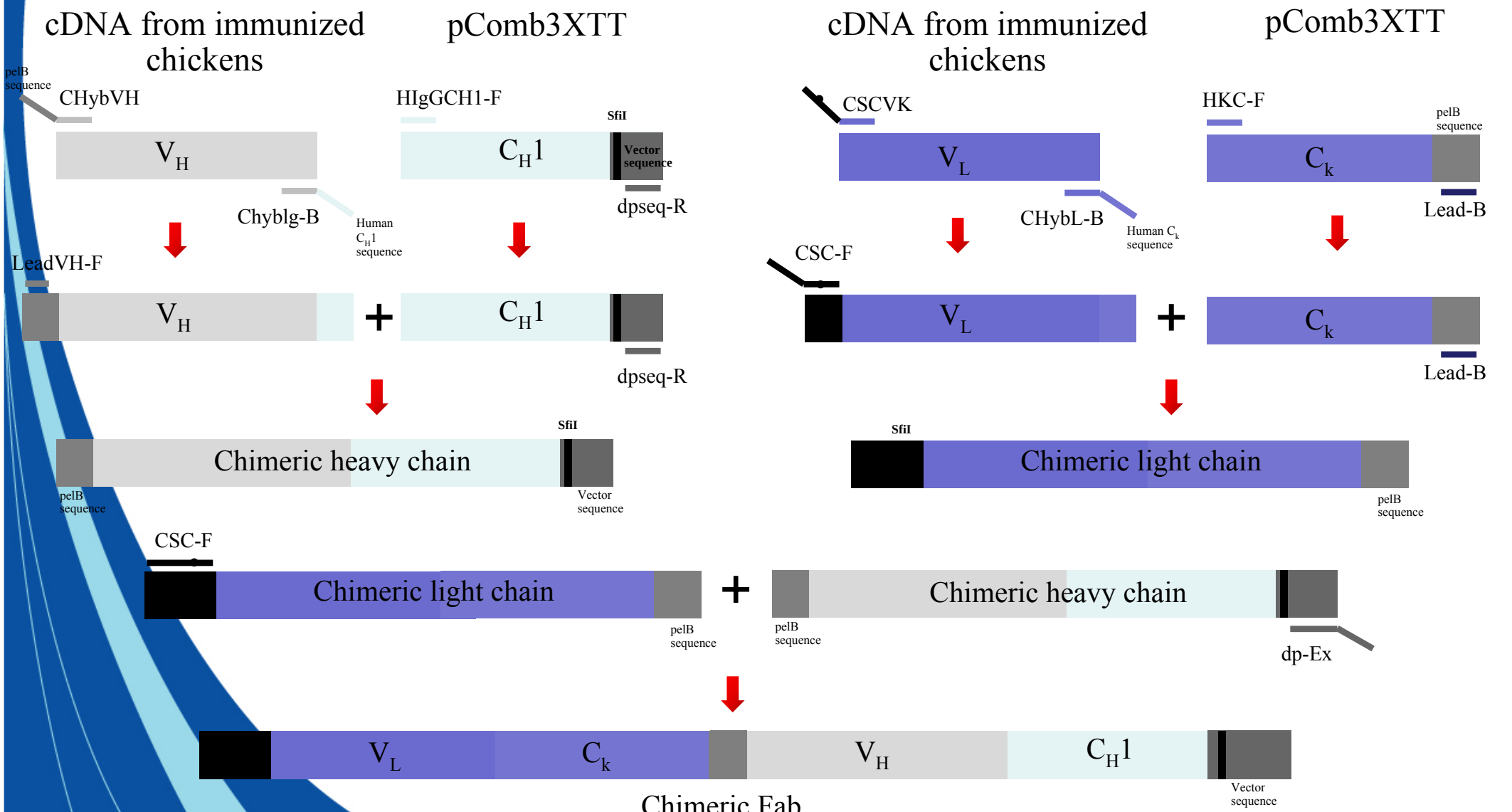
Construction of chimeric chicken/ Human Fab fragment



