FIG. 1

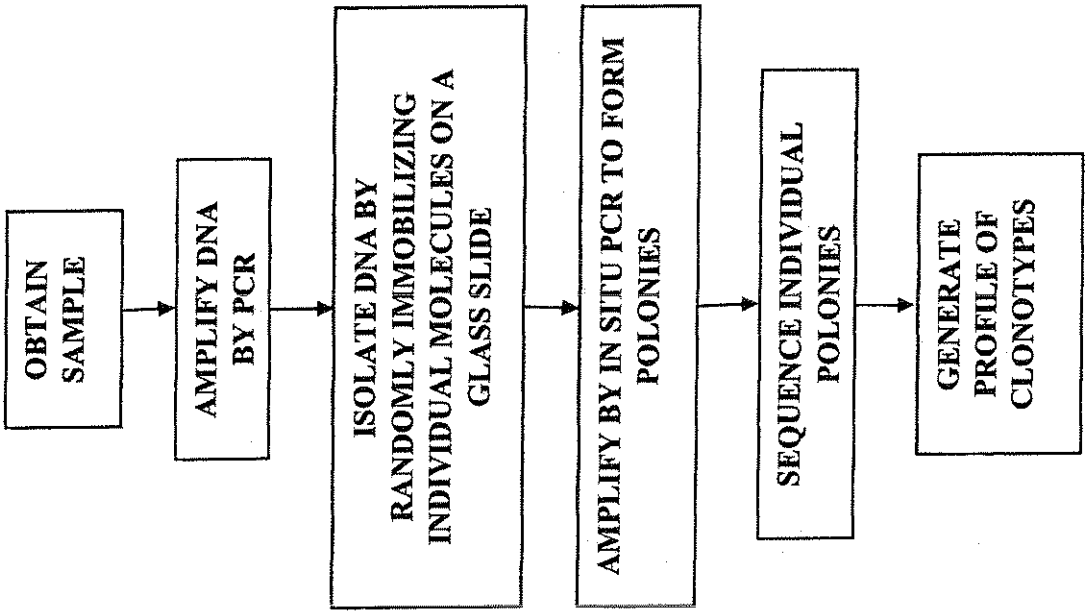

OBTAIN SAMPLE
AMPLIFY DNA BY PCR
ISOLATE DNA BY RANDOMLY IMMOBILIZING INDIVIDUAL MOLECULES ON A GLASS SLIDE
AMPLIFY BY IN SITU PCR TO FORM POLONIES
SEQUENCE INDIVIDUAL POLONIES
GENERATE PROFILE OF CLONOTYPES

FIG. 2

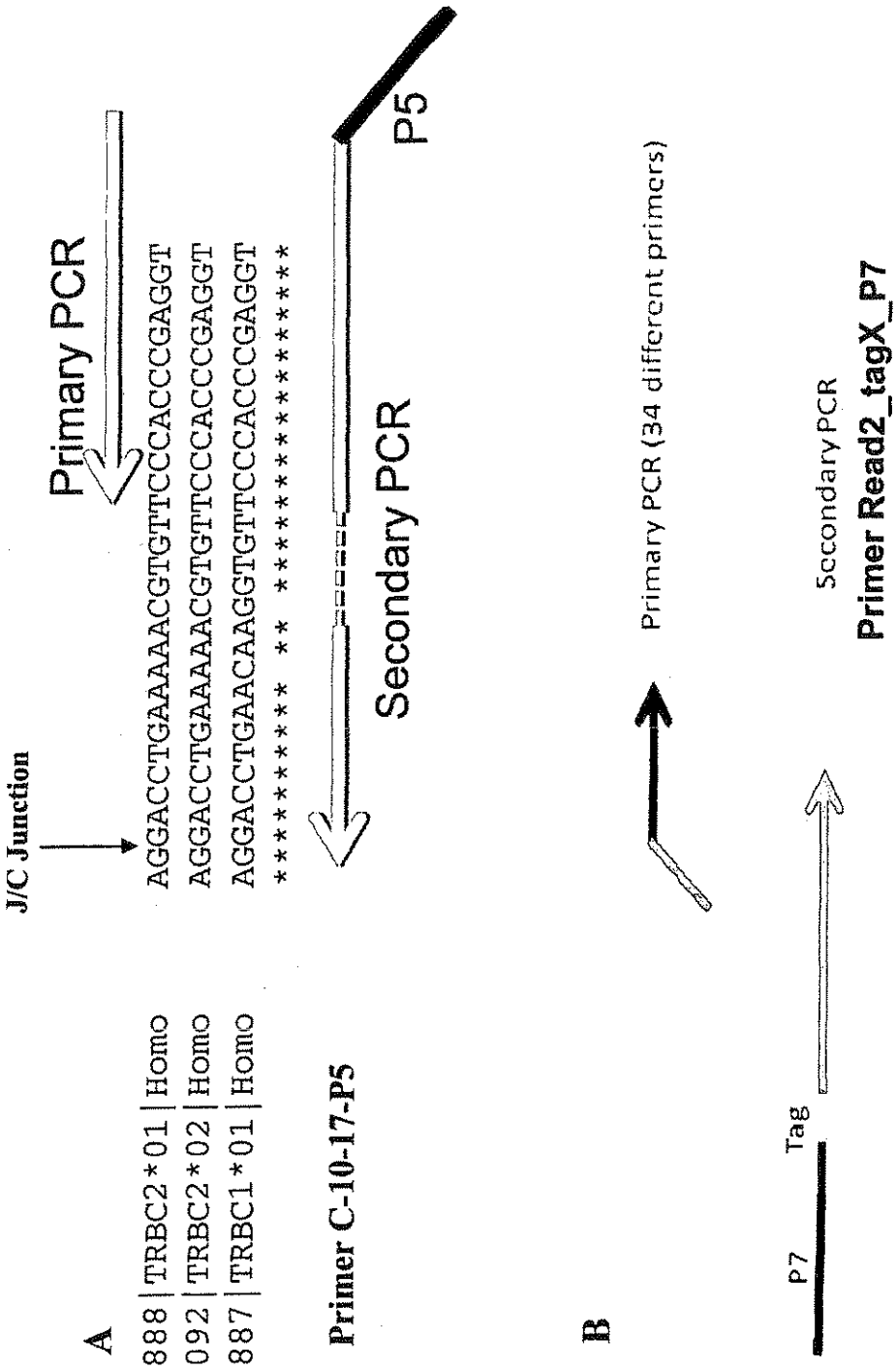

A
J/C Junction
M12888|TRBC2*01|Homo
L36092|TRBC2*02|Homo
M12887|TRBC1*01|Homo
AGGACCTGAAAAACGTGTTCCCACCCGAGGT
AGGACCTGAAAAACGTGTTCCCACCCGAGGT
AGGACCTGAACAAGGTGTTCCCACCCGAGGT
********** ** **********
Primer C-10-17-P5
Primary PCR
Secondary PCR
P5
B
Primary PCR (34 different primers)
Sccondary PCR
Primer Read2_tagX_P7
P7
Tag

FIG. 3



FIG. 4

A. BCR amplification- C side

Primary PCR

```
J00230|IGHG2*01|Homo    -CCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAG 49
AJ250170|IGHG2*02|Homo  -CCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAG 49
AL928742|IGHG2*06|Homo  -CCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAG 49
AF449617|IGHG2*04|Homo  -CCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAG 49
K01316|IGHG4*01|Homo    -CTTCCACCAAGGGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGAG 49
AL928742|IGHG4*04|Homo  -CTTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAG 49
                         * ********************* ********** **** ***** *** ***
```

Secondary PCR

P5

Sequencing primer

Cluster formation

FIG. 4 cont.
B. BCR amplification- V side
~-255
~-160
~-30
V segment
D seg.
J segment
Common 14 bp sequence
23 primers
33 primers
32 primers
Primary PCR
Secondary PCR
P7
Tag
Cluster formation
Sequencing primer

FIG. 5

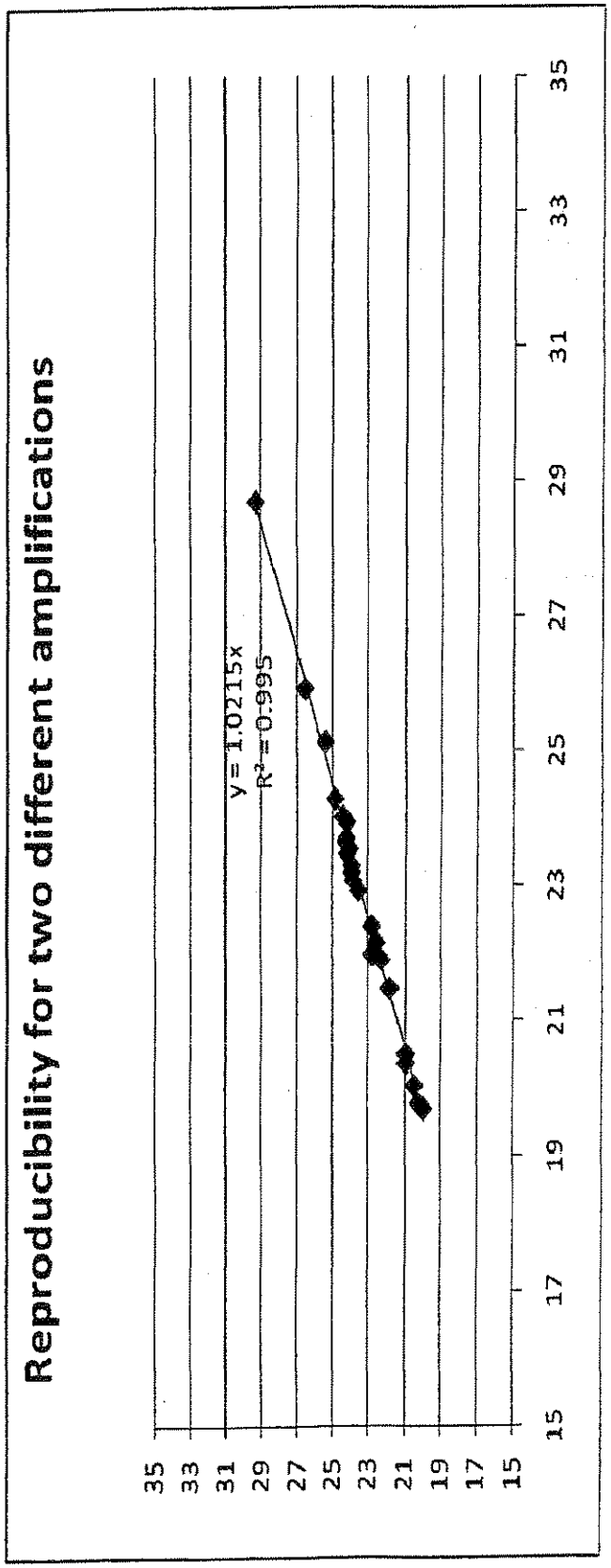

Reproducibility for two different amplifications
y = 1.0215x
R² = 0.995
15
17
19
21
23
25
27
29
31
33
35
Cycle number for amplified sample 1
Cycle number for amplified sample 2

