Figure 1 A

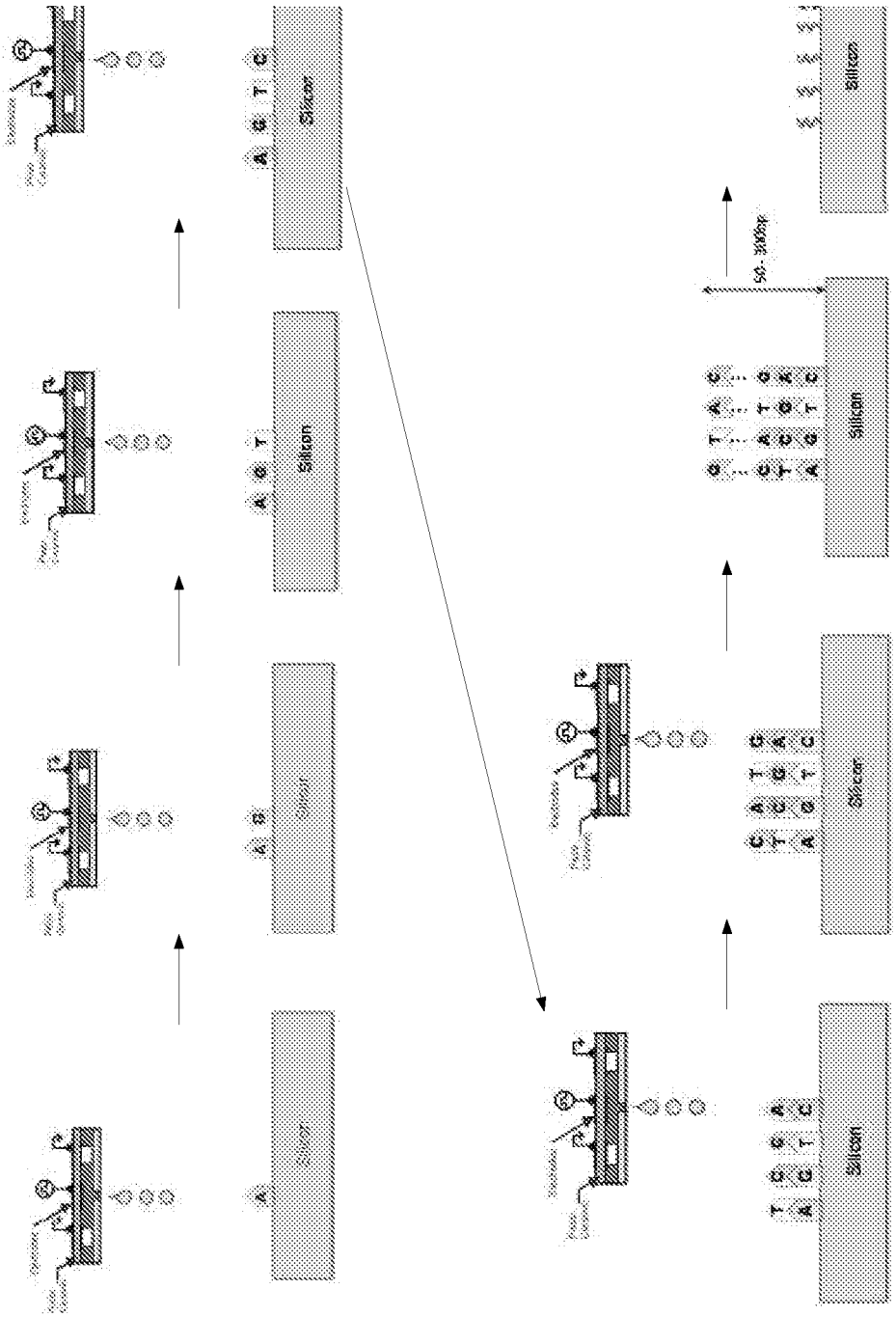

Silicon
Silicon
Silicon
Silicon
Silicon
Silicon
Silicon
Silicon

Figure 1 B

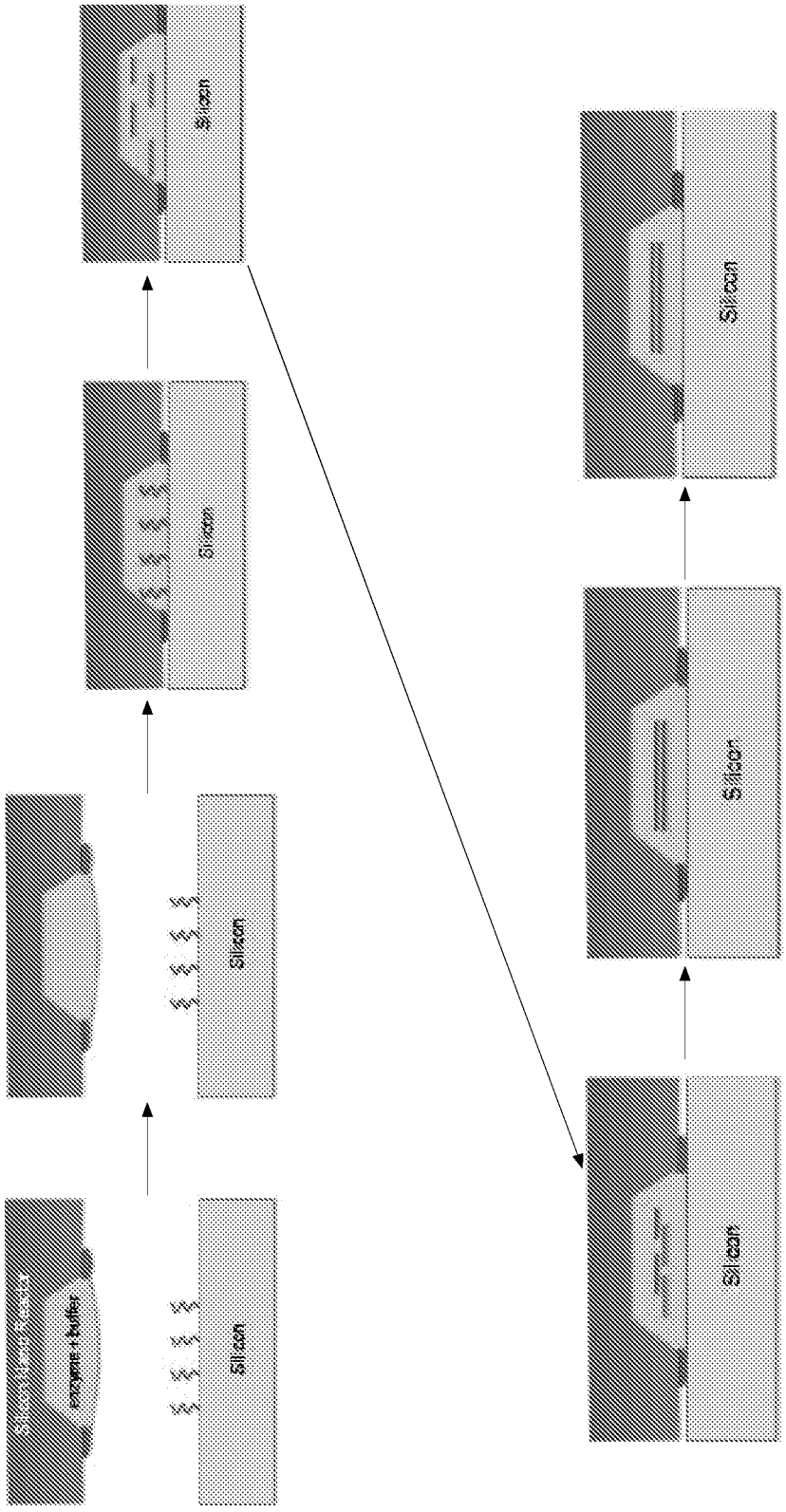


Figure 1 C

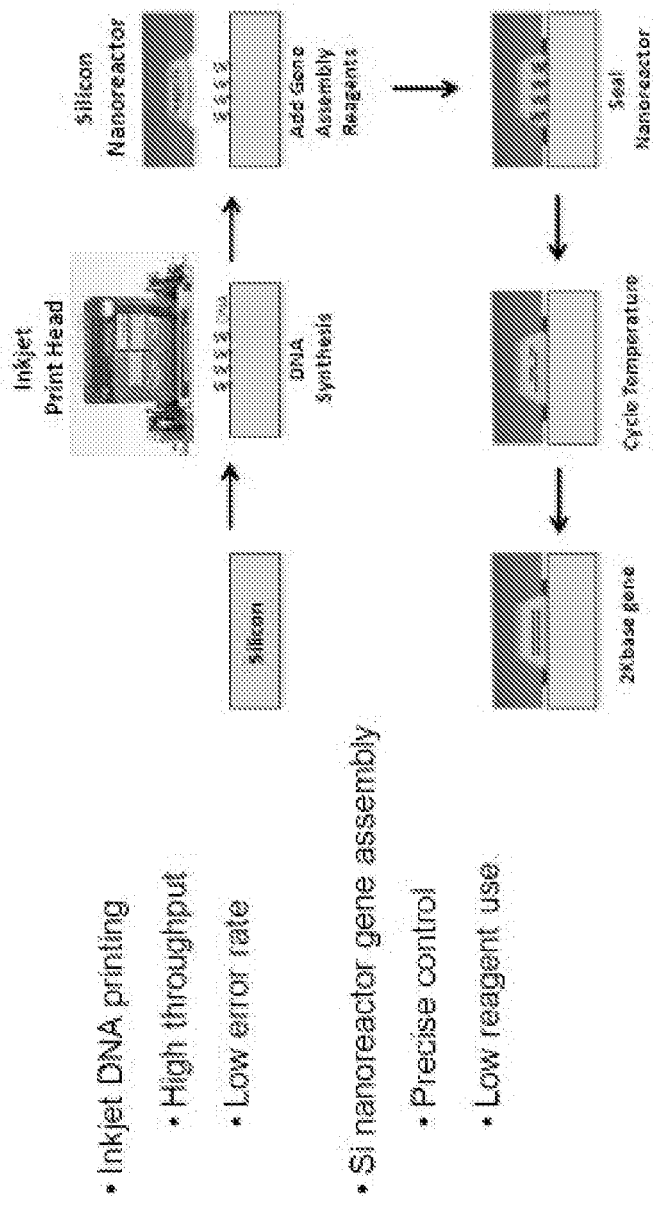

Gene Printing and NanoReactor Technology
• Inkjet DNA printing
• High throughput
• Low error rate
• Si nanoreactor gene assembly
• Precise control
• Low reagent use
Inkjet Print Head
Silicon Nanoreactor
Silicon
DNA Synthesis
Add Gene Assembly Reagents
2K base gene
Cycle Temperature
Seal Nanoreactor

Figure 2 A

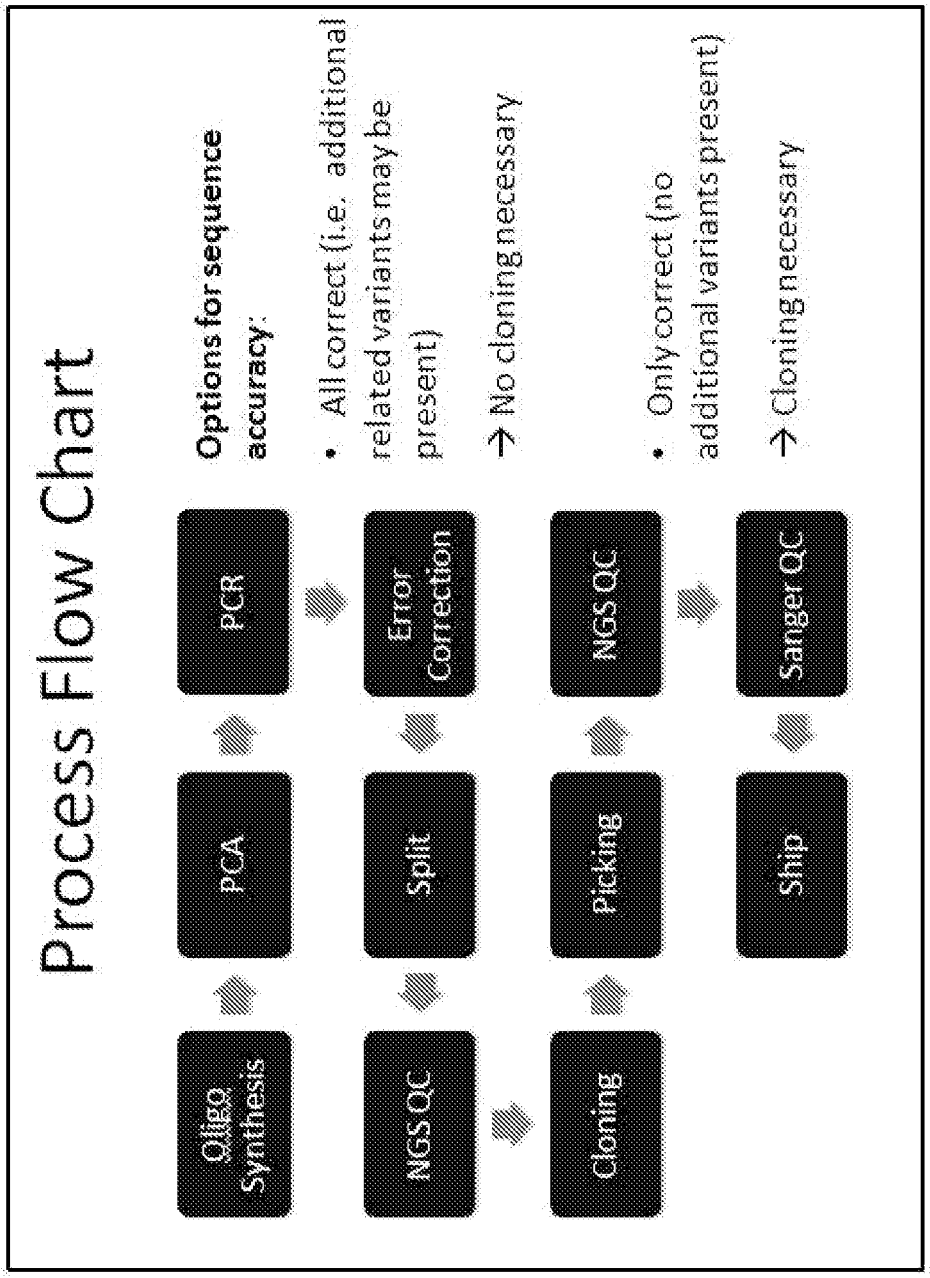

Process Flow Chart
Oligo Synthesis
PCA
PCR
Options for sequence accuracy:
• All correct (i.e. additional related variants may be present)
→ No cloning necessary
NGS QC
Split
Error Correction
Cloning
Picking
NGS QC
• Only correct (no additional variants present)
Ship
Sanger QC
→ Cloning necessary

