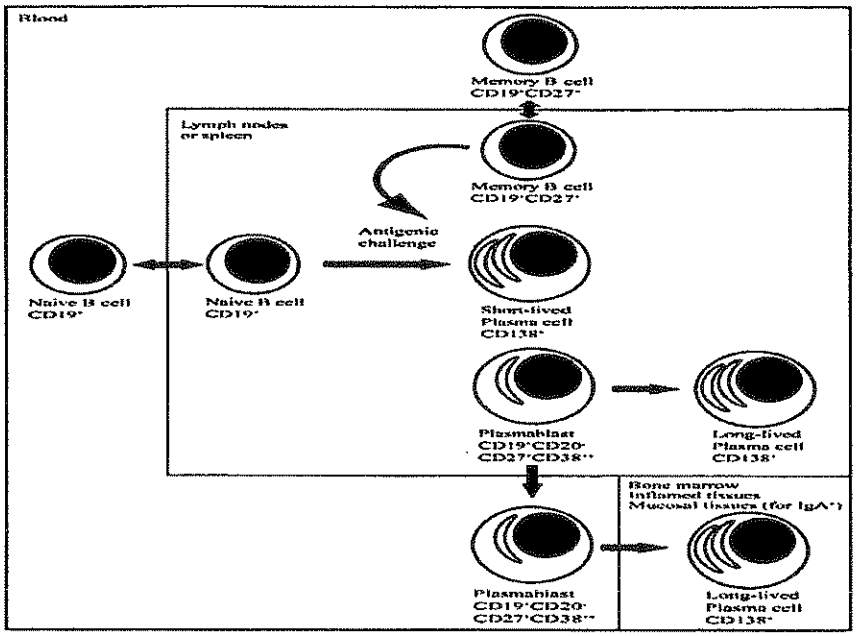
Figure 1


Blood
Memory B cell
CD19+CD27+
Lymph nodes
or spleen
Memory B cell
CD19+CD27+
Antigenic
challenge
Naive B cell
CD19+
Naive B cell
CD19+
Short-lived
Plasma cell
CD138+
Plasmablast
CD19+CD20-
CD27+CD38++
Long-lived
Plasma cell
CD138+
Bone marrow
Inflamed tissues
Mucosal tissues (for IgA+)
Plasmablast
CD19+CD20-
CD27+CD38++
Long-lived
Plasma cell
CD138+

1

Figure 2

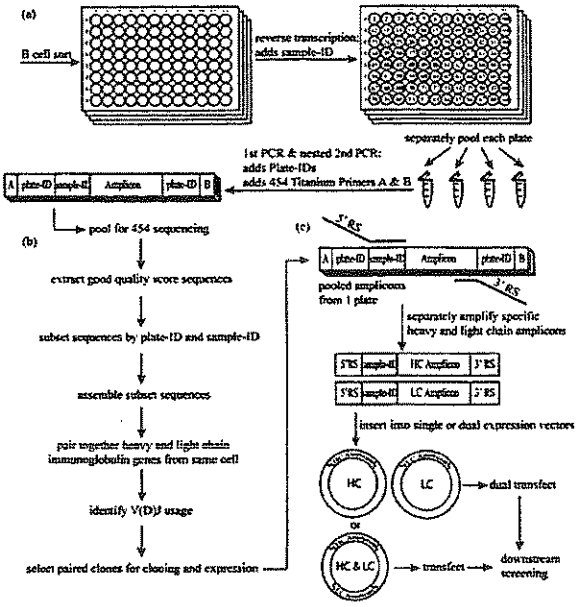

(a)
B cell sort
reverse transcription:
adds sample-ID
separately pool each plate
1st PCR & nested 2nd PCR:
adds Plate-IDs
adds 454 Titanium Primers A & B
A
plate-ID
sample-ID
Amplicon
plate-ID
B
pool for 454 sequencing
(b)
extract good quality score sequences
subset sequences by plate-ID and sample-ID
assemble subset sequences
pair together heavy and light chain immunoglobulin genes from same cell
identify V(D)J usage
select paired clones for cloning and expression
(c)
5' RS
pooled amplicons from 1 plate
3' RS
separately amplify specific heavy and light chain amplicons
5'RS
sample-ID
HC Amplicon
3' RS
LC Amplicon
insert into single or dual expression vectors
HC
LC
dual transfect
or
HC & LC
transfect
downstream screening

Figure 3

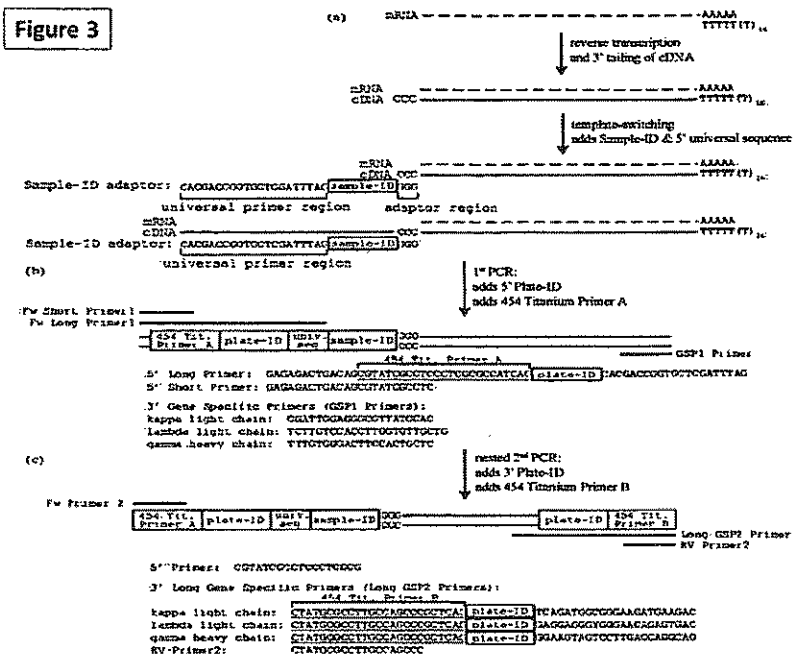

(a)
mRNA
AAAAA
TTTTT (T)
reverse transcription
and 3' tailing of cDNA
cDNA
CCC
template-switching
adds Sample-ID & 5' universal sequence
Sample-ID adaptor:
universal primer region
adaptor region
(b)
1st PCR:
adds 5' Plate-ID
adds 454 Titanium Primer A
Fw Short Primer1
Fw Long Primer1
454 Tit. Primer A
plate-ID
sample-ID
GSP1 Primer
5' Long Primer:
5' Short Primer:
3' Gene Specific Primers (GSP1 Primers):
kappa light chain:
lambda light chain:
gamma heavy chain:
(c)
nested 2nd PCR:
adds 3' Plate-ID
adds 454 Titanium Primer B
Fw Primer 2
454 Tit. Primer B
Long GSP2 Primer
RV Primer2
5'' Primer:
3' Long Gene Specific Primers (Long GSP2 Primers):
kappa light chain:
lambda light chain:
gamma heavy chain:
RV-Primer2:

Figure 4

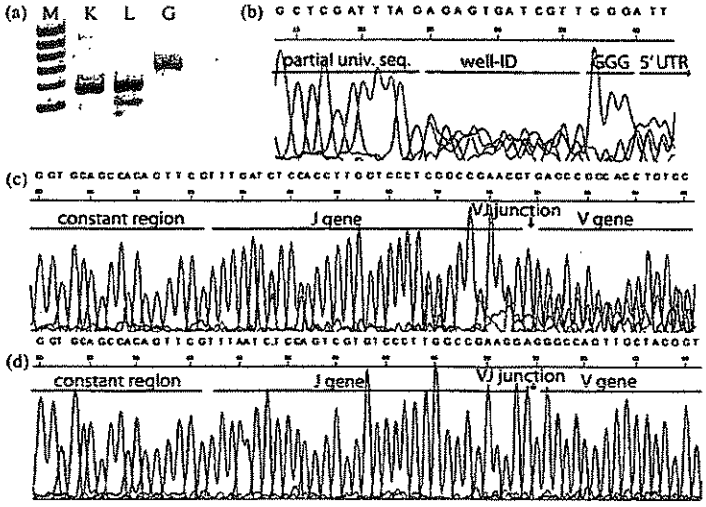

(a) M K L G
(b)
partial univ. seq.
well-ID
GGG 5' UTR
(c)
constant region
J gene
VJ junction
V gene
(d)
constant region
J gene
VJ junction
V gene