FIG. 6

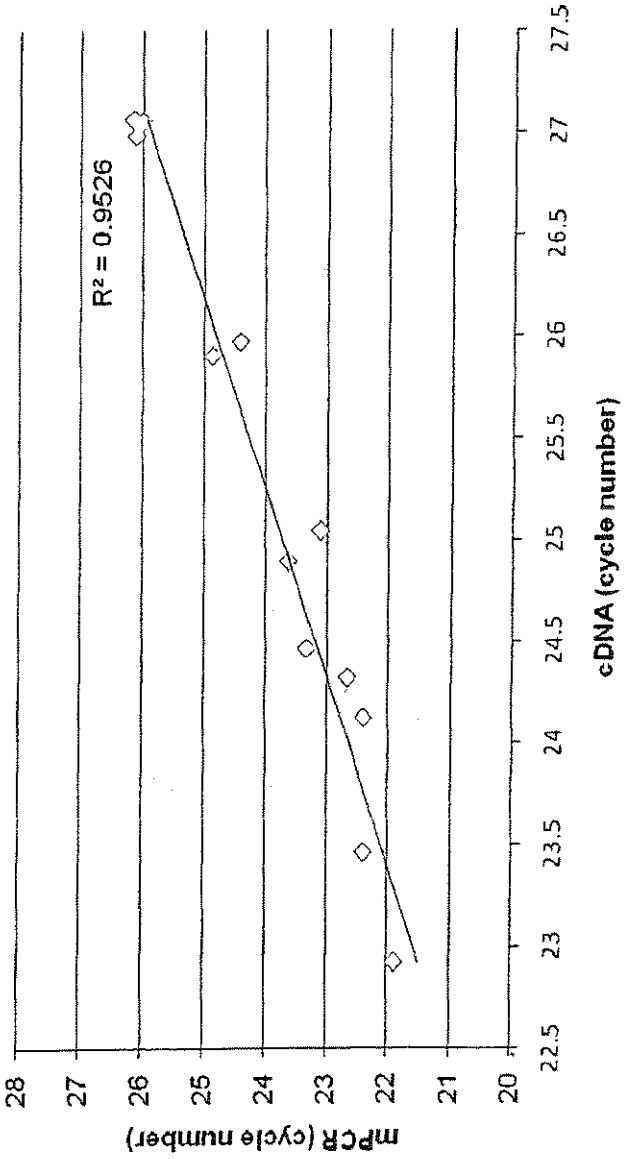

R² = 0.9526
mPCR (cycle number)
cDNA (cycle number)
28
27
26
25
24
23
22
21
20
22.5
23
23.5
24
24.5
25
25.5
26
26.5
27
27.5

FIG. 7

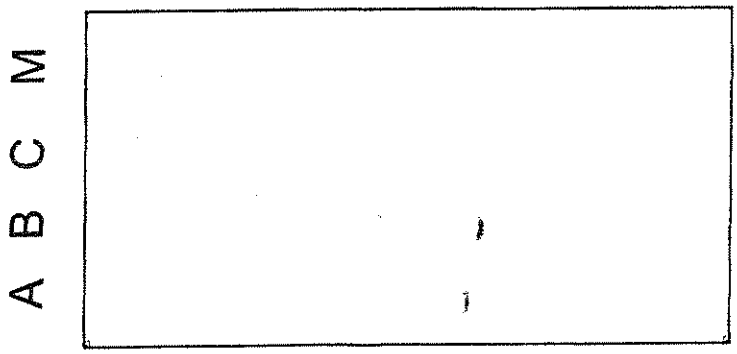
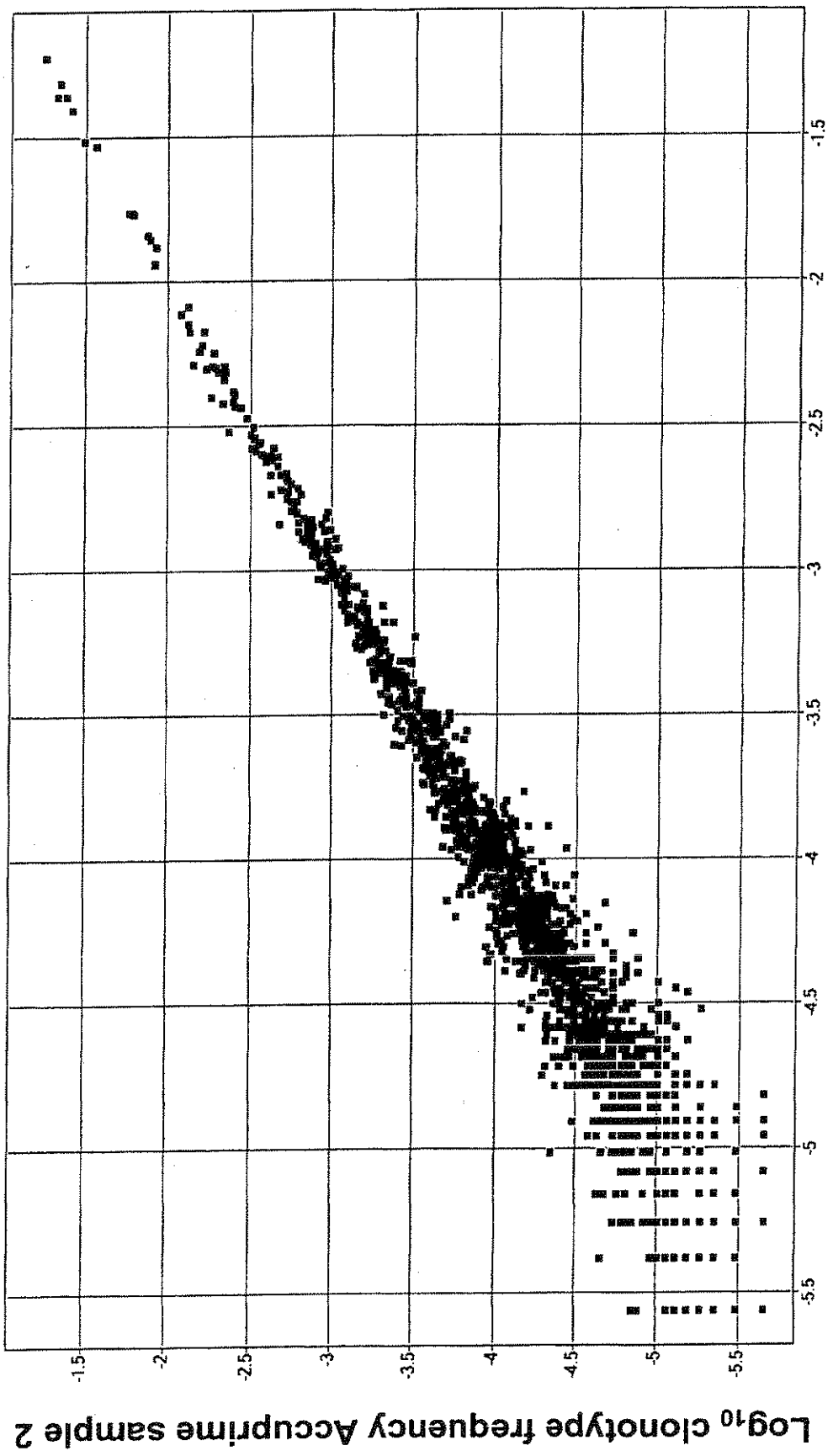


FIG. 8A


Log10 clonotype frequency Accuprime sample 2
Log10 clonotype frequency Accuprime sample 1
-1.5
-2
-2.5
-3
-3.5
-4
-4.5
-5
-5.5

