V_H Stripping from Cells by Restriction Digestion

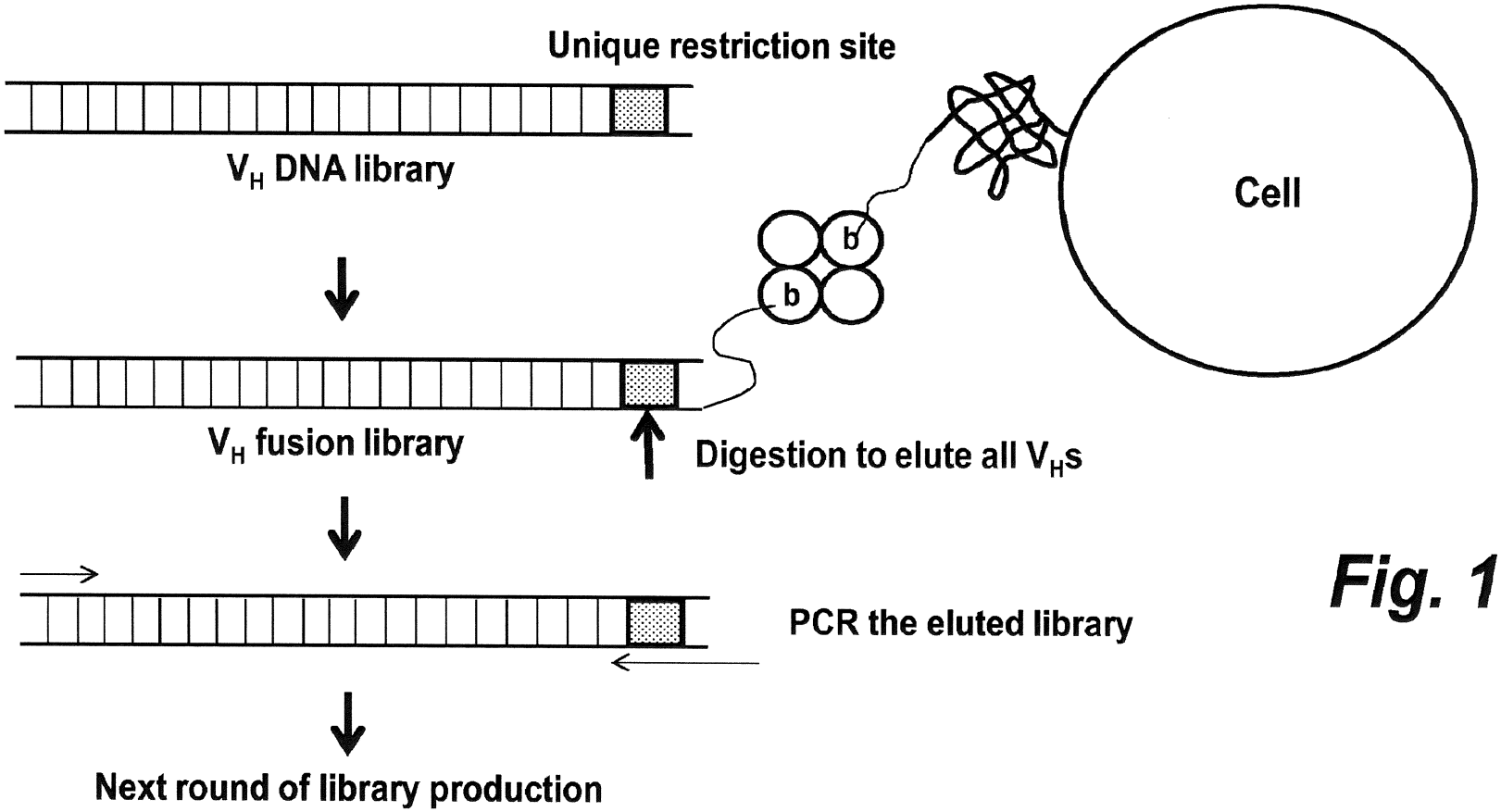


Fig. 1

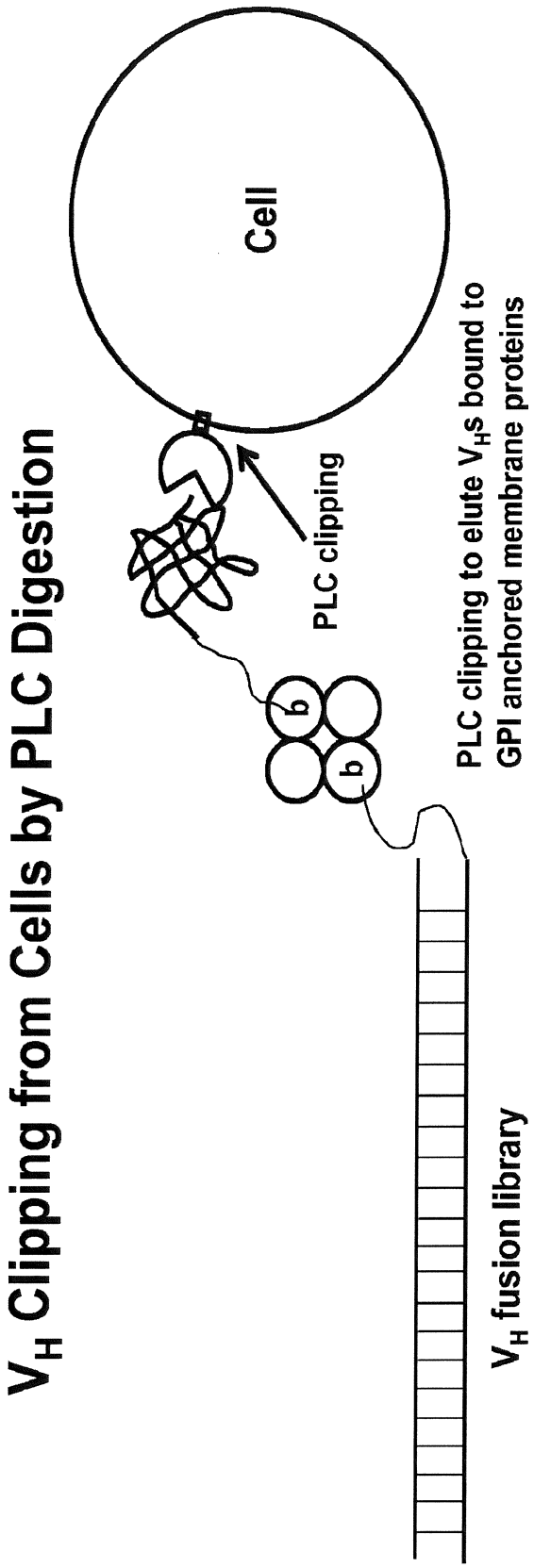
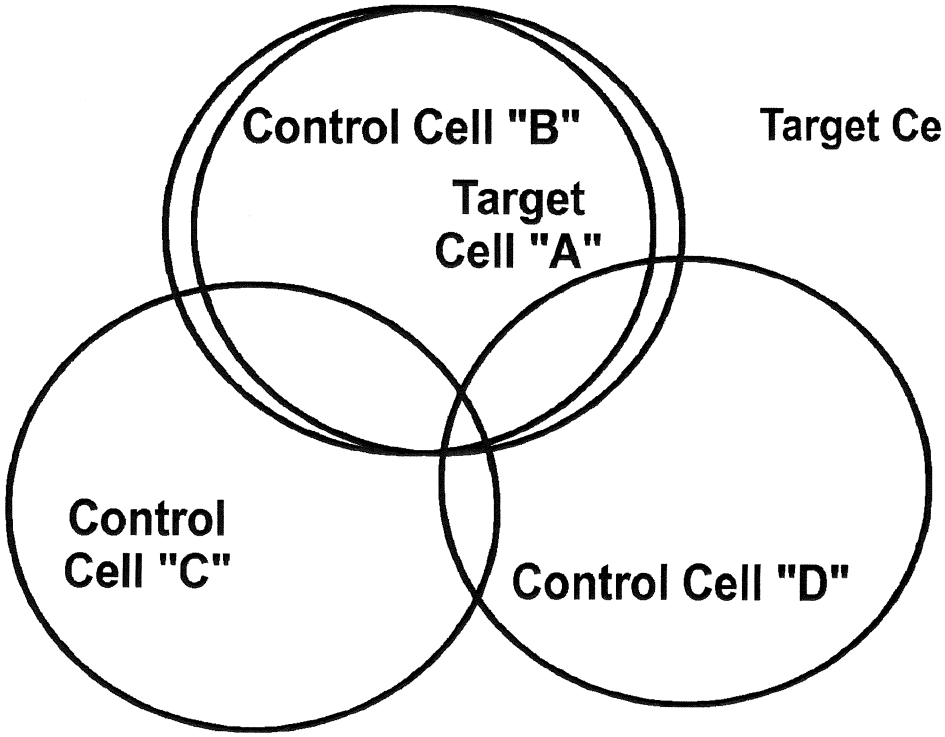

V_H Clipping from Cells by PLC Digestion
Cell
PLC clipping
PLC clipping to elute V_Hs bound to GPI anchored membrane proteins
V_H fusion library
PCR the eluted library
Next round of library production