Figure 5

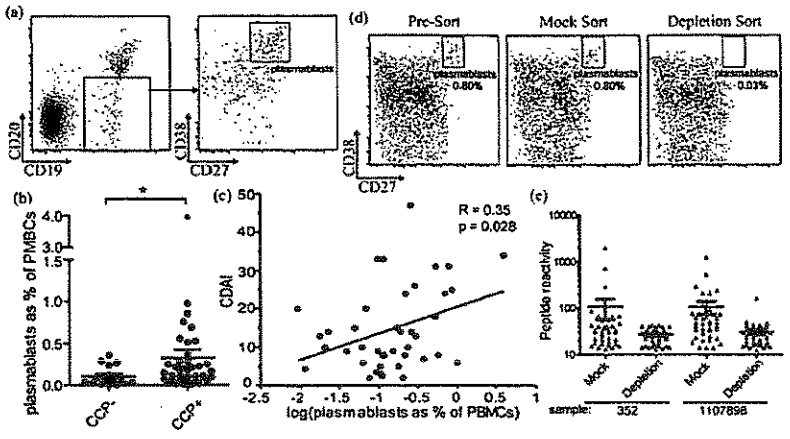

(a)
CD20
CD19
plasmablasts
CD38
CD27
(d)
Pre-Sort
Mock Sort
Depletion Sort
plasmablasts
0.80%
plasmablasts
0.80%
plasmablasts
0.03%
CD38
CD27
(b)
plasmablasts as % of PMBCs
CCP-
CCP+
(c)
CDAI
R = 0.35
p = 0.028
log(plasmablasts as % of PBMCs)
(c)
Peptide reactivity
Mock
Depletion
Mock
Depletion
sample:
352
1107898

Figure 6

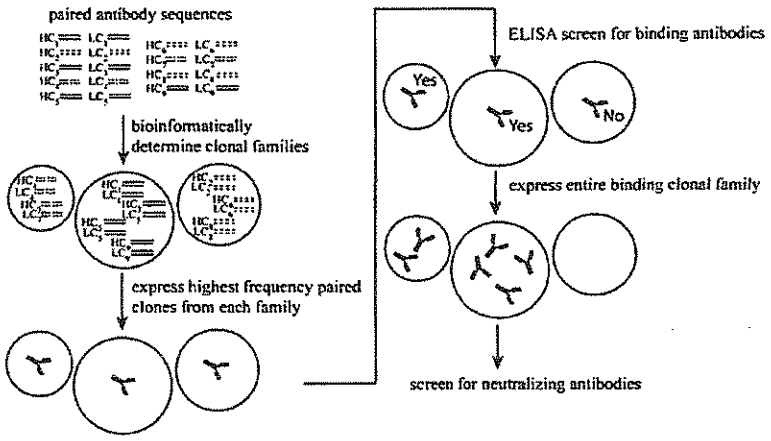
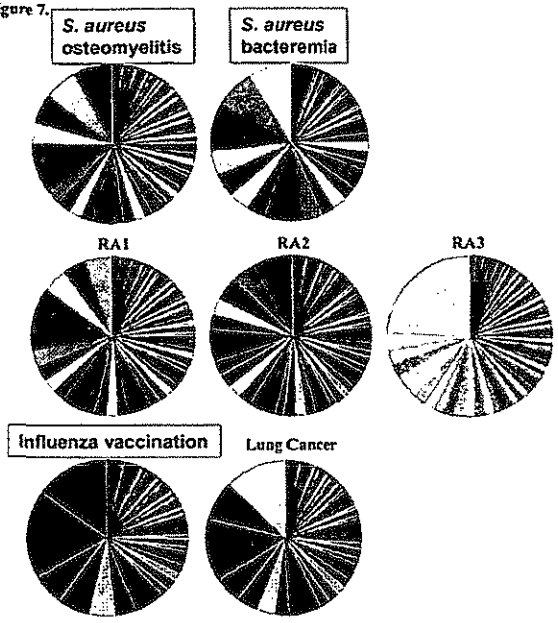
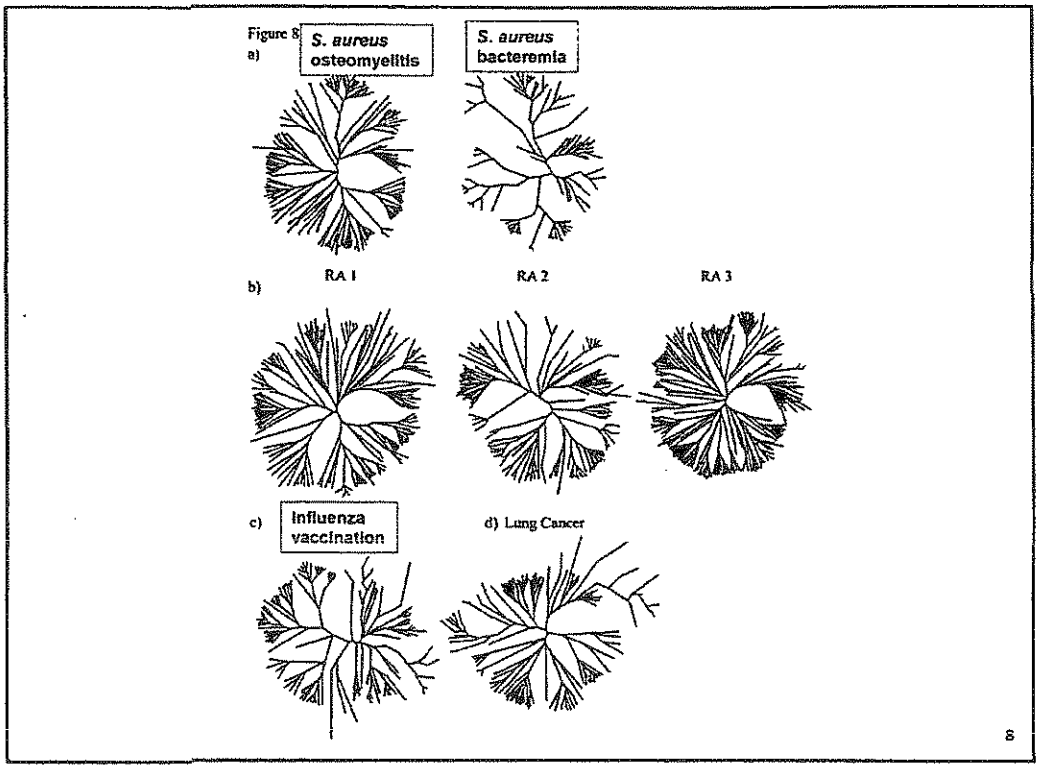

paired antibody sequences
bioinformatically
determine clonal families
express highest frequency paired
clones from each family
ELISA screen for binding antibodies
Yes
Yes
No
express entire binding clonal family
screen for neutralizing antibodies

Figure 7.


S. aureus
osteomyelitis
S. aureus
bacteremia
RA1
RA2
RA3
Influenza vaccination
Lung Cancer

7


Figure 8
a)
S. aureus osteomyelitis
S. aureus bacteremia
b)
RA 1
RA 2
RA 3
c)
Influenza vaccination
d) Lung Cancer
8

