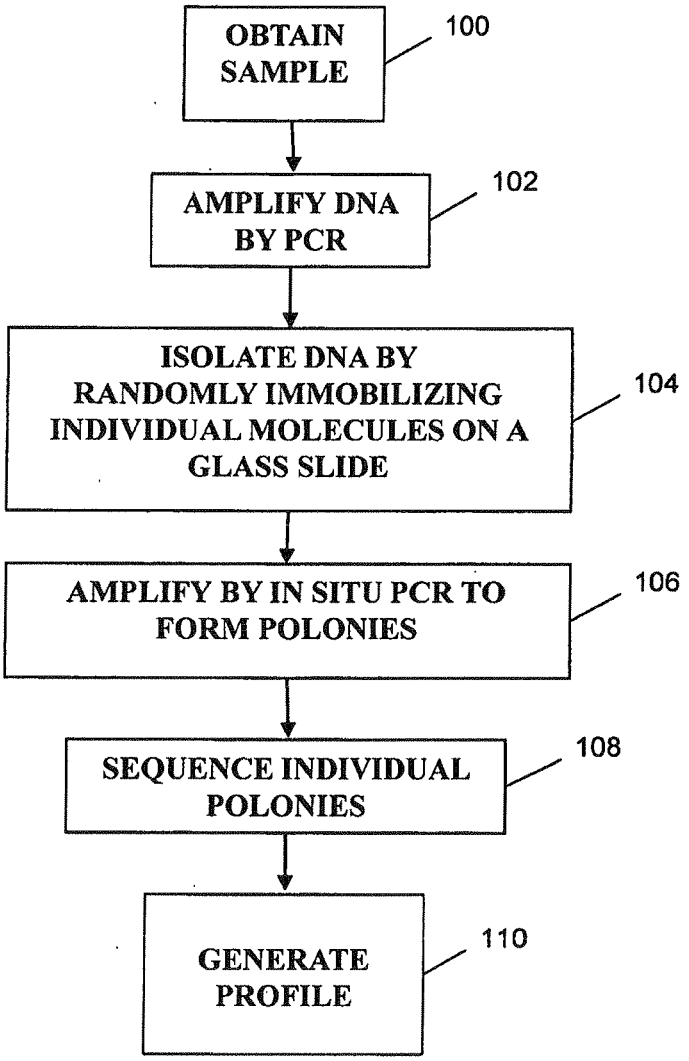

OBTAIN SAMPLE
100
AMPLIFY DNA BY PCR
102
ISOLATE DNA BY RANDOMLY IMMOBILIZING INDIVIDUAL MOLECULES ON A GLASS SLIDE
104
AMPLIFY BY IN SITU PCR TO FORM POLONIES
106
SEQUENCE INDIVIDUAL POLONIES
108
GENERATE PROFILE
110

Fig. 1A

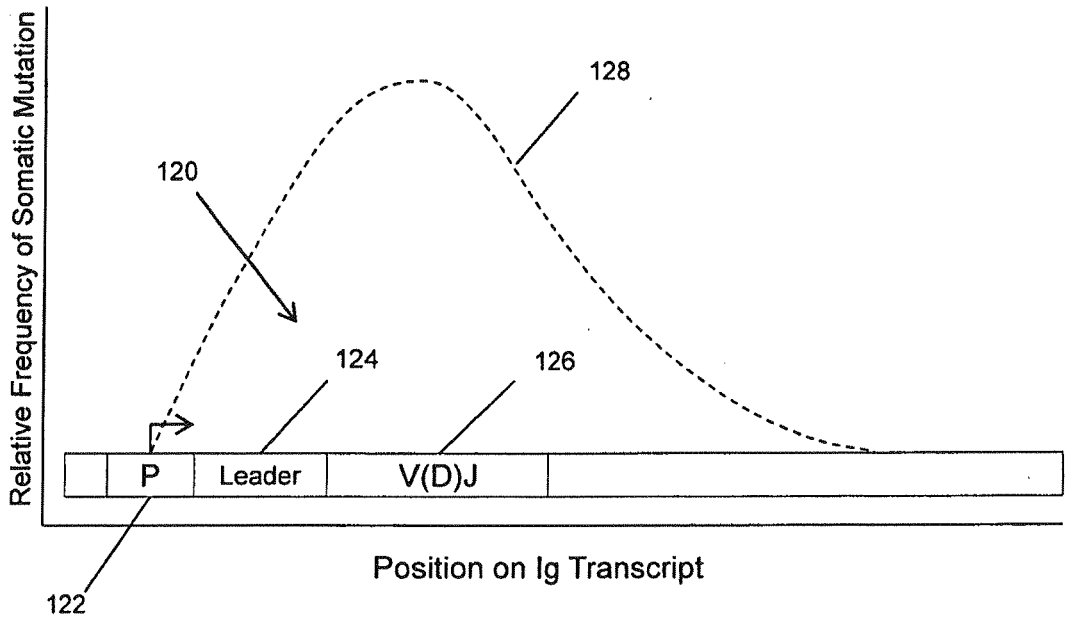

Relative Frequency of Somatic Mutation
128
120
124
126
P
Leader
V(D)J
122
Position on Ig Transcript