Figure 2 B

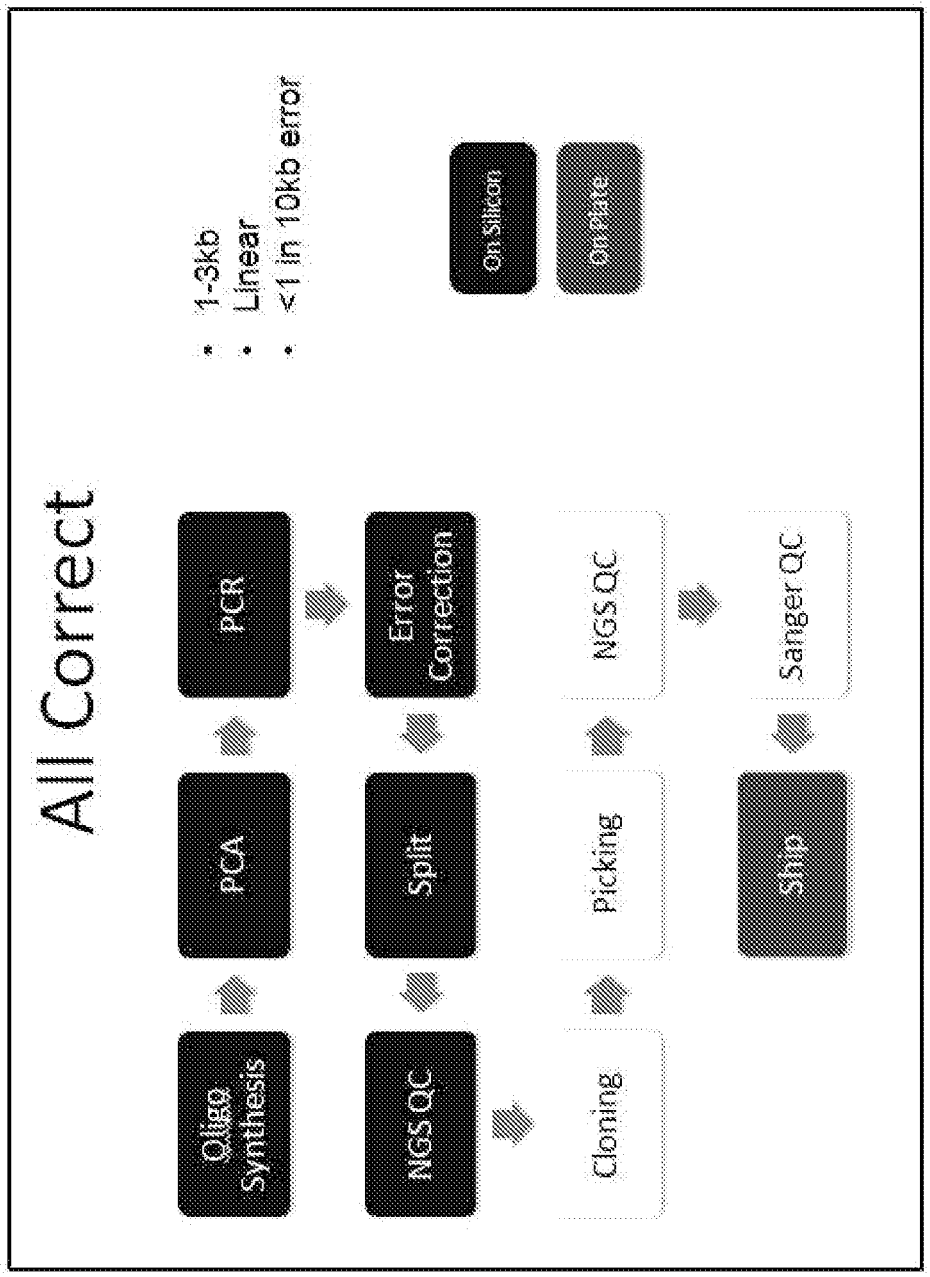

All Correct
1-3kb
Linear
<1 in 10kb error
Oligo Synthesis
PCA
PCR
Error Correction
Split
NGS QC
Cloning
Picking
NGS QC
Ship
Sanger QC
On Silicon
On Plate

Figure 2 C

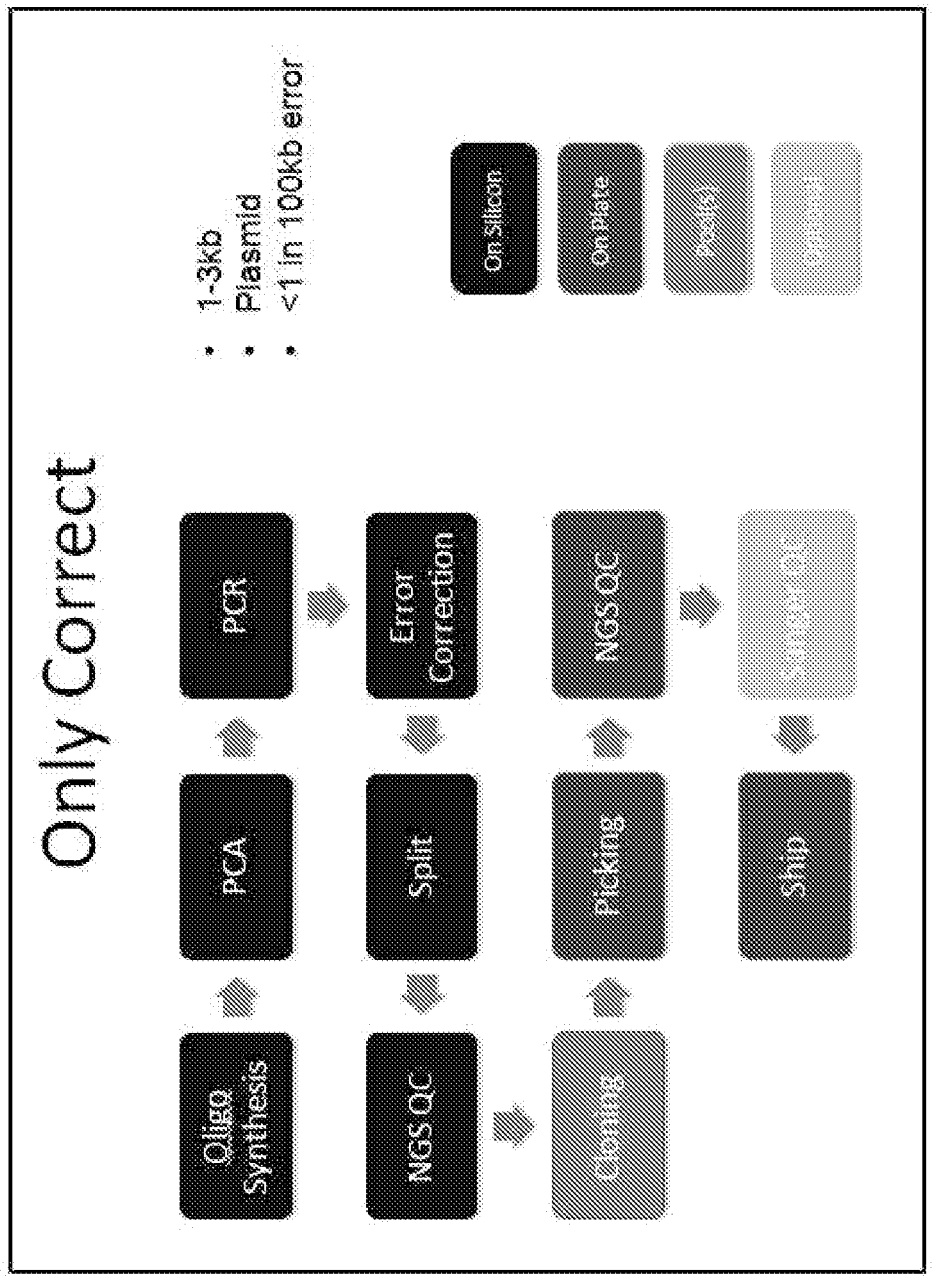

Only Correct
1-3kb
Plasmid
<1 in 100kb error
Oligo Synthesis
PCA
PCR
Error Correction
Split
NGS QC
Cloning
Picking
NGS QC
Ship
On Silicon
On Plate
Assay