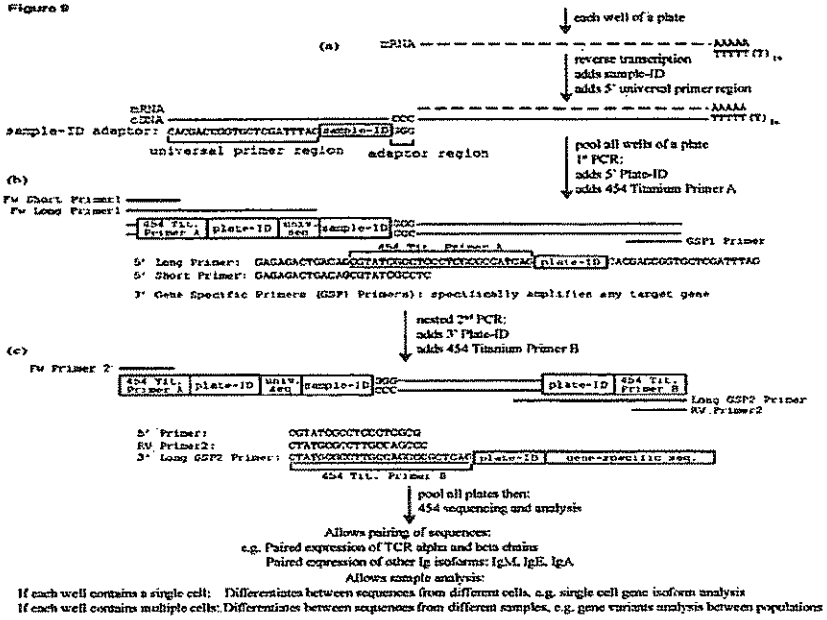

Figure 9
each well of a plate
(a)
mRNA
AAAAA
TTTTT (T) 15
reverse transcription
adds sample-ID
adds 5' universal primer region
mRNA
cDNA
CCC
GGG
sample-ID adaptor:
sample-ID
universal primer region
adaptor region
pool all wells of a plate
1st PCR:
adds 5' Plate-ID
adds 454 Titanium Primer A
(b)
Fw Short Primer:
Fw Long Primer:
454 Tit. Primer A
plate-ID
univ. seq
sample-ID
GSP1 Primer
5' Long Primer:
5' Short Primer:
3' Gene Specific Primers (GSP Primers): specifically amplifies any target gene
nested 2nd PCR:
adds 3' Plate-ID
adds 454 Titanium Primer B
(c)
Fw Primer 2:
454 Tit. Primer B
Long GSP2 Primer
RV Primer2
5' Primer:
RV Primer2:
3' Long GSP2 Primer:
gene-specific seq.
454 Tit. Primer B
pool all plates then:
454 sequencing and analysis
Allows pairing of sequences:
e.g. Paired expression of TCR alpha and beta chains
Paired expression of other Ig isoforms: IgM, IgE, IgA
Allows sample analysis:
If each well contains a single cell: Differentiates between sequences from different cells, e.g. single cell gene isoform analysis
If each well contains multiple cells: Differentiates between sequences from different samples, e.g. gene variants analysis between populations
9

Figure 10

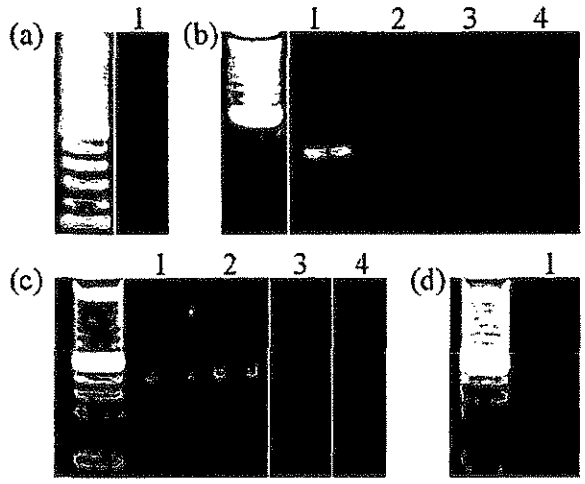
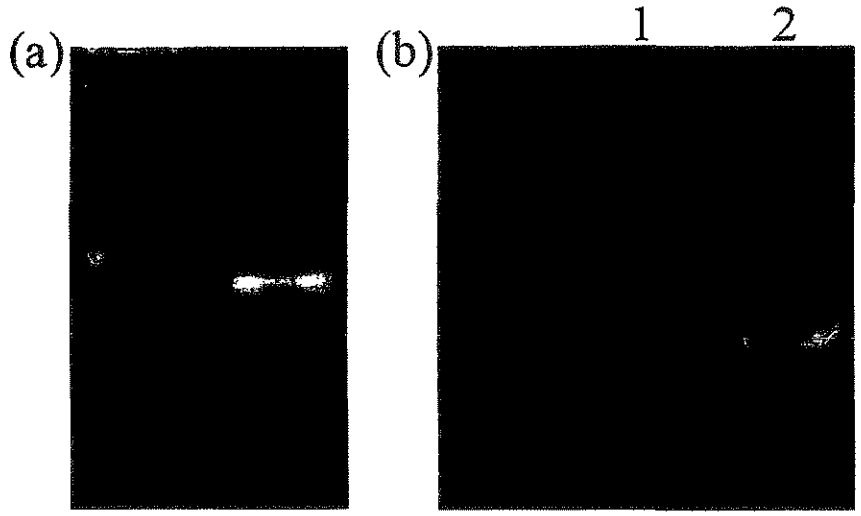
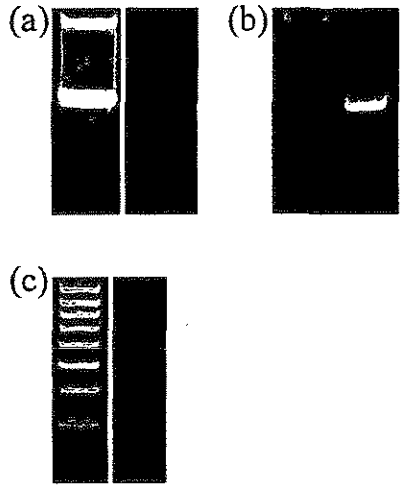


Figure 11



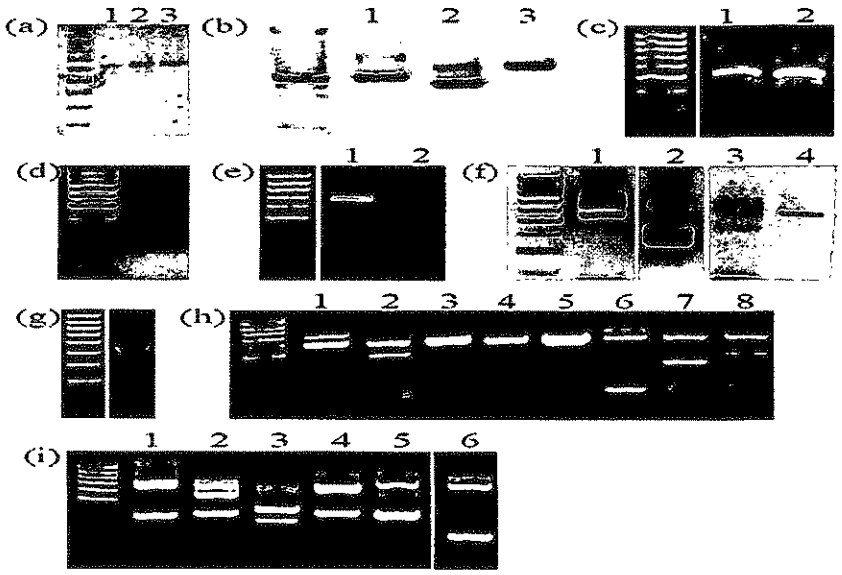
11

Figure 12



12

Figure 13



13

Figure 14

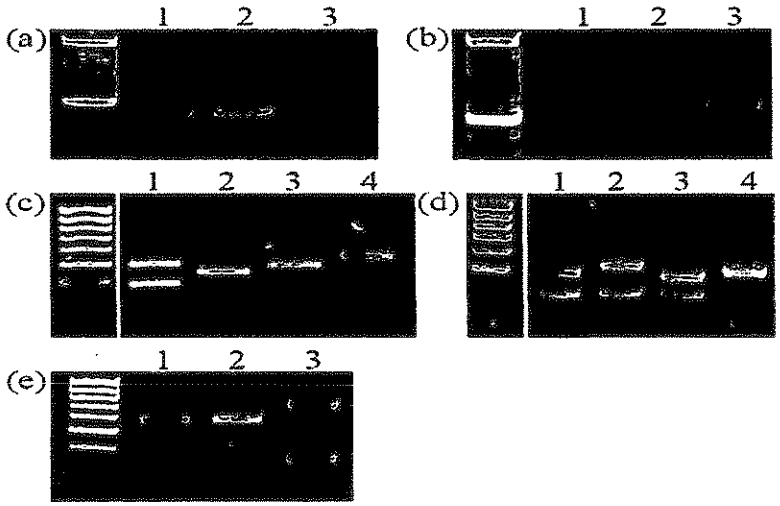
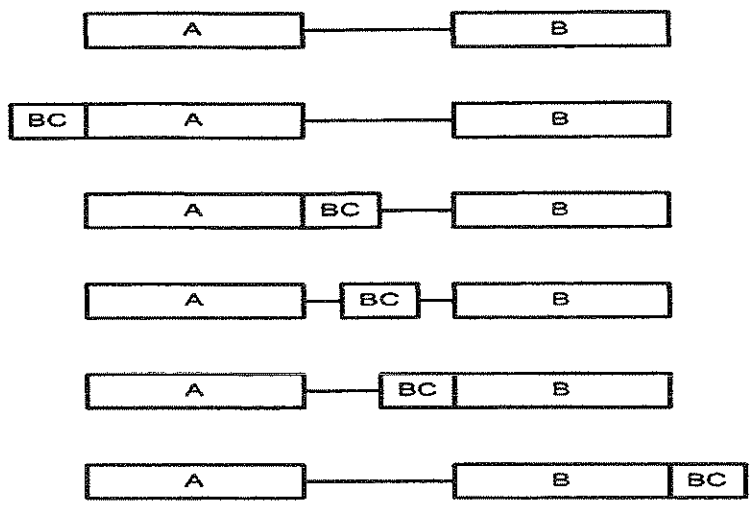
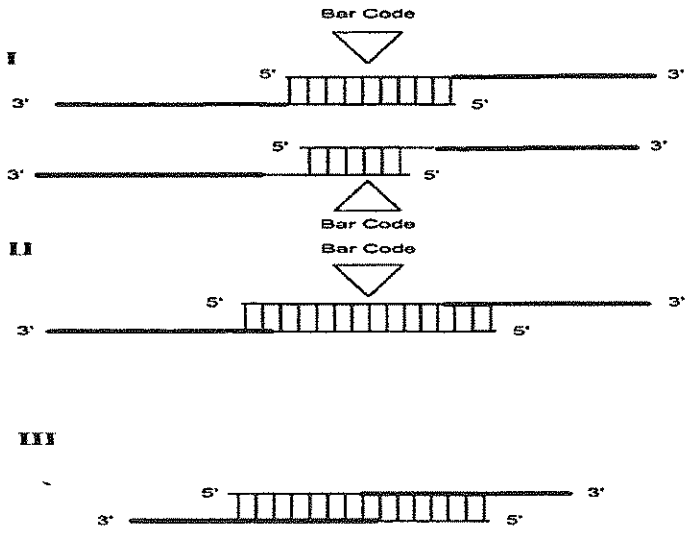