Fig. 2

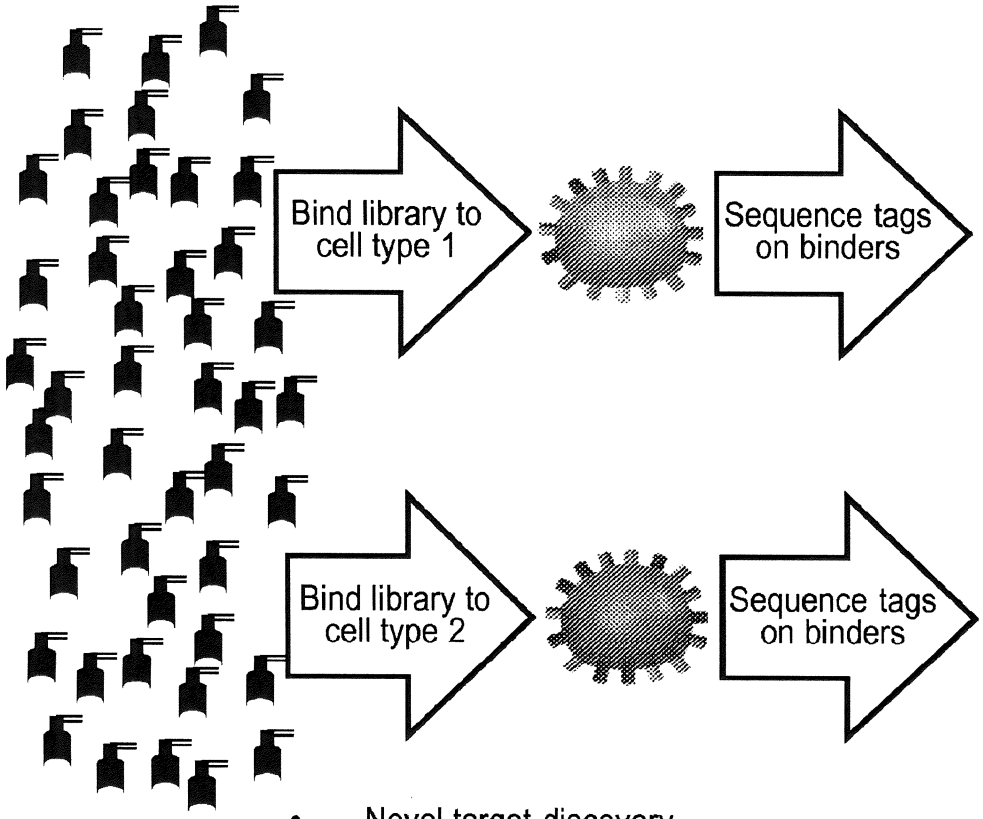
Identification of Binders to Target Cell "A" that do not Bind Control Cells B, C or D



Target Cell "A" is Closely Related to Control Cell B

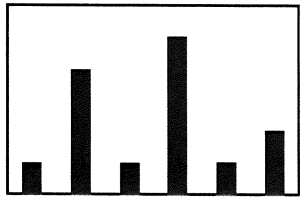
Fig. 3

Live Cell Selection and Deep Sequencing to Identify Target Cell Specific Target Epitopes



Deep Sequencing Results

GATCCCTCGA	CGCCCGGACTC
CGCCCGGACTC	TTCCCGATCCGA
ACGTGTCTACC	AACCCAATCCC
GACACCGGGCCC	GATCCCTCGA
TTCCCGATCCGA	CTACTCCTACC
ACGTGTCTACC	ACGTGTCTACC
CTACTCCTACC	CCTCCTTCGACC
GACACCGGGCCC	CGCCCGGACTC



GATCCCTCGA	CGCCCGGACTC
CGCCCGGACTC	CCGGCTTTCCAA
CCGGCTTTCCAA	AACCCAATCCC
GACACCGGGCCC	CGGCTTCCTGAT
TTCCCGATCCGA	CTACTCCTACC
CCGGCTTTCCAA	CGGCTTCCTGAT
CTACTCCTACC	CCTCCTTCGACC
GACACCGGGCCC	CGCCCGGACTC



Most antibody sequences are retrieved by both cell types, but a few are specific to one type or the other (highlighted)

- Novel target discovery
- Functional discovery (internalization, etc.)
- Enabled by proprietary methods to parse binders on cells with deep sequence analysis