Figure 3
Oligo Printer

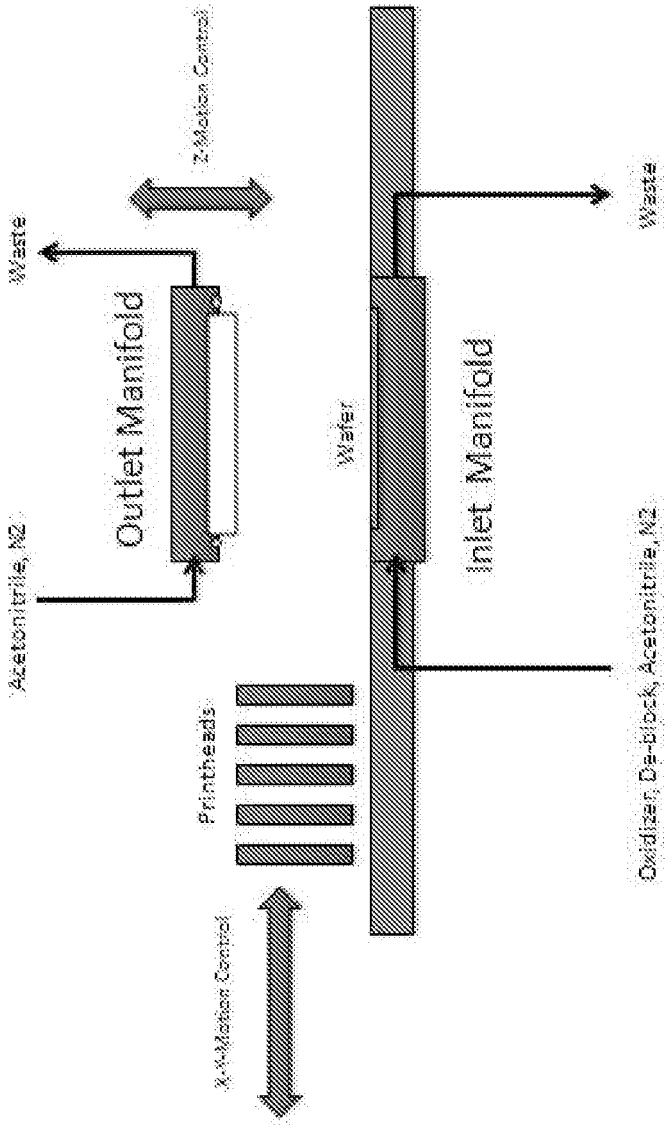


Figure 4

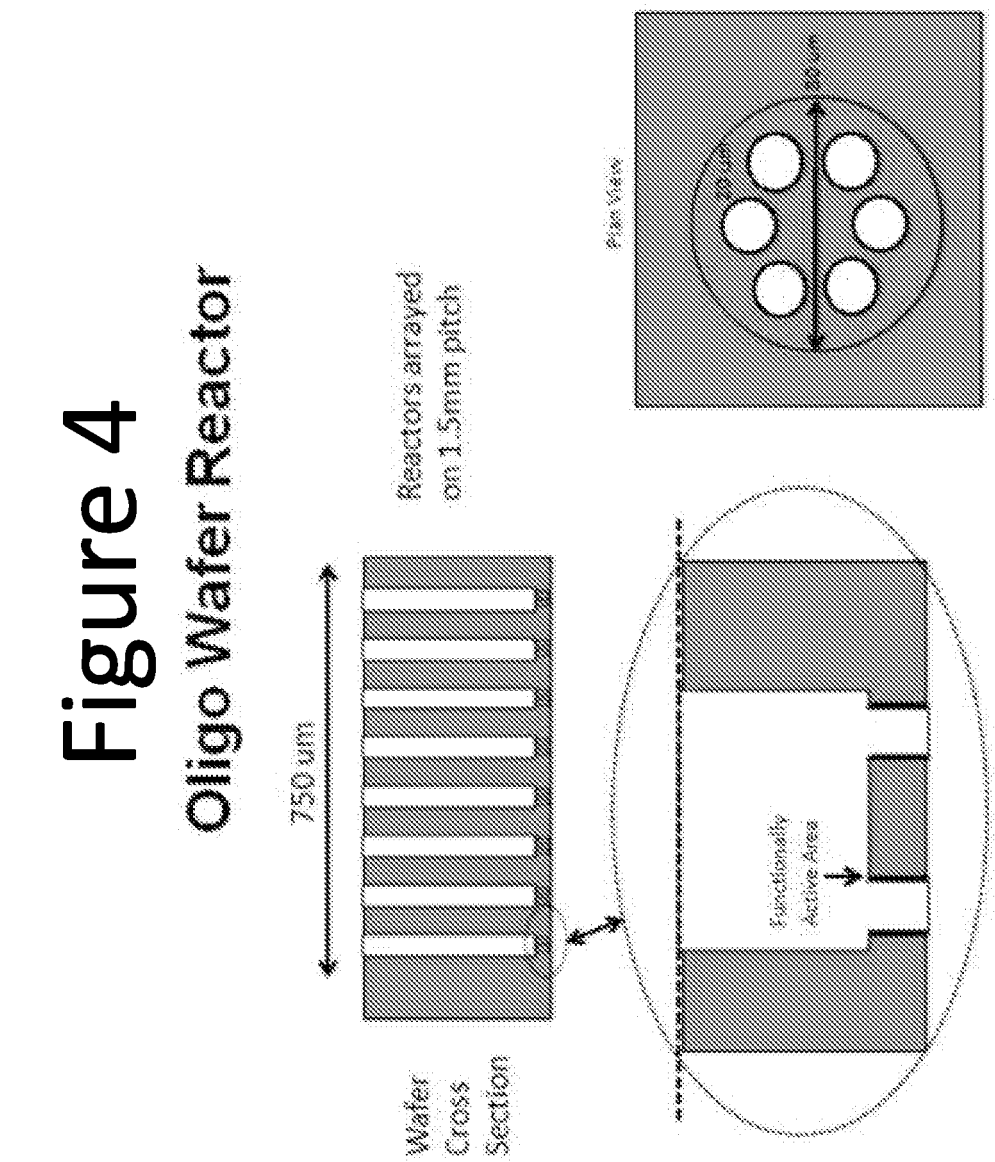

Oligo Wafer Reactor
750 um
Reactors arrayed on 1.5mm pitch
Wafer Cross Section
Functionality Active Area
Top View

Figure 5

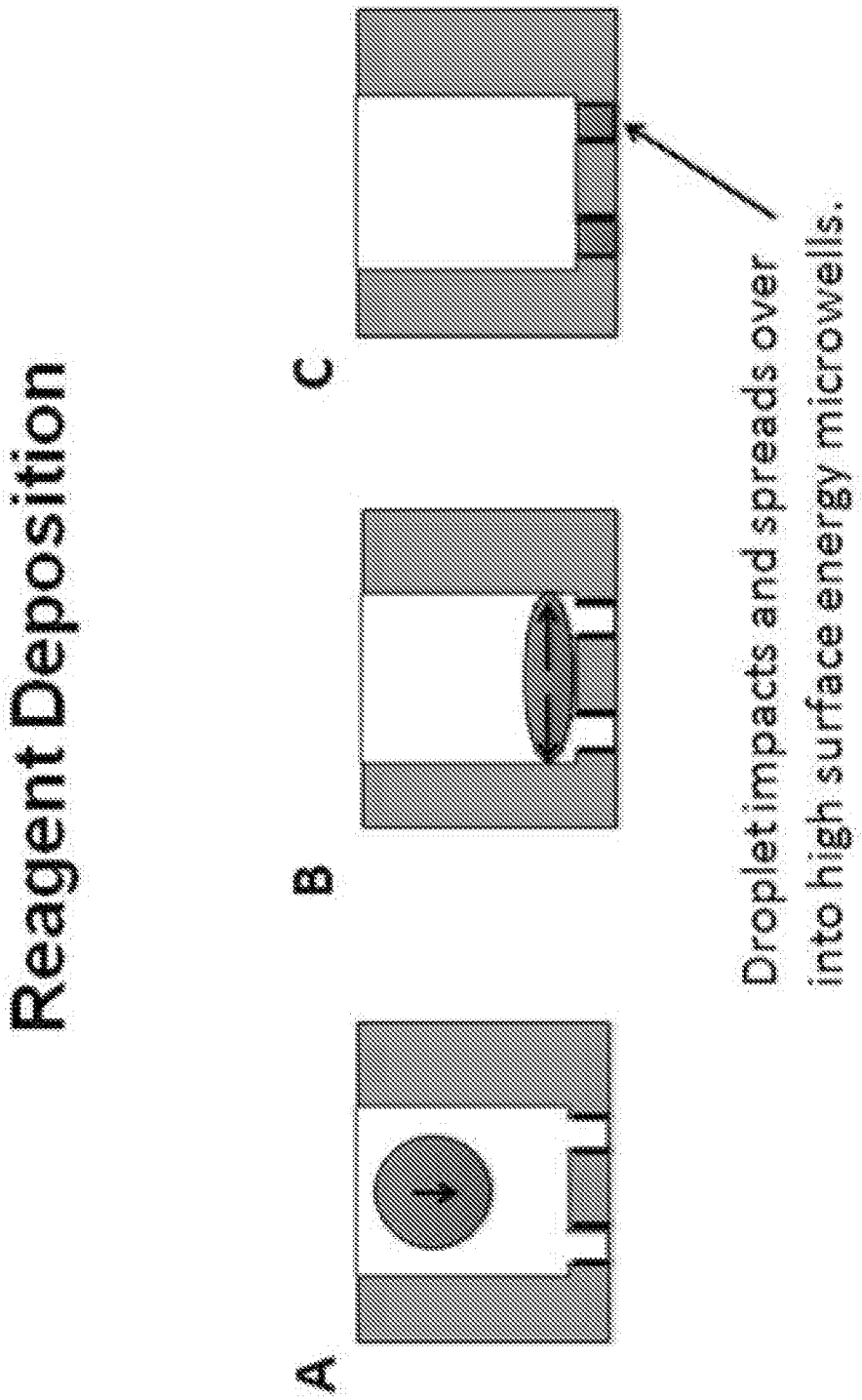

Reagent Deposition
A
B
C
Droplet impacts and spreads over
into high surface energy microwells.