Figure 15



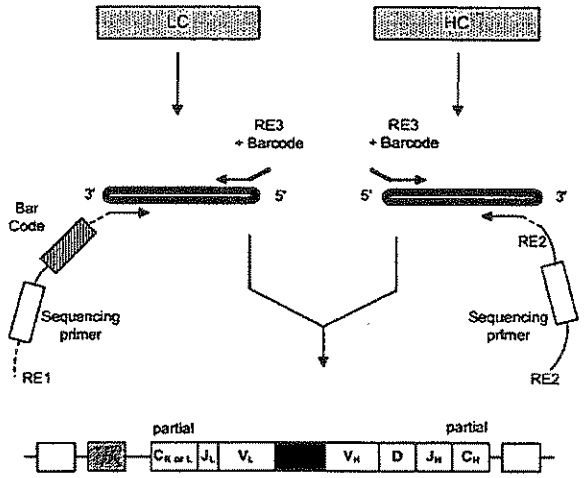
15

Figure 16



16

Figure 17


LC
HC
RE3
+ Barcode
RE3
+ Barcode
3'
5'
5'
3'
Bar
Code
RE2
Sequencing
primer
Sequencing
primer
RE1
RE2
partial
partial
$C_{K \text{ or } L}$
J_L
V_L
V_H
D
J_H
C_H

17

Figure 18

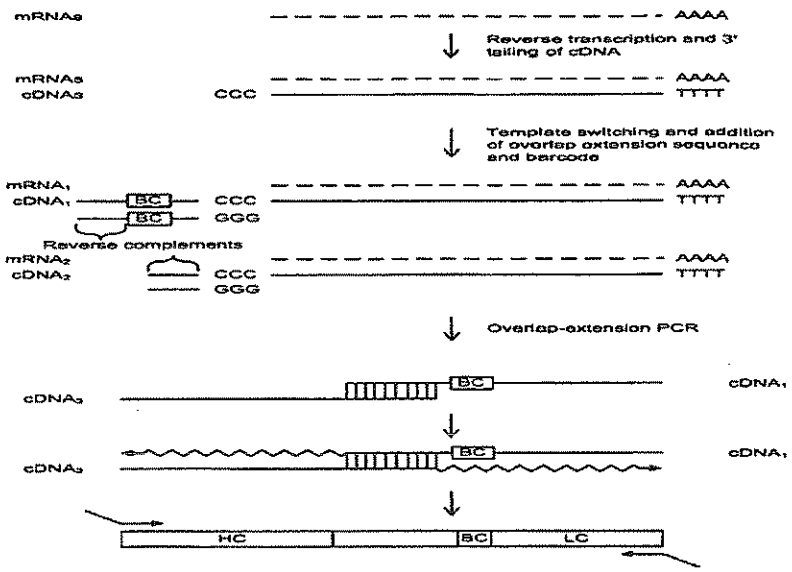

mRNAs
AAAA
Reverse transcription and 3' tailing of cDNA
mRNAs
cDNAs
CCC
AAAA
TTTT
Template switching and addition of overlap extension sequence and barcode
mRNA1
cDNA1
BC
CCC
AAAA
TTTT
GGG
Reverse complements
mRNA2
cDNA2
CCC
GGG
Overlap-extension PCR
cDNA3
cDNA1
cDNA3
cDNA1
HC
BC
LC

Figure 19

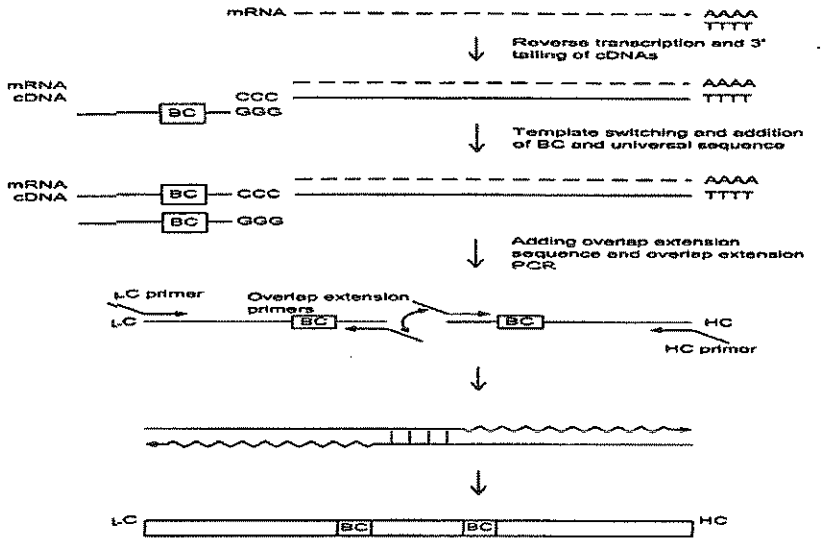

mRNA
AAAA
TTTT
Reverse transcription and 3' tailing of cDNAs
mRNA
cDNA
BC
CCC
GGG
AAAA
TTTT
Template switching and addition of BC and universal sequence
mRNA
cDNA
BC
CCC
BC
GGG
AAAA
TTTT
Adding overlap extension sequence and overlap extension PCR
LC primer
LC
Overlap extension primers
BC
BC
HC
HC primer
LC
BC
BC
HC

Figure 20

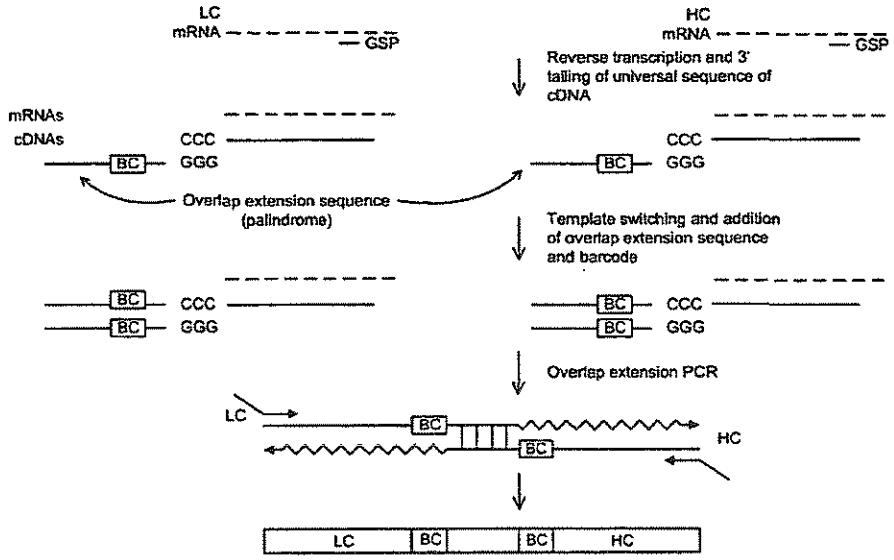
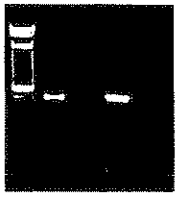