Fig. 4

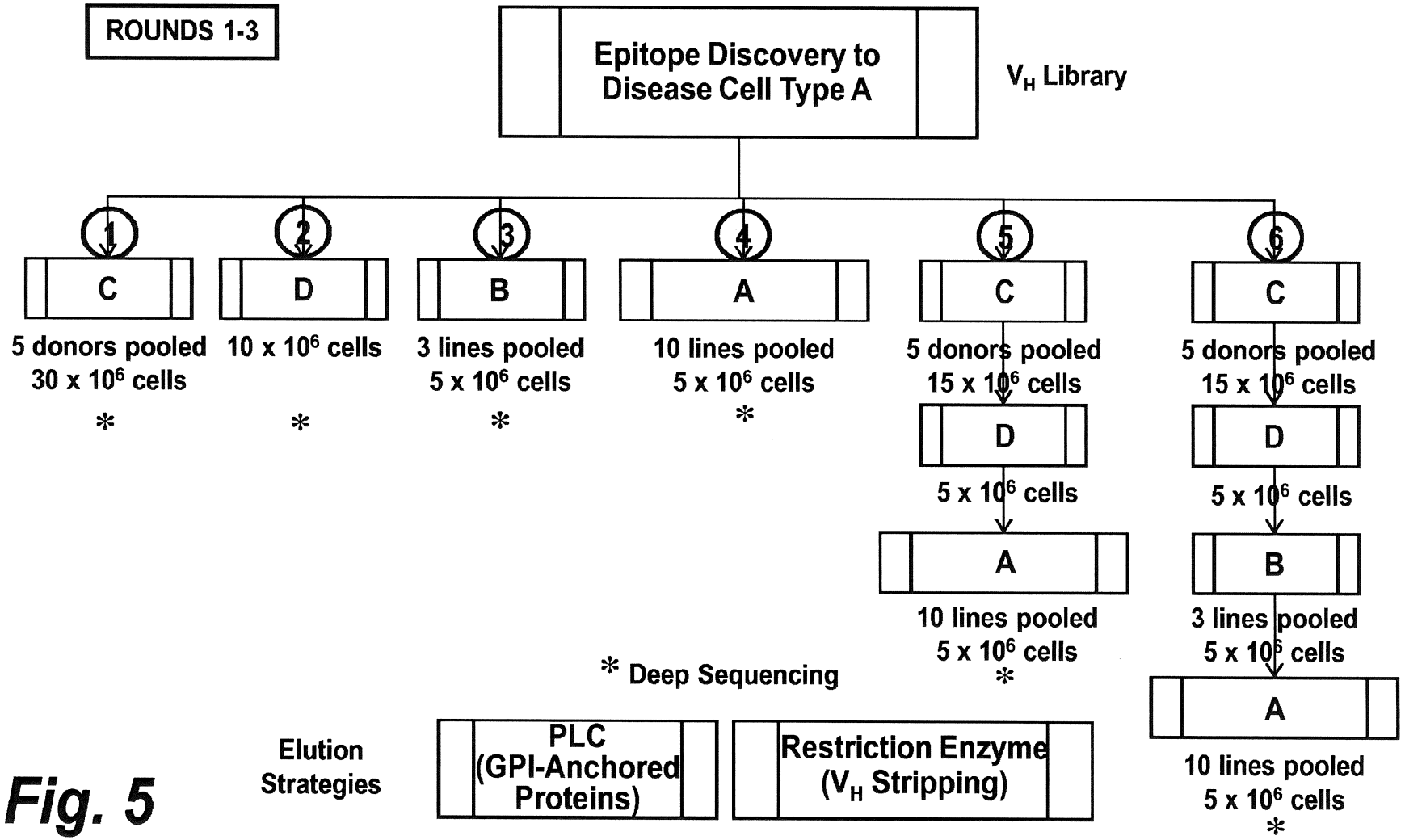

ROUNDS 1-3
Epitope Discovery to Disease Cell Type A
V_H Library
1
2
3
4
5
6
C
D
B
A
C
C
5 donors pooled
30 x 10^6 cells
10 x 10^6 cells
3 lines pooled
5 x 10^6 cells
10 lines pooled
5 x 10^6 cells
5 donors pooled
15 x 10^6 cells
5 donors pooled
15 x 10^6 cells
D
D
5 x 10^6 cells
5 x 10^6 cells
A
B
10 lines pooled
5 x 10^6 cells
3 lines pooled
5 x 10^6 cells
A
10 lines pooled
5 x 10^6 cells
* Deep Sequencing
Elution Strategies
PLC (GPI-Anchored Proteins)
Restriction Enzyme (V_H Stripping)