Fig. 1B

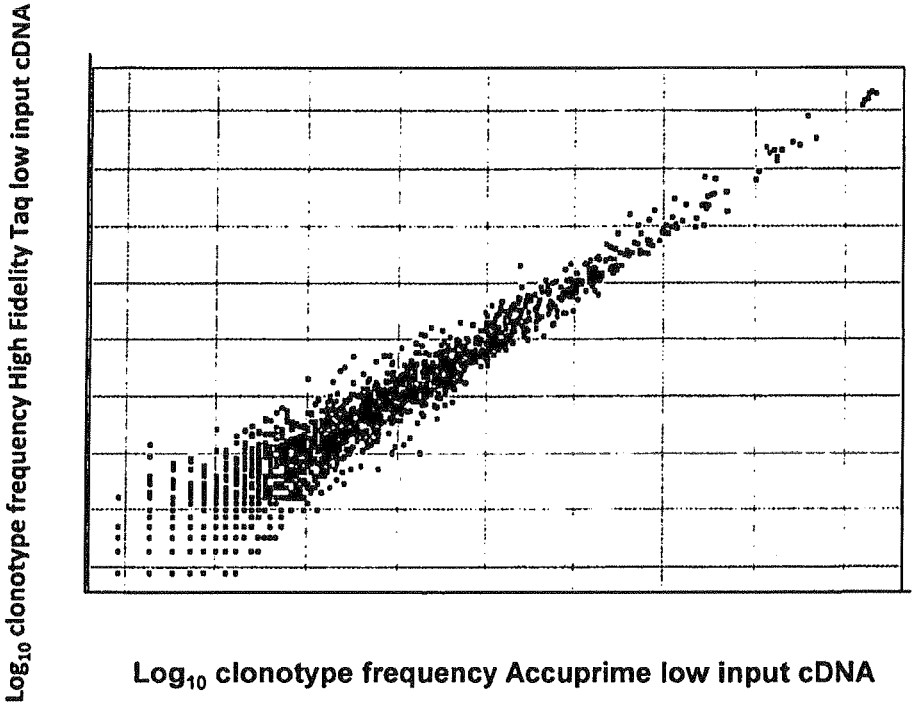

Log10 clonotype frequency High Fidelity Taq low input cDNA
Log10 clonotype frequency Accuprime low input cDNA