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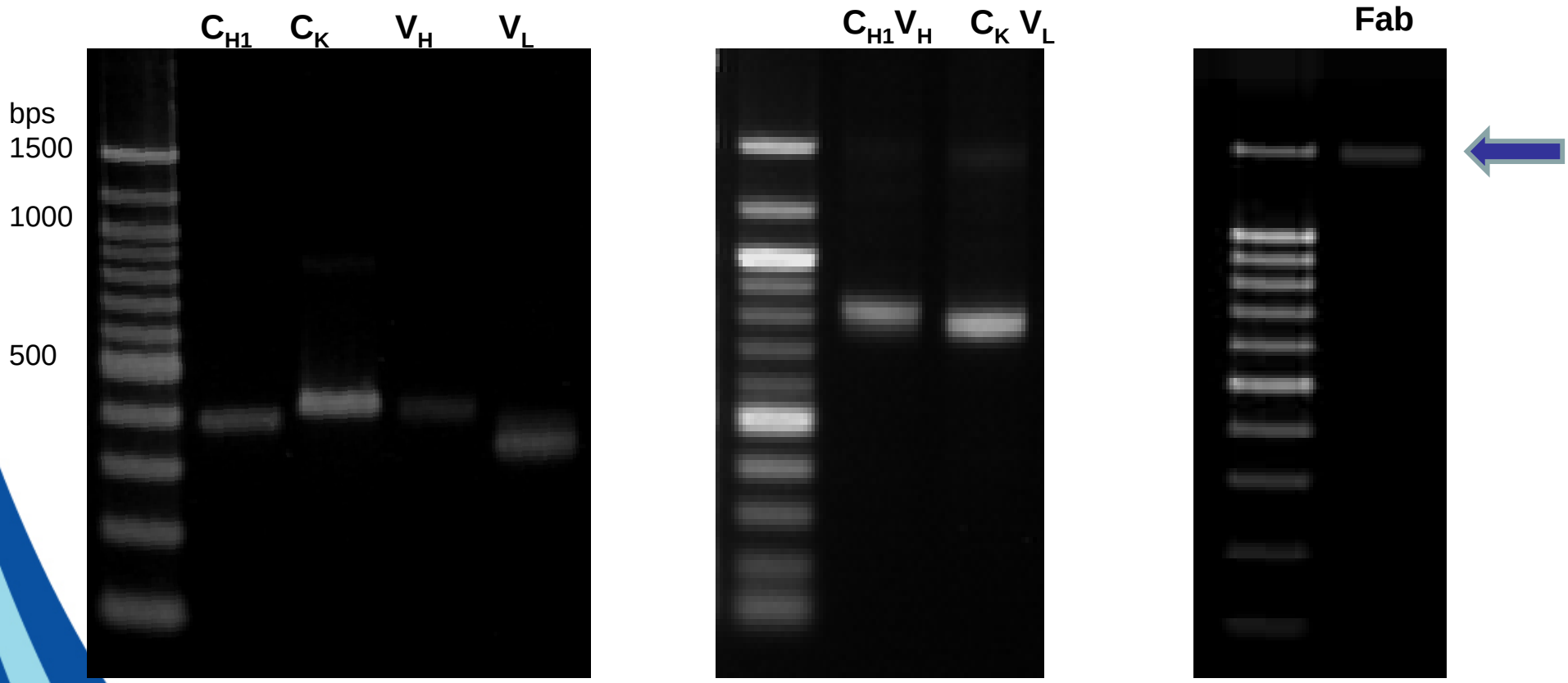
Results



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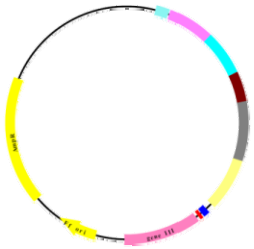
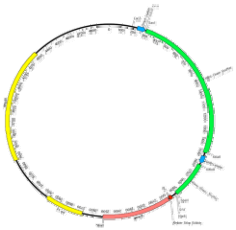
Construction of phage libraries



Chimeric Fab

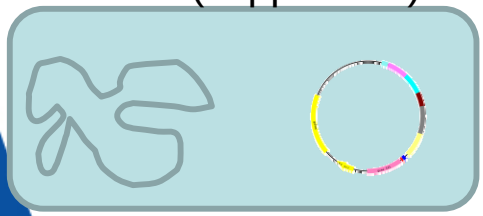
+

pComb3XSS



Electroporate at 2.5 kV, 25 µF, and 200 Ω

ER2738 (suppressor)



5 ml SOC medium

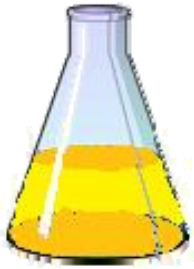
37 ºC, 250 rpm, 1 hr.

10 ml SB, Tet (final conc. 10 µg/ml)

37 ºC, 250 rpm, 1 hr.

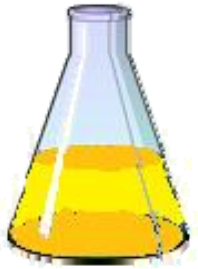
2 ml of VCSM13 helper phage (10^{12}-10^{13} pfu/ml)

183 ml SB, Carb (final conc. 46.25 µg/ml)



37 ºC, 250 rpm, 1.5-2 hr.

