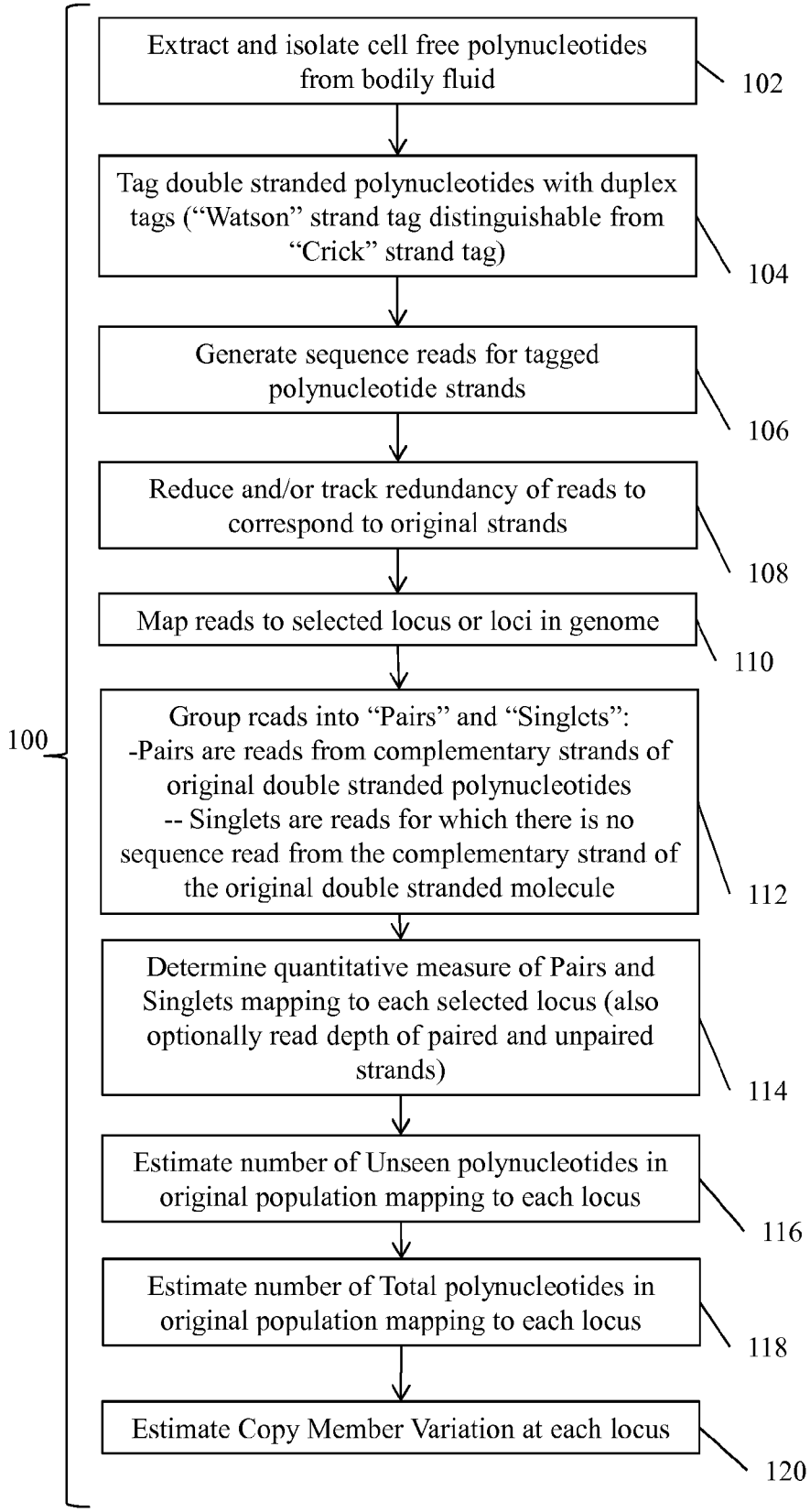
Extract and isolate cell free polynucleotides from bodily fluid
102
Tag double stranded polynucleotides with duplex tags ("Watson" strand tag distinguishable from "Crick" strand tag)
104
Generate sequence reads for tagged polynucleotide strands
106
Reduce and/or track redundancy of reads to correspond to original strands
108
Map reads to selected locus or loci in genome
110
Group reads into "Pairs" and "Singlets":
-Pairs are reads from complementary strands of original double stranded polynucleotides
-- Singlets are reads for which there is no sequence read from the complementary strand of the original double stranded molecule
112
Determine quantitative measure of Pairs and Singlets mapping to each selected locus (also optionally read depth of paired and unpaired strands)
114
Estimate number of Unseen polynucleotides in original population mapping to each locus
116
Estimate number of Total polynucleotides in original population mapping to each locus
118
Estimate Copy Member Variation at each locus
120
100