Fig. 7C

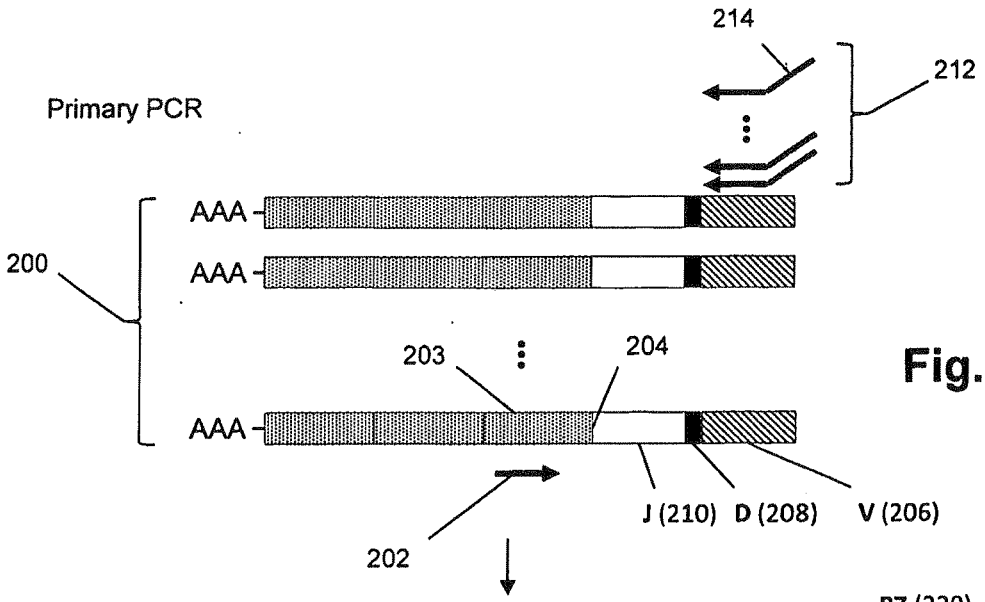


Fig. 2A

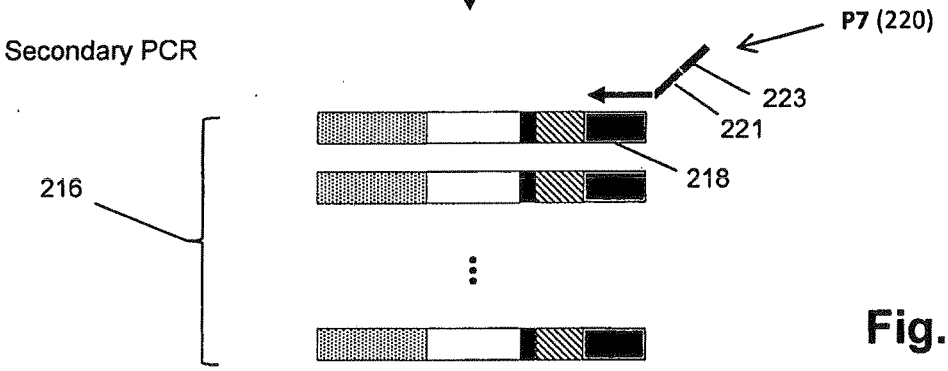


Fig. 2B

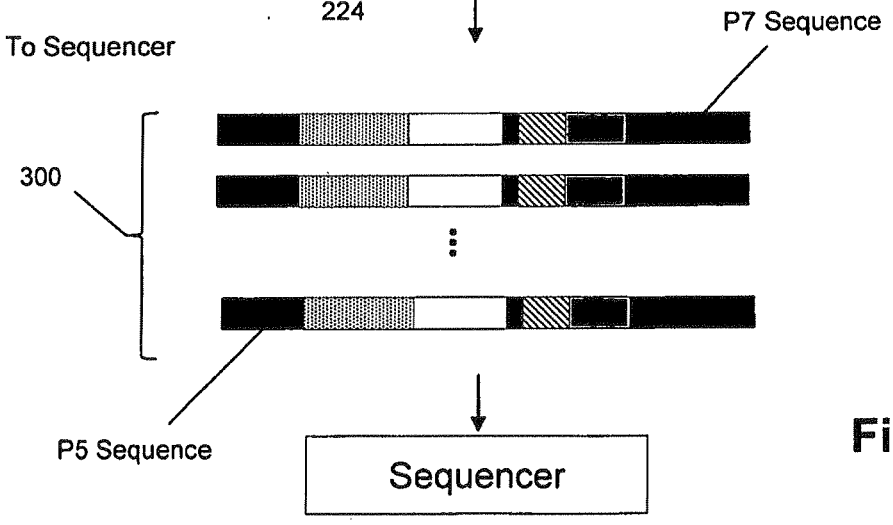


Fig. 3A

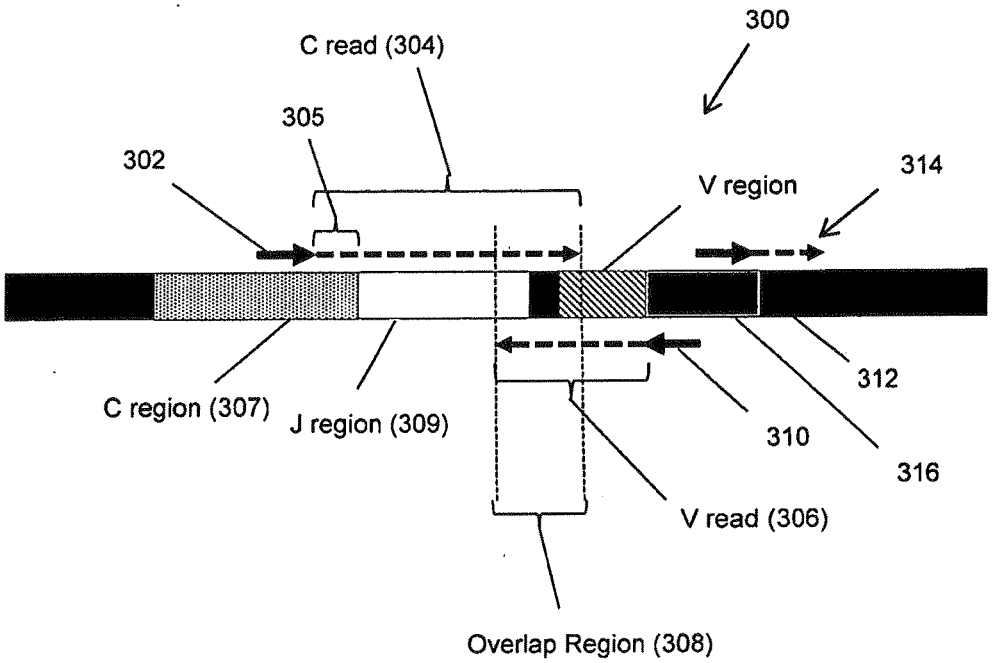

300
C read (304)
305
302
V region
314
C region (307)
J region (309)
312
310
316
V read (306)
Overlap Region (308)