Fig. 5

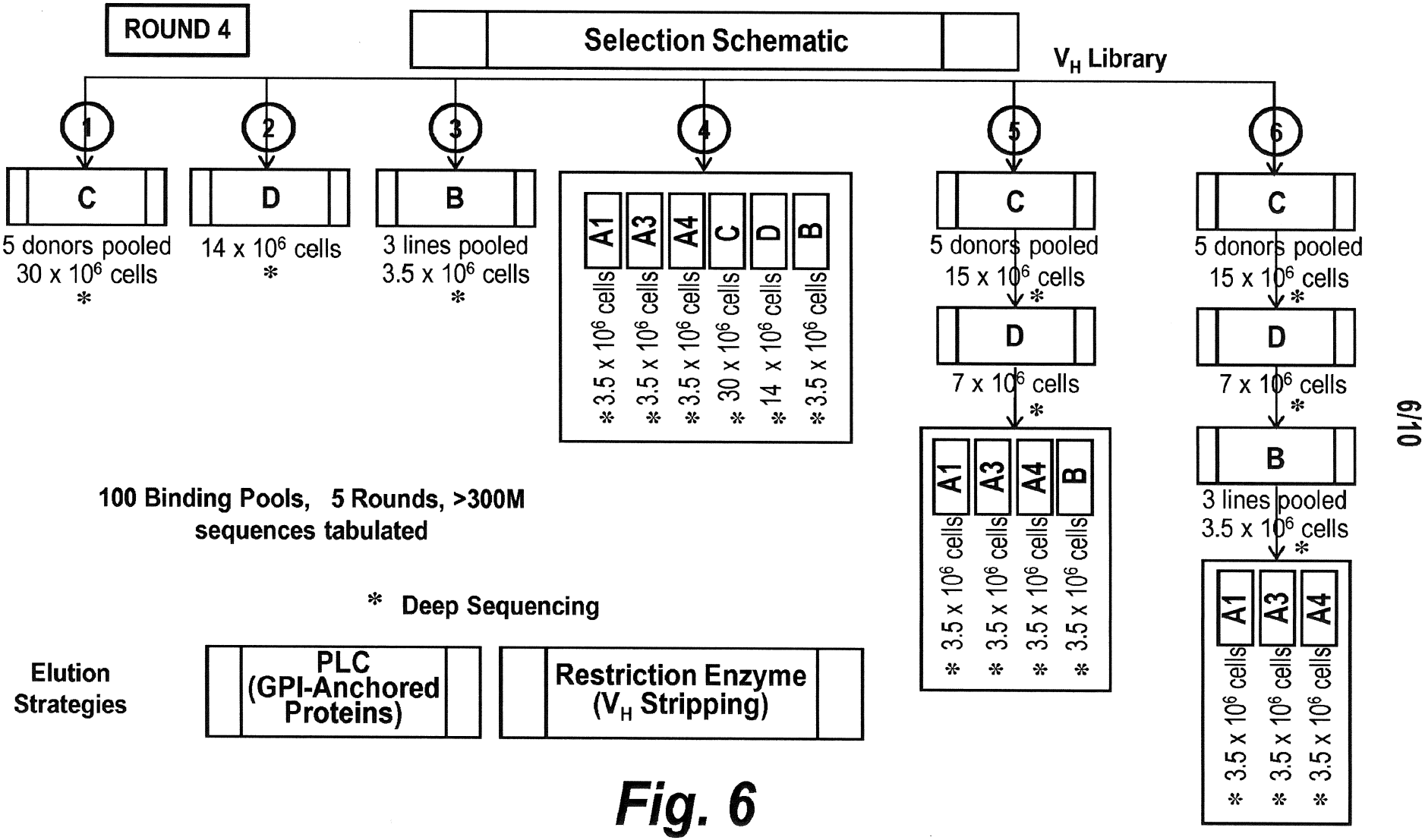