LC
mRNA
GSP
HC
mRNA
GSP
Reverse transcription and 3' tailing of universal sequence of cDNA
mRNAs
cDNAs
CCC
BC
GGG
Overlap extension sequence (palindrome)
Template switching and addition of overlap extension sequence and barcode
Overlap extension PCR
LC
HC
BC
LC
BC
BC
HC

Figure 21



Sequences:	
Univ_seq_2	AACGCGTGACGAGAGACTGACAG
VK	ATG AGGSTCCCYGCTCAGCTGCTG G
VL	GGT CCTGGGCCCAGT CTGCCCTG
IgKC_v3_barcoded	AGGCCCTTACGACTGCGTCTTGAACAATACCAGATGGCGGGAAGATGAAGAC
IgLC_v5_barcoded	AGGCCCTTACGACTGCGTCTTG AACAATACCTCCCGGGTAGAAGTCAC
Fixed_PCR3	AGGCCCTTACGACTGCGTCTTG

FIGURE 22

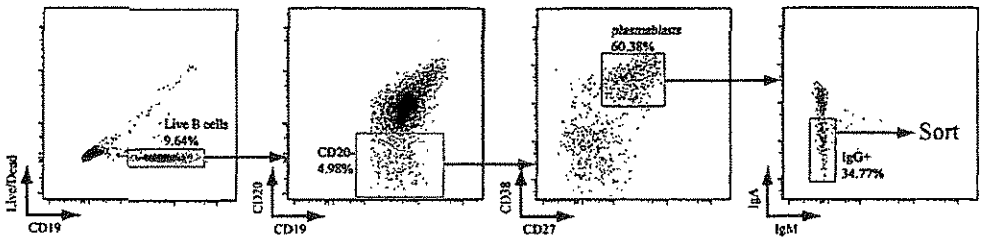

Live B cells
9.64%
LiveDead
CD19
CD20+
4.98%
CD20
CD19
plasmablasts
60.38%
CD38
CD27
Sort
IgG+
34.77%
IgA
IgM

FIGURE 23

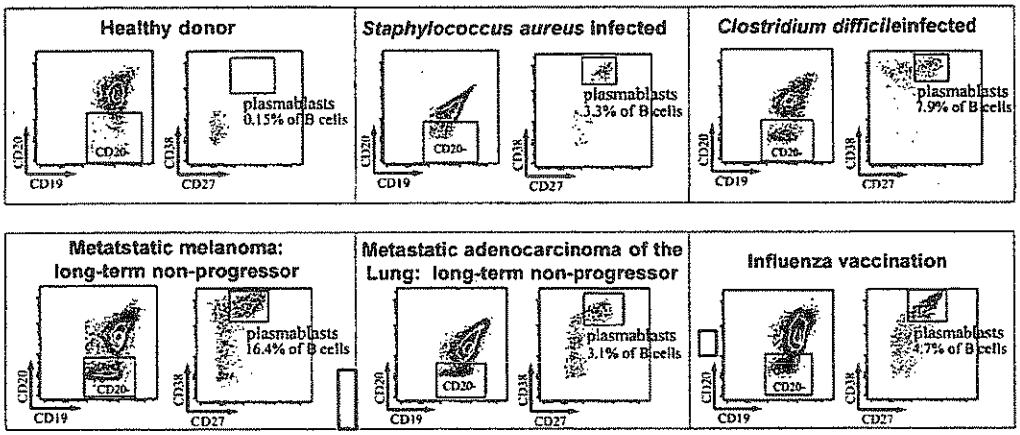

Healthy donor
plasmablasts
0.15% of B cells
CD20-
CD20
CD19
CD38
CD27
Staphylococcus aureus infected
plasmablasts
3.3% of B cells
CD20-
CD20
CD19
CD38
CD27
Clostridium difficile infected
plasmablasts
7.9% of B cells
CD20-
CD20
CD19
CD38
CD27
Metatstatic melanoma:
long-term non-progressor
plasmablasts
16.4% of B cells
CD20-
CD20
CD19
CD38
CD27
Metastatic adenocarcinoma of the
Lung: long-term non-progressor
plasmablasts
3.1% of B cells
CD20-
CD20
CD19
CD38
CD27
Influenza vaccination
plasmablasts
4.7% of B cells
CD20-
CD20
CD19
CD38
CD27

FIGURE 24

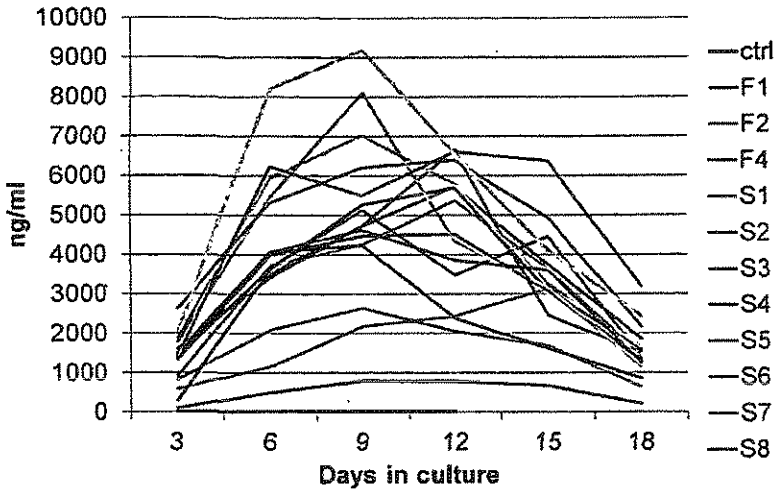

10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
ng/ml
3
6
9
12
15
18
Days in culture
ctrl
F1
F2
F4
S1
S2
S3
S4
S5
S6
S7
S8

FIGURE 25A

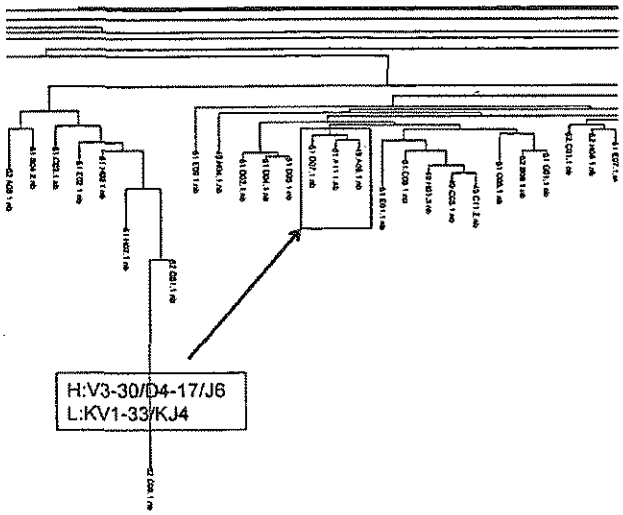


FIGURE 25B

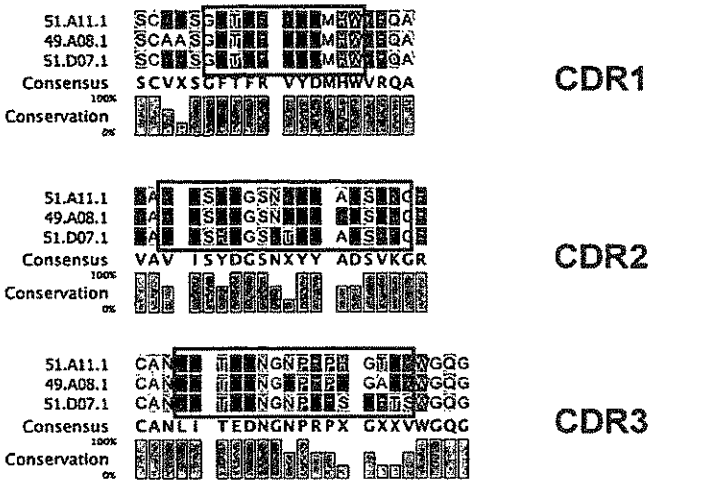
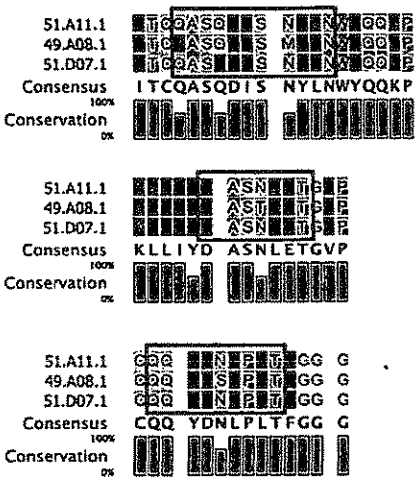

