Construction and Selection of Fab antibody library specific to hemagglutinin antigen of H5N1 Avian Influenza Virus

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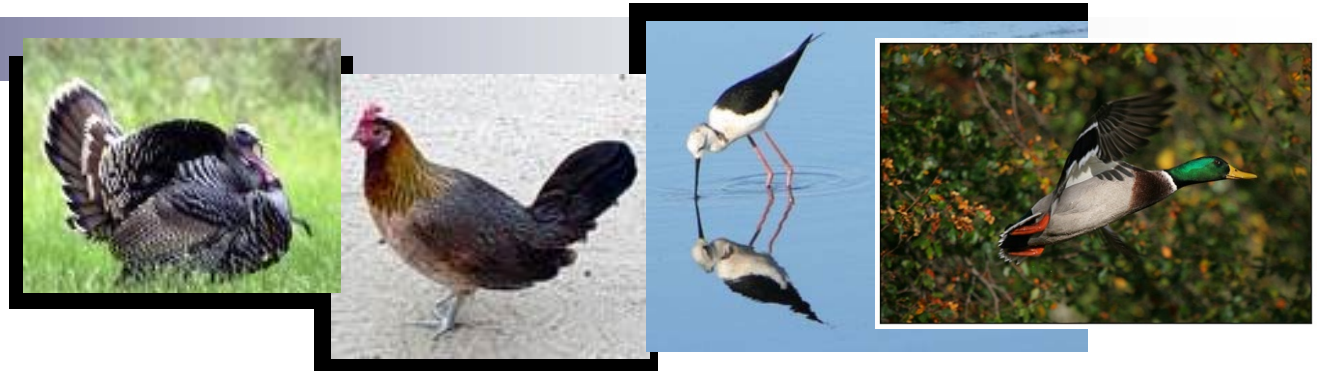
Topics

- Rationale and Objective
- Materials & Methods
- Results
- Conclusion and further study

Rationale



- HPAI H5N1 avian influenza virus cause adverse impact on Economic and Public health.
- Most infected human cases have history of close contact with infected chicken
- Rapid detection of H5N1 avian influenza virus in both human and chickens is needed
- Diagnostic test development for H5N1 avian influenza virus is required, and set as high priority national policy
- Monoclonal antibody (Mab) is major reagent for making Diagnostic test

Objective

1. Construction of Fab antibody library specific to H5N1 Avian Influenza virus
2. Selection of Fab antibody specific to Hemagglutinin antigen
3. Production of monoclonal antibody specific to Hemagglutinin (H5)
4. Further develop as diagnostic test for H5N1

Materials & Methods

Immunized with dead H5N1 (treated with beta-propiolactone) and serum titer checked (titer at 1:64)