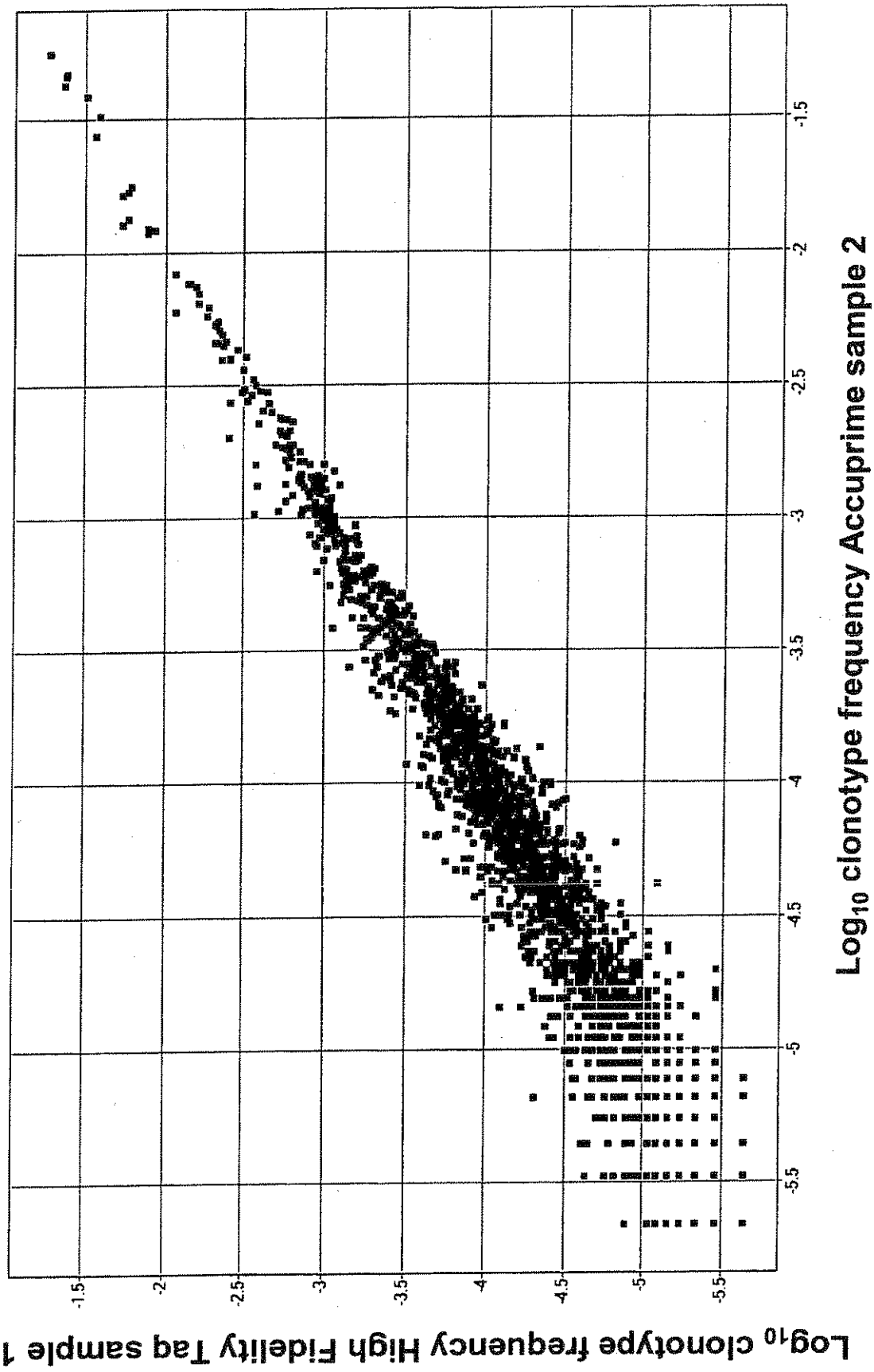

Log10 clonotype frequency High Fidelity Taq sample 1
Log10 clonotype frequency Accuprime sample 2
-1.5
-2
-2.5
-3
-3.5
-4
-4.5
-5
-5.5

FIG. 8B

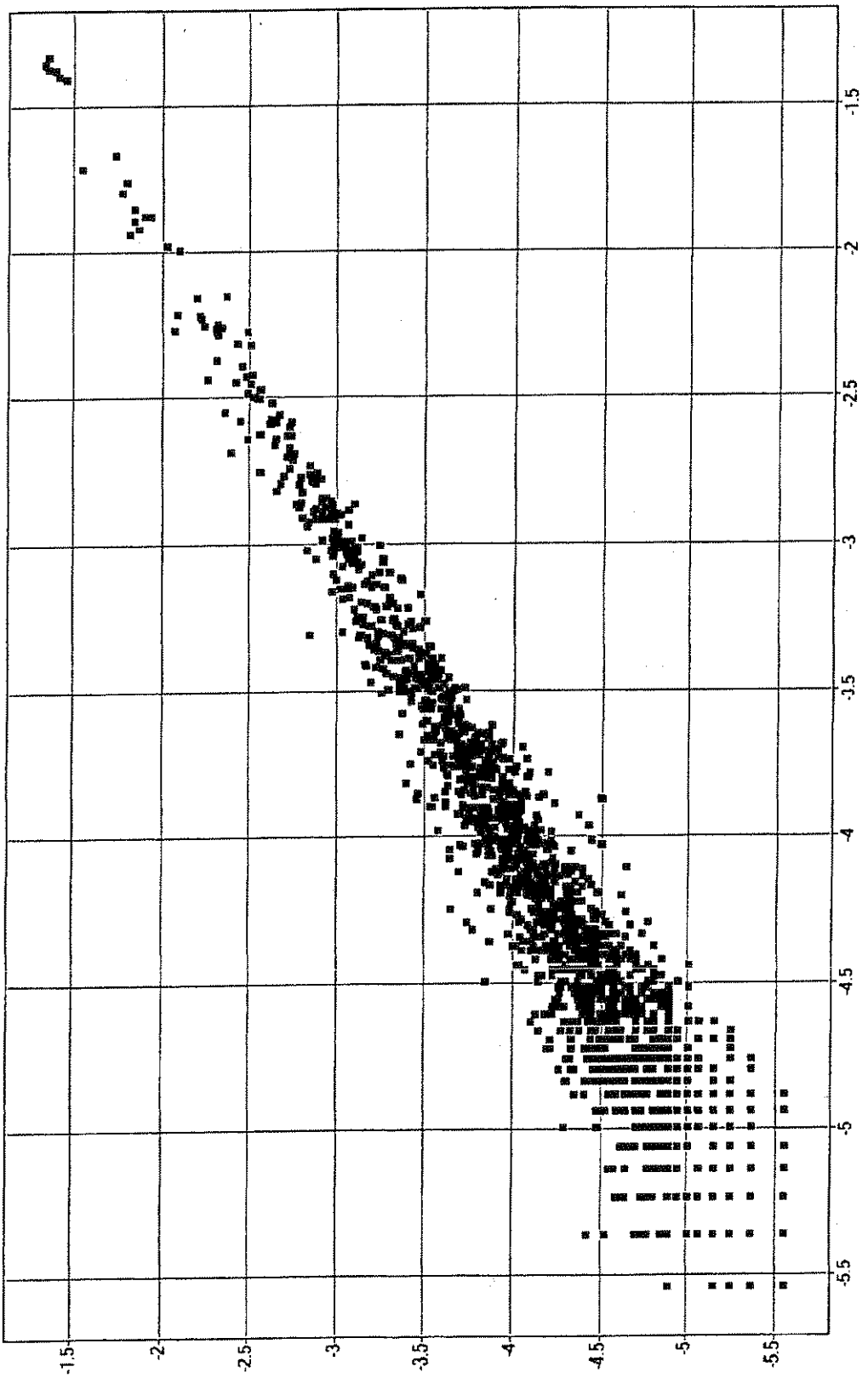

Log10 clonotype frequency High Fidelity Taq low input cDNA
Log10 clonotype frequency Accuprime low input cDNA
-1.5
-2
-2.5
-3
-3.5
-4
-4.5
-5
-5.5

FIG. 8C

FIG. 9

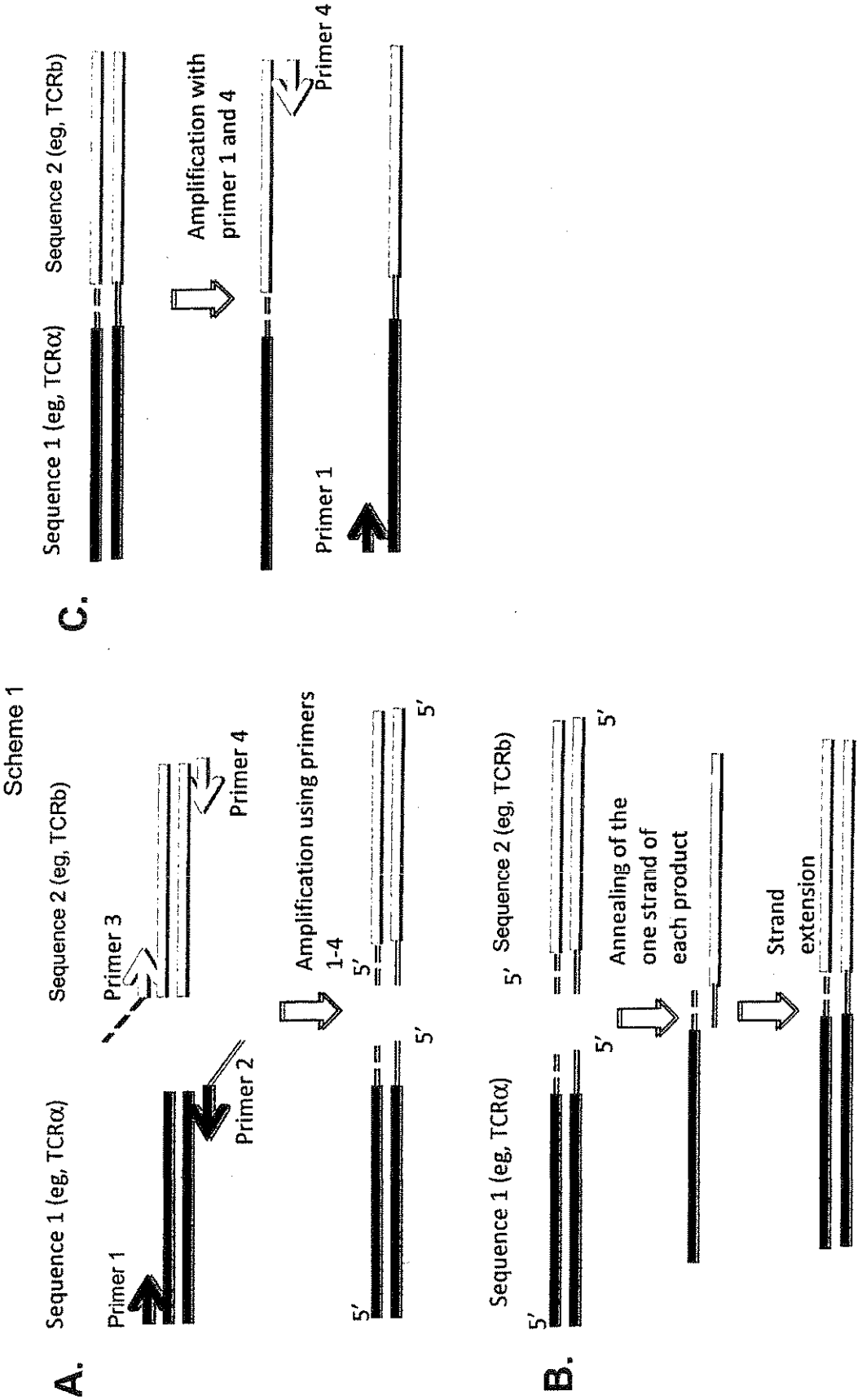

Scheme 1
A.
Sequence 1 (eg, TCRα)
Sequence 2 (eg, TCRb)
Primer 1
Primer 2
Primer 3
Primer 4
Amplification using primers 1-4
5′
5′
5′
5′
B.
Sequence 1 (eg, TCRα)
5′
5′
Sequence 2 (eg, TCRb)
5′
5′
Annealing of the one strand of each product
Strand extension
C.
Sequence 1 (eg, TCRα)
Sequence 2 (eg, TCRb)
Amplification with primer 1 and 4
Primer 1
Primer 4