Fig. 1

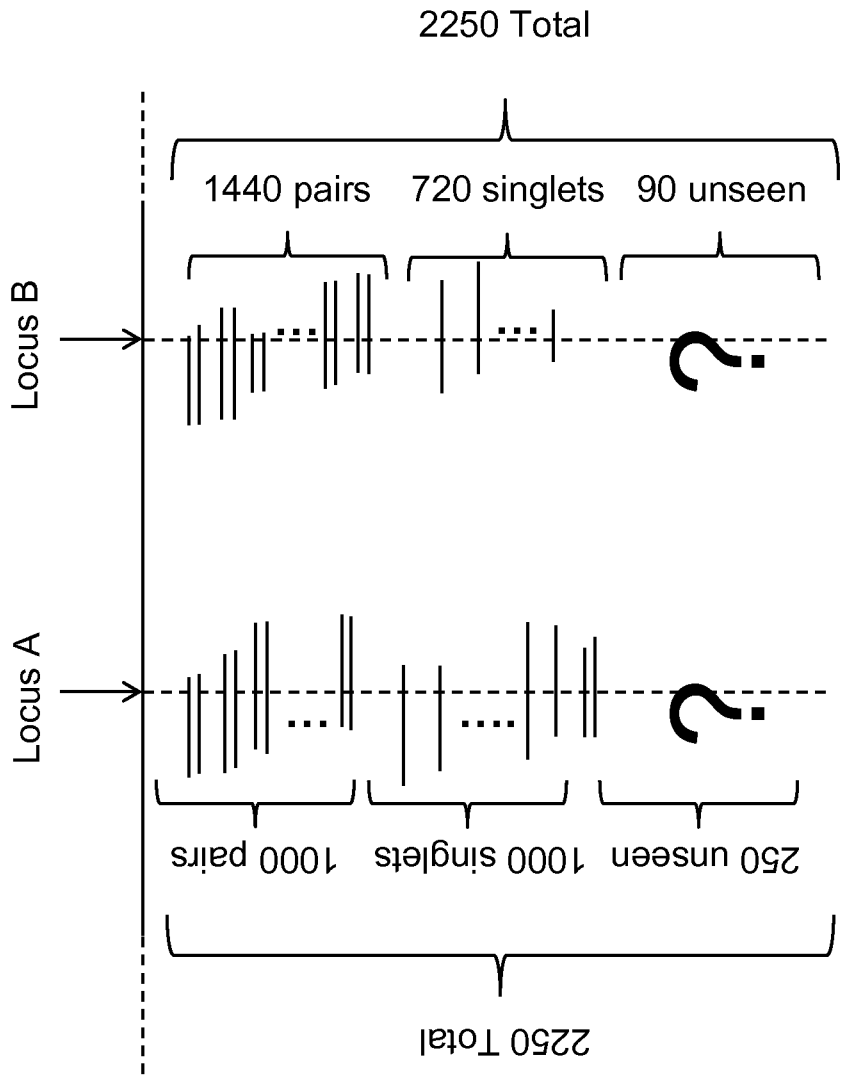
2250 Total
1440 pairs
720 singlets
90 unseen
Locus B
Locus A
1000 pairs
1000 singlets
250 unseen
2250 Total