RNA isolation

cDNA synthesis

Fab amplification

Library construction

Selection

Fab amplification

RNA

cDNA

VH

V λ

pComb3XTT

Cκ

CH1

Sense Extension Primer

V λ

Cκ

pelB Sequence

Lead-B
Reverse Primer

Lead VH

VH

CH1

dpseq
Reverse Primer

Sense Extension Primer

Chimeric Light Chain

Fd

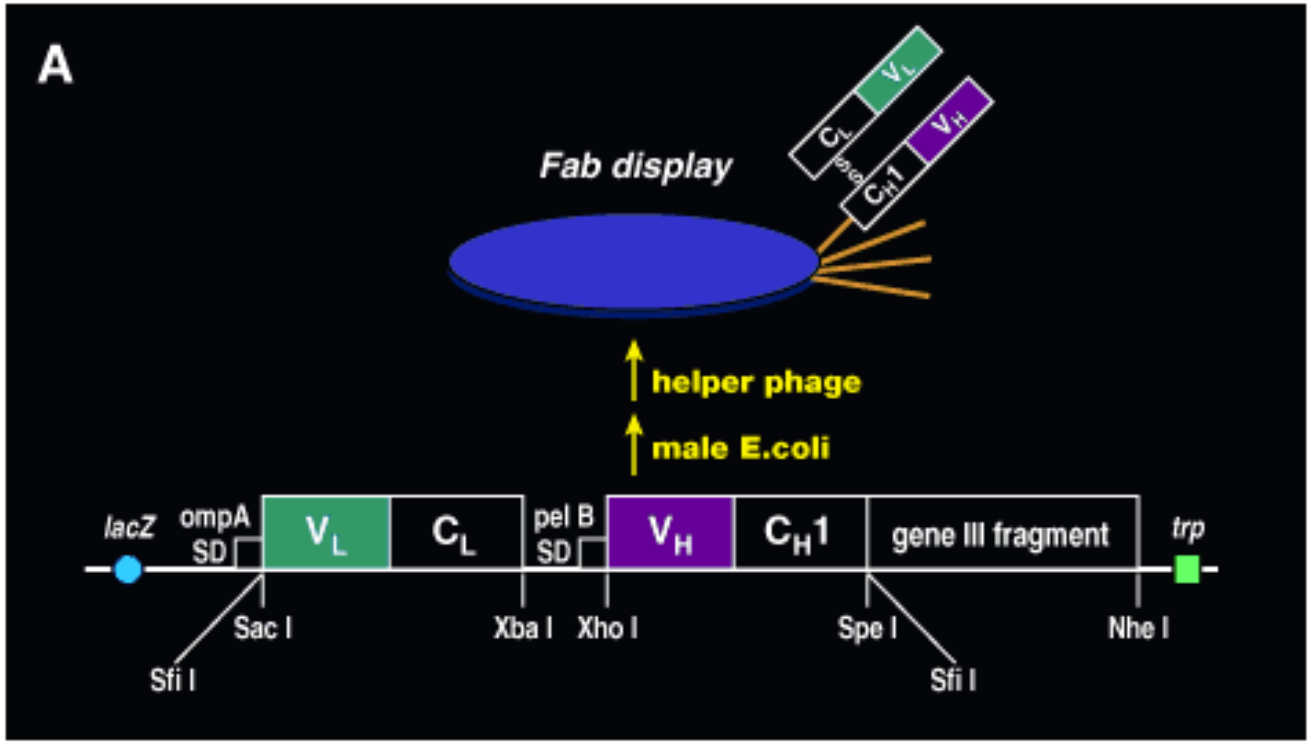
**dpEx Reverse
Extension Primer**

Fab fragment

Fab Library construction

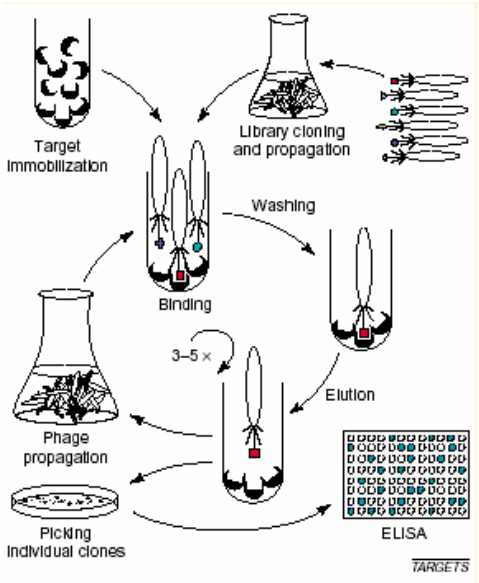
Fab and pComb3Xss *Sfi*I digestion and Ligation