Fig. 3B

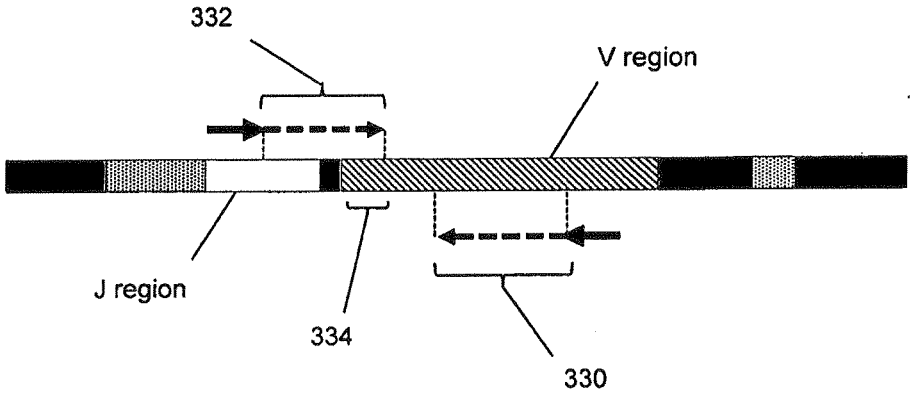

332
V region
J region
334
330

Fig. 3C

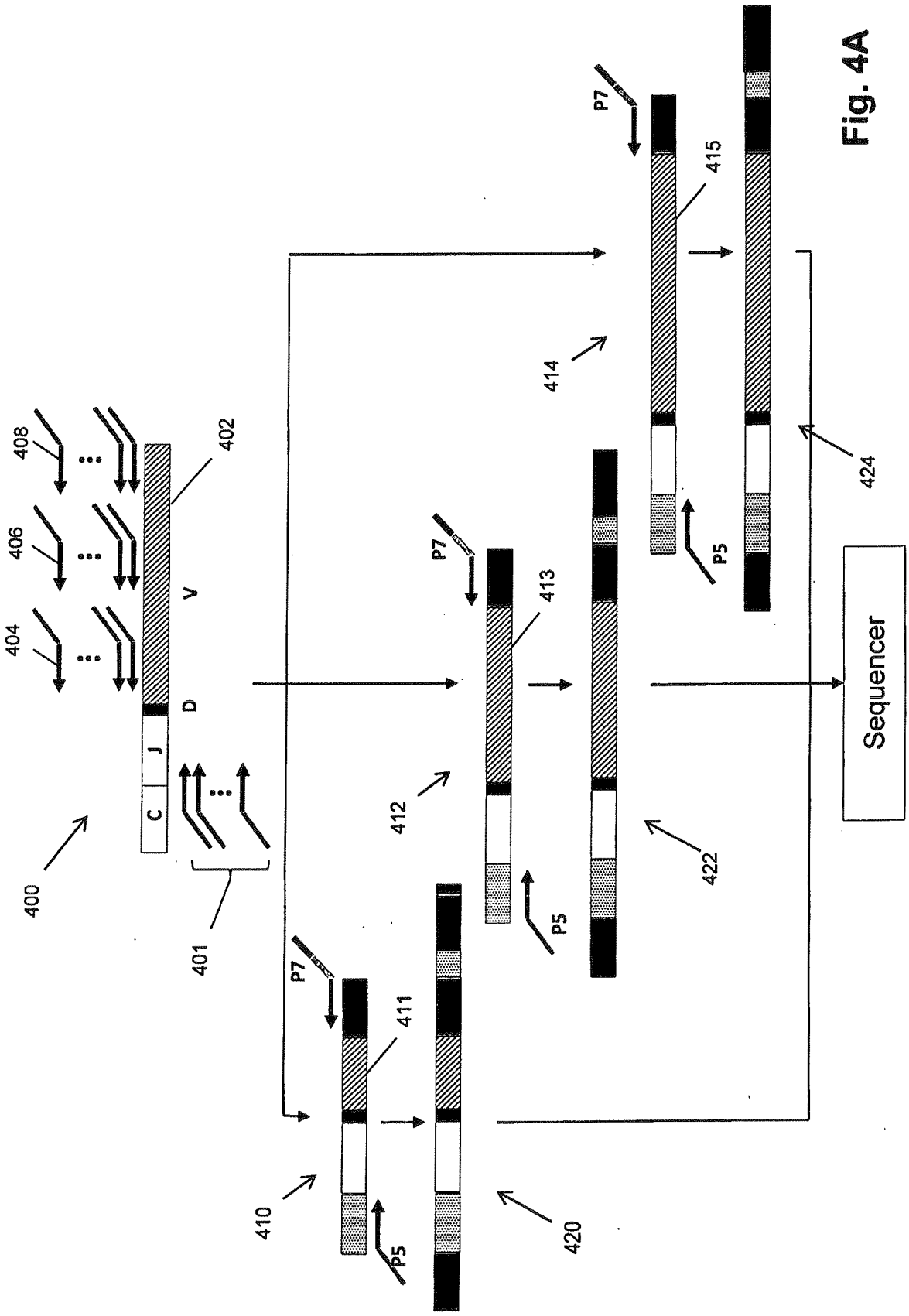

400
401
402
404
406
408
V
D
J
C
410
411
412
413
414
415
420
422
424
P5
P7
Sequencer

Fig. 4A

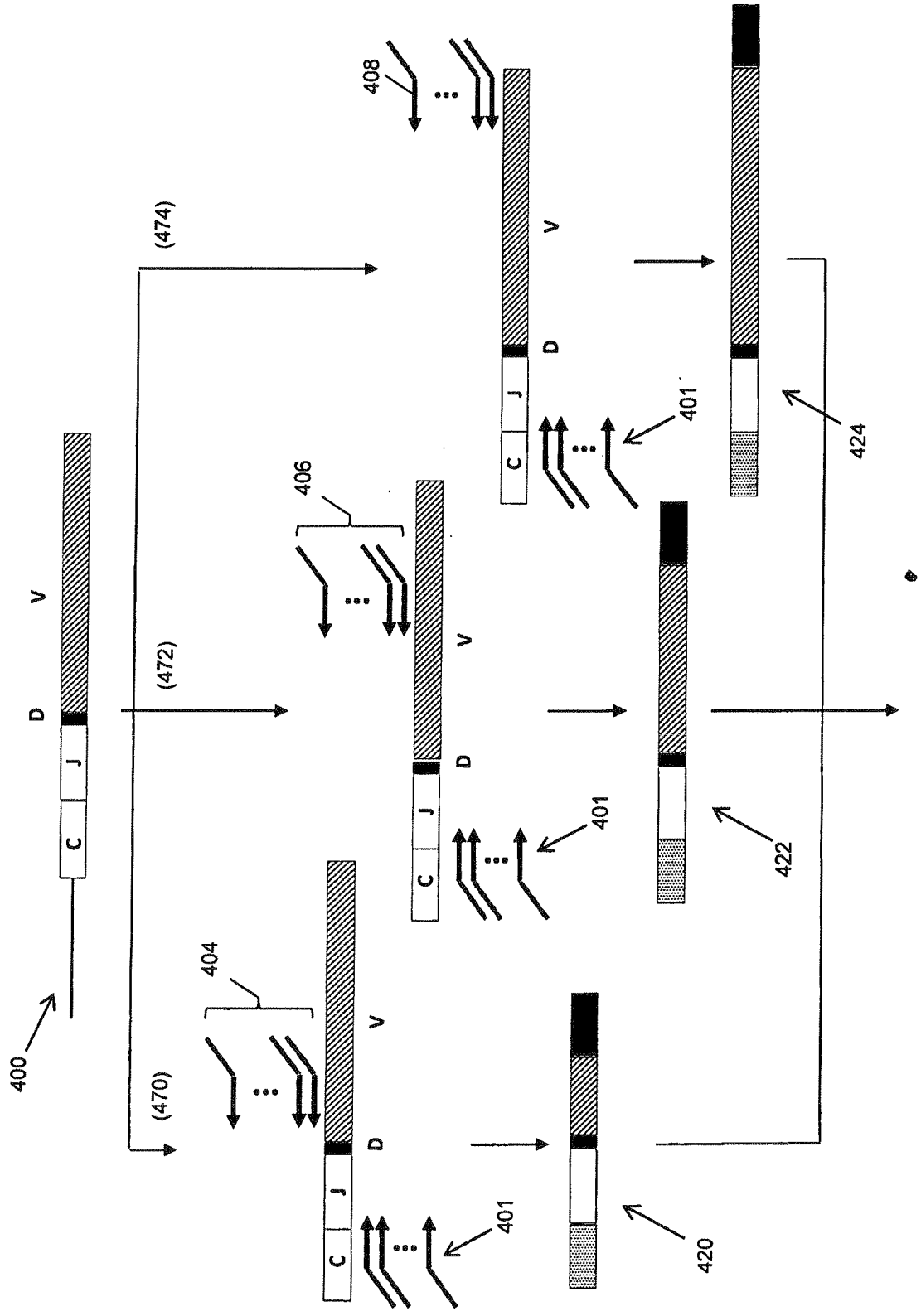
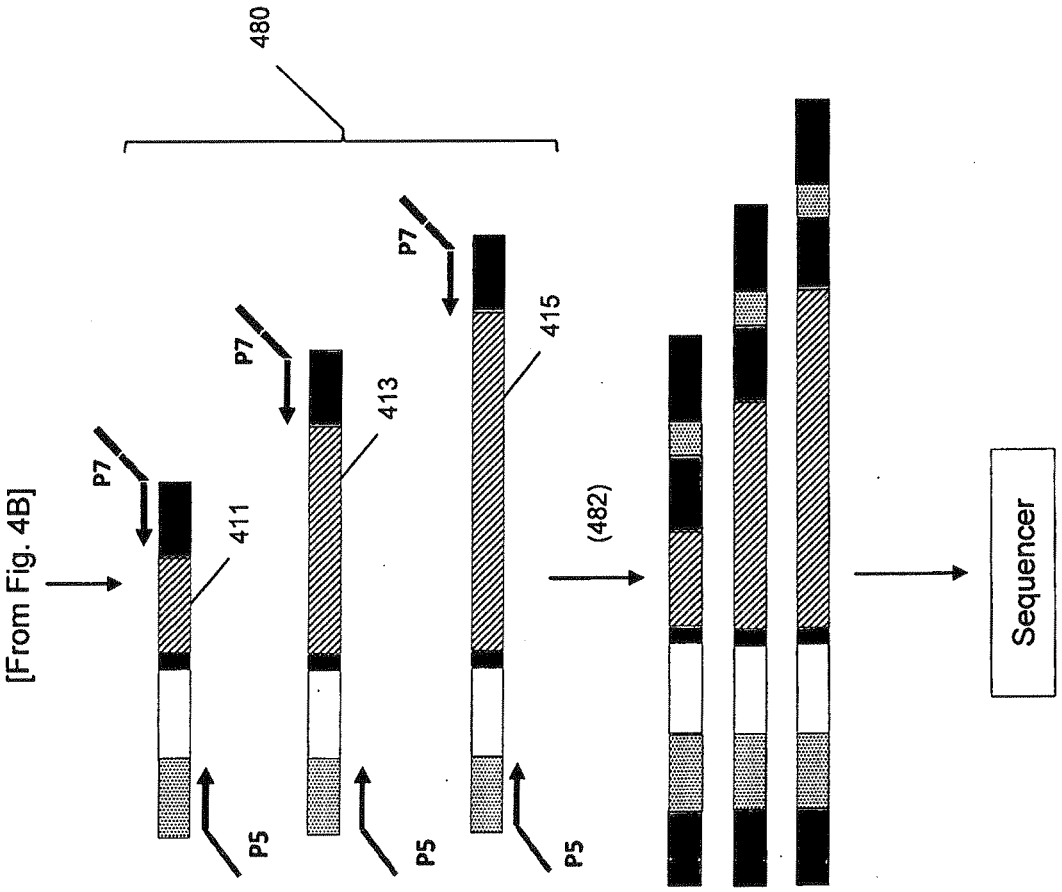