FIG. 10

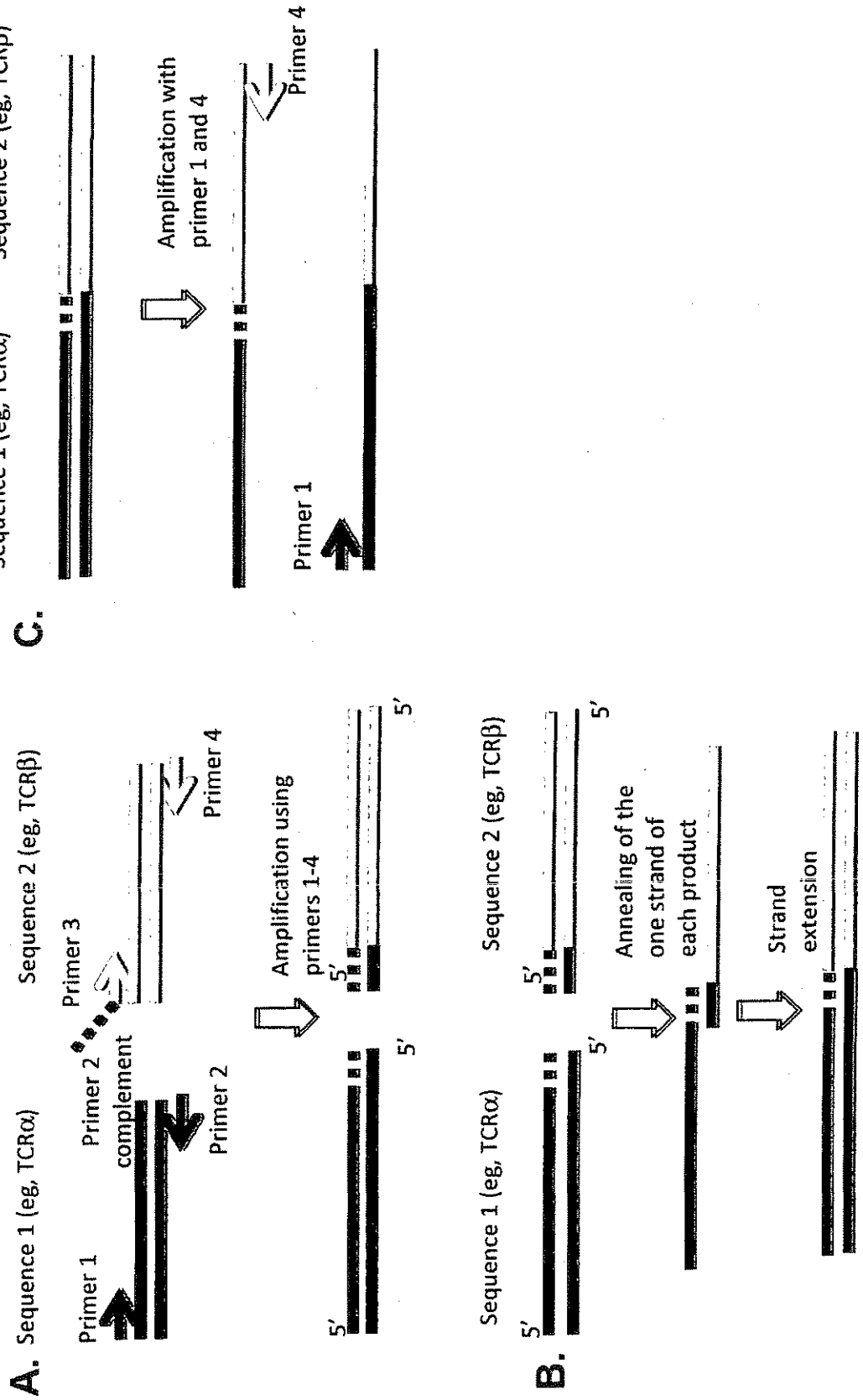

Scheme 1(a)
A.
Sequence 1 (eg, TCRα)
Sequence 2 (eg, TCRβ)
Primer 1
Primer 2
Primer 3
complement
Primer 2
Primer 4
Amplification using primers 1-4
5′
5′
5′
5′
B.
Sequence 1 (eg, TCRα)
Sequence 2 (eg, TCRβ)
5′
5′
5′
5′
Annealing of the one strand of each product
Strand extension
C.
Sequence 1 (eg, TCRα)
Sequence 2 (eg, TCRβ)
Amplification with primer 1 and 4
Primer 4
Primer 1

FIG. 11

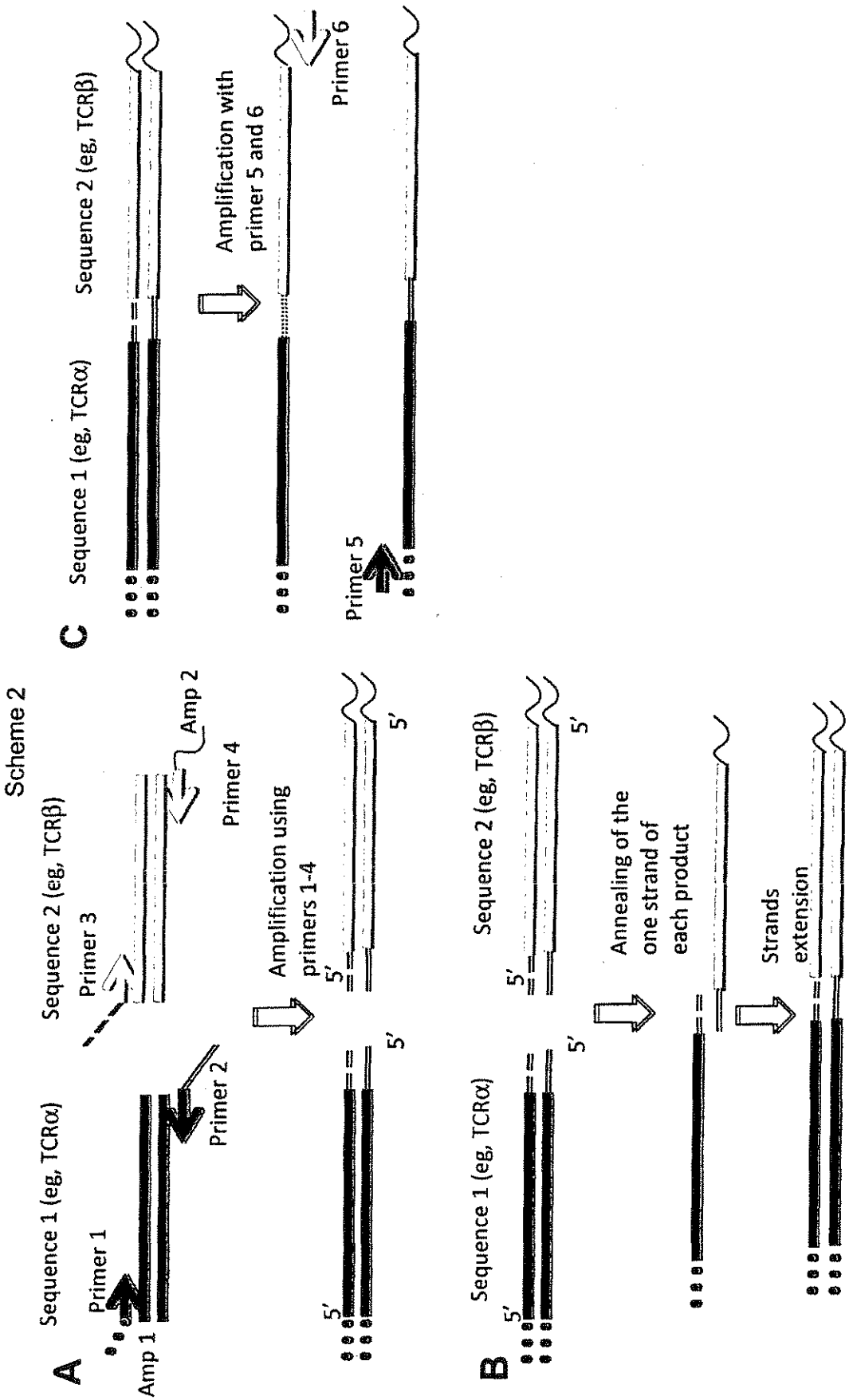

Scheme 2
A
Sequence 1 (eg, TCRα)
Primer 1
Amp 1
Primer 2
Sequence 2 (eg, TCRβ)
Primer 3
Amp 2
Primer 4
Amplification using primers 1-4
5′
5′
5′
5′
B
Sequence 1 (eg, TCRα)
5′
Sequence 2 (eg, TCRβ)
5′
5′
Annealing of the one strand of each product
Strands extension
C
Sequence 1 (eg, TCRα)
Sequence 2 (eg, TCRβ)
Amplification with primer 5 and 6
Primer 6
Primer 5