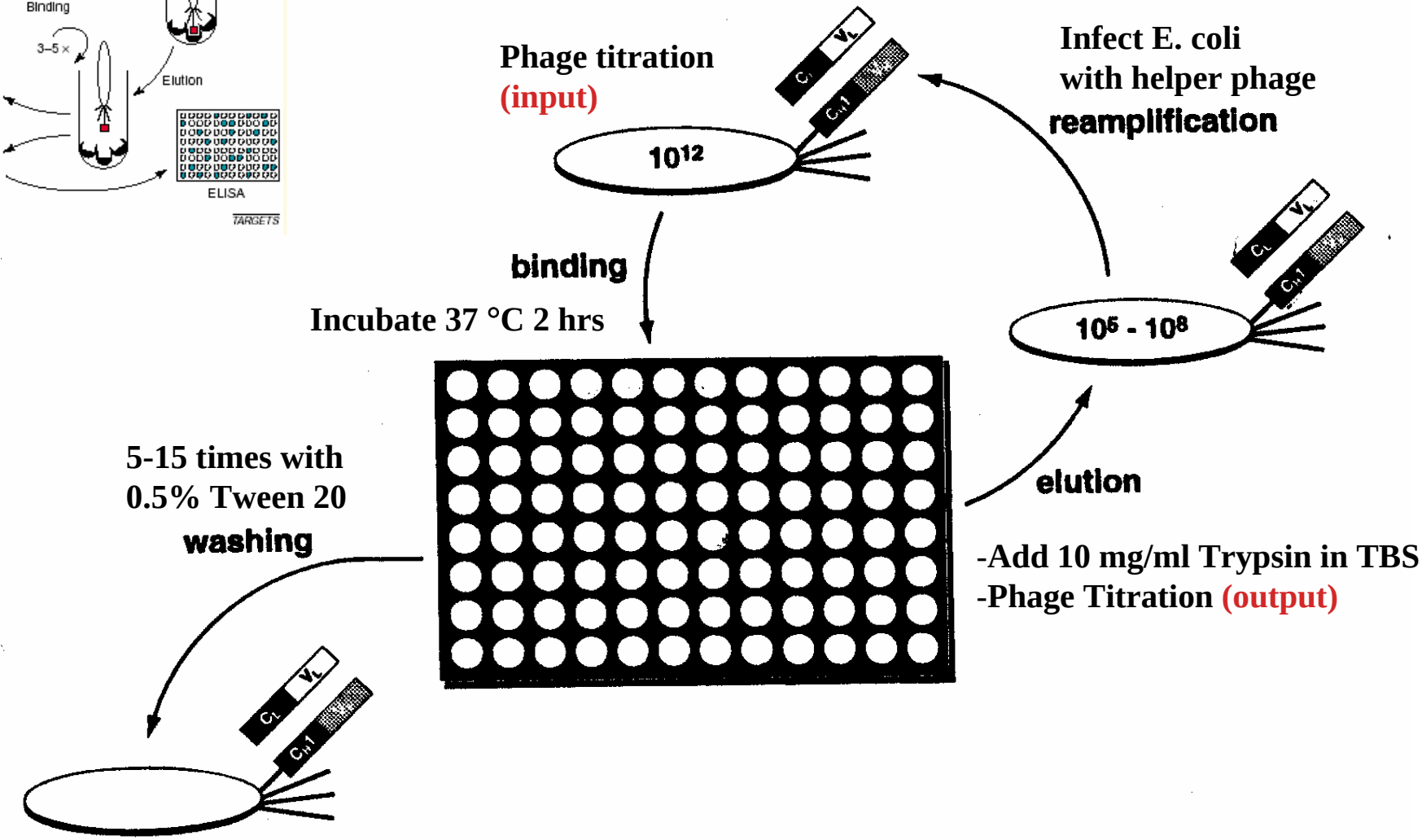
Transform to ER cell



Fab fragment

Selection (panning)





Selection (panning)

1. **stringency increased**

	Ag coated (ng)	Incubation time	Washing time
1st round	100	2 hrs	5
2ndround	100	1 hr	5
3rd round	50	1 hr	5
4th round	20	45 mins	5

2. **no stringency increased**

	Ag coated (ng)	Incubation time	Washing time
1st round	100	2 hrs	5
2ndround	100	2 hrs	5
3rd round	100	2 hrs	5
4th round	100	2 hrs	5

Fab ELISA (binding specificity test)

- Phage pool Fab expression

TOP10F' cell culture

↓

infection of phage pool in 1:1 phage-to-cell ratio

↓

Incubate for 15 min at RT

↓

Add Carbenicillin and incubate at 30 °C O/N

- Single clone Fab expression

Pick single clone from 3rd and 4th pan plate

↓

Inoculate in SB media + Carb

↓

Incubate at 30 °C O/N

Fab ELISA (binding specificity test)

- **Phage pool Fab expression**
- **Single clone Fab expression**

Dilute the O/N culture (1:100) in SB media + Carb

↓

Incubate at 30 °C until OD_{600} ~ 0.7

↓

Add IPTG to 0.5 mM (final conc.)

↓

Incubate at 30 °C O/N

Fab ELISA (binding specificity test)

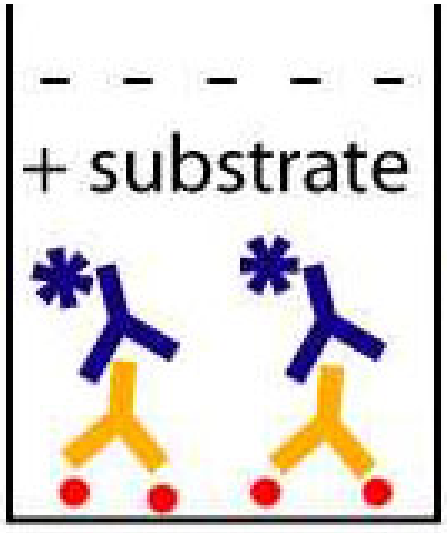
Sonication for breaking the cell

↓

Centrifuge at 3,500 rpm for 20 min

↓

Use Fab supernatant for ELISA



Signal development (ABTS)

⇧

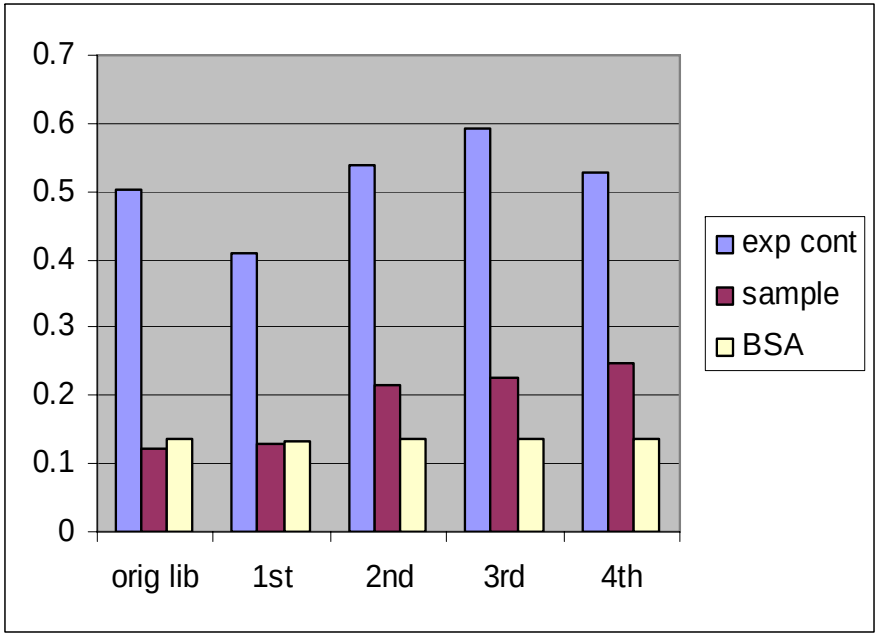
Anti-HA antibody –HRP

⇧

Fab supernatant

⇧

H5 antigen



Results

1. Panning result – stringency increased

	Input phage	Output phage
1st round	1.1×10^{12}	3.0×10^{6}
2nd round	7.5×10^{11}	2.0×10^{5}
3rd round	6.25×10^{11}	1.8×10^{6}
4th round	5×10^{11}	3.2×10^{5}