Kan (final conc. 70 µg/ml)



37 ºC, 250 rpm, o/n

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Construction of phage libraries

Collect supernatant by centrifuge at 3,000 xg for 15 min at 4ºC

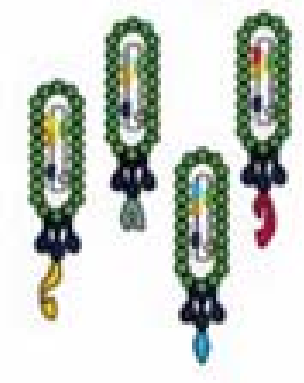
4% (w/v) PEG, 3% (w/v) of NaCl

Incubate on ice for 30 min

Centrifuge at 15,000 xg for 15 min at 4ºC

Resuspend the phage pellet in 2 ml of 1% (w/v) BSA in TBS

Centrifuge at 13,000 xg for 5 min at 4ºC and pass the supernatant through to 0.2-µm filter



freshly phage libraries can be used for panning step

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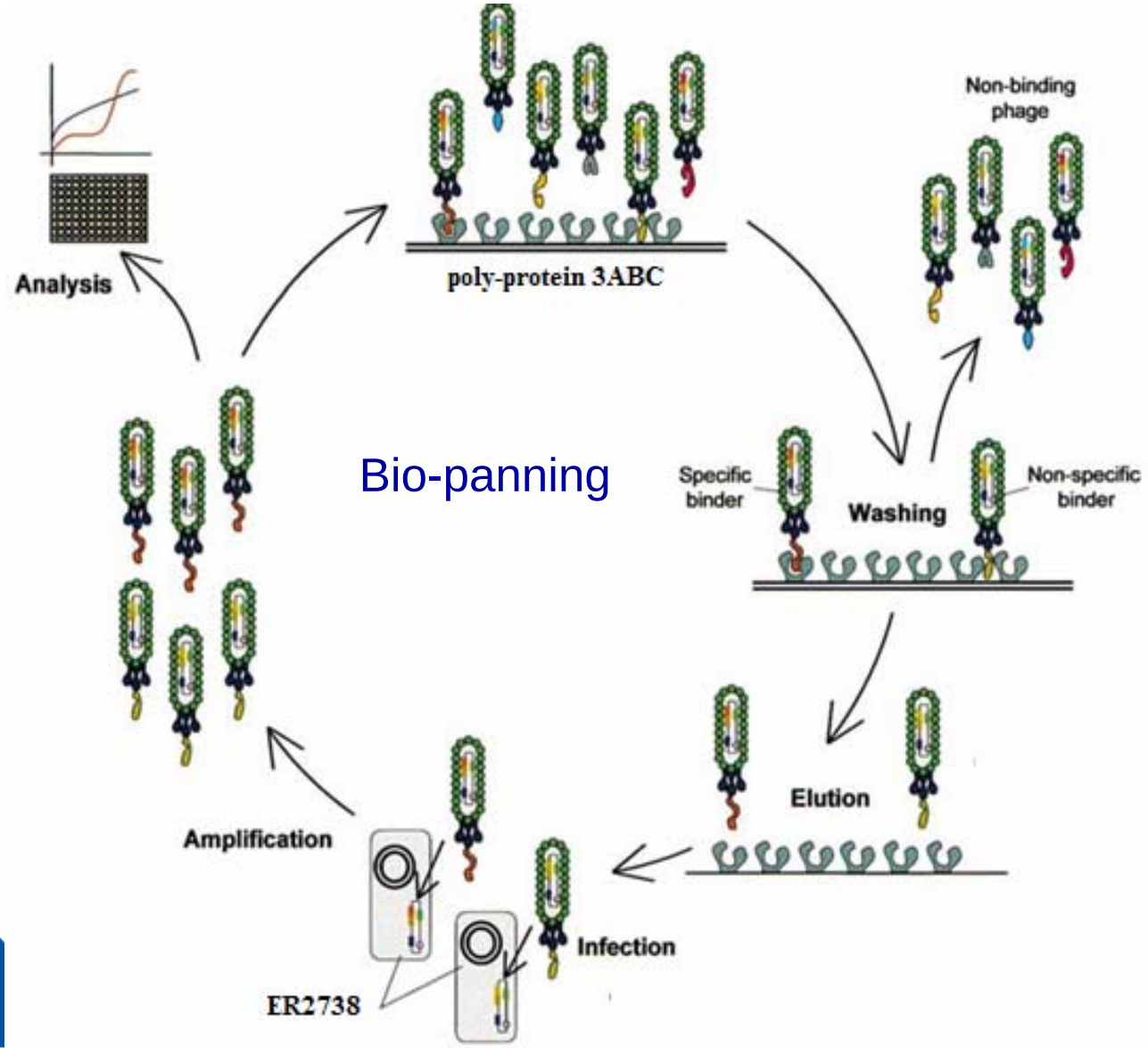
Selection of phage libraries