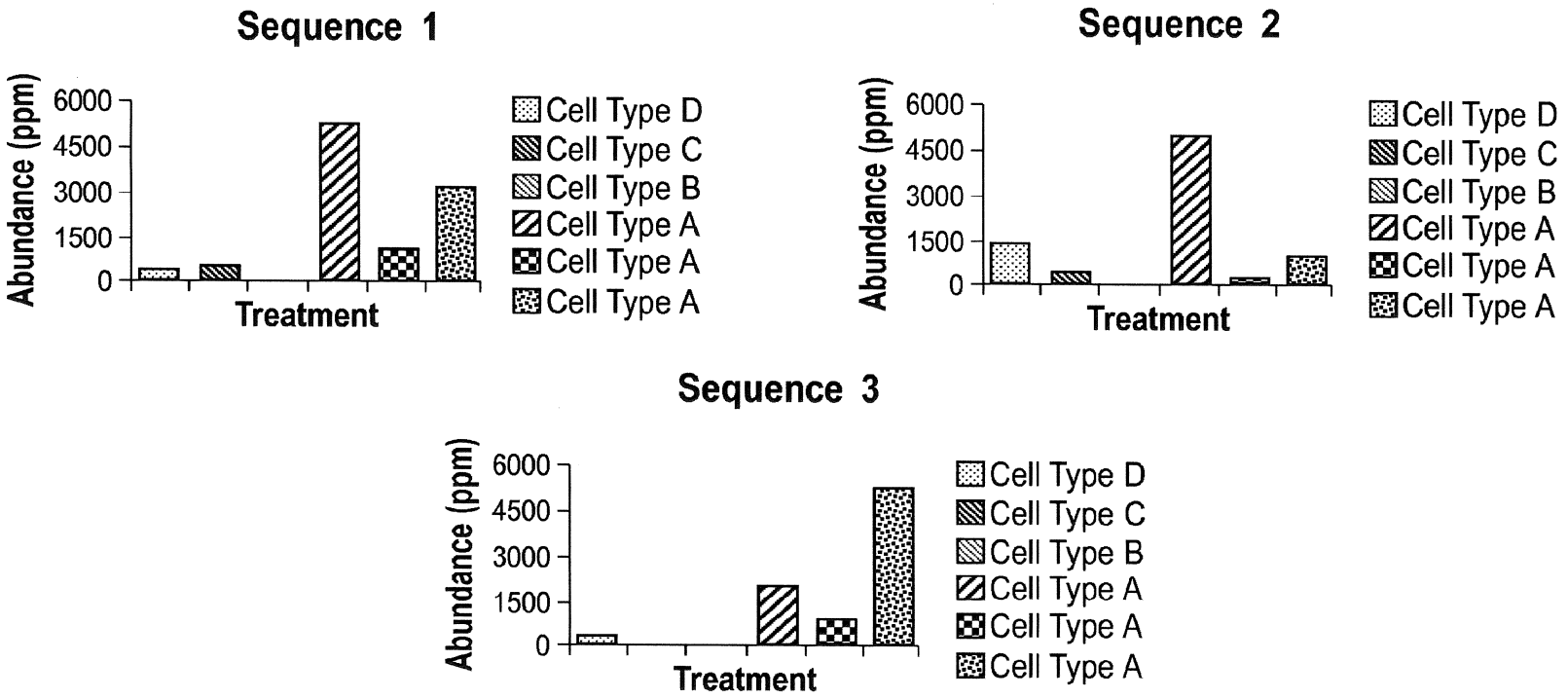