Fig. 2

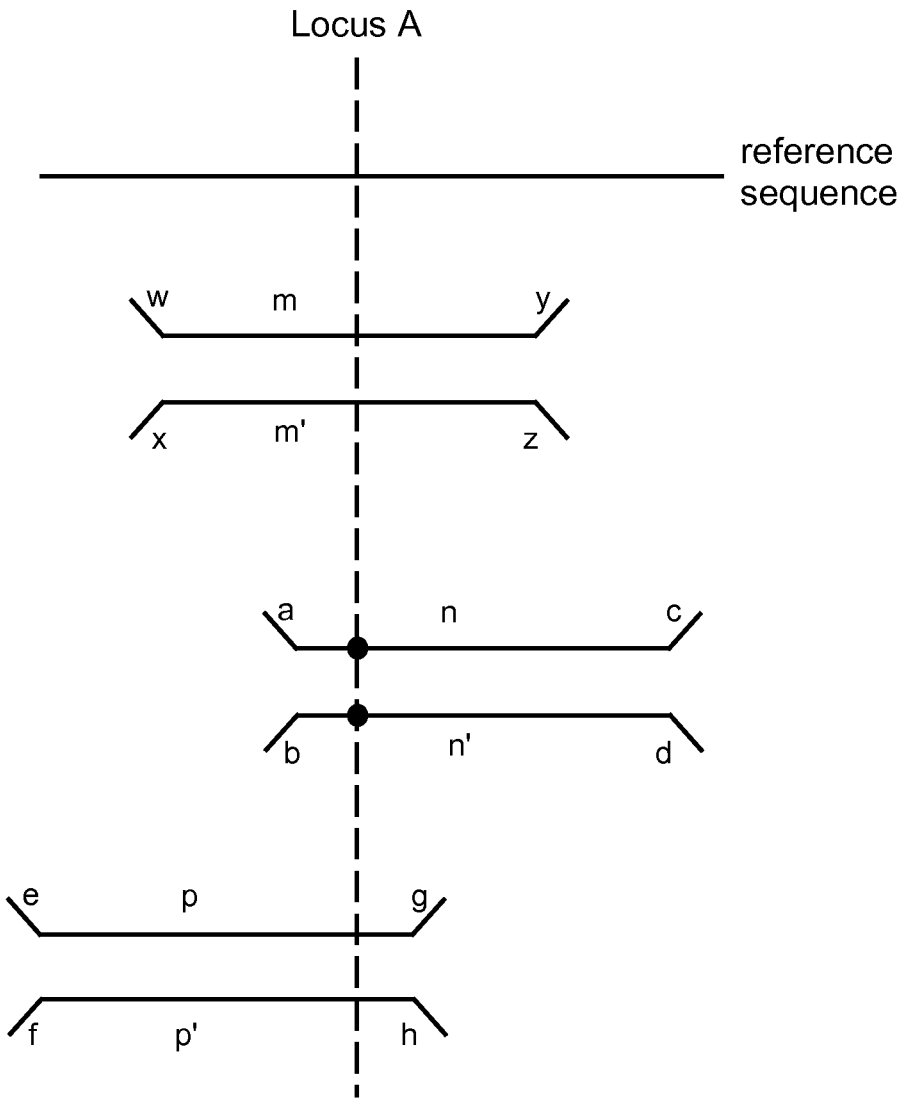

Fig. 3

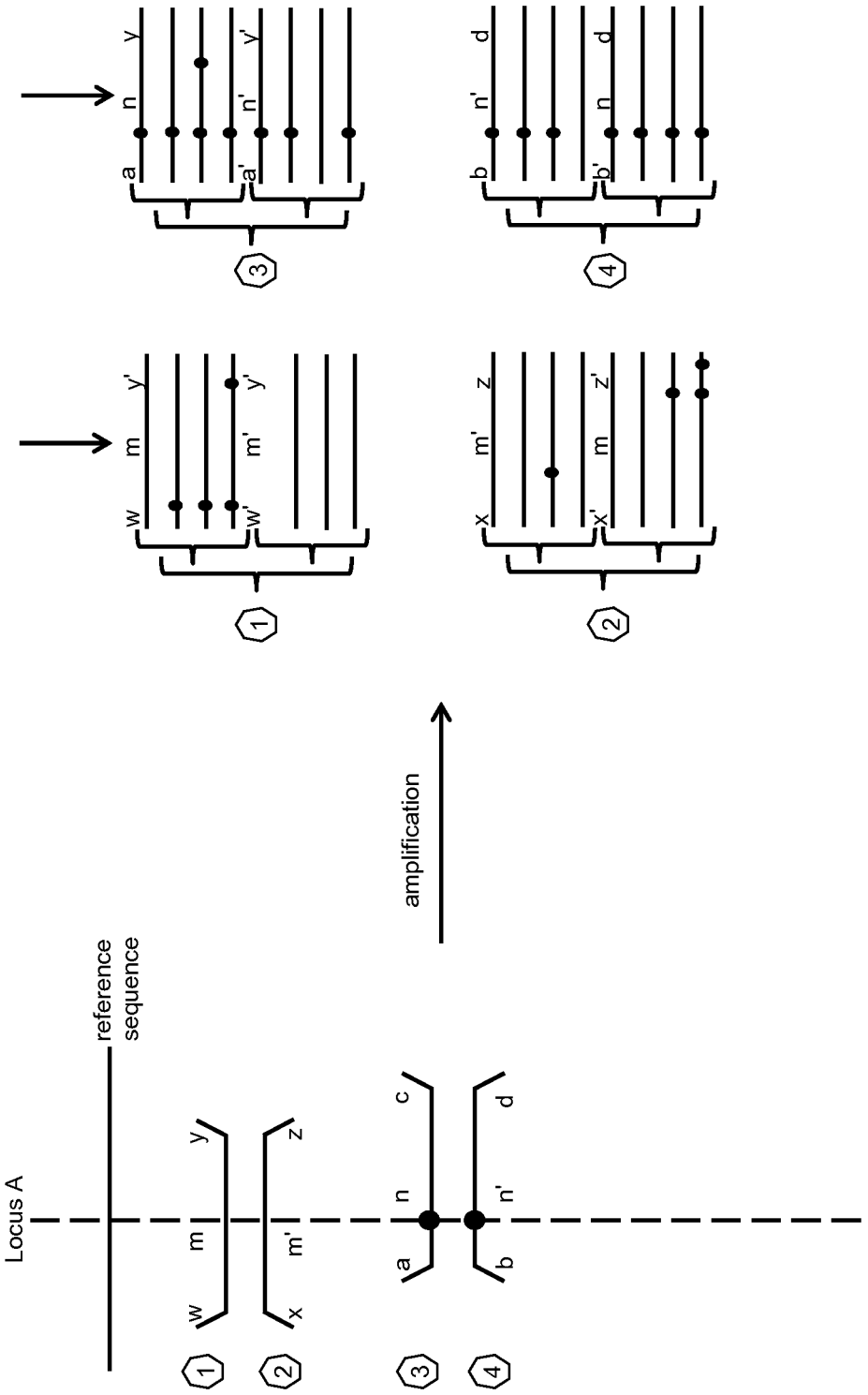
y
n
a
y'
n'
a'
3
d
n'
b
d
n
b'
4
y'
m
w
y'
m'
w'
1
z
m'
x
z'
m
x'
2
amplification
reference sequence
Locus A
y
m
w
z
m'
x
c
n
a
d
n'
b
1
2
3
4