Willats, 2002

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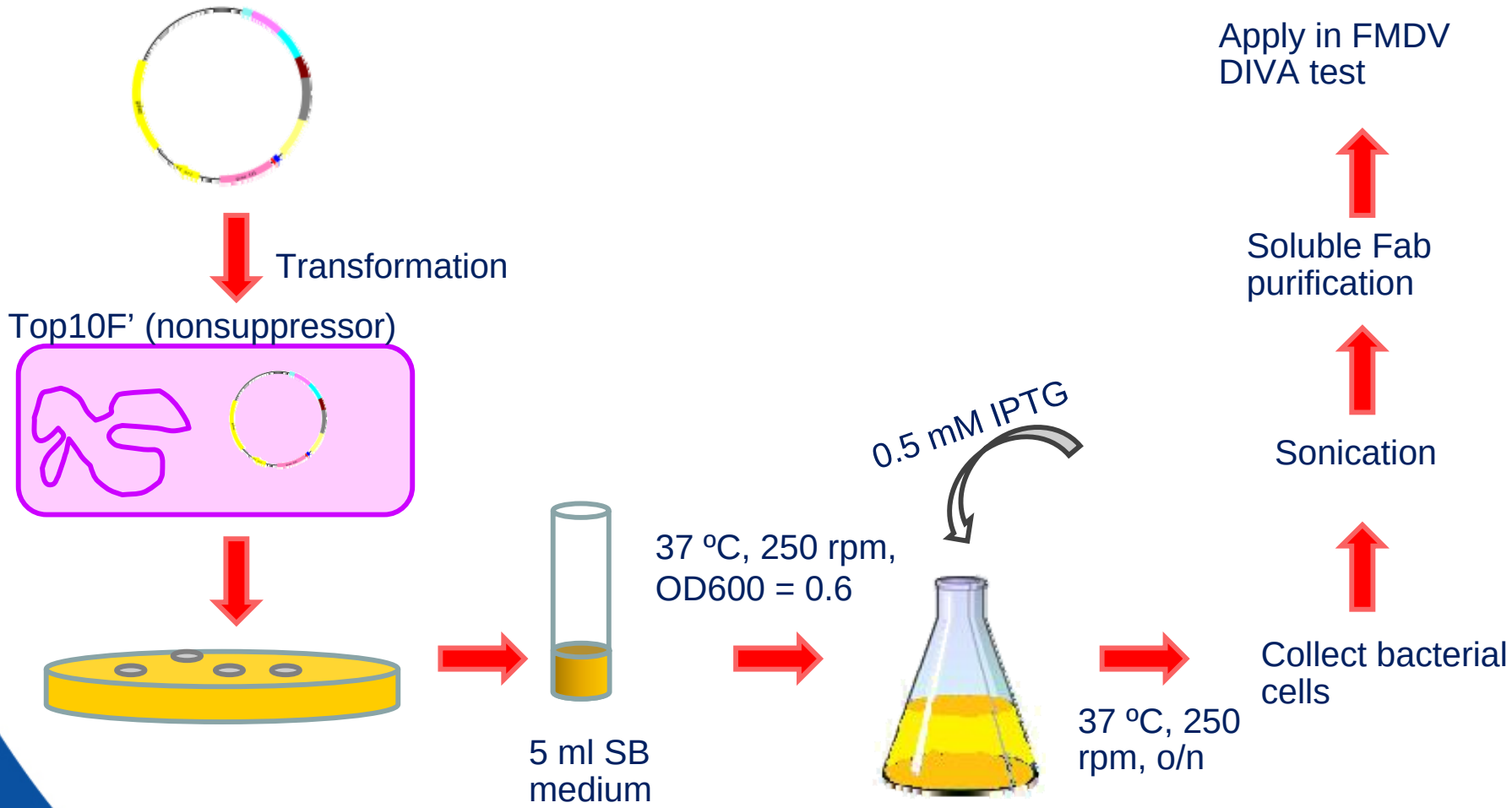
Production of soluble Fab



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Conclusions

- In this study, the recombinant mu3ABC was expressed without degradation
- The recombinant 3ABC protein can be reacted strongly and specifically with anti-FMDV from infected cattle

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Conclusions

- Fab fragment could be amplified from spleens of immunized chickens and will be further constructed Fab mAbs

- The NSP 3ABC of FMDV and the Fab mAbs may be further applied as diagnostic reagents for distinguishing between FMDV infected and vaccinated animals

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Acknowledgement

- The Royal Golden Jubilee Ph.D program (RGJ), Thailand

- National Research Council of Thailand (NRCT)

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Thank you for your attention