FIG. 12A

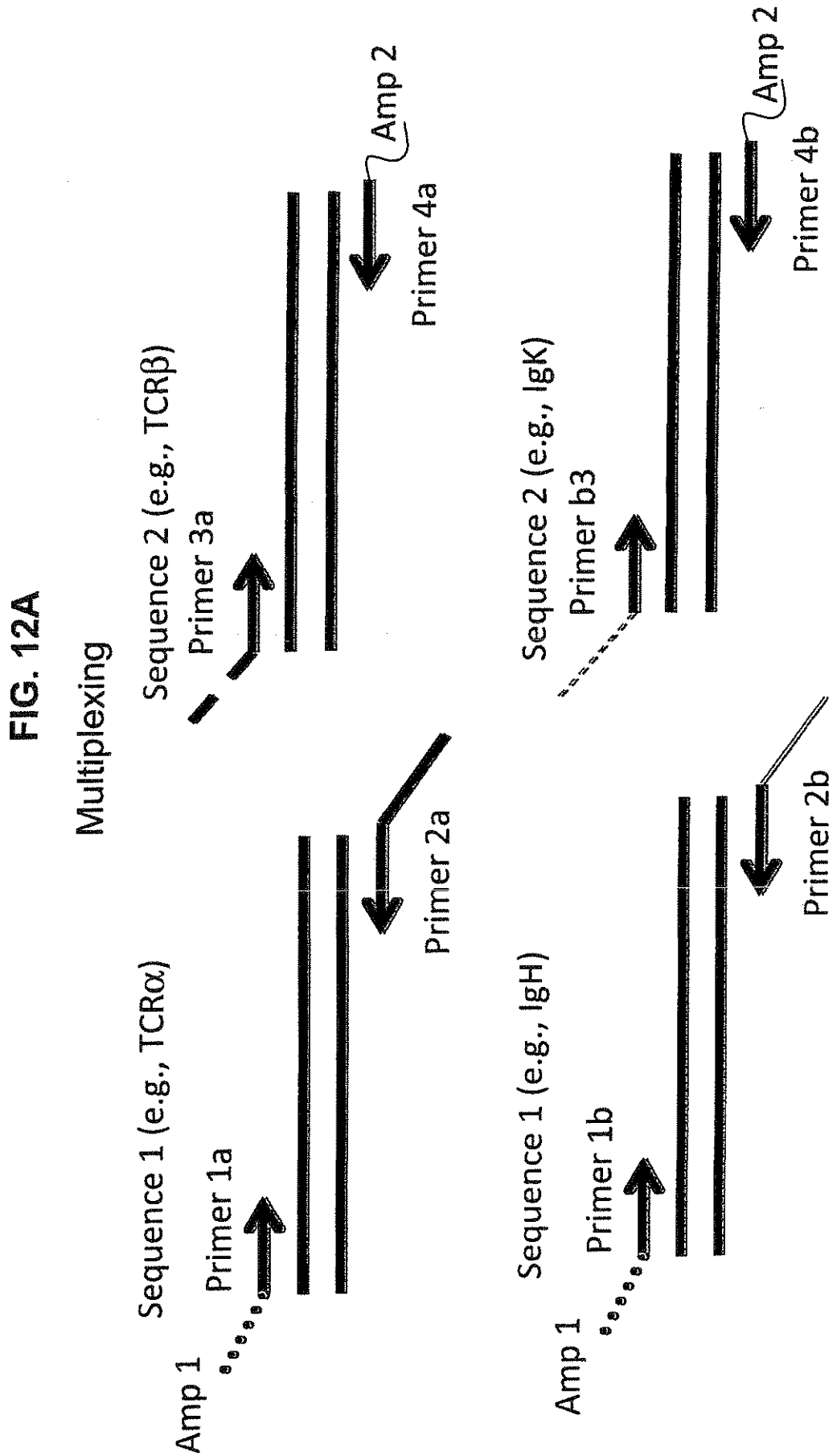

Multiplexing
Amp 1
Sequence 1 (e.g., TCRα)
Primer 1a
Primer 2a
Sequence 2 (e.g., TCRβ)
Primer 3a
Amp 2
Primer 4a
Amp 1
Sequence 1 (e.g., IgH)
Primer 1b
Primer 2b
Sequence 2 (e.g., IgK)
Primer b3
Amp 2
Primer 4b

FIG. 12B

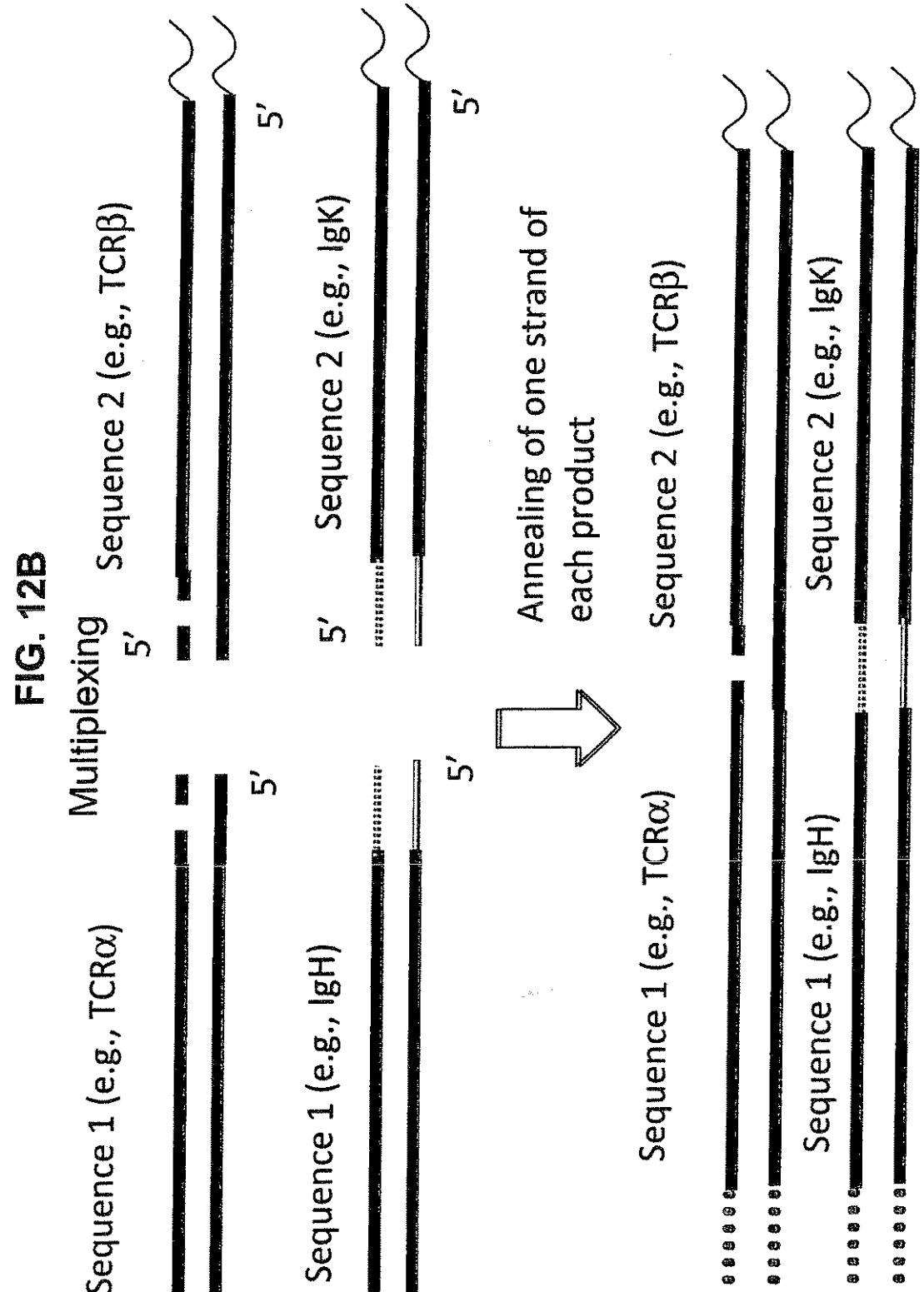

Multiplexing
Sequence 1 (e.g., TCRα)
Sequence 2 (e.g., TCRβ)
Sequence 1 (e.g., IgH)
Sequence 2 (e.g., IgK)
5′
Annealing of one strand of each product
Sequence 1 (e.g., TCRα)
Sequence 2 (e.g., TCRβ)
Sequence 1 (e.g., IgH)
Sequence 2 (e.g., IgK)