Fig. 4A

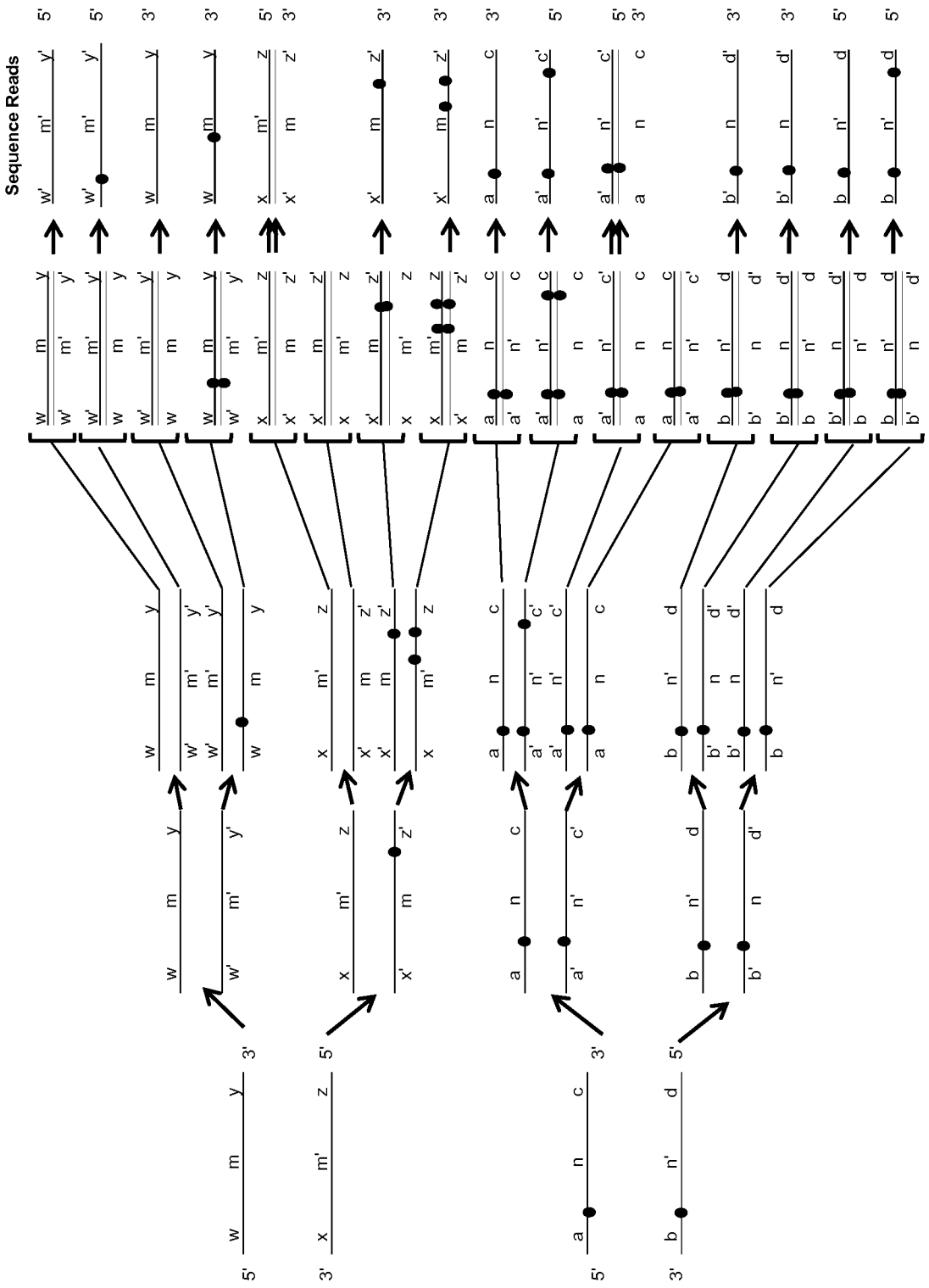

Fig. 4B

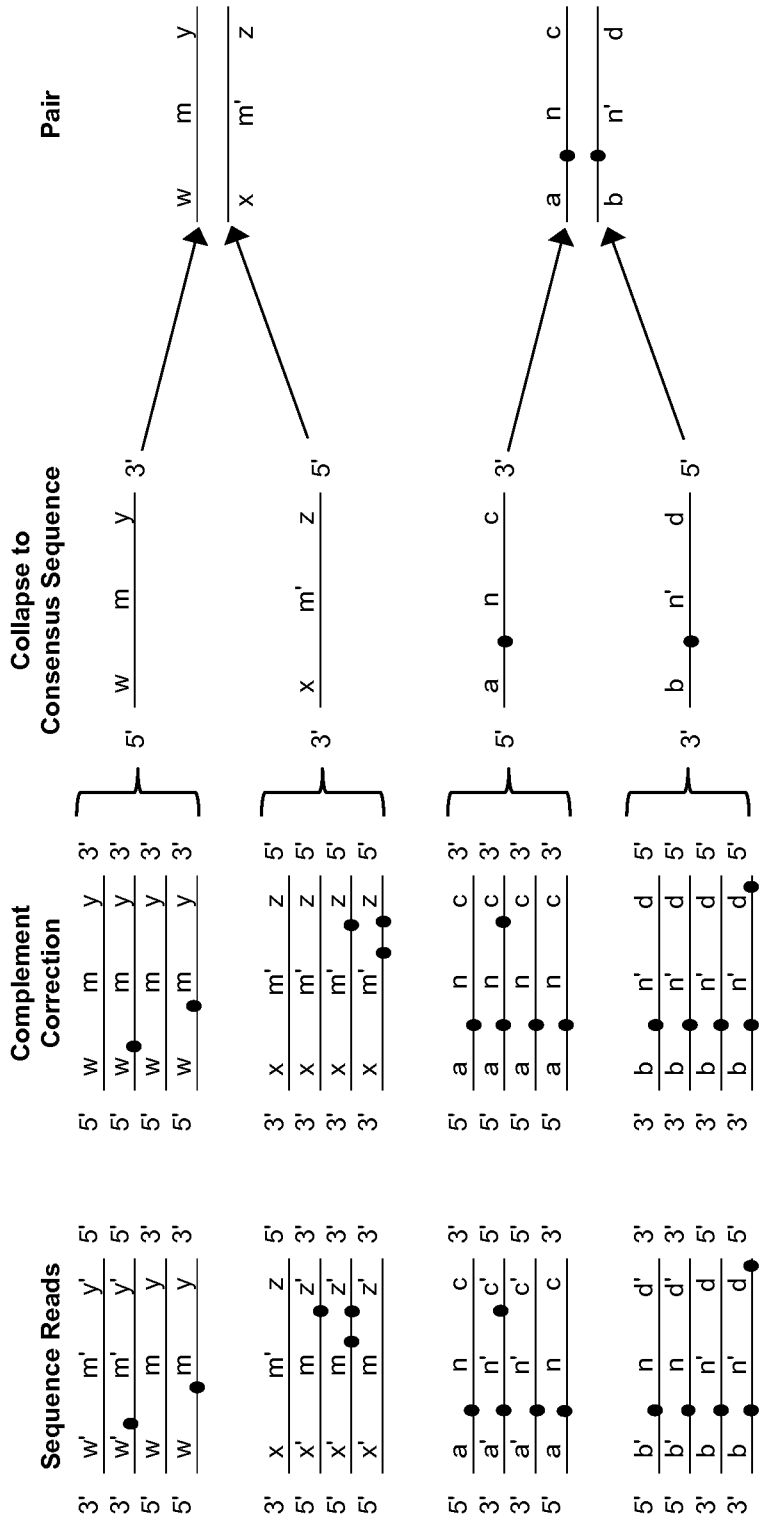
Sequence Reads
Complement Correction
Collapse to Consensus Sequence
Pair