51.A11.1
49.A08.1
51.D07.1
Consensus
Conservation
CDR1
CDR2
CDR3

FIGURE 25C



CDR1

CDR2

CDR3

FIGURE 26

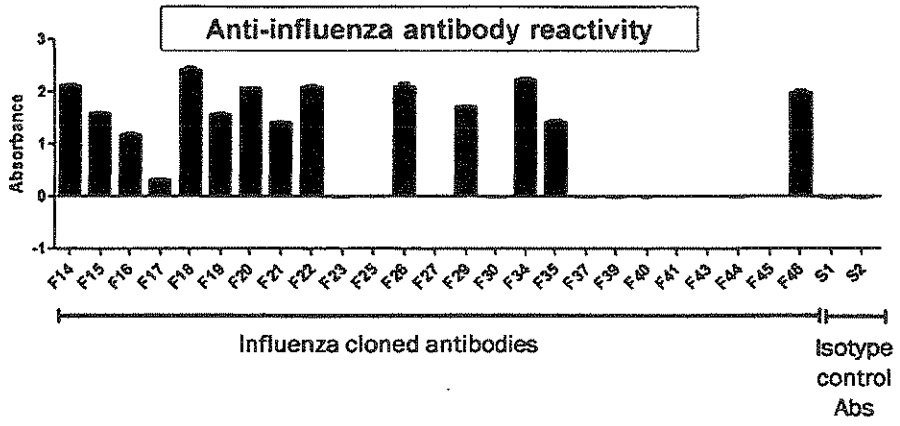

Anti-influenza antibody reactivity
Absorbance
3
2
1
0
-1
F14
F15
F16
F17
F18
F19
F20
F21
F22
F23
F25
F26
F27
F29
F30
F34
F35
F37
F39
F40
F41
F43
F44
F45
F48
S1
S2
Influenza cloned antibodies
Isotype control Abs

FIGURE 27

Antibody	Bound H3 Perth?	Bound H1 California?	Ka	Kd	K_D
FLU 14	Y	N	7.12E+05	1.13E-02	3.04E-08
FLU 16	Y	N	5.35E+05	5.86E-03	1.36E-08
FLU 18	Y	N	2.89E+05	1.60E-03	6.00E-09
FLU 19	Y	N	1.96E+05	5.08E-02	2.59E-07
FLU 20	Y	N	3.77E+05	6.28E-02	4.62E-07
FLU 22	Y	N	1.39E+05	6.75E-03	5.38E-08
FLU 26	Y	N	5.24E+05	1.52E-04	3.13E-10
FLU 34	Y	N	1.40E+05	1.26E-02	9.25E-08
FLU 35	Y	N	1.96E+04	2.35E-02	1.20E-06
FLU 46	Y	N	3.39E+04	4.13E-02	1.24E-06
FLU 21	N	Y	2.23E+05	5.29E-02	2.38E-07
FLU 15	N	N	--	--	--
FLU 17	N	N	--	--	--
FLU 29	N	N	--	--	--

FIGURE 28

Antibody tested	Neutralization of Infectivity	
	H1N1- A/California/07/2009	H3N2 A/Perth/16/2009
mAb F15	No	No
mAb F16	No	Yes, Neutralizes at ≤1.5 µg/ml
mAb F18	No	Yes, Neutralizes at ≤1.5 µg/ml
mAb F19	No	Yes, Neutralizes at 3.1 µg/ml
mAb F20	No	Yes, Neutralizes at 12.5 µg/ml
mAb F21	Yes, Neutralizes at ≥ 1.5 µg/ml	Yes, Neutralizes at 100 µg/ml
Polyclonal Control	Yes, Neutralizes at ≥1/128 diln	Yes, Neutralizes at ≥1/128 diln

FIGURE 29

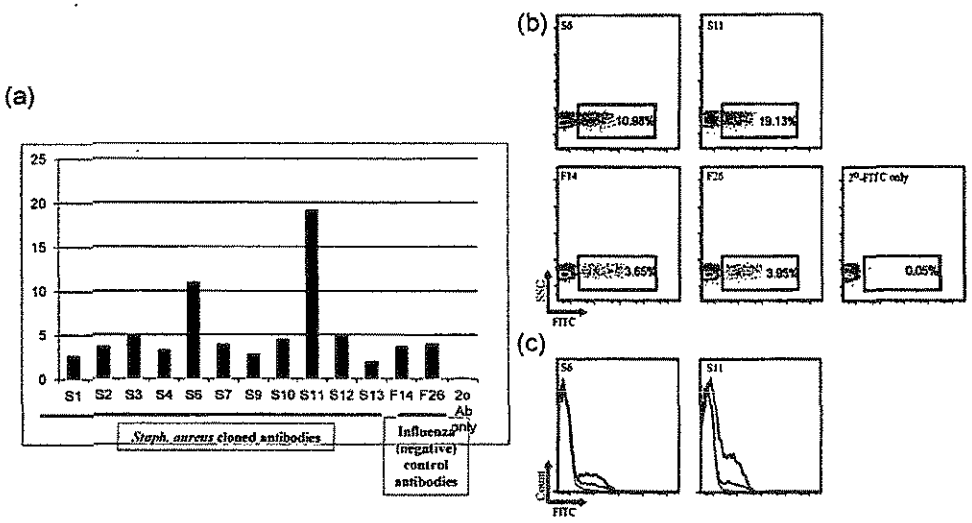

(a)
25
20
15
10
5
0
S1 S2 S3 S4 S5 S7 S9 S10 S11 S12 S13 F14 F26 2o Ab only
Staph. aureus cloned antibodies
Influenza (negative) control antibodies
(b)
S6
10.98%
S11
19.13%
F14
3.65%
F26
3.95%
2°-FITC only
0.05%
SSC
FITC
(c)
S6
S11
Count
FITC

FIGURE 30

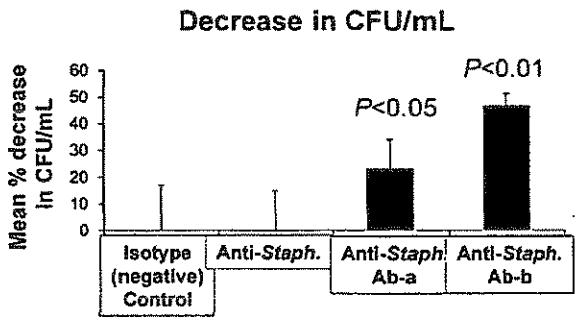

Decrease in CFU/mL
Mean % decrease in CFU/mL
60
50
40
30
20
10
0
P<0.05
P<0.01
Isotype (negative) Control
Anti-Staph.
Anti-Staph. Ab-a
Anti-Staph. Ab-b

FIGURE 31

Antibody	Target Accession #	Protein Name
S04	Q6G7S2	Phenol-soluble modulin alpha 1 peptide
S04	P0C7Y8	Delta-hemolysin

FIGURE 32

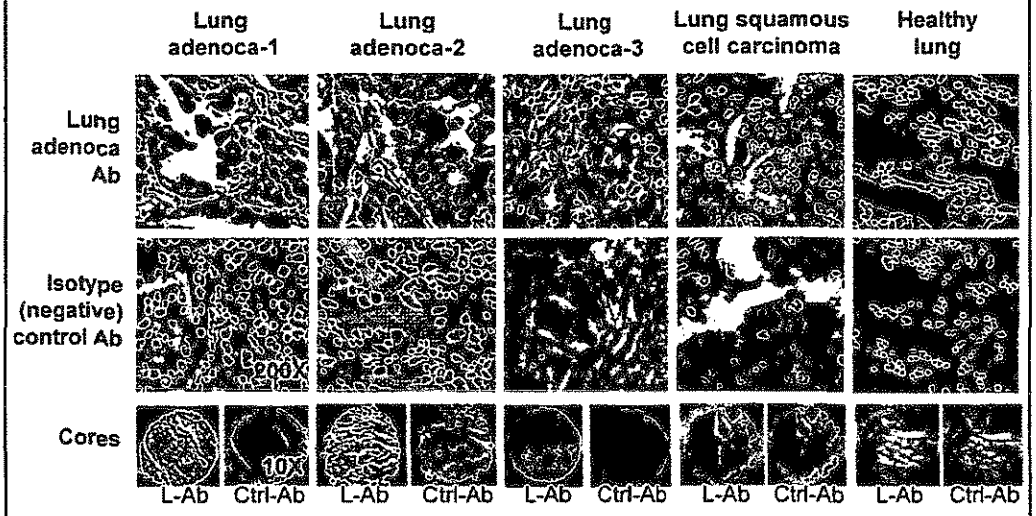

Lung adenoca-1
Lung adenoca-2
Lung adenoca-3
Lung squamous cell carcinoma
Healthy lung
Lung adenoca Ab
Isotype (negative) control Ab
200X
Cores
10X
L-Ab
Ctrl-Ab
L-Ab
Ctrl-Ab
L-Ab
Ctrl-Ab
L-Ab
Ctrl-Ab
L-Ab
Ctrl-Ab