FIG. 13A

Linking 3 pieces

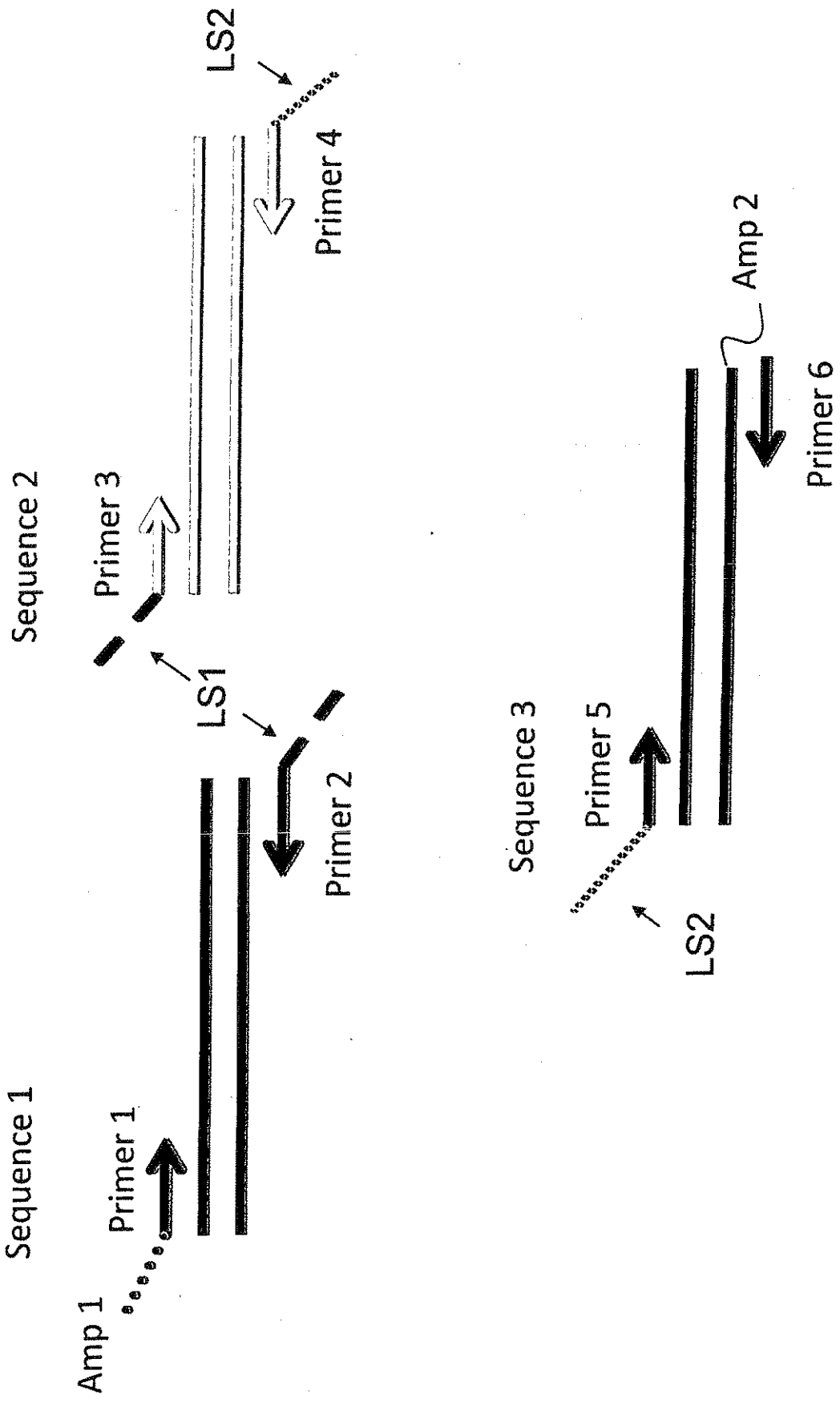


FIG. 13B

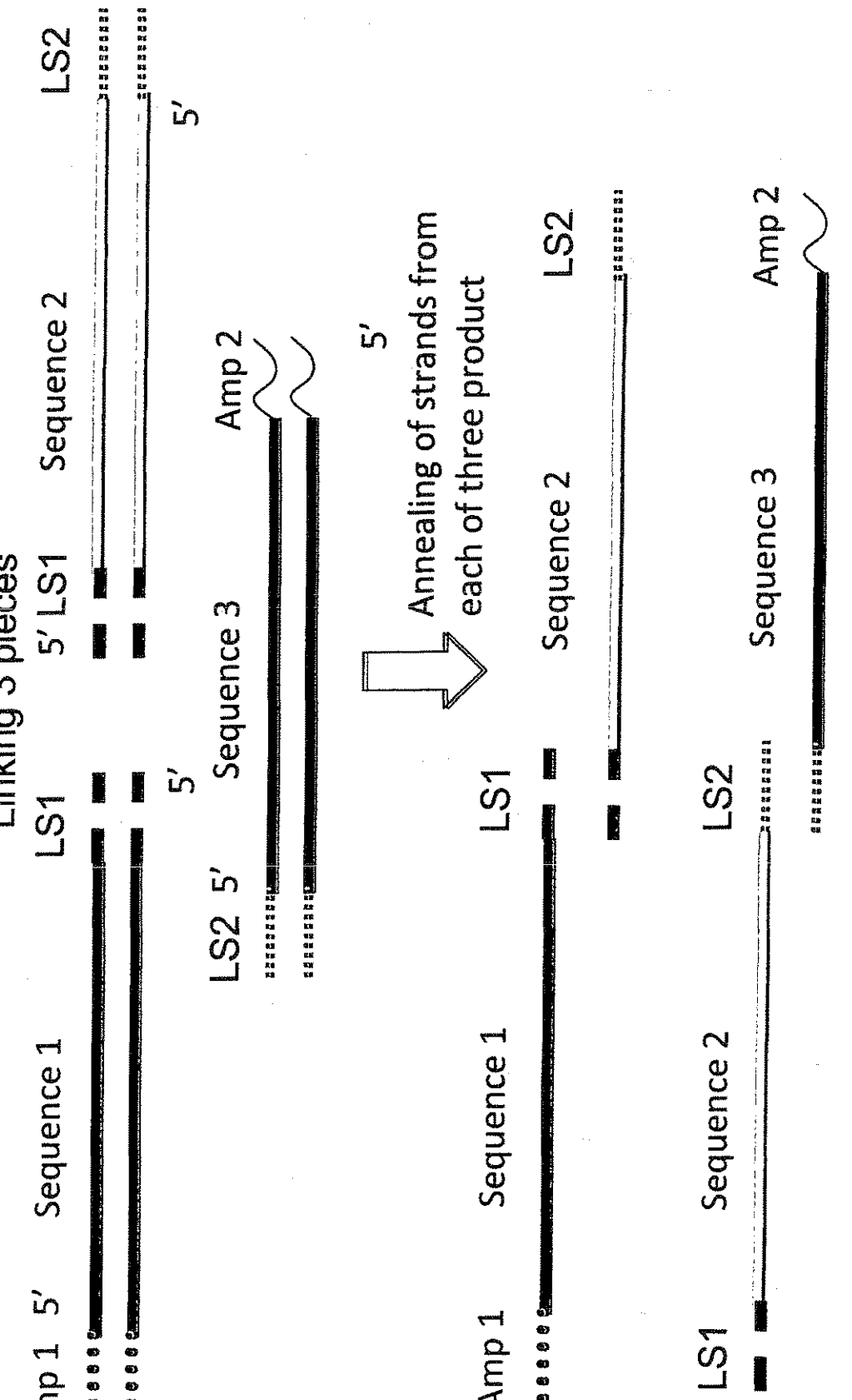

Linking 3 pieces
Amp 1
5′
Sequence 1
LS1
5′
LS1
Sequence 2
LS2
5′
LS2
5′
Sequence 3
Amp 2
5′
Annealing of strands from each of three product
Amp 1
Sequence 1
LS1
Sequence 2
LS2
LS1
Sequence 2
LS2
Sequence 3
Amp 2