- **46 clones were selected from 3rd round panning**
- **20 clones were selected from 4th round panning**

Results

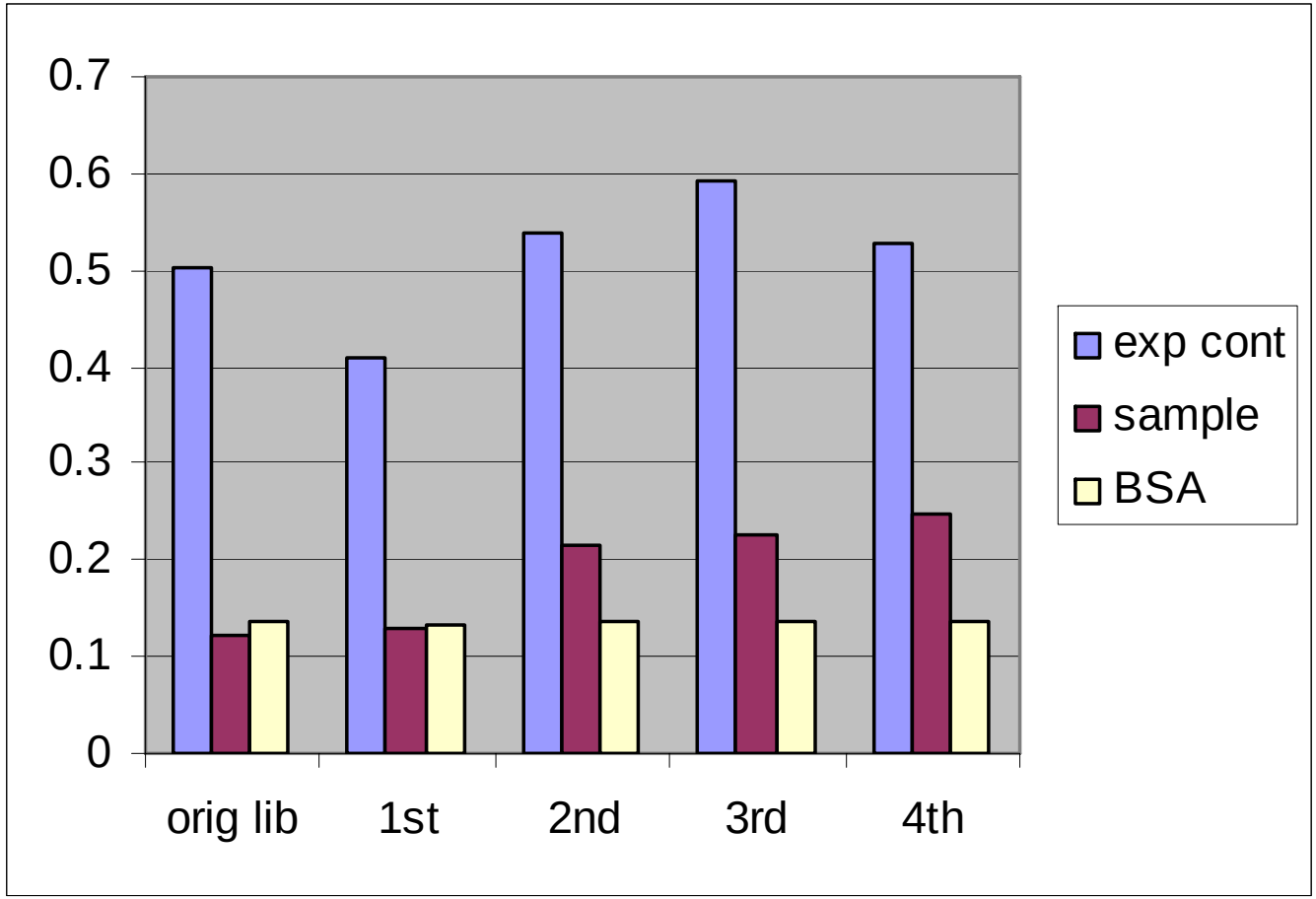
2. Panning result – no stringency increased

	Input phage	Output phage
1st round	**1.5×10^{12}**	**2.5×10^{5}**
2nd round	**1.25×10^{12}**	**8.0×10^{5}**
3rd round	**8.75×10^{11}**	**1.8×10^{6}**
4th round	**1.25×10^{11}**	**3.5×10^{6}**

- **10 clones were selected from 3rd round panning**
- **10 clones were selected from 4th round panning**