FIGURE 33

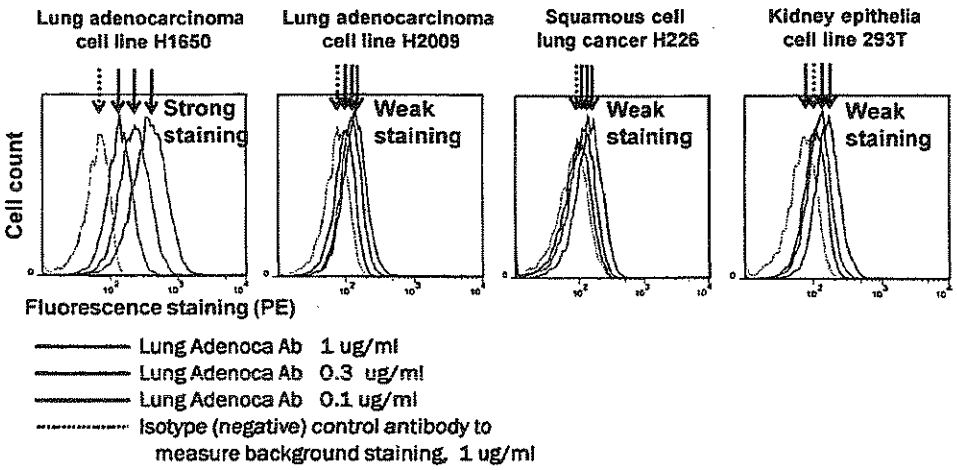

Lung adenocarcinoma cell line H1650
Lung adenocarcinoma cell line H2009
Squamous cell lung cancer H226
Kidney epithelia cell line 293T
Strong staining
Weak staining
Weak staining
Weak staining
Cell count
Fluorescence staining (PE)
Lung Adenoca Ab 1 ug/ml
Lung Adenoca Ab 0.3 ug/ml
Lung Adenoca Ab 0.1 ug/ml
Isotype (negative) control antibody to measure background staining, 1 ug/ml

FIGURE 34

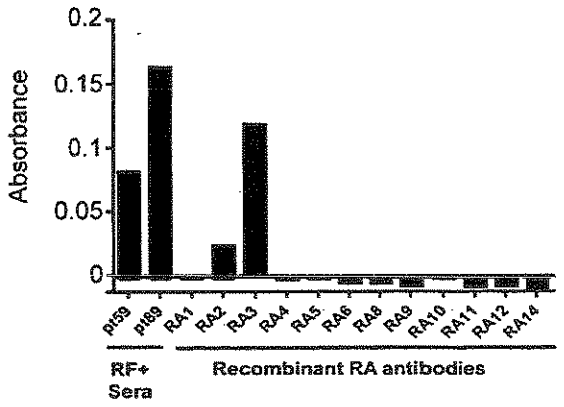
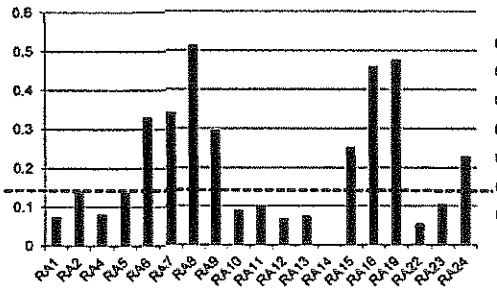

0.2
0.15
0.1
0.05
0
Absorbance
pt59
pt89
RA1
RA2
RA3
RA4
RA5
RA6
RA8
RA9
RA10
RA11
RA12
RA14
RF+ Sera
Recombinant RA antibodies

FIGURE 35

(a)


0.6
0.5
0.4
0.3
0.2
0.1
0
RA1
RA2
RA4
RA5
RA6
RA7
RA8
RA9
RA10
RA11
RA12
RA13
RA14
RA15
RA16
RA19
RA22
RA23
RA24

(b)

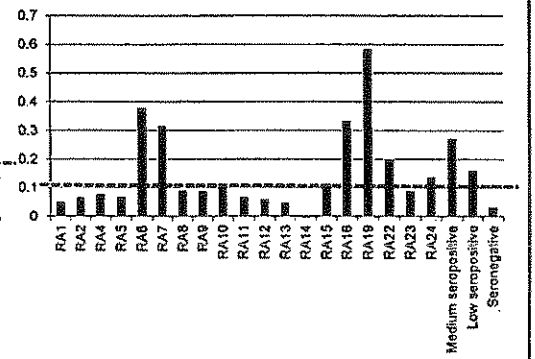

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
RA1
RA2
RA4
RA5
RA6
RA7
RA8
RA9
RA10
RA11
RA12
RA13
RA14
RA15
RA16
RA19
RA22
RA23
RA24
Medium seropositive
Low seropositive
Seronegative

FIGURE 36

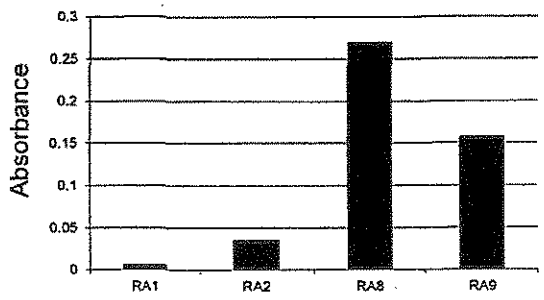


FIGURE 37

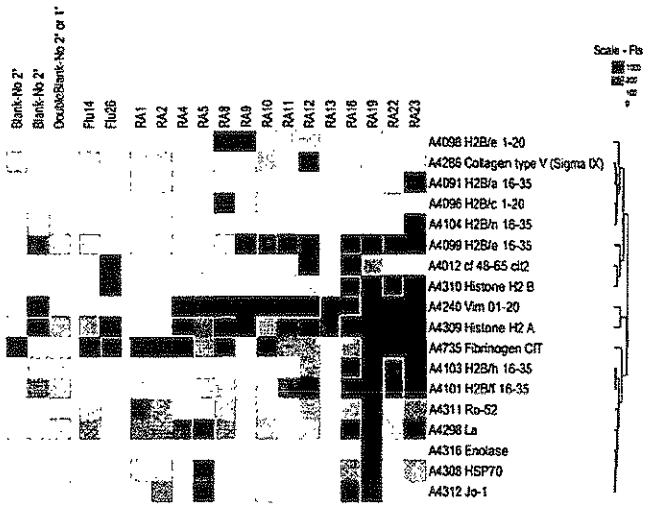

Blank-No 2°
Blank-No 2°
DoubleBlank-No 2° or 1°
Flu14
Flu26
RA1
RA2
RA4
RA5
RA8
RA9
RA10
RA11
RA12
RA13
RA16
RA19
RA22
RA23
A4098 H2B/e 1-20
A4286 Collagen type V (Sigma IX)
A4091 H2B/a 16-35
A4096 H2B/c 1-20
A4104 H2B/n 16-35
A4099 H2B/e 16-35
A4012 cf 48-65 clt2
A4310 Histone H2 B
A4240 Vim 01-20
A4309 Histone H2 A
A4735 Fibrinogen CIT
A4103 H2B/h 16-35
A4101 H2B/f 16-35
A4311 Ro-52
A4298 La
A4316 Enolase
A4308 HSP70
A4312 Jo-1