FIG. 13C

Linking 3 pieces

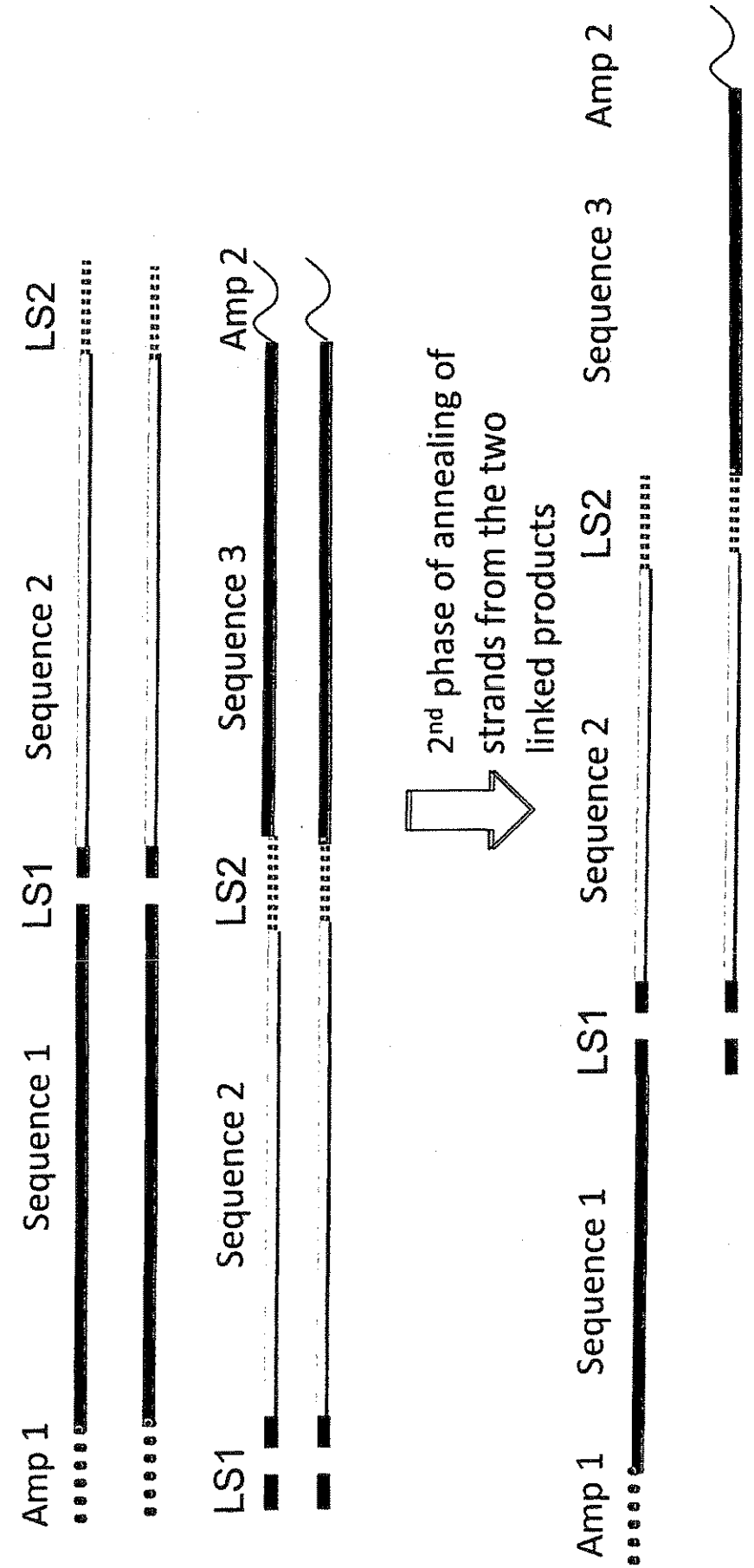


FIG. 13D

Linking 3 pieces

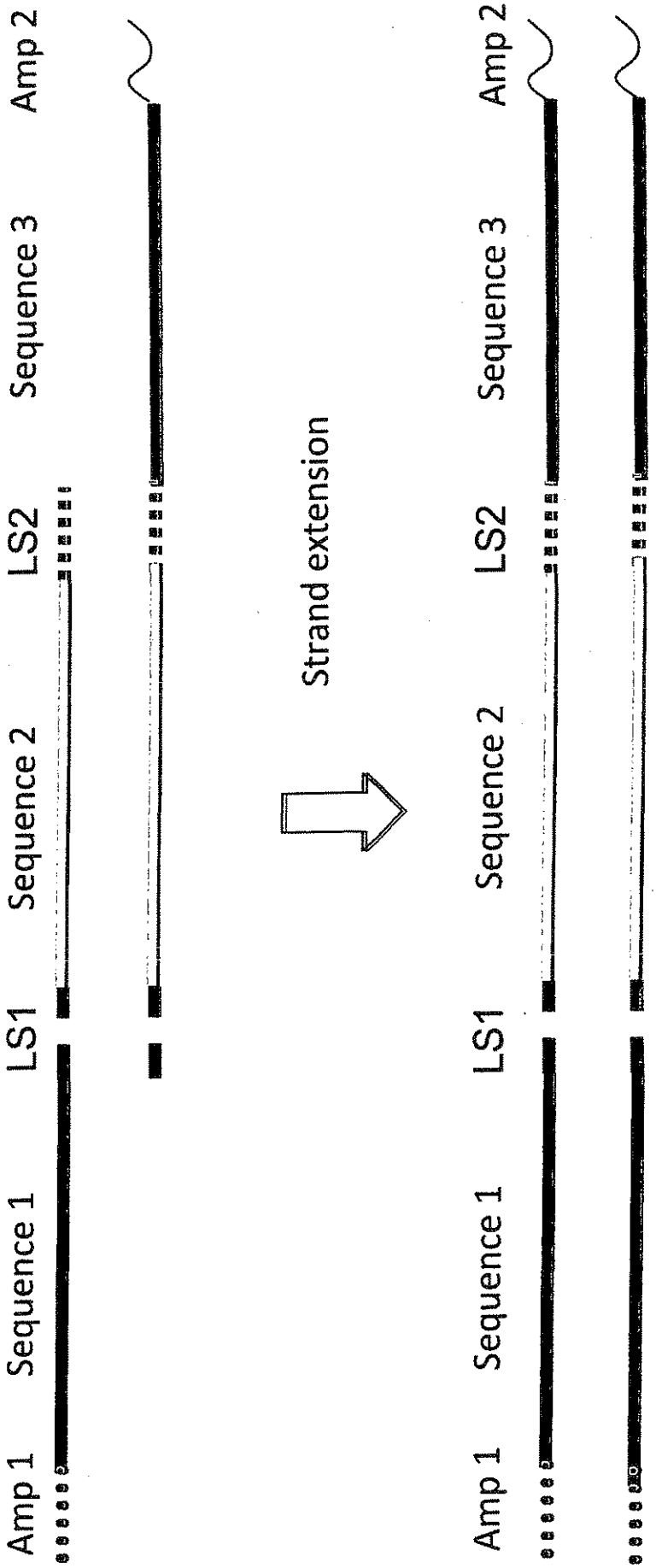


FIG. 14

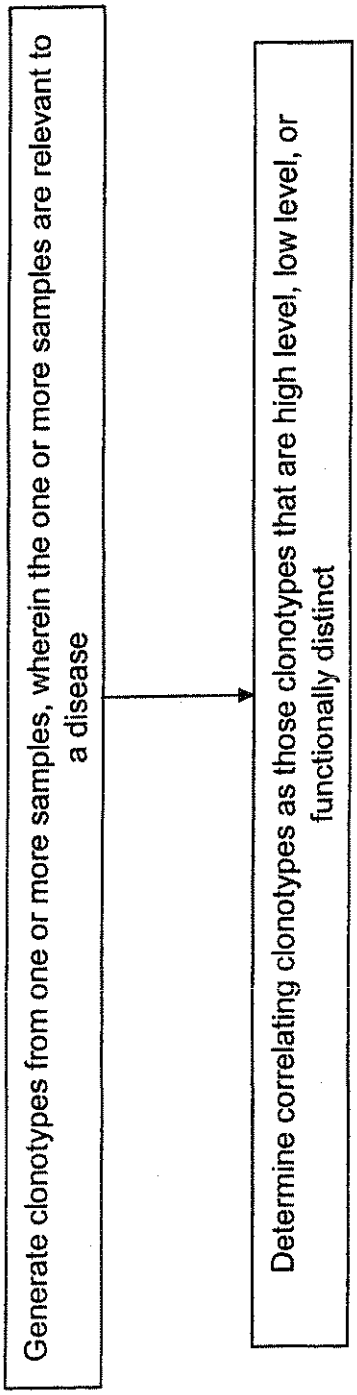

Discover correlating clonotypes using a calibration test
Generate clonotypes from one or more samples, wherein the one or more samples are relevant to a disease
Determine correlating clonotypes as those clonotypes that are high level, low level, or functionally distinct

FIG. 15

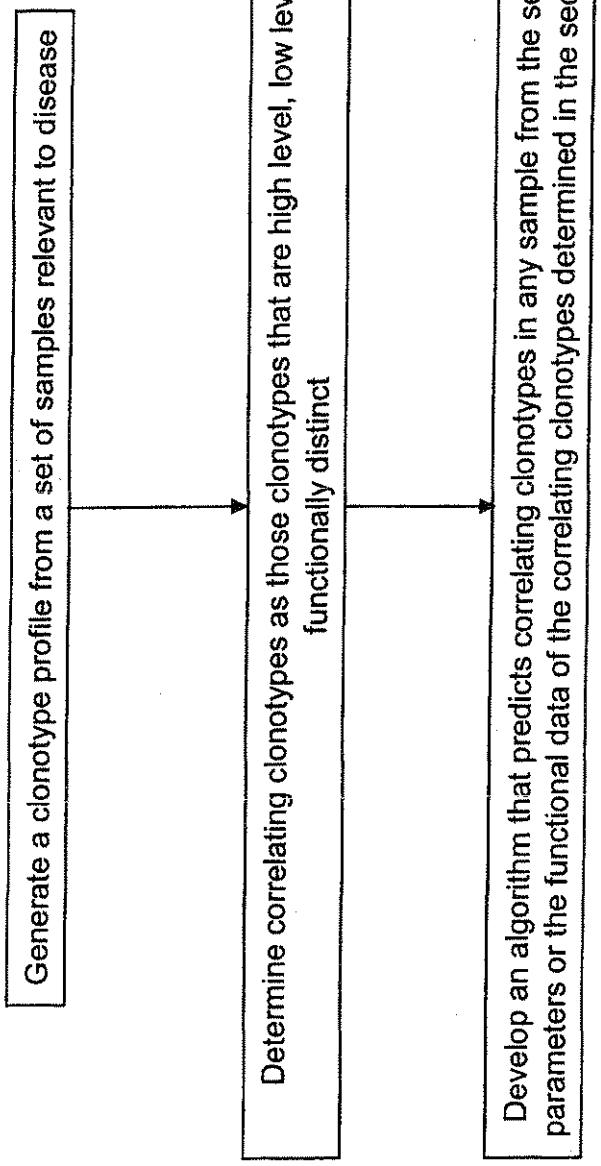

Discover correlating clonotypes using a population study
Generate a clonotype profile from a set of samples relevant to disease
Determine correlating clonotypes as those clonotypes that are high level, low level, or functionally distinct
Develop an algorithm that predicts correlating clonotypes in any sample from the sequence parameters or the functional data of the correlating clonotypes determined in the second step

FIG. 16

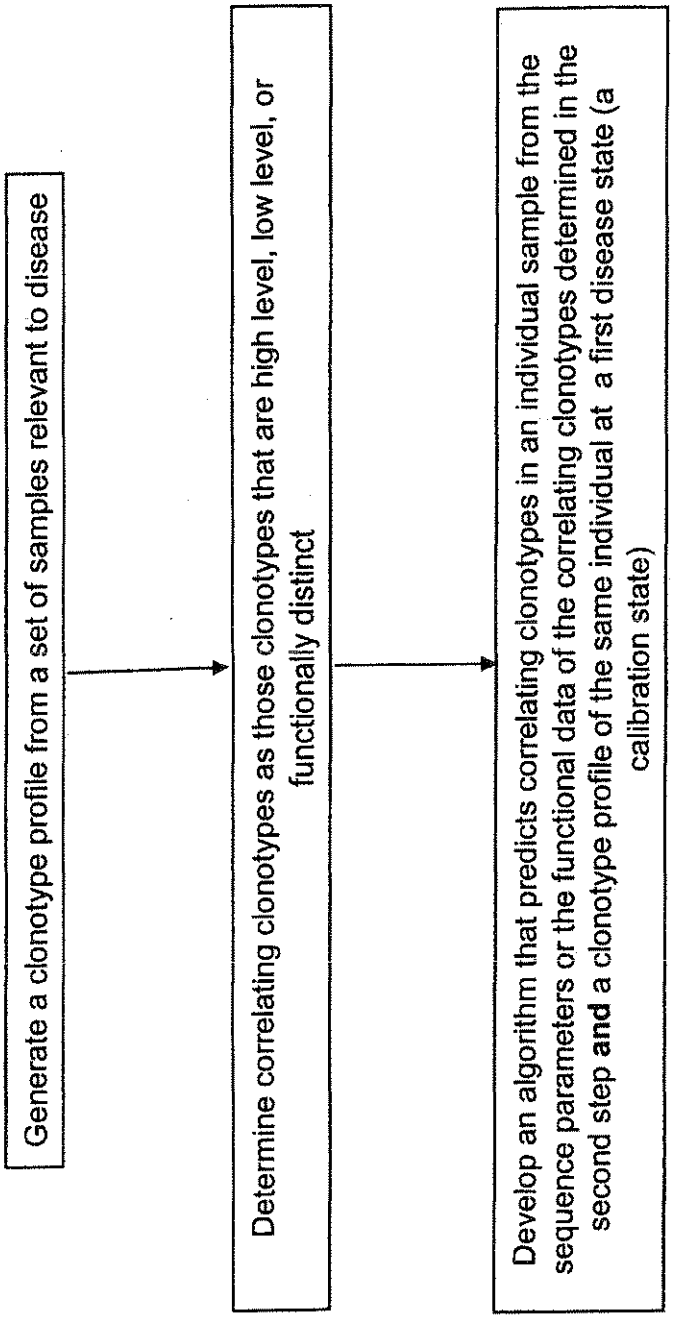

Discover correlating clonotypes using a population study and calibration test
Generate a clonotype profile from a set of samples relevant to disease
Determine correlating clonotypes as those clonotypes that are high level, low level, or functionally distinct
Develop an algorithm that predicts correlating clonotypes in an individual sample from the sequence parameters or the functional data of the correlating clonotypes determined in the second step and a clonotype profile of the same individual at a first disease state (a calibration state)