Fig. 4C

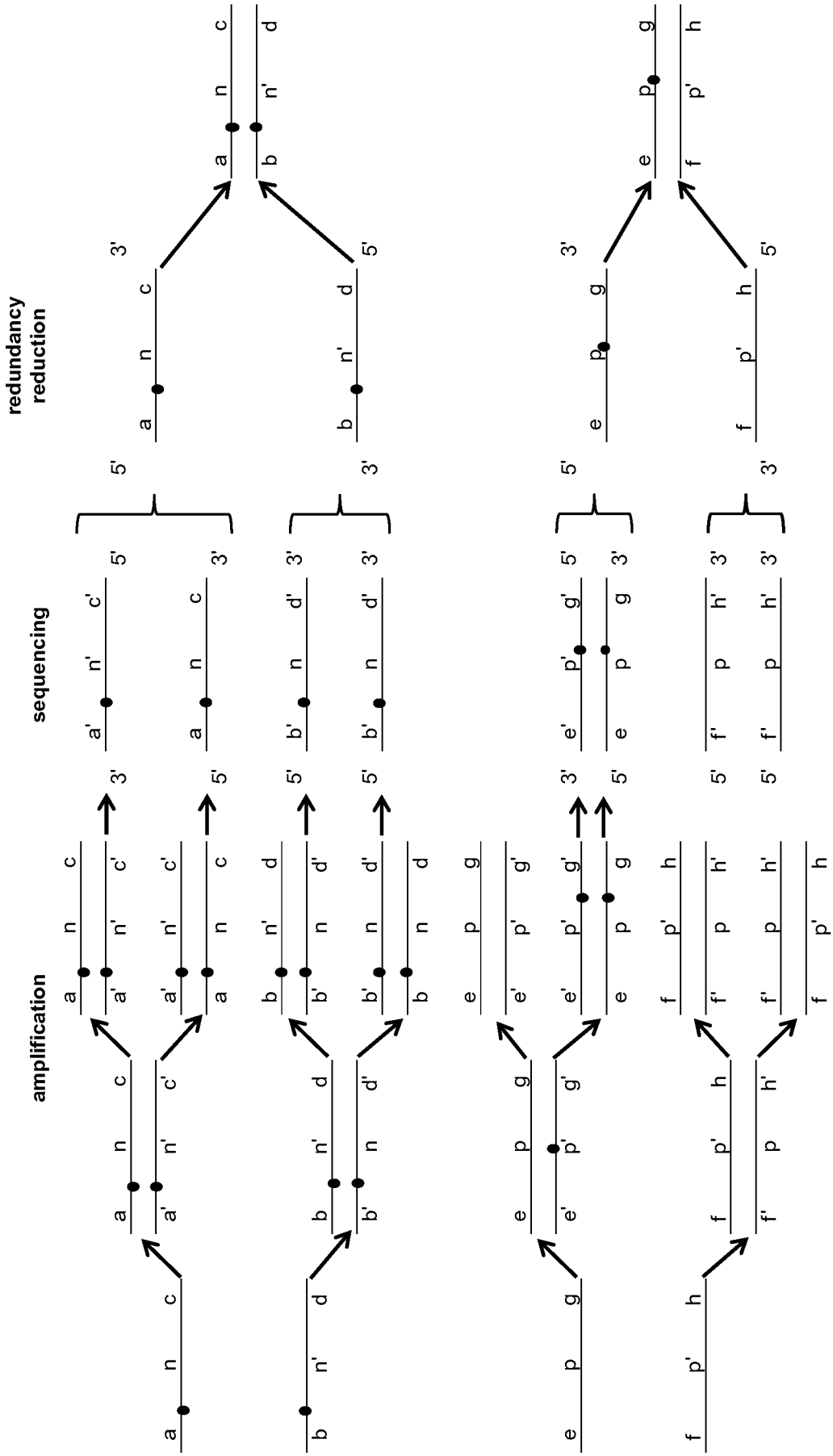

Fig. 5

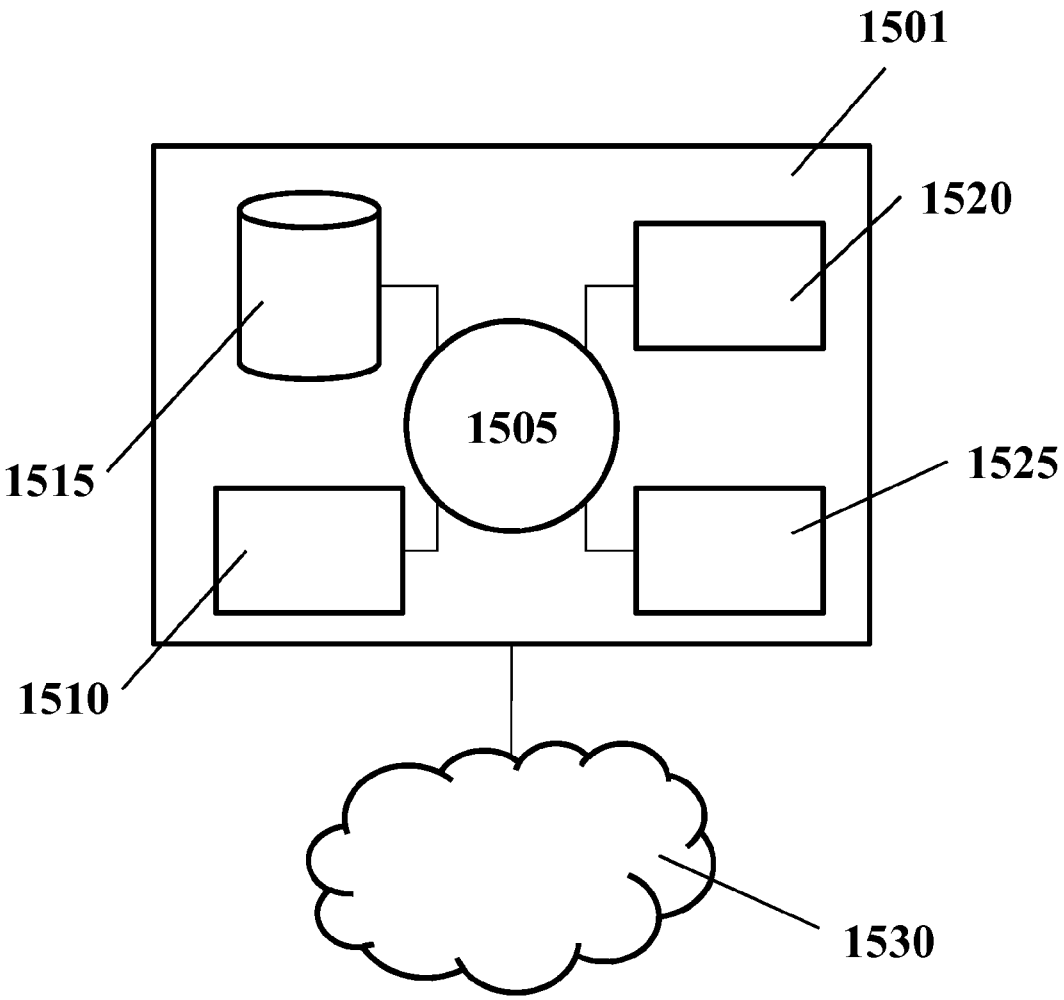

Fig. 6

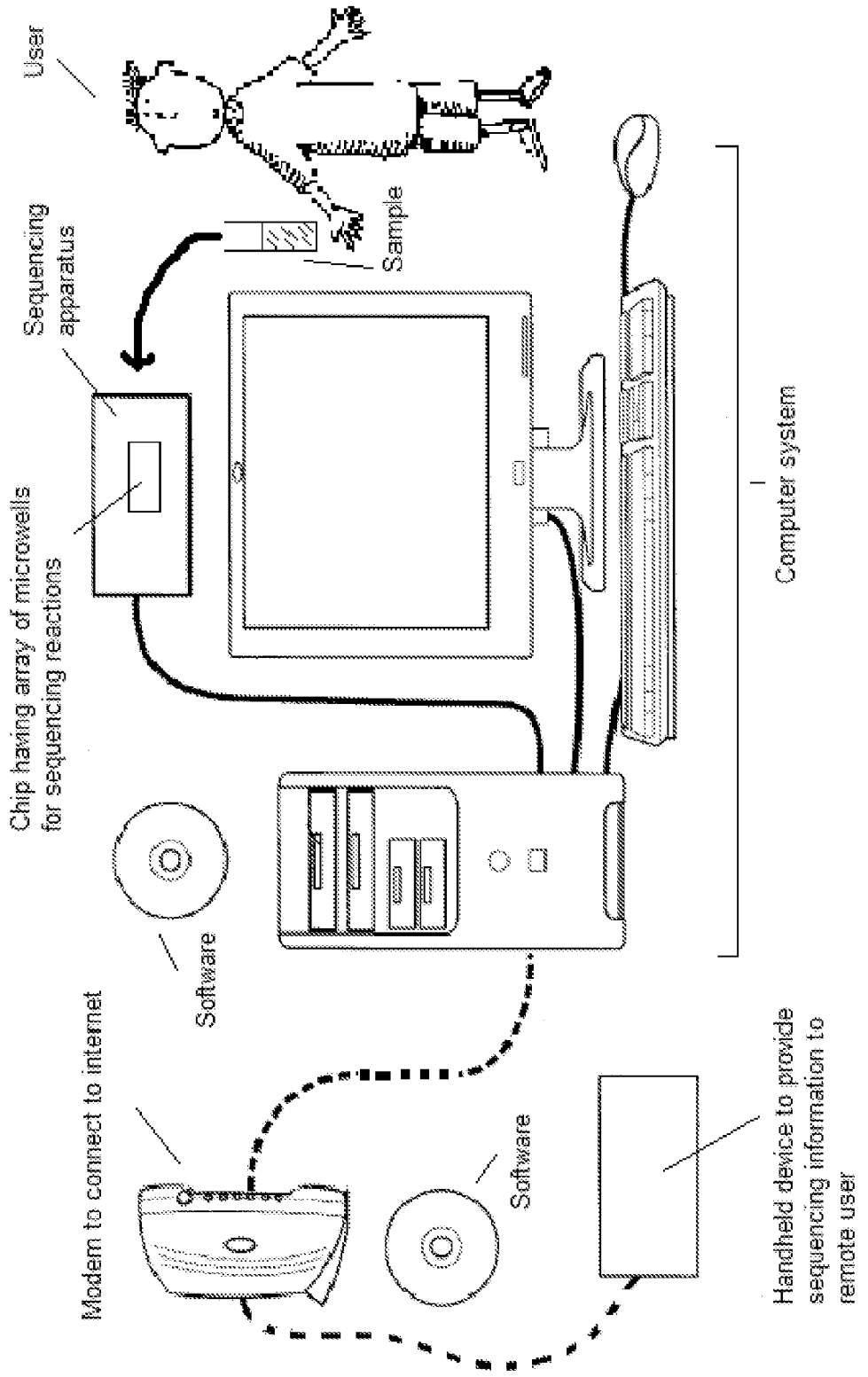
User
Sample
Sequencing apparatus
Chip having array of microwells for sequencing reactions
Computer system
Software
Modem to connect to internet
Software
Handheld device to provide sequencing information to remote user

Fig. 7

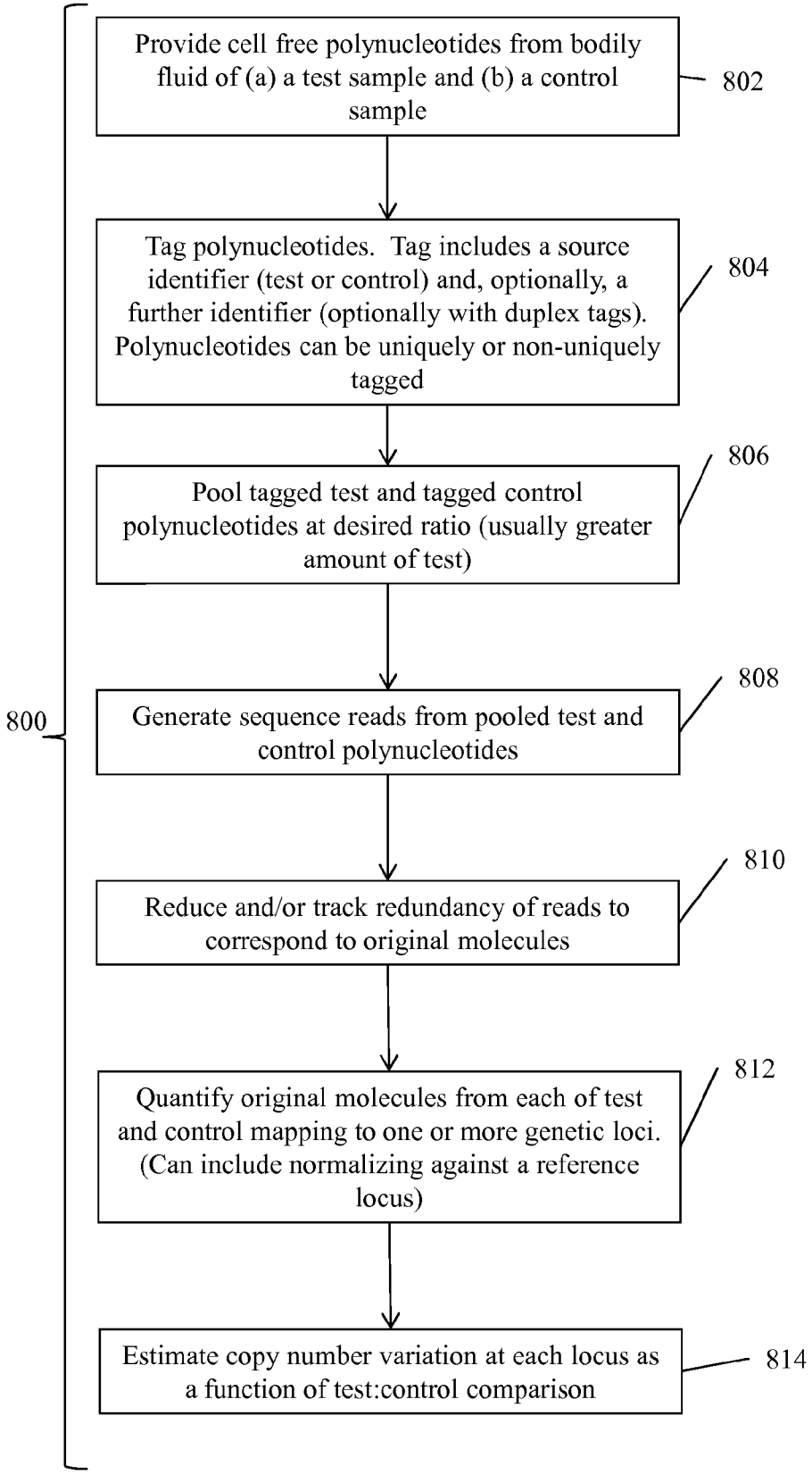
Provide cell free polynucleotides from bodily fluid of (a) a test sample and (b) a control sample
802
Tag polynucleotides. Tag includes a source identifier (test or control) and, optionally, a further identifier (optionally with duplex tags). Polynucleotides can be uniquely or non-uniquely tagged
804
Pool tagged test and tagged control polynucleotides at desired ratio (usually greater amount of test)
806
Generate sequence reads from pooled test and control polynucleotides
808
800
Reduce and/or track redundancy of reads to correspond to original molecules
810
Quantify original molecules from each of test and control mapping to one or more genetic loci. (Can include normalizing against a reference locus)
812
Estimate copy number variation at each locus as a function of test:control comparison
814