Fig. 4B

Fig. 4C



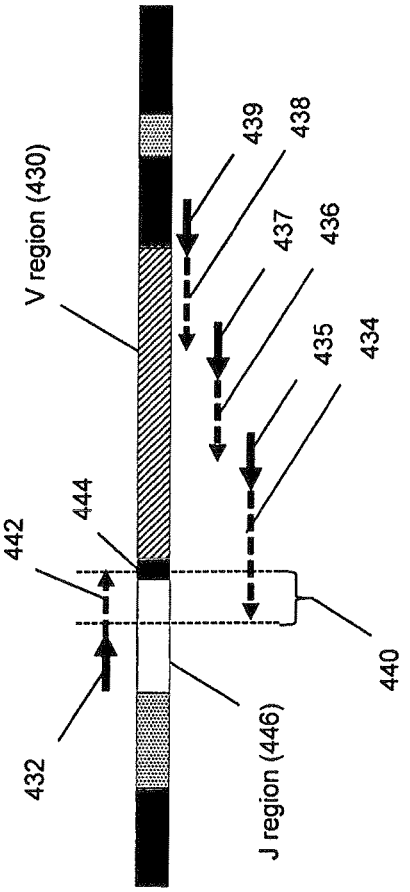

V region (430)
439
438
437
436
435
434
442
444
440
432
J region (446)

Fig. 4D

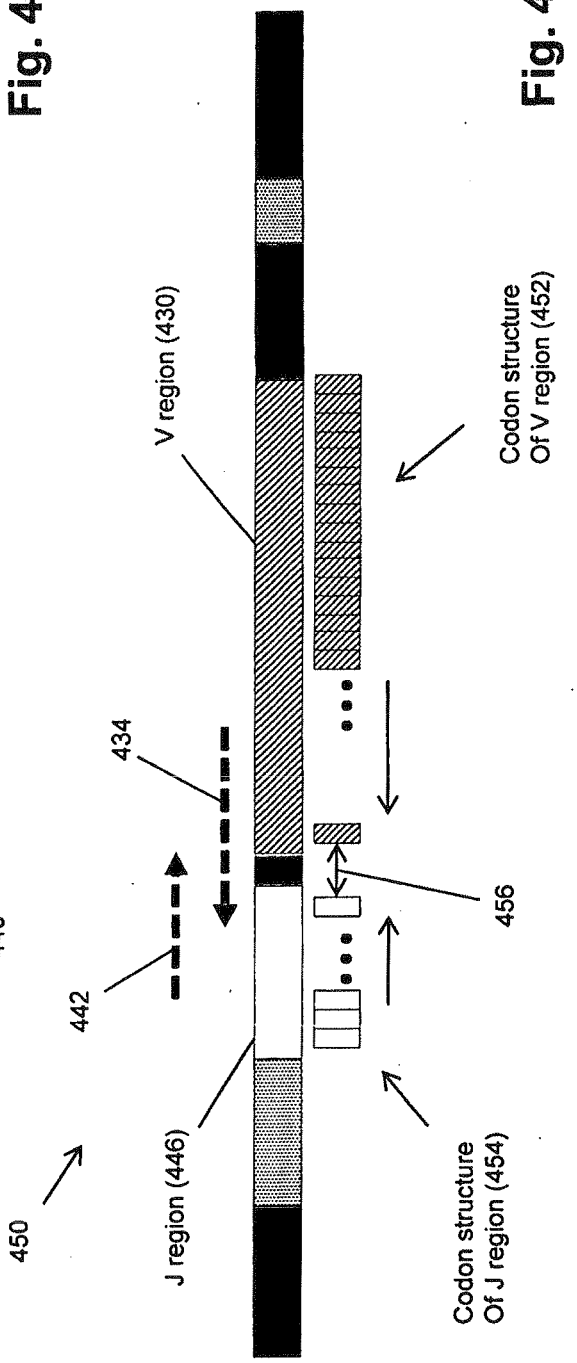

V region (430)
Codon structure
Of V region (452)
434
442
456
450
J region (446)
Codon structure
Of J region (454)