Figure 6A

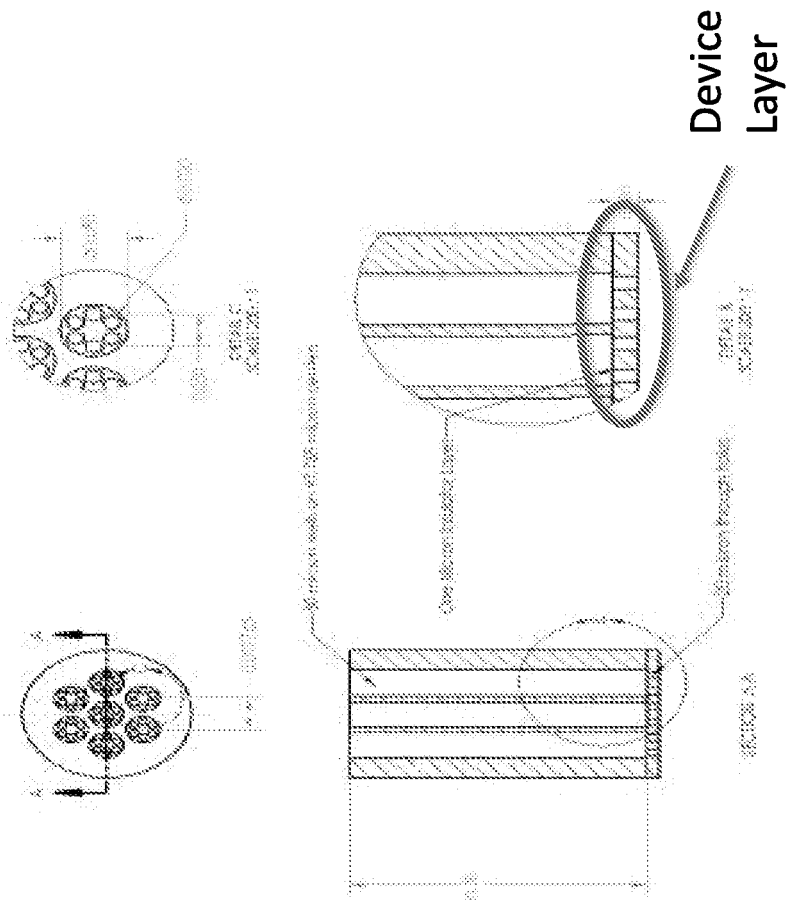


Figure 6B

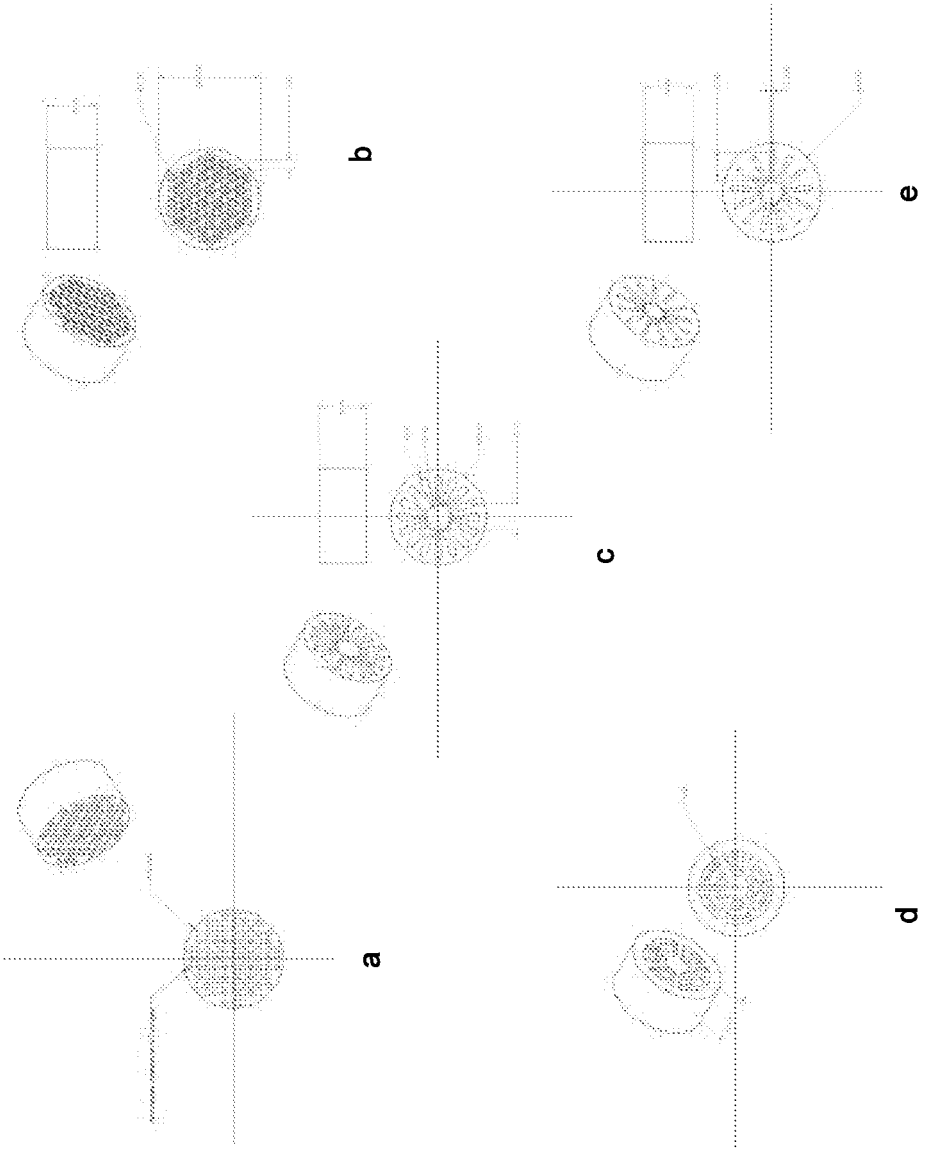


Figure 6C
Wafer Layout

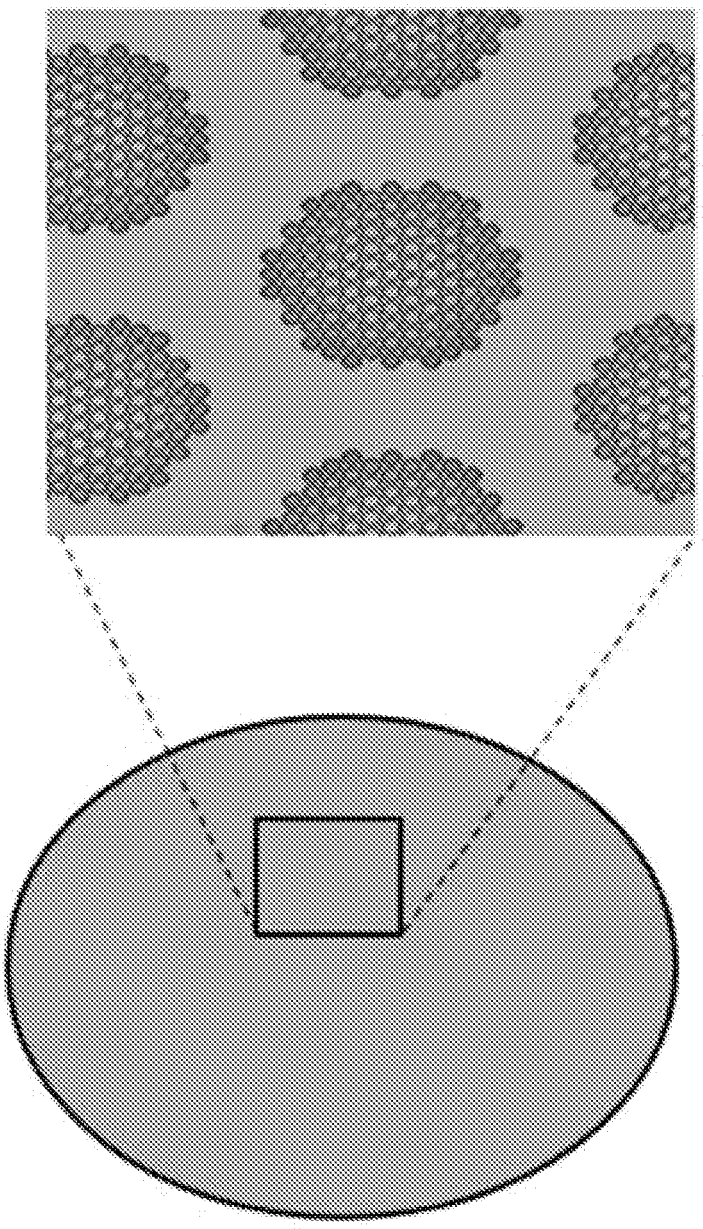


Figure 7

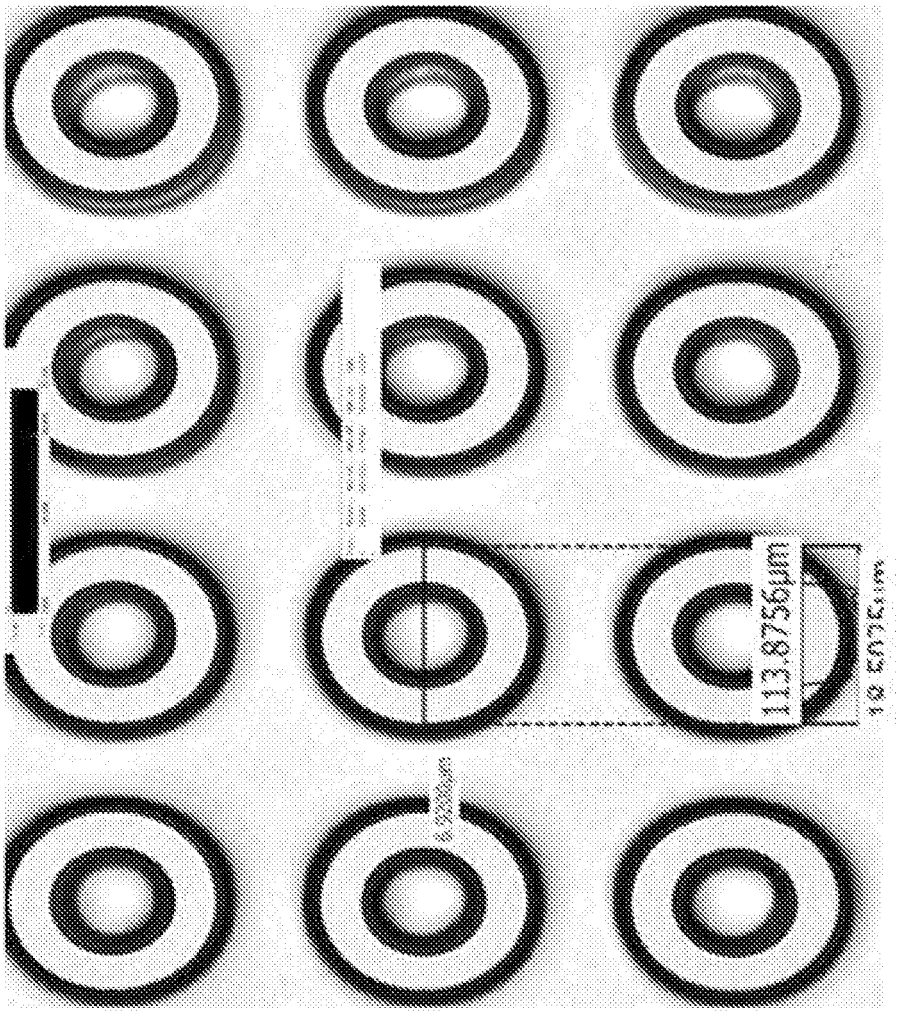


Figure 8

Process Workflow

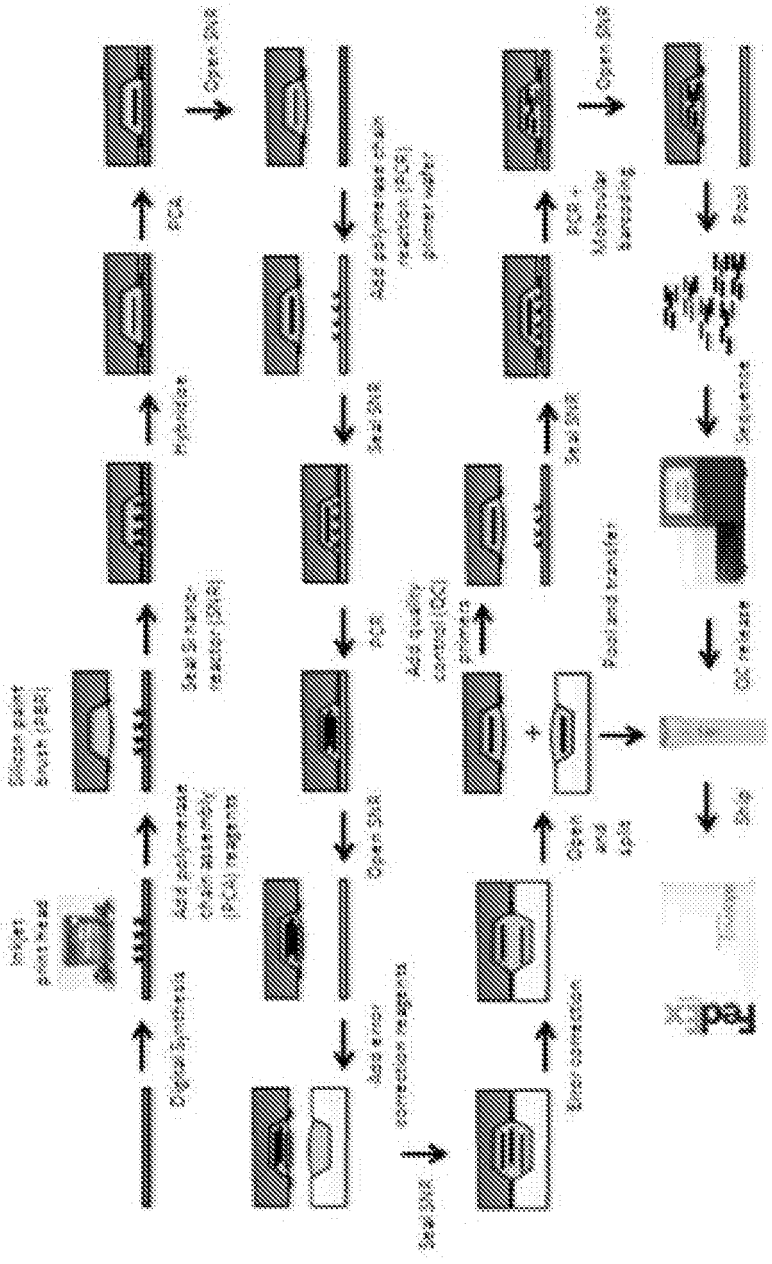


Figure 9

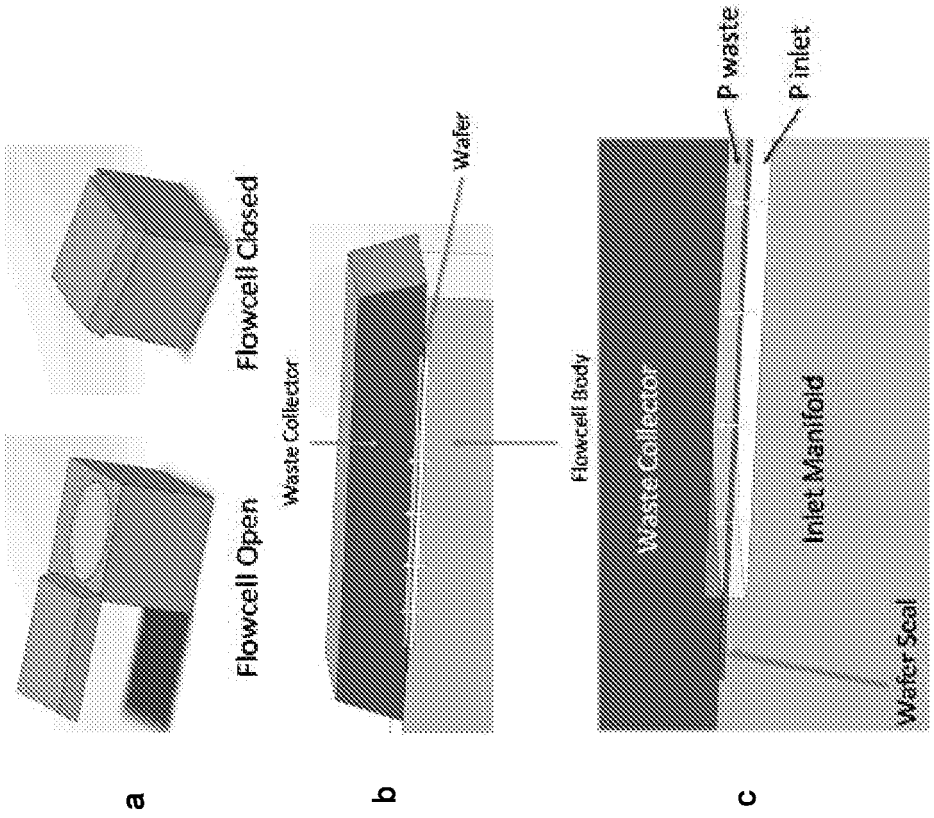

Flowcell Open
Flowcell Closed
a
Waste Collector
Wafer
Flowcell Body
b
P waste
P inlet
Waste Collector
Inlet Manifold
Wafer Seal
c

Figure 10

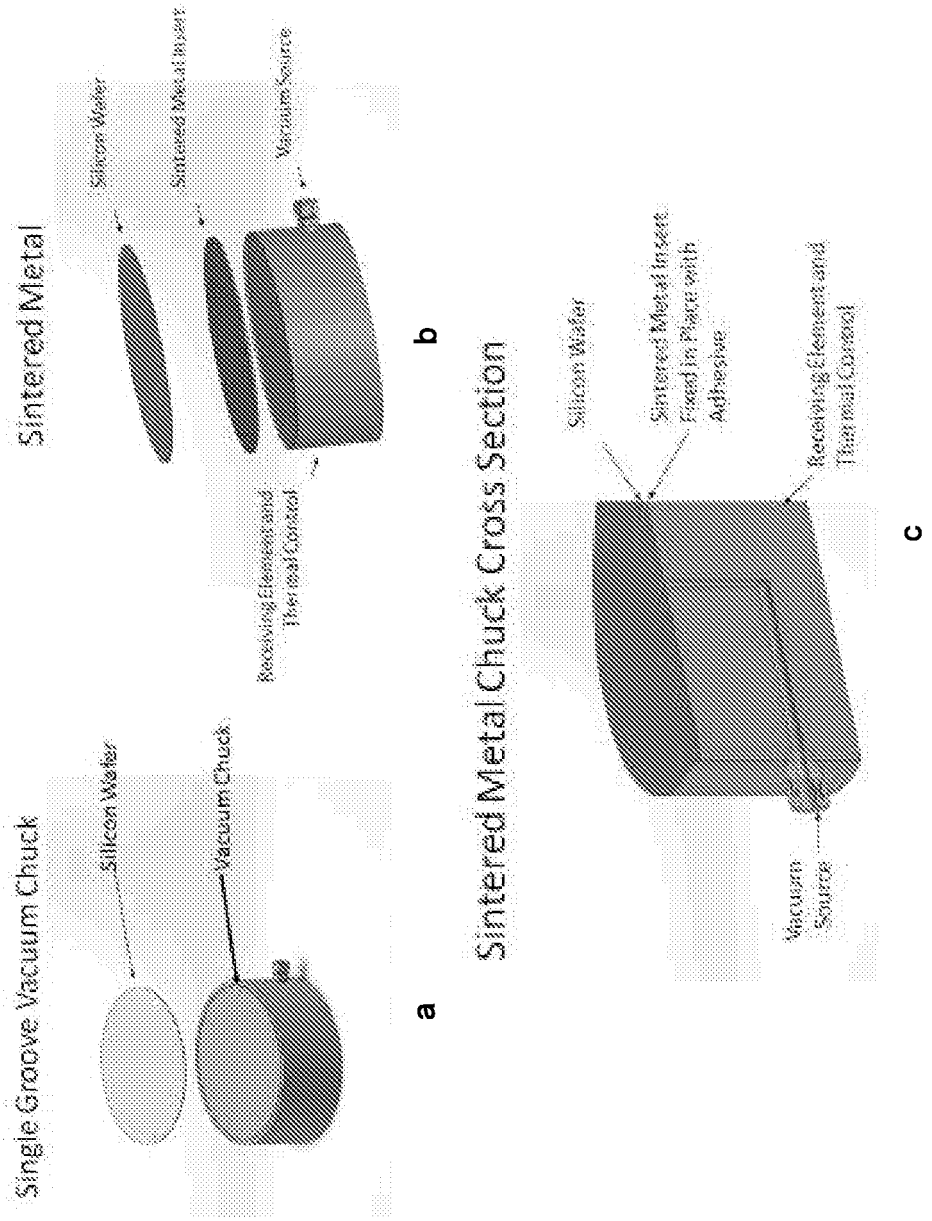

Single Groove Vacuum Chuck
Silicon Wafer
Vacuum Chuck
a
Sintered Metal
Silicon Wafer
Sintered Metal Insert
Vacuum Source
Receiving Element and Thermal Control
b
Sintered Metal Chuck Cross Section
Silicon Wafer
Sintered Metal Insert Fixed in Place with Adhesive
Receiving Element and Thermal Control
Vacuum Source
c