FIG. 17

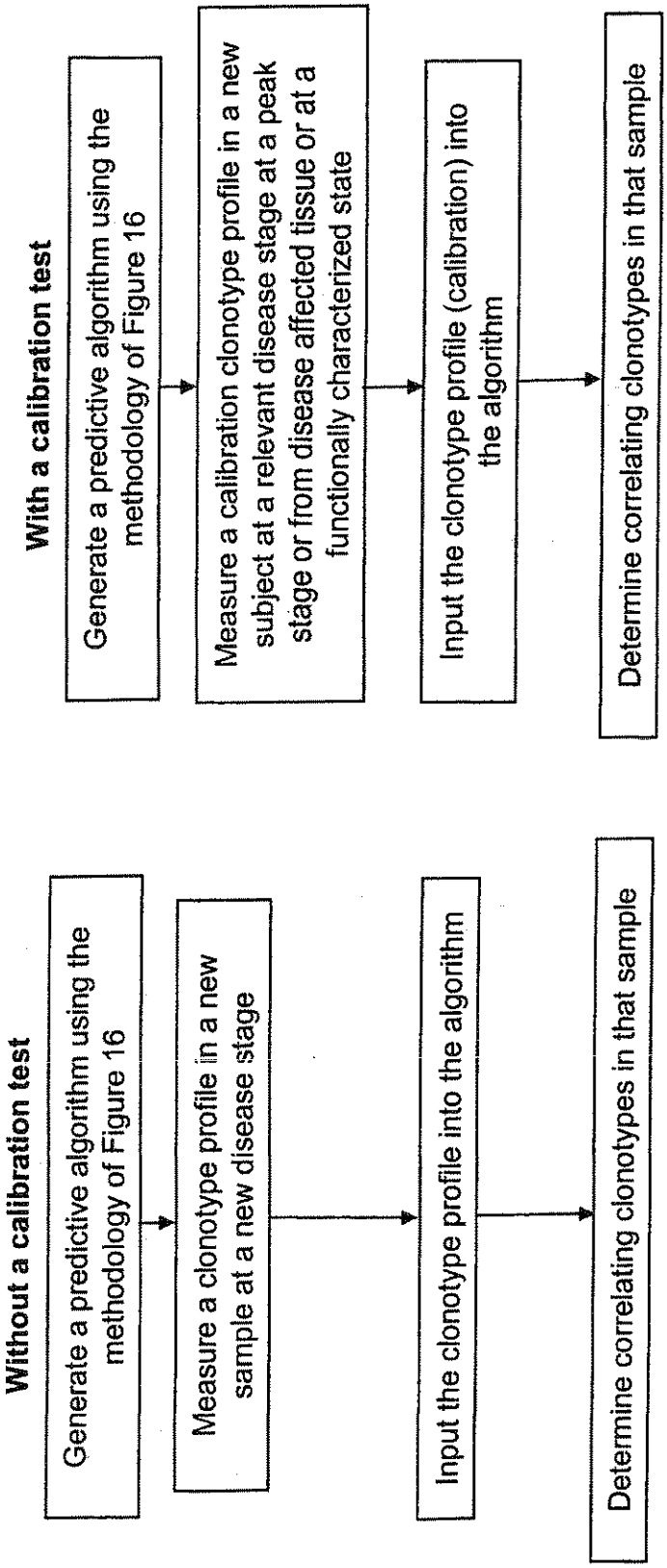

Algorithms that can predict correlating clonotypes in a sample
Without a calibration test
Generate a predictive algorithm using the methodology of Figure 16
Measure a clonotype profile in a new sample at a new disease stage
Input the clonotype profile into the algorithm
Determine correlating clonotypes in that sample
With a calibration test
Generate a predictive algorithm using the methodology of Figure 16
Measure a calibration clonotype profile in a new subject at a relevant disease stage at a peak stage or from disease affected tissue or at a functionally characterized state
Input the clonotype profile (calibration) into the algorithm
Determine correlating clonotypes in that sample

FIG. 18

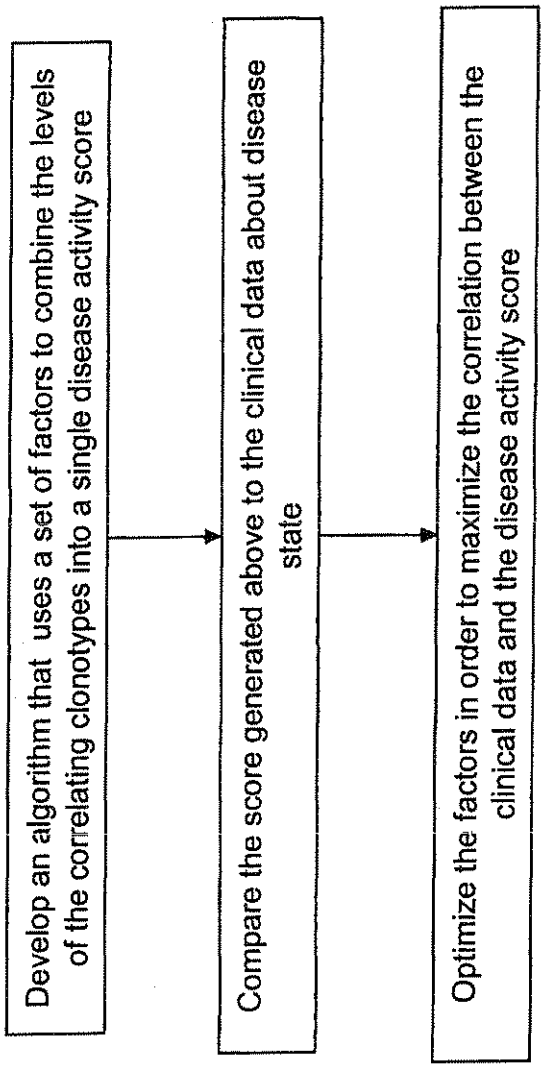

Monitoring algorithm (Calculating Immune Load)
Develop an algorithm that uses a set of factors to combine the levels of the correlating clonotypes into a single disease activity score
Compare the score generated above to the clinical data about disease state
Optimize the factors in order to maximize the correlation between the clinical data and the disease activity score

FIG. 19

Monitoring Test without using a calibration test

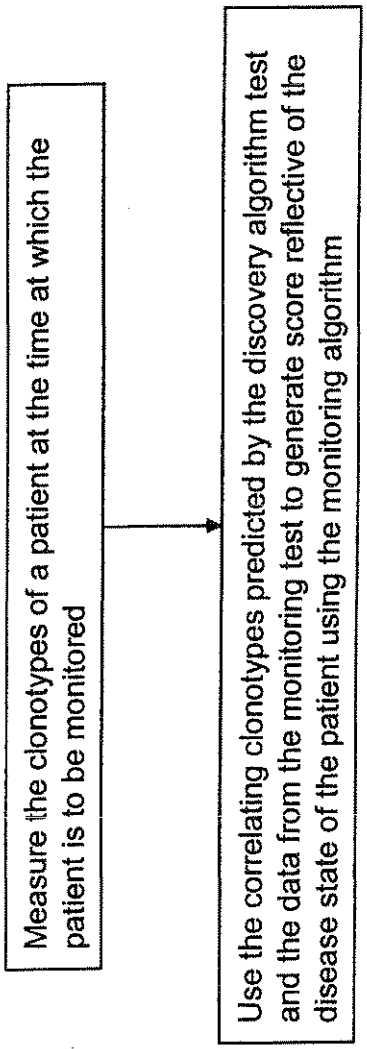
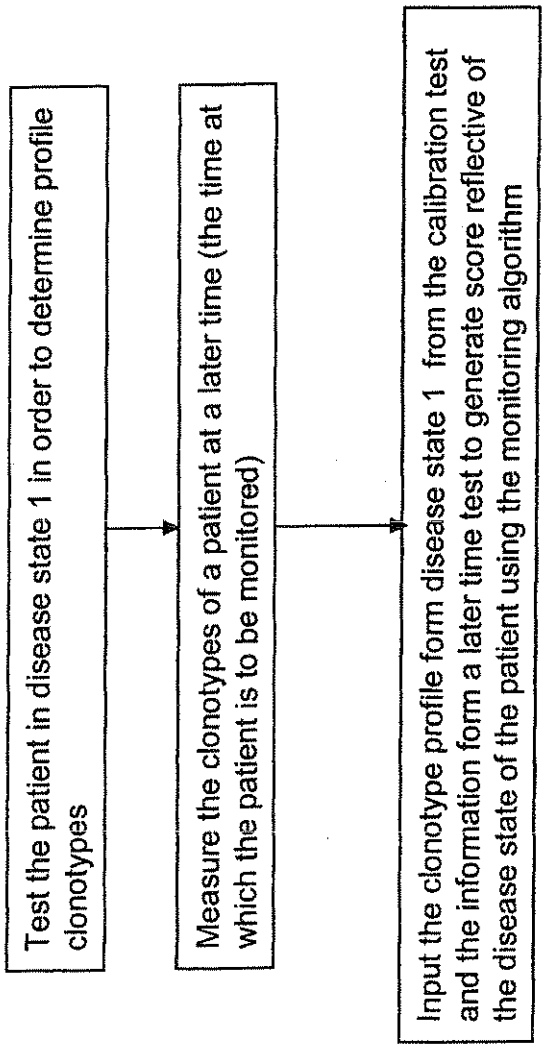


FIG. 20


Monitoring Test using a calibration test
Test the patient in disease state 1 in order to determine profile clonotypes
Measure the clonotypes of a patient at a later time (the time at which the patient is to be monitored)
Input the clonotype profile form disease state 1 from the calibration test and the information form a later time test to generate score reflective of the disease state of the patient using the monitoring algorithm