Results

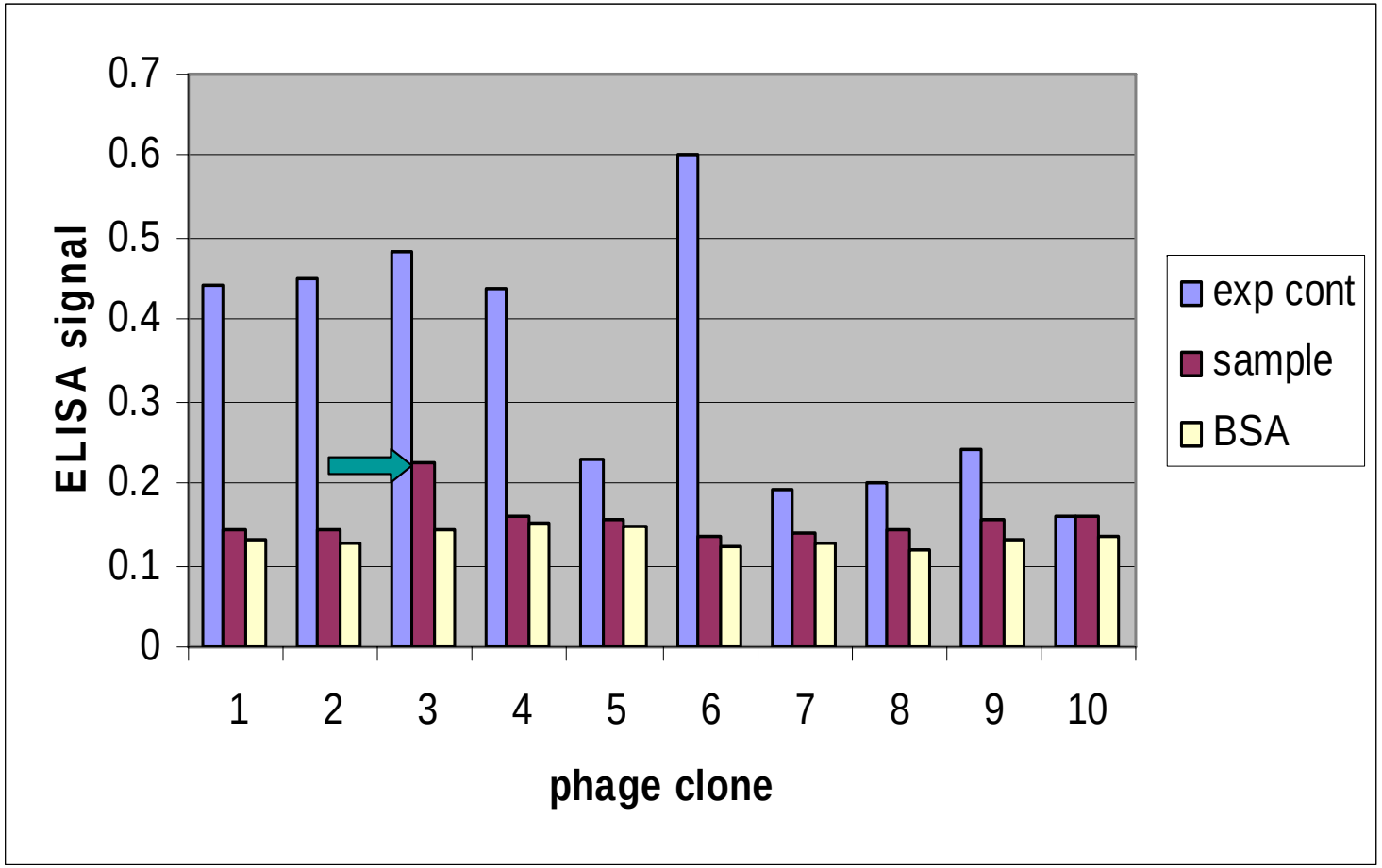
3. Fab ELISA – phage pool Fab



Results

Fab ELISA from some of single clones from 3rd round panning

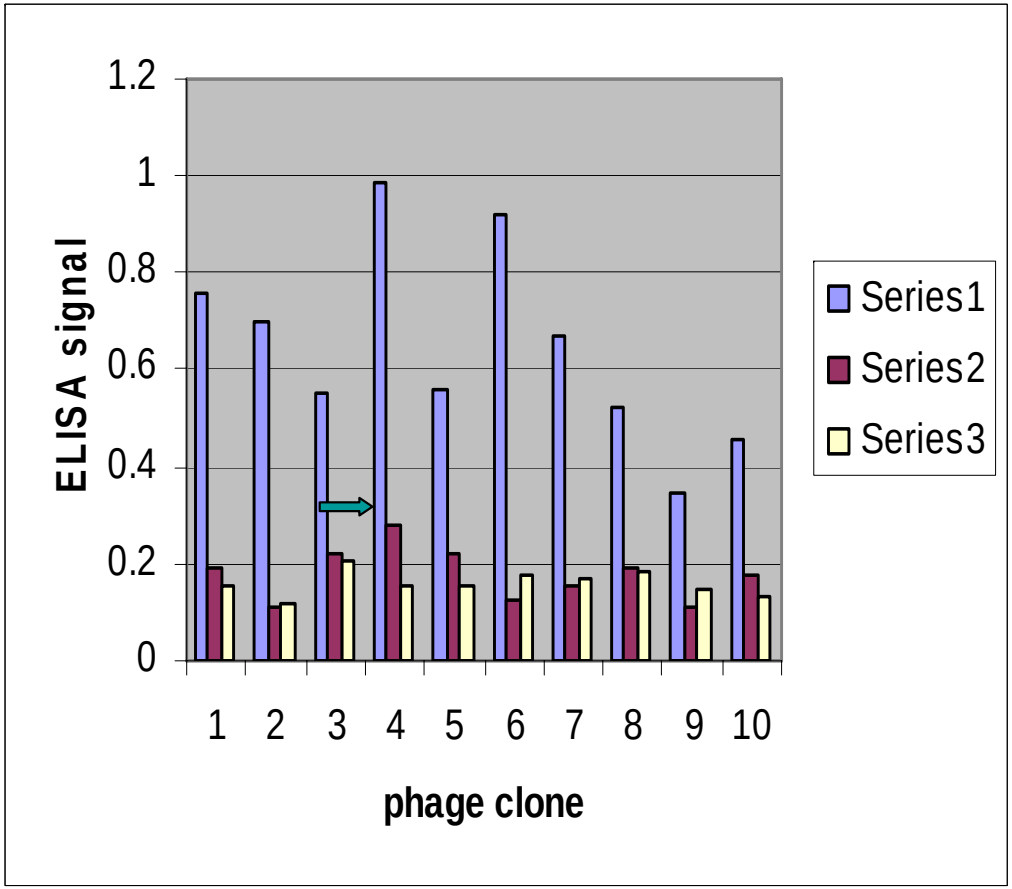
(increased of stringency)