Figure 11

Standard Phosphoramidite Chemistry

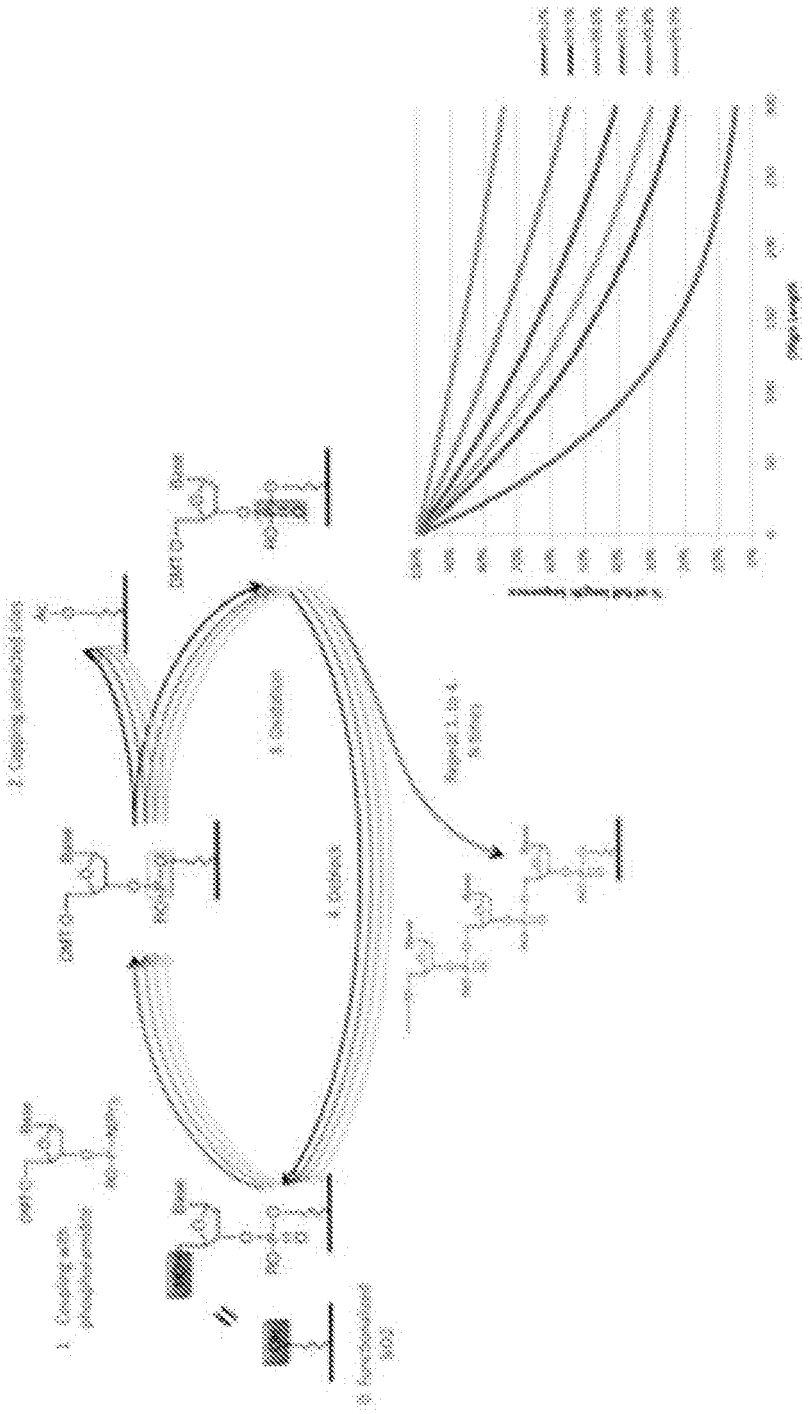


Figure 12

Polymerase Chain Assembly

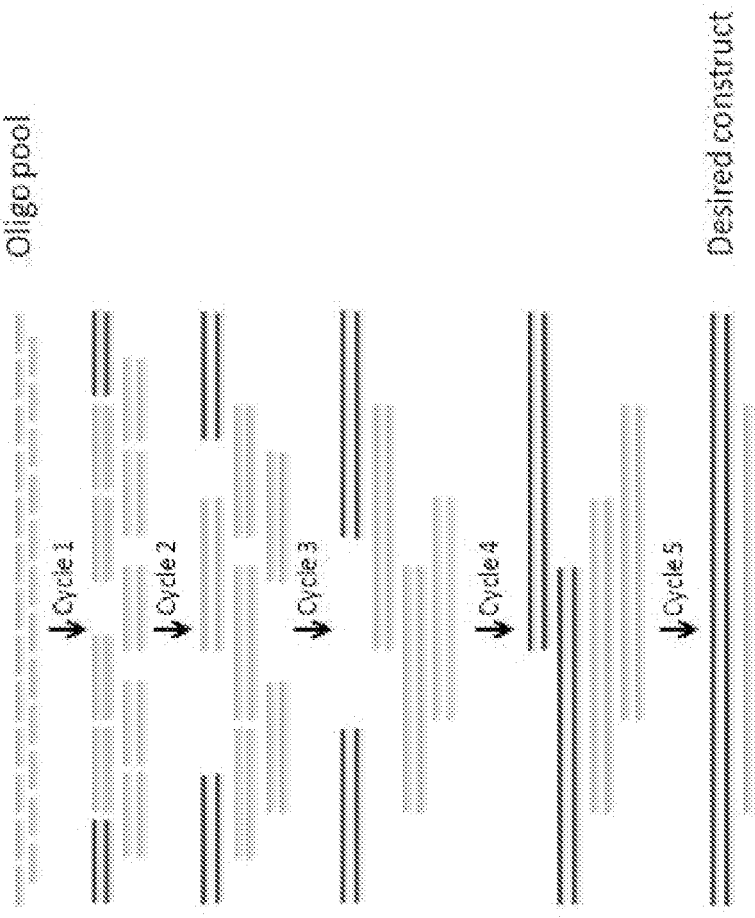


Figure 13
Advantage of Long Oligos

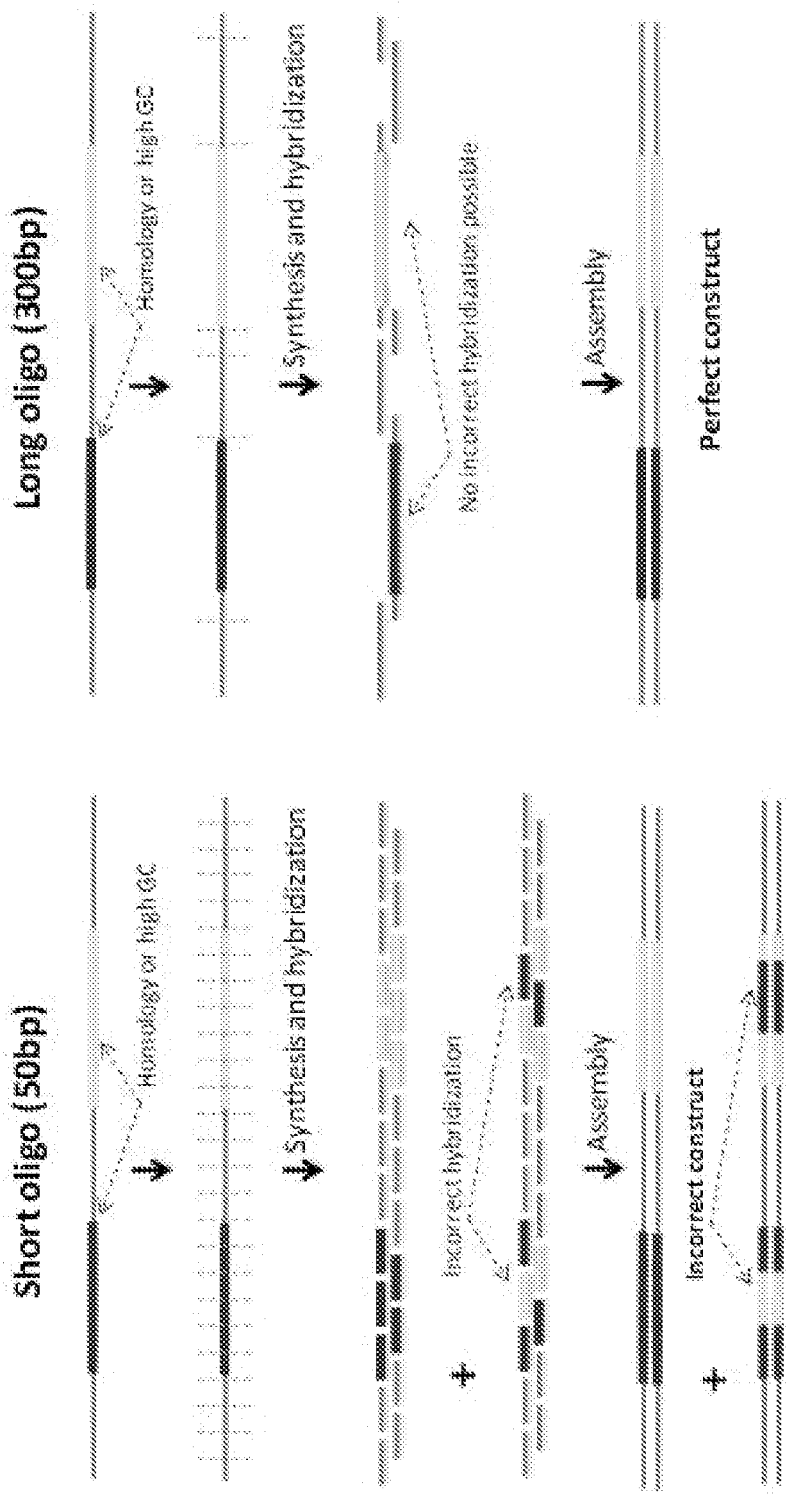


Figure 14

PCA + Gibson: 2-10Kb assembly

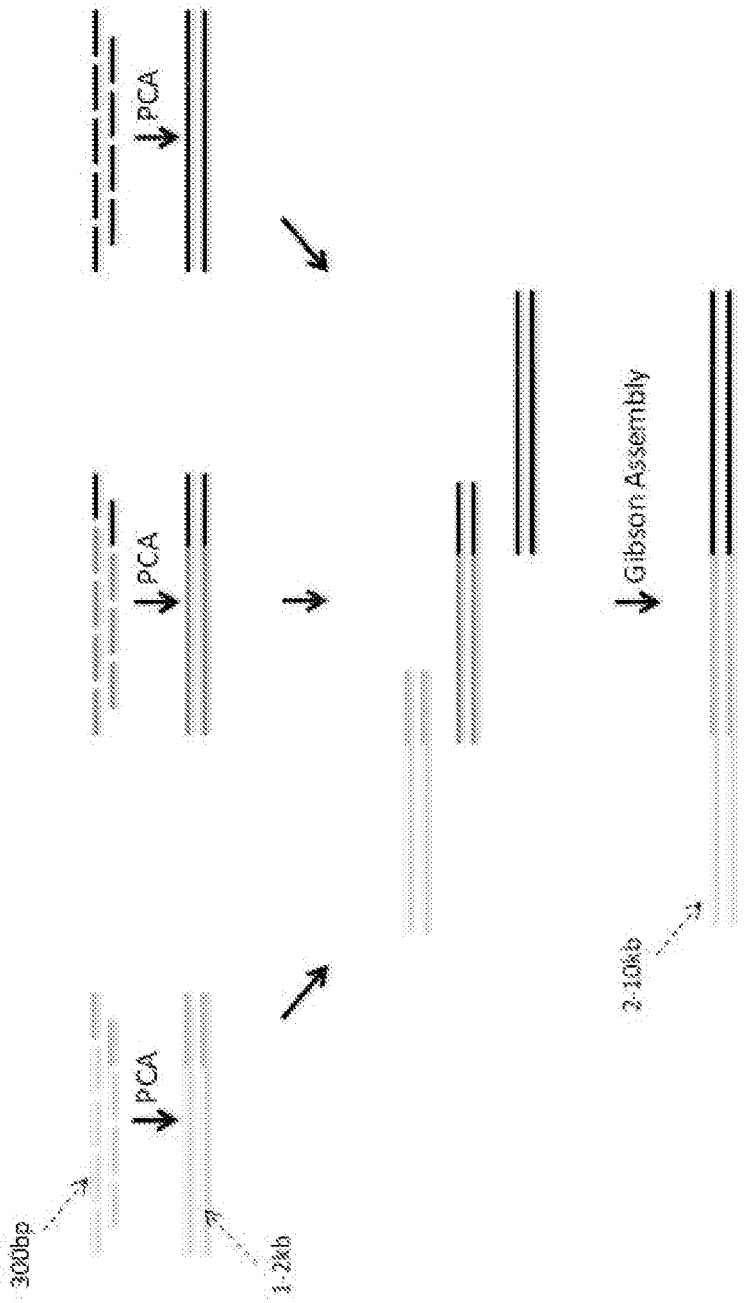


Figure 15

Error Correction: High Error Rate

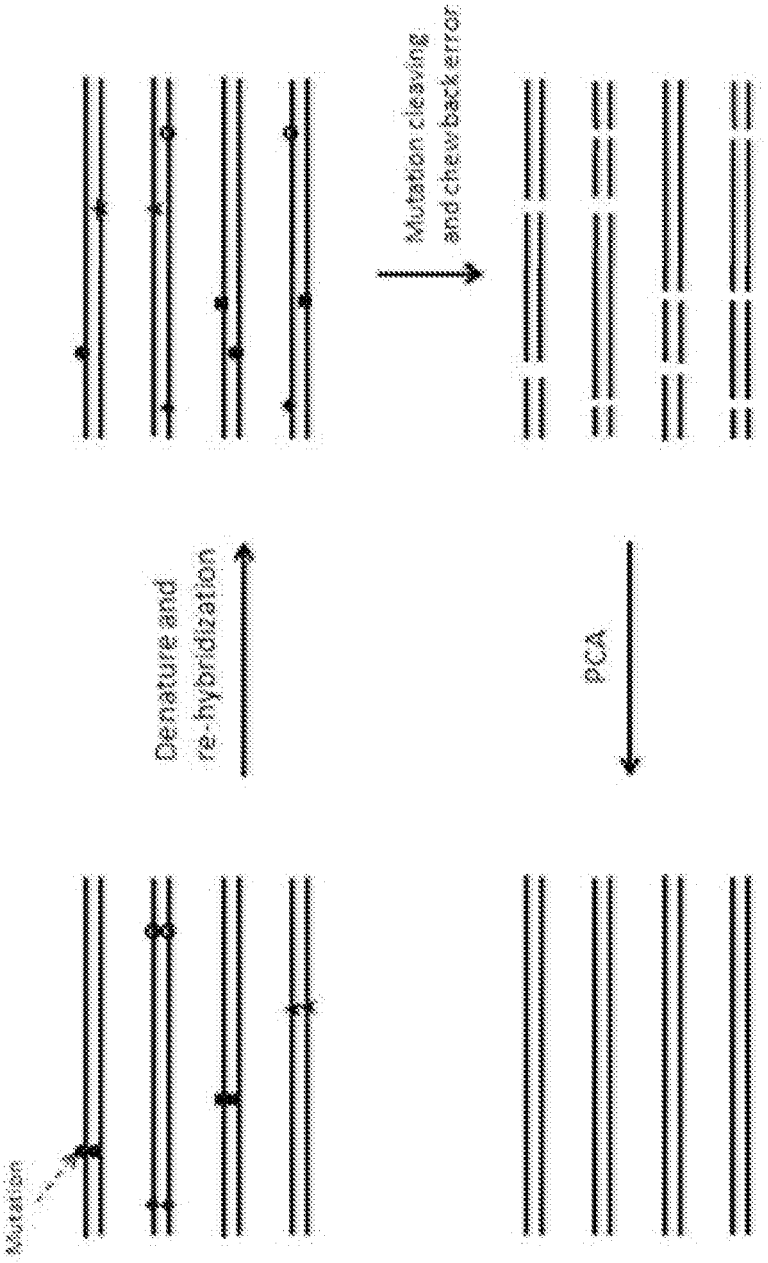