Fig. 4E

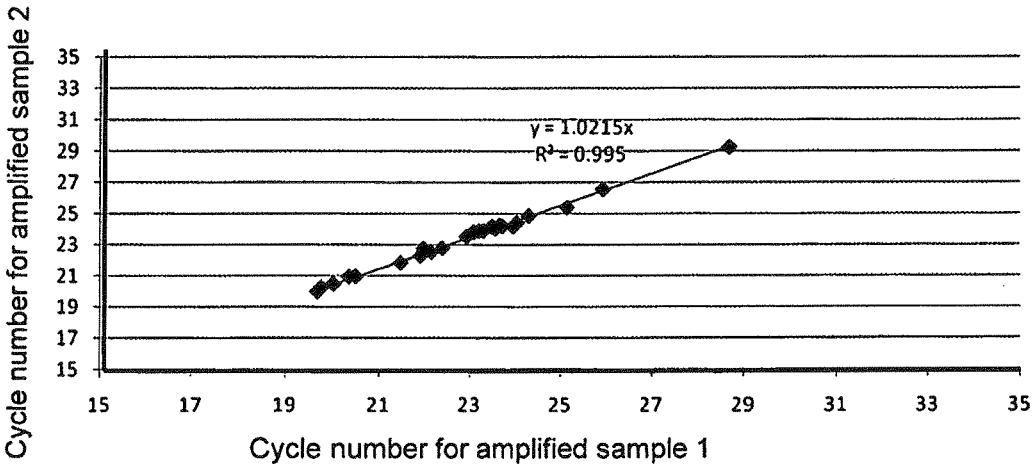

Cycle number for amplified sample 2
35
33
31
29
27
25
23
21
19
17
15
y = 1.0215x
R² = 0.995
15
17
19
21
23
25
27
29
31
33
35
Cycle number for amplified sample 1

Fig. 5

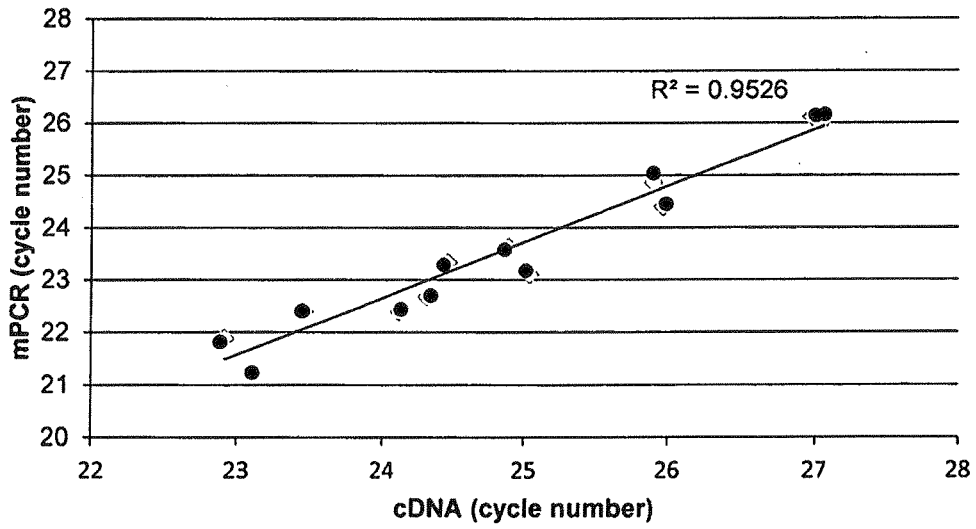

mPCR (cycle number)
28
27
26
25
24
23
22
21
20
R² = 0.9526
22
23
24
25
26
27
28
cDNA (cycle number)

Fig. 6

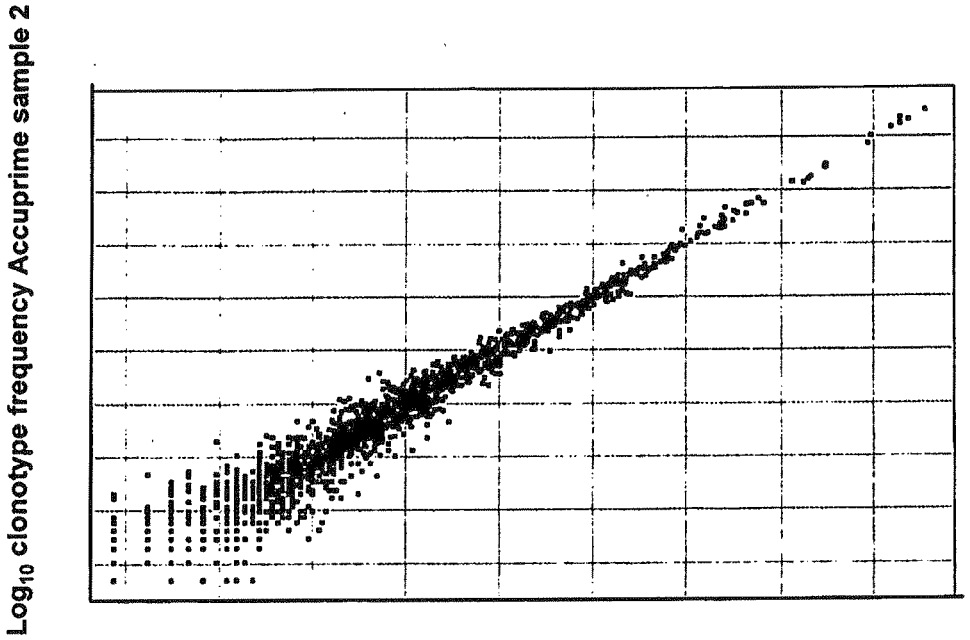

Log10 clonotype frequency Accuprime sample 2
Log10 clonotype frequency Accuprime sample 1