ROUND 4
Selection Schematic
V_H Library
1
2
3
4
5
6
C
D
B
A1
A3
A4
C
D
B
5 donors pooled
30 x 10^6 cells
*
14 x 10^6 cells
*
3 lines pooled
3.5 x 10^6 cells
*
* 3.5 x 10^6 cells
* 3.5 x 10^6 cells
* 3.5 x 10^6 cells
* 30 x 10^6 cells
* 14 x 10^6 cells
* 3.5 x 10^6 cells
5 donors pooled
15 x 10^6 cells
*
7 x 10^6 cells
*
* 3.5 x 10^6 cells
* 3.5 x 10^6 cells
* 3.5 x 10^6 cells
* 3.5 x 10^6 cells
5 donors pooled
15 x 10^6 cells
*
7 x 10^6 cells
*
3 lines pooled
3.5 x 10^6 cells
*
* 3.5 x 10^6 cells
* 3.5 x 10^6 cells
* 3.5 x 10^6 cells
100 Binding Pools, 5 Rounds, >300M sequences tabulated
* Deep Sequencing
Elution Strategies
PLC (GPI-Anchored Proteins)
Restriction Enzyme (V_H Stripping)

Fig. 6

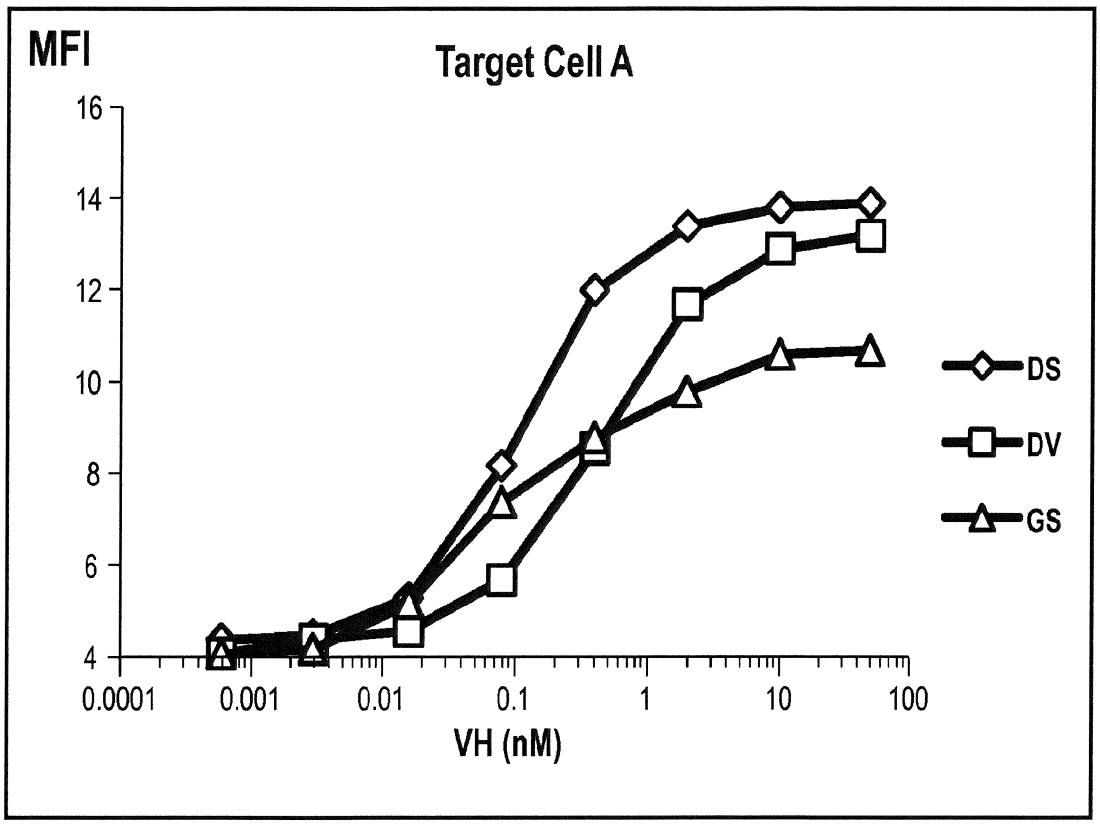
Deep Sequence Analysis Predicts Cell Binding Specificity of Selected V_Hs



These clones enrich through 4 rounds of selection and predict binding selectivity to Cell Type A

Fig. 7

High Affinity Binding of V_Hs to Cell Type A



Clone	EC_{50} (pM)
DS	110
DV	430
GS	85

Fig. 8

Selections Yielded Specific Binders to Each Closely Related Cell Type

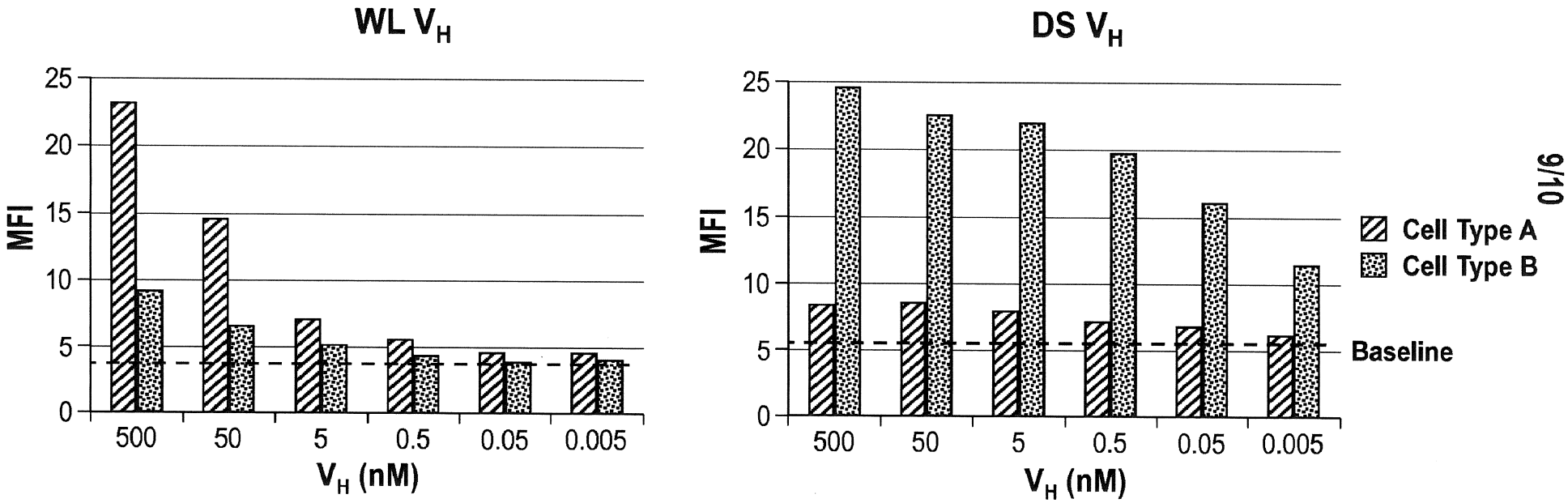


Fig. 9

Three Distinct V_Hs That Bind Selectively to Target Cell A, and not Control Cells B, C and D