Figure 16

Error Correction: Low Error Rate

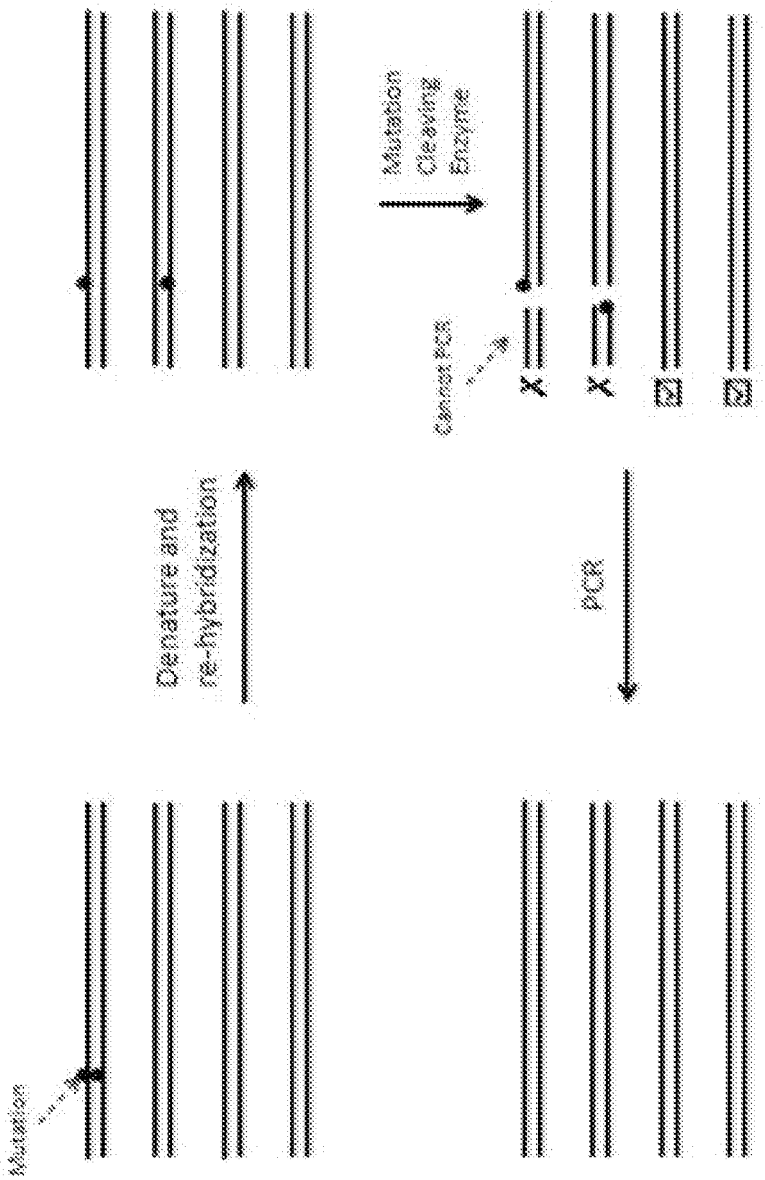


Figure 17

Molecular Barcoding & NGS QC

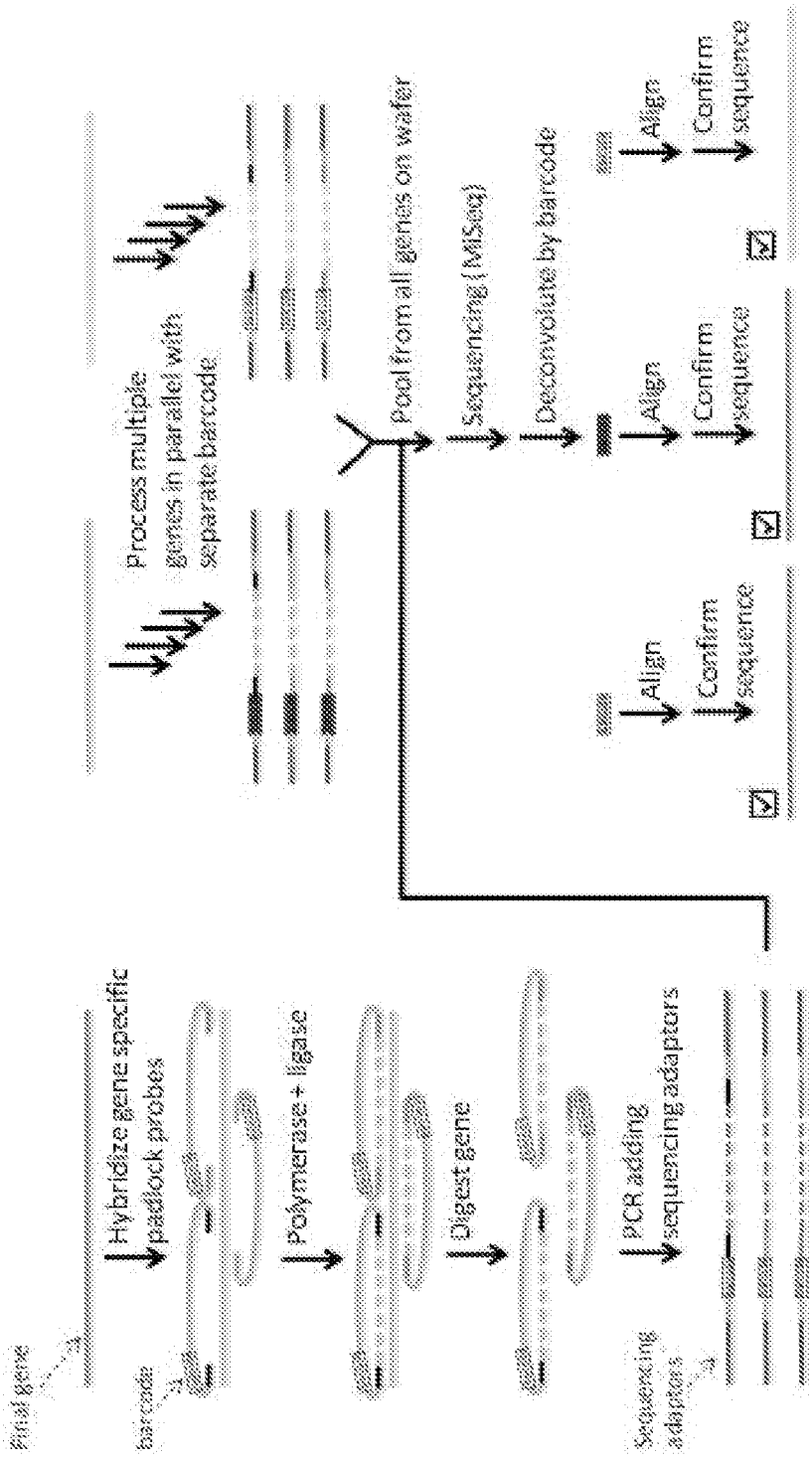


Figure 18

Inkjet Assembly

- Inkjet assembly of 10 heads
- Inkjet heads have Silicon orifice plates with 256 nozzles on 254um centers
- 100 micron fly height
- Each head needs access to each well that traverses