Fig. 8

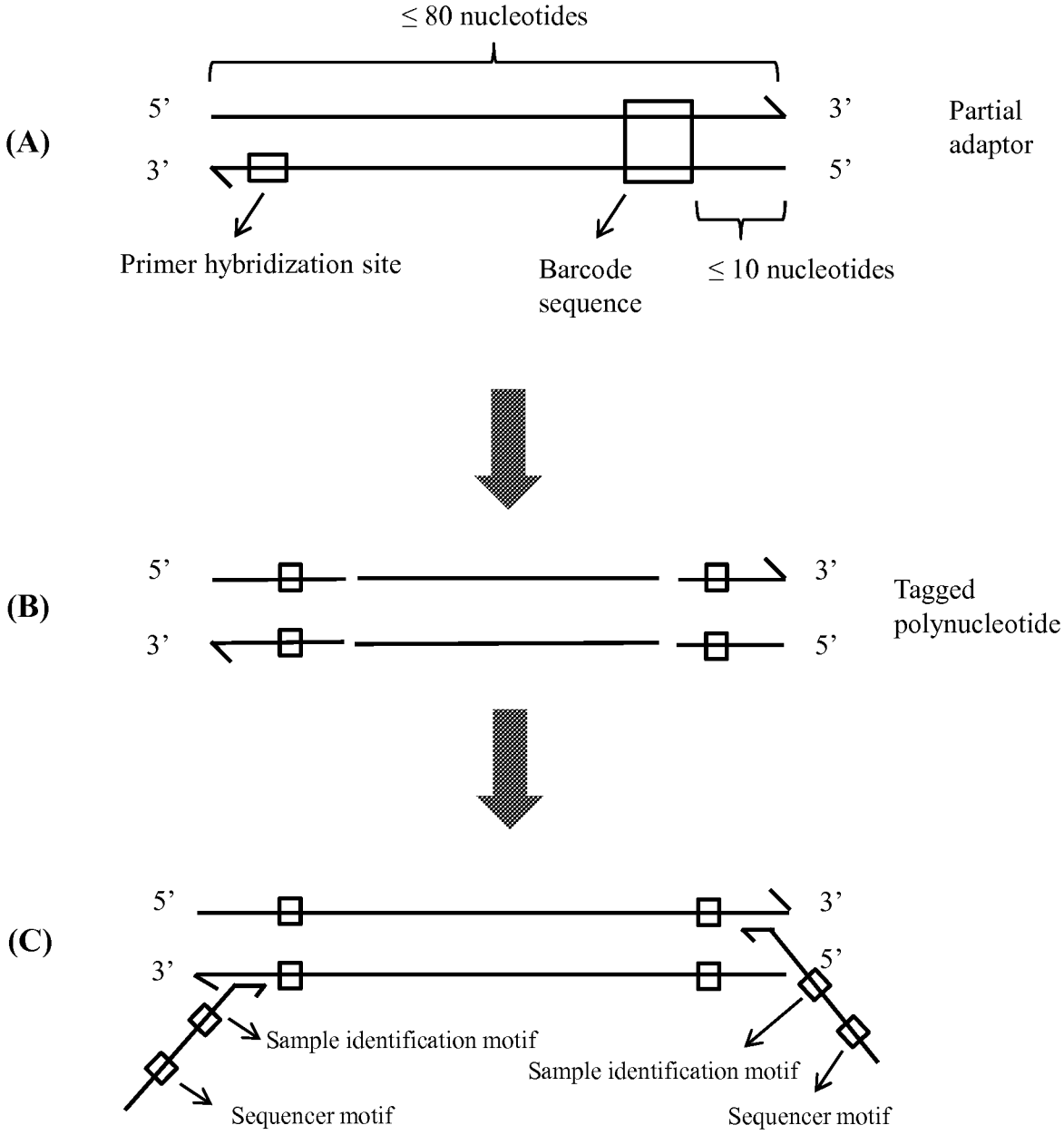
≤ 80 nucleotides
(A)
5'
3'
3'
5'
Partial adaptor
Primer hybridization site
Barcode sequence
≤ 10 nucleotides
(B)
5'
3'
3'
5'
Tagged polynucleotide
(C)
5'
3'
3'
5'
Sample identification motif
Sample identification motif
Sequencer motif
Sequencer motif

Fig. 9