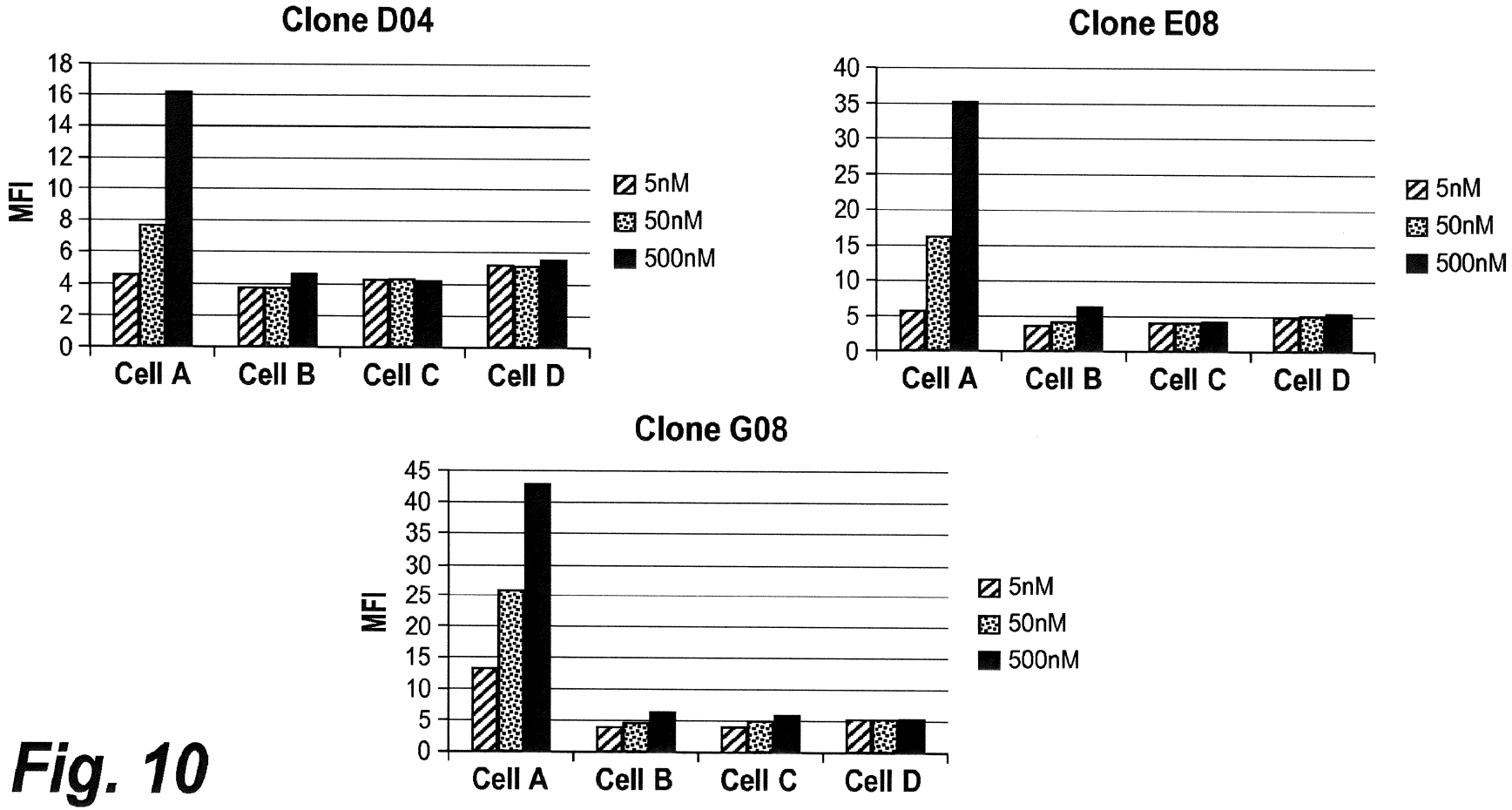


Fig. 10