Figure 19

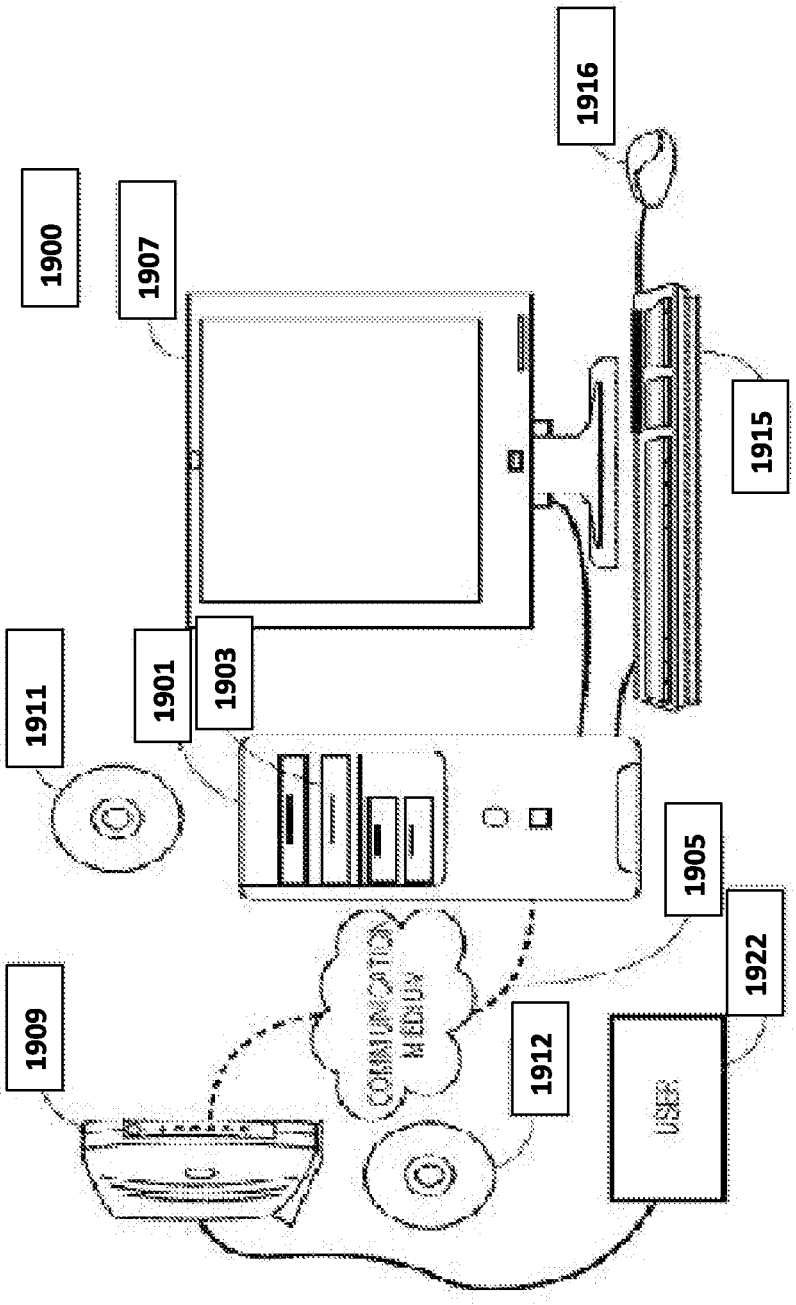


Figure 20

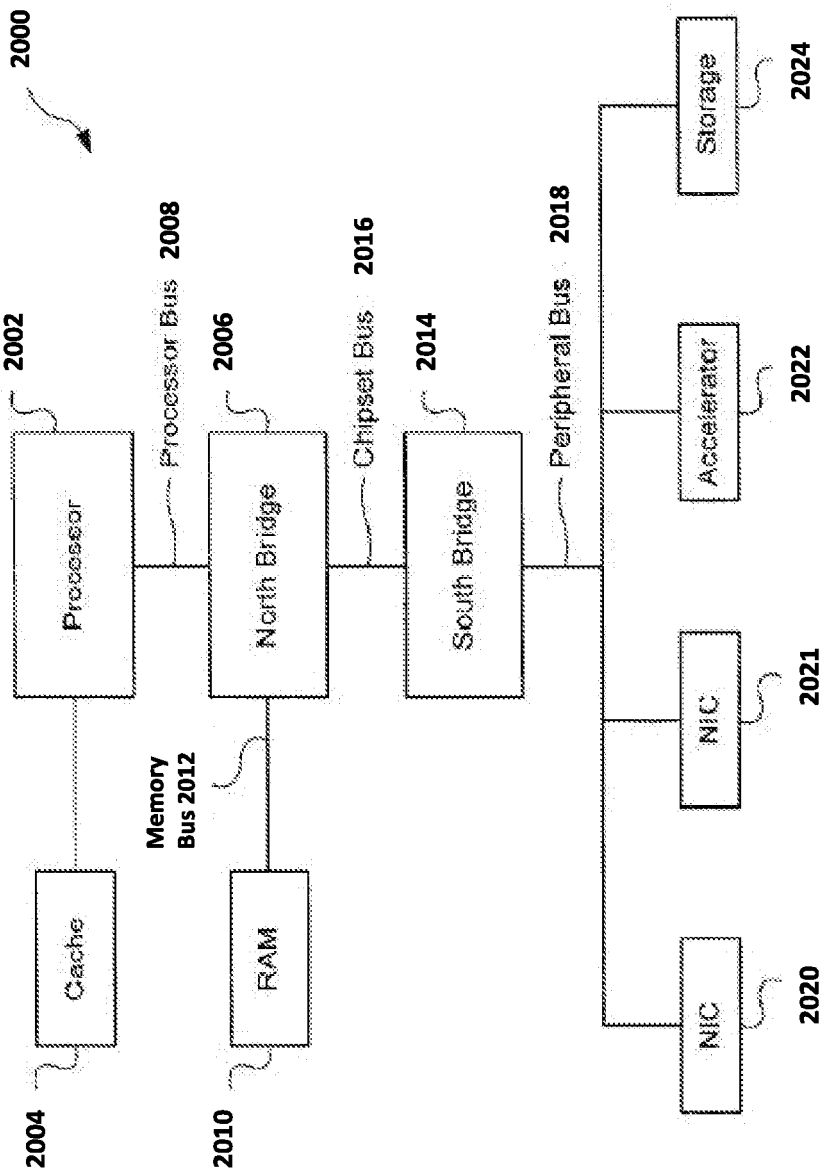

2000
2002
Processor
Processor Bus 2008
2006
North Bridge
Chipset Bus 2016
2014
South Bridge
Peripheral Bus 2018
Storage
2024
Accelerator
2022
NIC
2021
NIC
2020
Cache
2004
Memory Bus 2012
RAM
2010

Figure 21

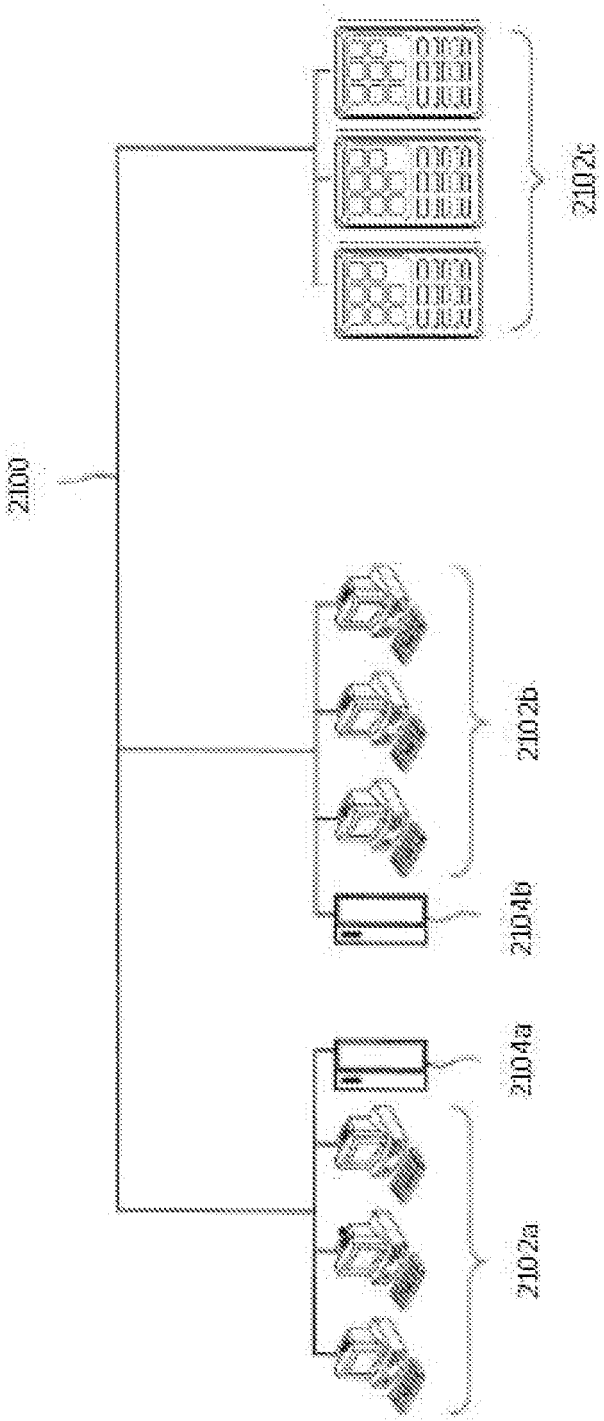


Figure 22

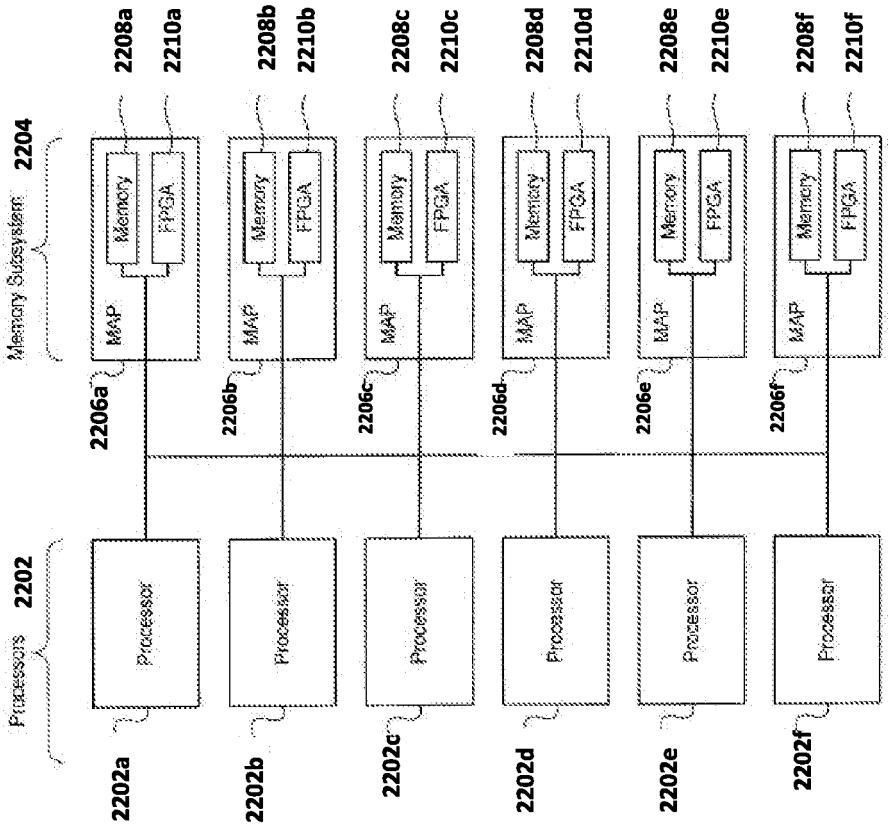

Processors 2202
Memory Subsystem 2204
Processor
MAP
Memory
FPGA
2202a
2202b
2202c
2202d
2202e
2202f
2206a
2206b
2206c
2206d
2206e
2206f
2208a
2208b
2208c
2208d
2208e
2208f
2210a
2210b
2210c
2210d
2210e
2210f

Figure 23

Front-End Processing

1. SOI substrate with oxide
2. Handle-side photolithography
3. Handle-side deep RIE
4. Photoresist strip
5. Device-side photolithography
6. Device-side deep RIE
7. Photoresist & oxide strip
8. Oxide growth

Figure 24
Back-End Processing

1. Front-end processing result
2. Inkjet photoresist
3. Oxygen plasma etch back
4. Passive functionalization
5. Photoresist strip
6. Active functionalization