Fig. 7A

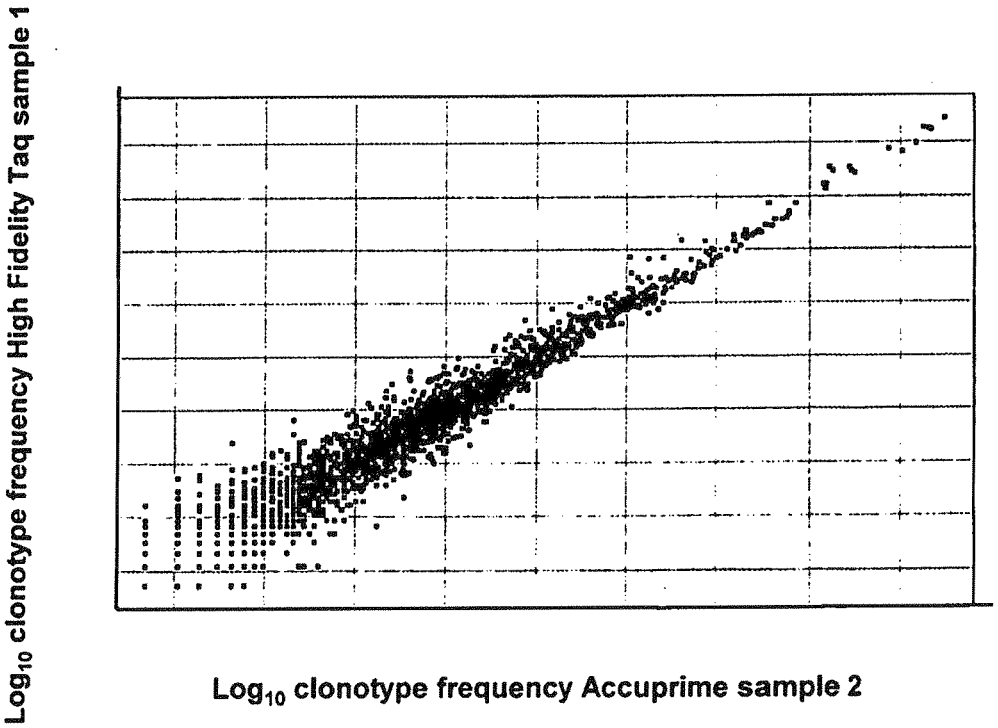
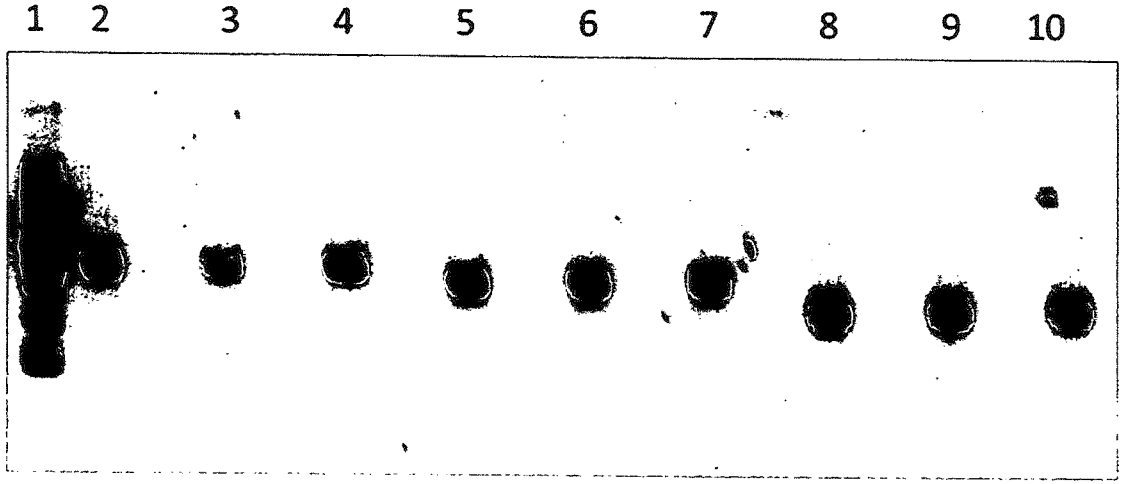
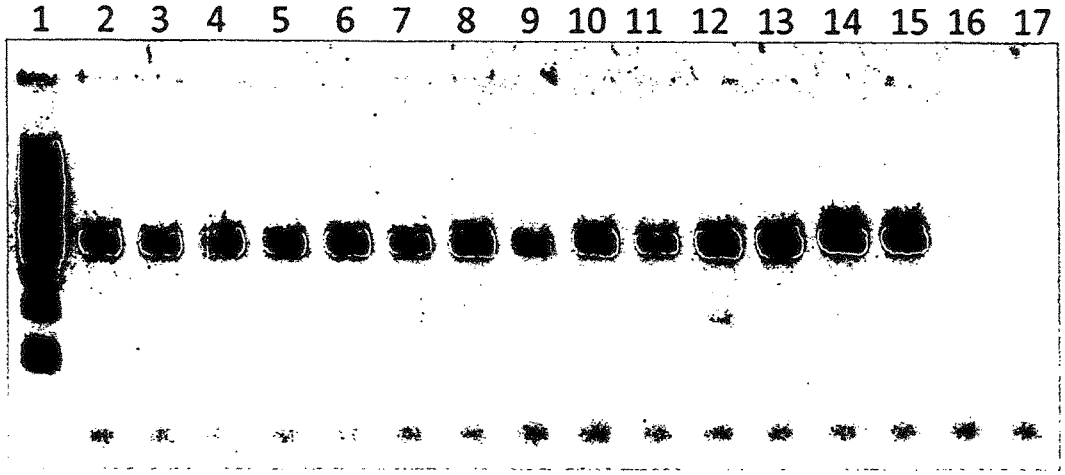

Log10 clonotype frequency High Fidelity Taq sample 1
Log10 clonotype frequency Accuprime sample 2