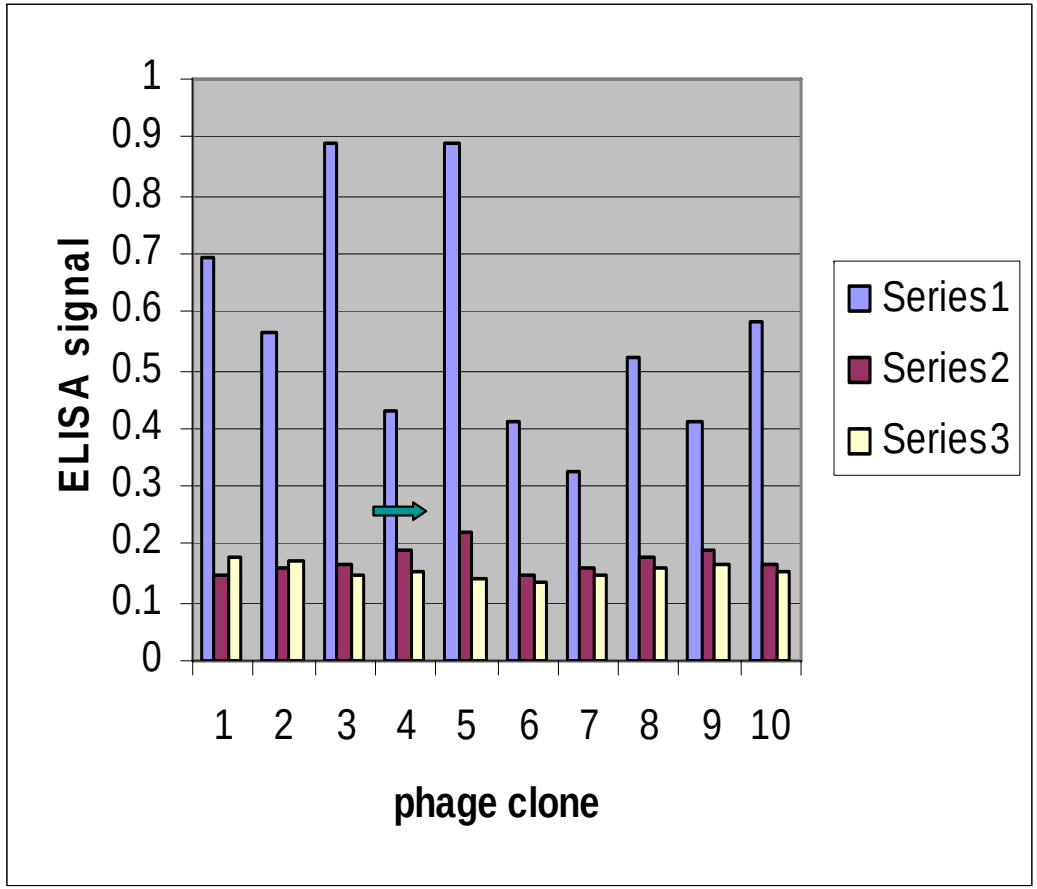
1 binder were obtained (1/46)

Results

- **Fab ELISA from some of single clones from 3rd and 4th round panning (no increased of stringency)**
- **2 binders were obtained (1/10), (1/10)**



3rd pan



4th pan

Conclusion

- Three binders were obtained, but not strong enough
  - Original source of B cell from chicken spleen
    - Chicken serum titer is not high enough (1:64)
  - Small size of library (library construction problems)

Further study

- Immunized the H5N1 antigen
- Construct new library, Panning
- Screening more clones
- Repeat ELISA