FIGURE 38

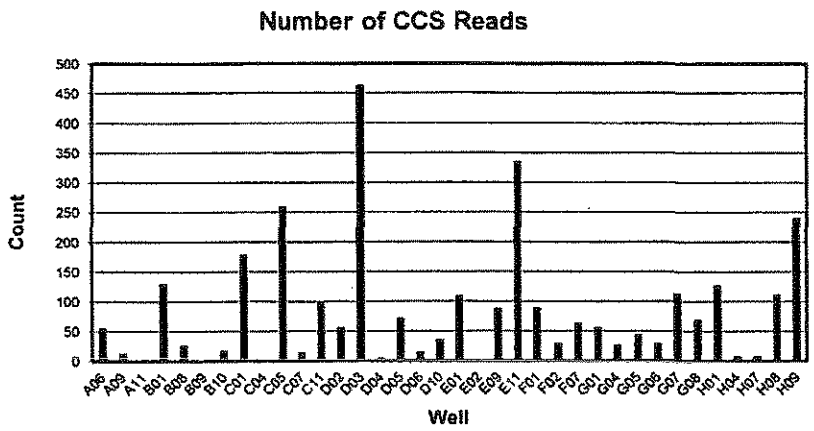

Number of CCS Reads
Count
500
450
400
350
300
250
200
150
100
50
0
A06
A09
A11
B01
B08
B09
B10
C01
C04
C05
C07
C11
D02
D03
D04
D05
D06
D10
E01
E02
E09
E11
F01
F02
F07
G01
G04
G05
G06
G07
G08
H01
H04
H07
H08
H09
Well

FIGURE 39

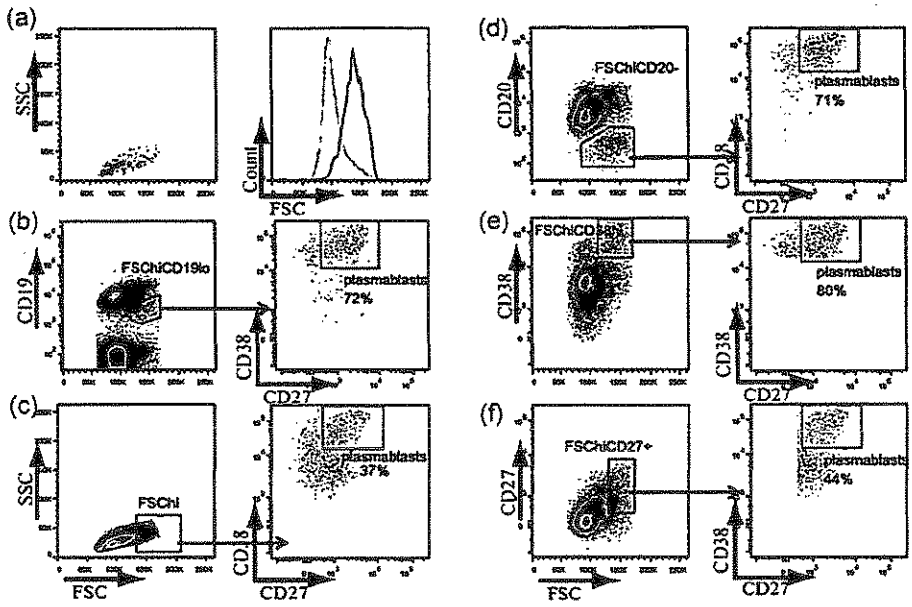

(a)
SSC
Count
FSC
(b)
CD19
FSChiCD19lo
plasmablasts
72%
CD38
CD27
(c)
SSC
FSChi
FSC
plasmablasts
37%
CD38
CD27
(d)
CD20
FSChiCD20-
plasmablasts
71%
CD38
CD27
(e)
CD38
FSChiCD38+
plasmablasts
80%
CD38
CD27
(f)
CD27
FSChiCD27+
plasmablasts
44%
CD38
FSC
CD27

FIGURE 40

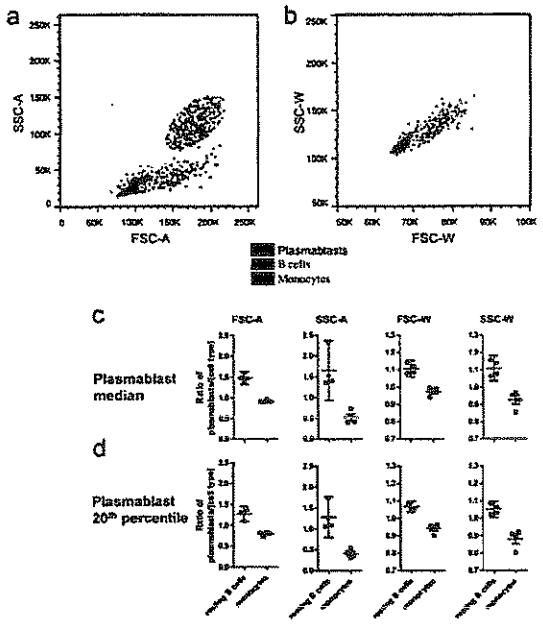

a
b
SSC-A
FSC-A
SSC-W
FSC-W
Plasmablasts
B cells
Monocytes
c
Plasmablast median
d
Plasmablast 20th percentile
FSC-A
SSC-A
FSC-W
SSC-W

FIGURE 41

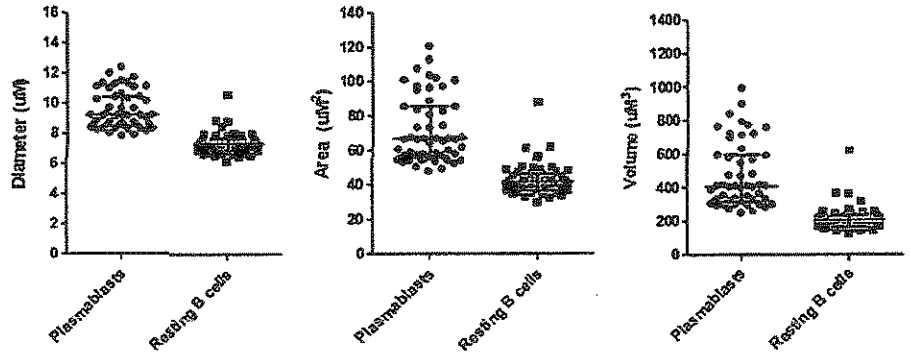

Diameter (um)
16
14
12
10
8
6
4
2
0
Plasmablasts
Resting B cells
Area (um²)
140
120
100
80
60
40
20
0
Plasmablasts
Resting B cells
Volume (um³)
1400
1200
1000
800
600
400
200
0
Plasmablasts
Resting B cells

FIGURE 42

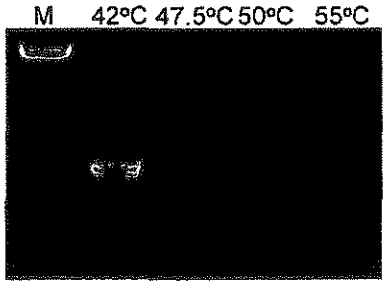


FIGURE 43



FIGURE 44

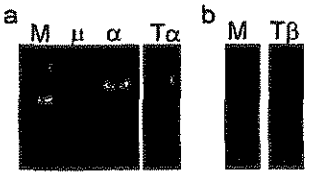


FIGURE 45

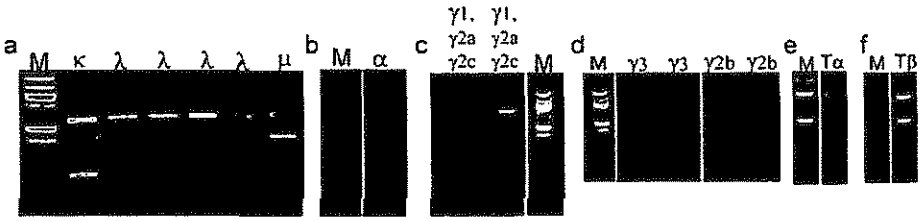
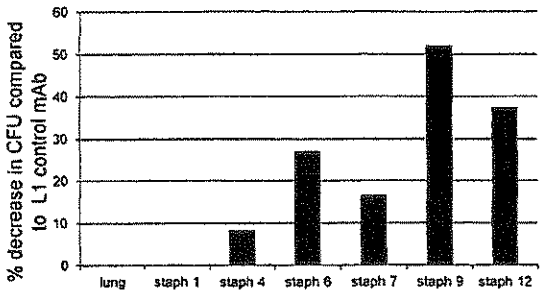


FIGURE 46


% decrease in CFU compared to L1 control mAb
60
50
40
30
20
10
0
lung
staph 1
staph 4
staph 6
staph 7
staph 9
staph 12