Fig. 7B



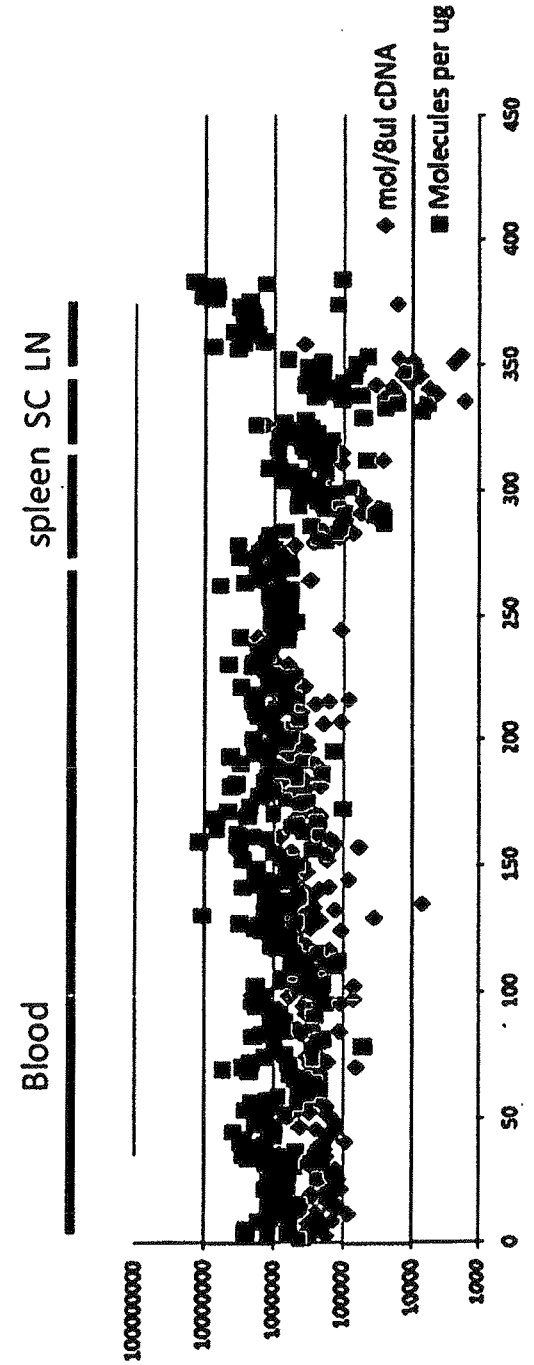
Amplification products after 2 stage PCRs with the 3 sets of V segment primers in stage 1 are shown. All use the J primers in the first stage. Lane 1 is the 100 bp size ladder (NEB), 2-4 amplification with primer set A, 5-7 with primer set B, and 8-10 with primer set C. The 3 different lanes for each primer set is for 3 different V segment primer concentrations. Since the second stage PCR primers are the same for all three pools, we have also shown that three products of the first stage PCR can be pooled to be amplified in the second stage PCR.

Fig. 8A



Amplification products after 2 stage PCRs with the J primers and the A set of V segment primers in stage 1 are shown. Lane 1 is the 100 bp size ladder (NEB), 2-9 use different amount of input blood genomic DNA (lane2-3: 250 ng, 4-5 and 10-11: 500 ng, 6-7: 1µg, and 8-9: 2.5 µg). Lanes 12-13 use 250 ng of spleen genomic DNA. Lanes 14-15 use 250 ng of the Raji B cell line (positive control), and lane 16-17 use 250 ng of Jurkat T cell line (negative control). The two reactions for each genomic DNA concentration use different primer concentrations.

Fig. 8B



Real time PCR using standards with known numbers of molecules were used to determine the number of TCRß molecules in the input DNA. The dark colored dots depict the number of molecules that were ultimately amplified in the multiplex PCR, and the light colored dots show the number of molecules per µg RNA assuming a perfect 1:1 relationship between RNA and cDNA. (SC is spinal cord and LN is lymph node).

Fig. 9

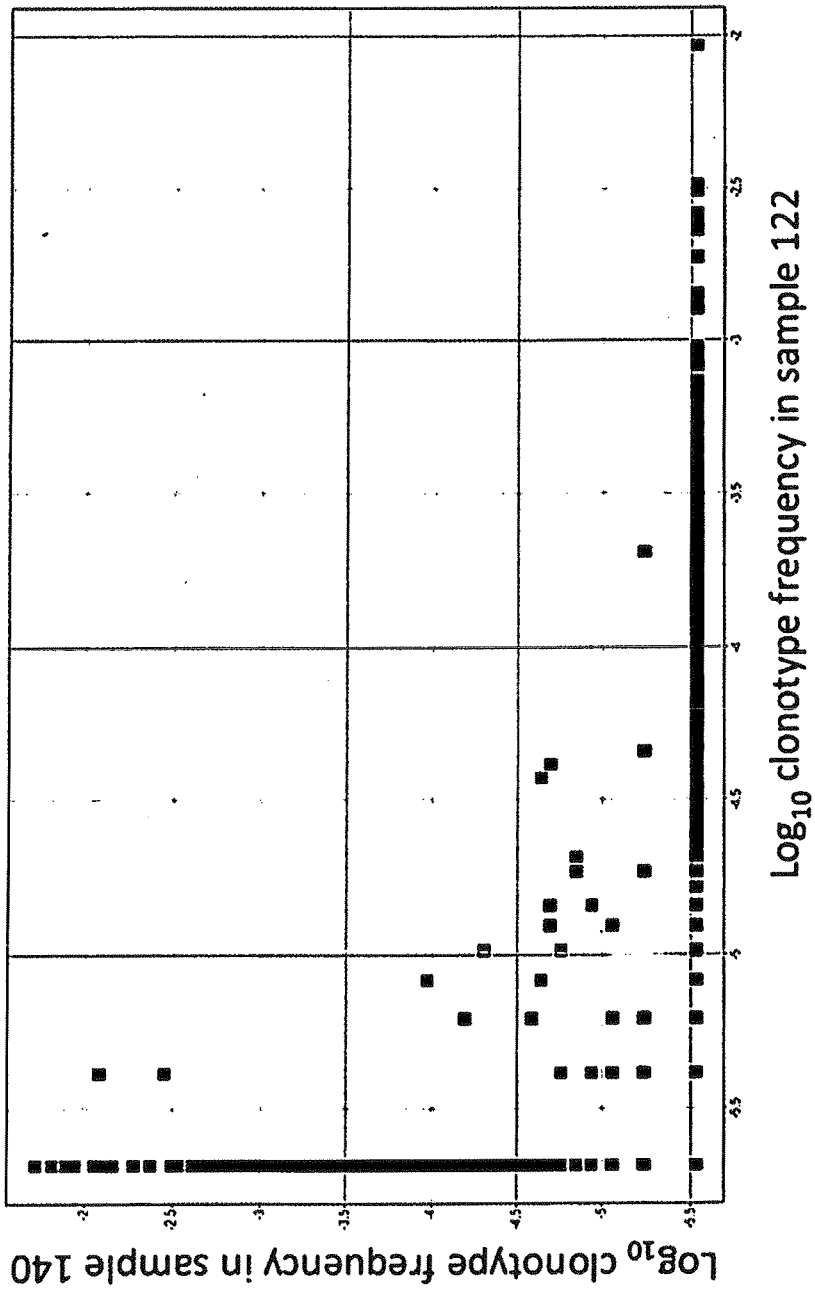

Log10 clonotype frequency in sample 140
Log10 clonotype frequency in sample 122

Fig. 10