Acknowledgement

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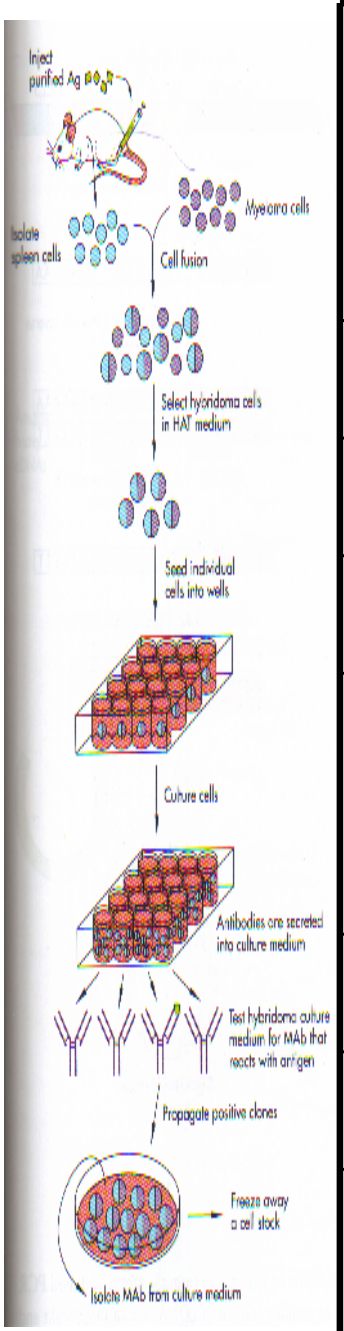
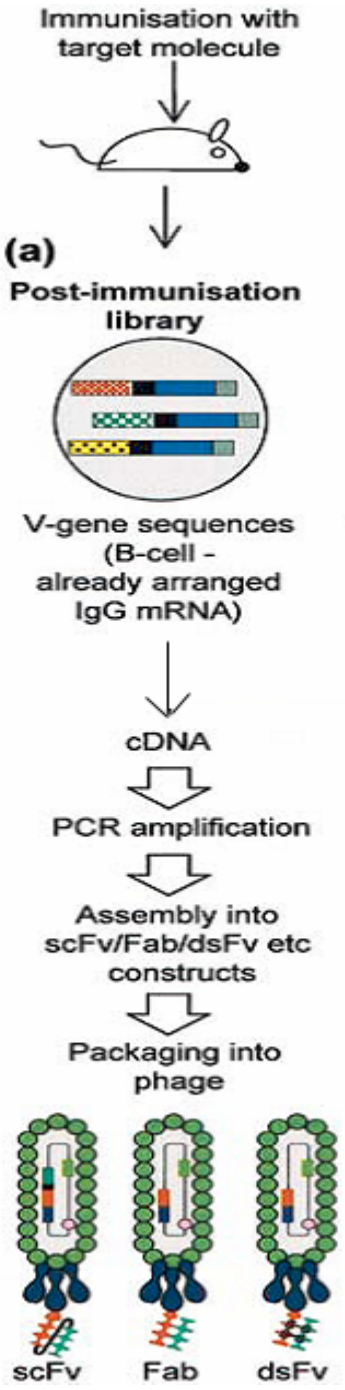
- Commission on Higher Education, Thailand
- The Scripps Research Institute, USA.


THANK YOU

Technology for producing antibody



Pro&con	Hybridoma technology 1970 (Tissue culture based)	Phage display technology 1990 (Genetic engineering)
Process	In vivo	In vivo / In vitro
Antigen required	milligrams	micrograms
Time required	2-3 months	2-3 weeks
Sensitivity, & Specificity Stability	high moderate	high high
Labor intensive	high	moderate
Reagent Cost	high	Low
Equipment Cost	high	Low



Antibody Library

scFv	Fab
Better tolerated by bacteria	More difficult to synthesize
Less likely to be degraded	More likely to be degraded
Can form dimers	No dimerization
Single protein molecules	Two protein molecules
Less stable	More stable
A fraction of expressed scFv can be non-functional	Tends to be more functional
DNA insert up to 700 bps	DNA insert > 1500 bps

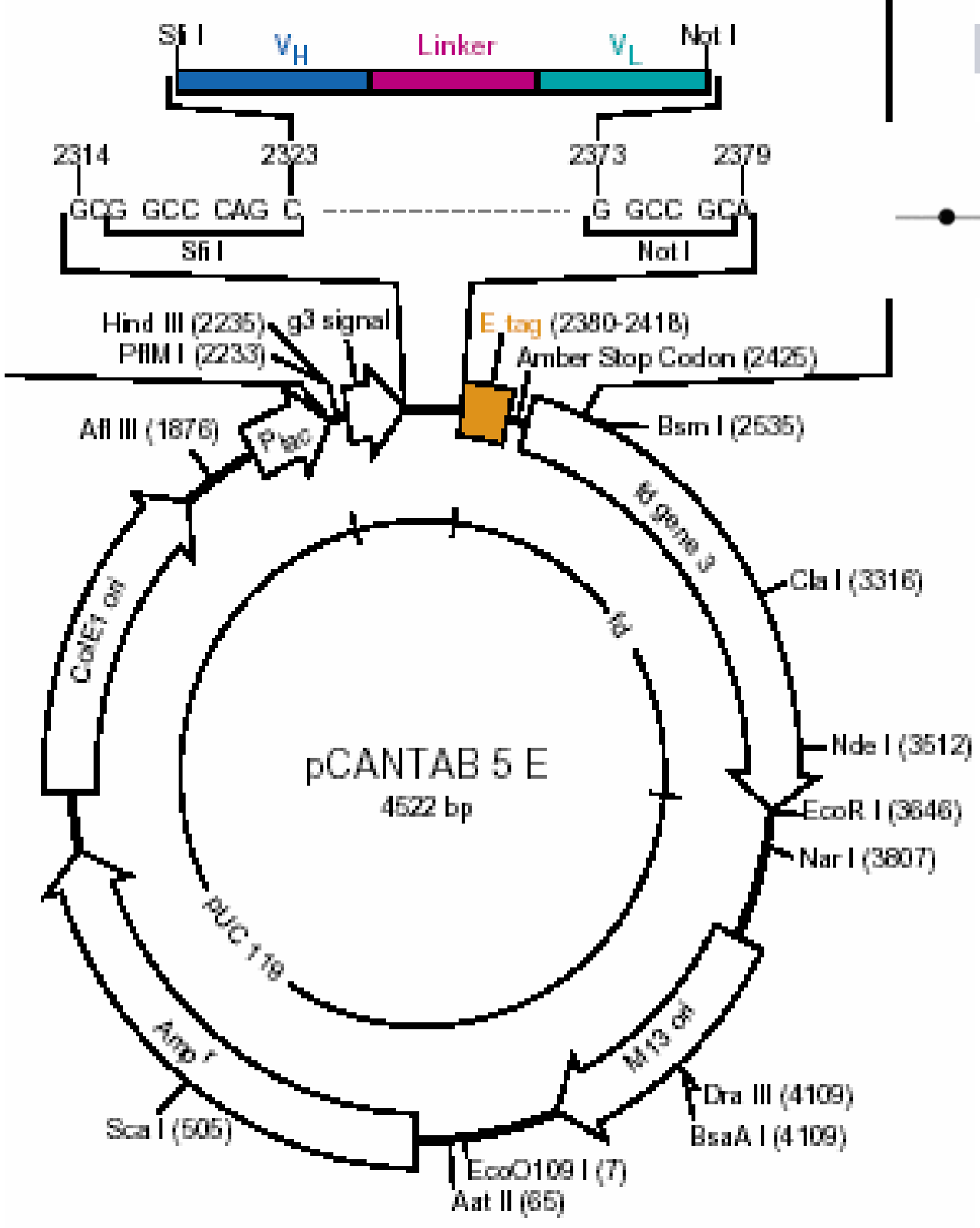
Dimerization can be reduced by increasing linker peptide to be more than 20 amino acids

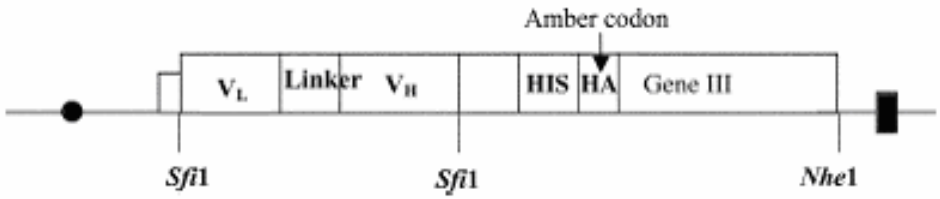
Influenza test kit in the market

Name	Type	Detect	Sense/Spec (%)
Accepta (UK)	Immuno chromatography	A + B	99/88
		A	
Innova Biotech (TH)		A	
Glumpine (Jordan)		H5	

Application of engineered antibody from phage display

	Clinical Application	*References*
Diagnostic test	***Constructed scFv against WSSV with high specificity for diagnostic kit development***	***Dai et al., 2003***
Pathogen neutralizing	***Antibody binding (Fab) can directly and effectively block the activity of pathogens, such as, Chlamydia trachomatis***	***Wilson et al., 2004***
Antiviral Therapy	***Synagis, humanized antibody used for the prevention of severe respiratory syncytial virus***	***Meanwell and Krystal, 2000***
Cancer therapy	***Blocking angiogenesis to prevent the establishment and growth of tumors***	***Sanz et al., 2002; Marty et al., 2002***
Intracellular antibodies	***Ab fragment can be expressed as intracellular proteins, typically scFv, termed intrabodies, and equipped with targeting signals either to neutralize intracellular gene product or target cellular pathway, e.g. huntingtin can be down-regulated by antibodies***	***Lecerf et al., 2001***


Sfi I
V_H
Linker
V_L
Not I
2314
2323
2373
2379
GCG GCC CAG C
G GCC GCA
Sfi I
Not I
Hind III (2235)
PIIM I (2233)
g3 signal
E tag (2380-2418)
Amber Stop Codon (2425)
Aff III (1876)
P_{lac}
Bsm I (2535)
fd gene 3
Cla I (3316)
ColE1 ori
fd
pCANTAB 5 E
4522 bp
Nde I (3512)
EcoR I (3646)
Nar I (3807)
pUC 119
M13 ori
Amp r
Dra III (4109)
BsaA I (4109)
Sca I (505)
EcoO109 I (7)
Aat II (65)


Amber codon
V_L
Linker
V_H
HIS
HA
Gene III
Sfi1
Sfi1
Nhe1