Figure 25A

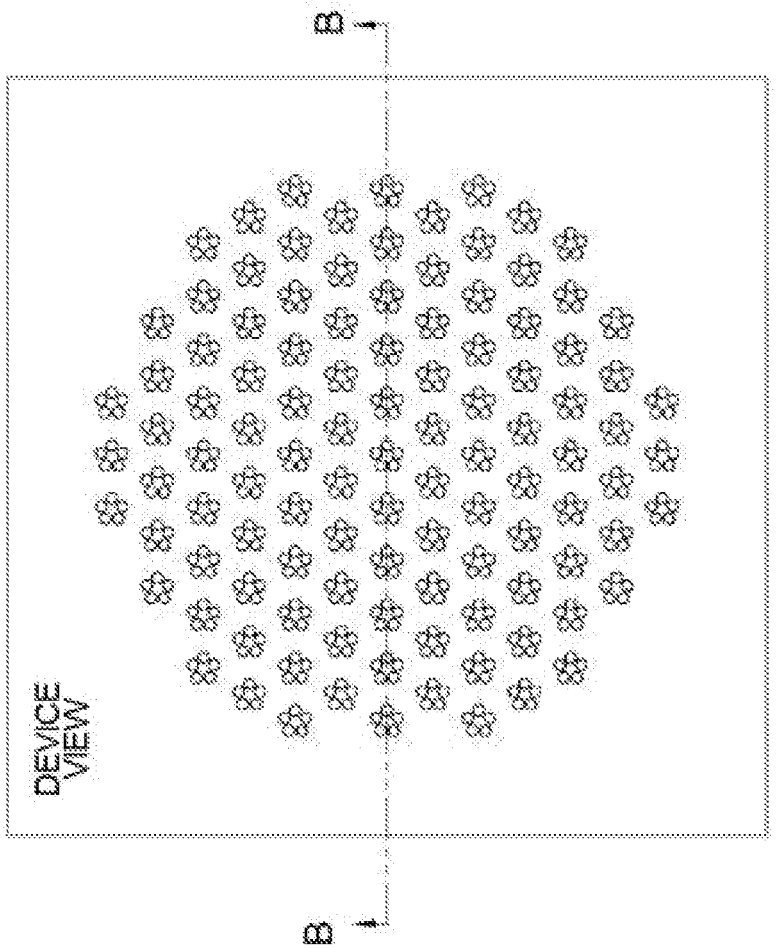


Figure 25B

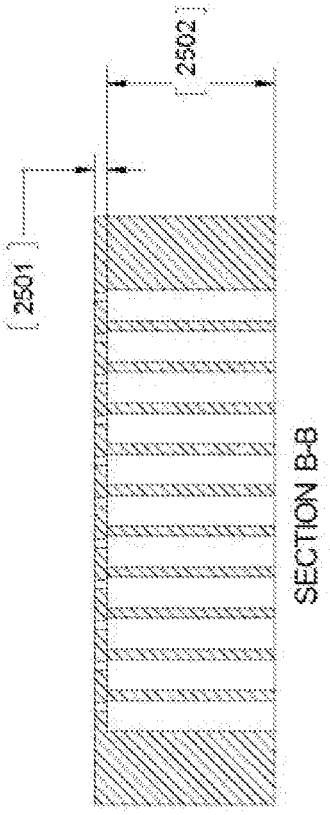


Figure 25C

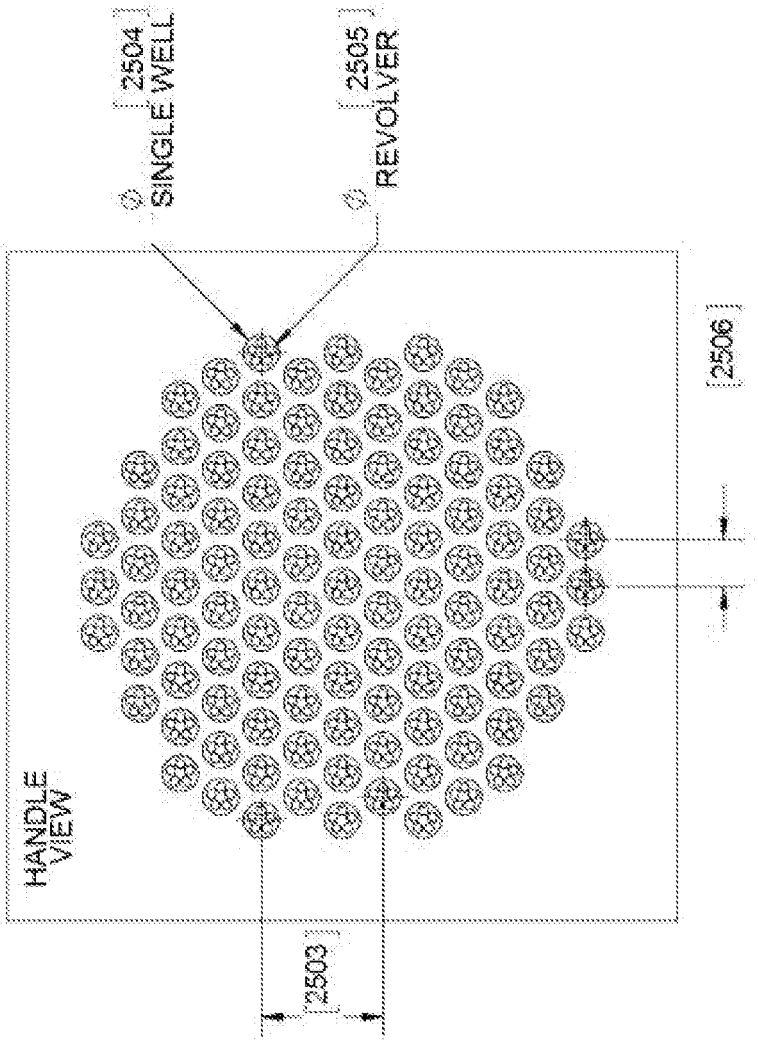


Figure 25D

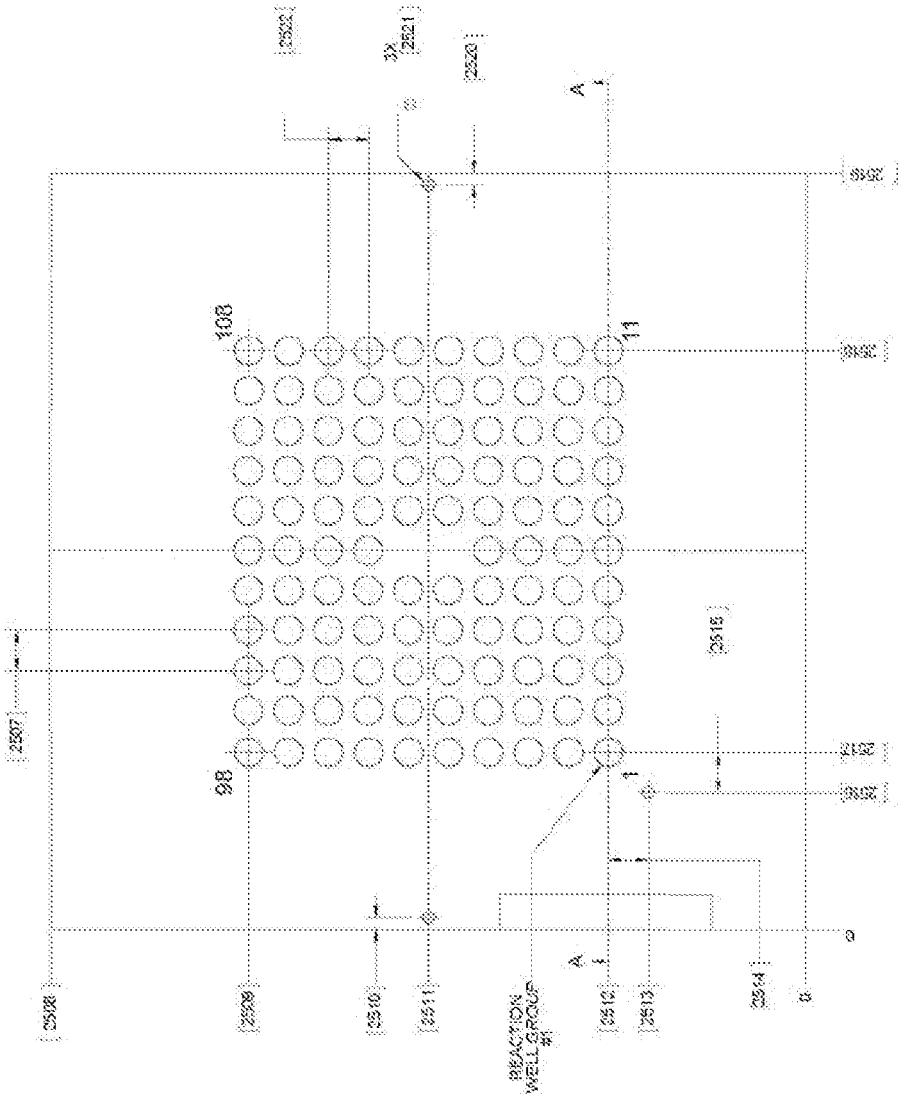


Figure 25E

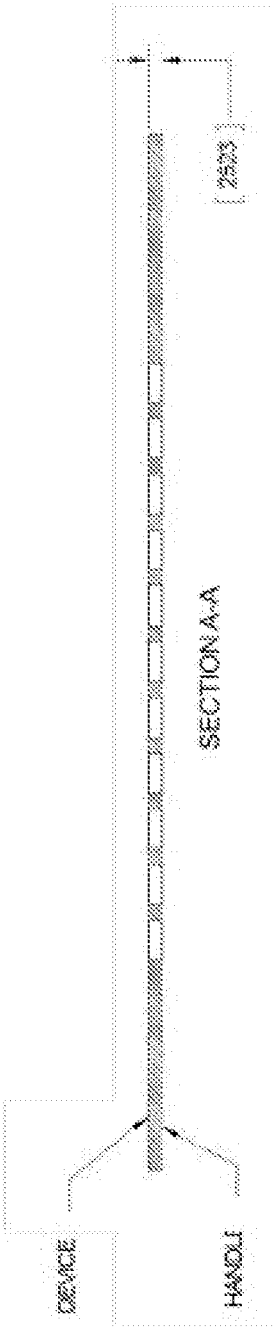


Figure 25F

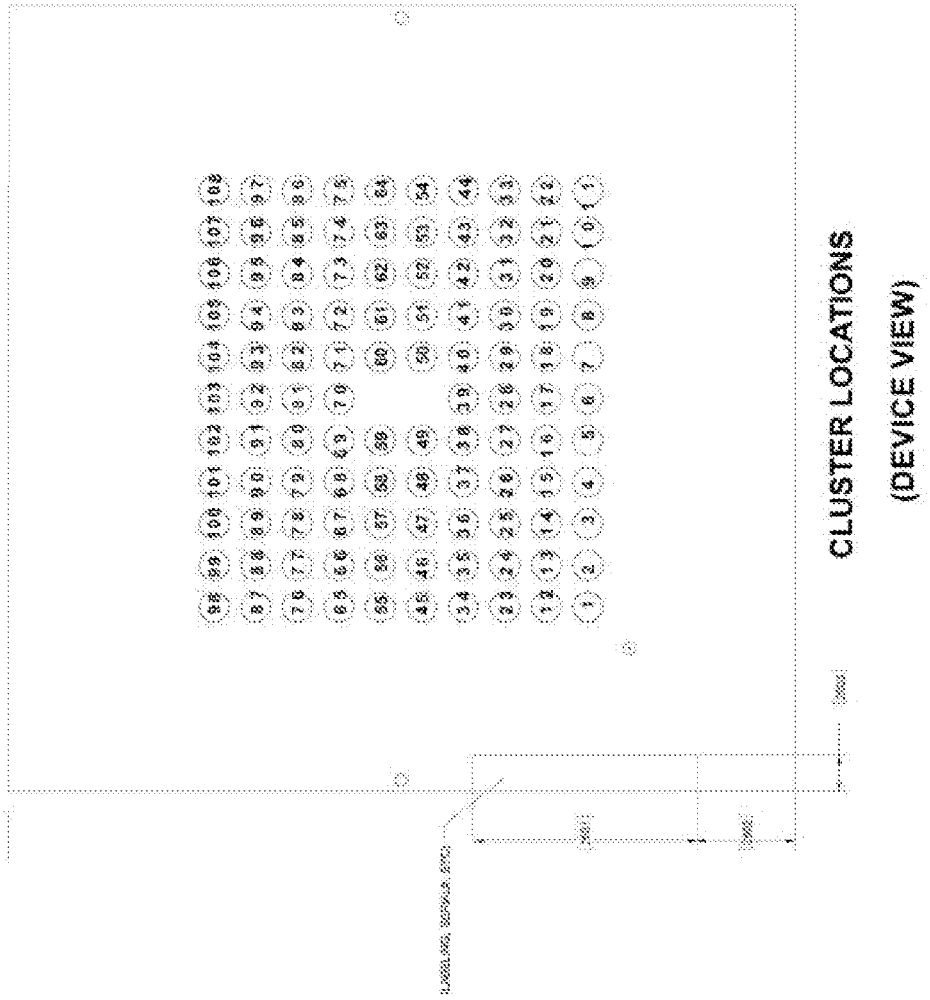
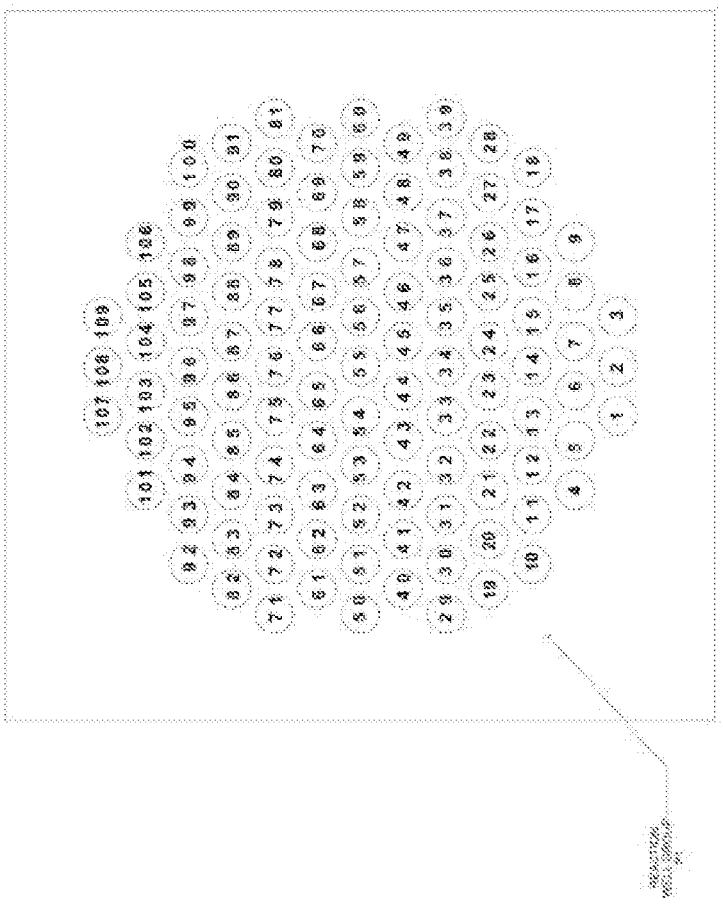


Figure 25G



WELL LOCATIONS
WITHIN INDIVIDUAL CLUSTER

(DEVICE VIEW)

Figure 26A

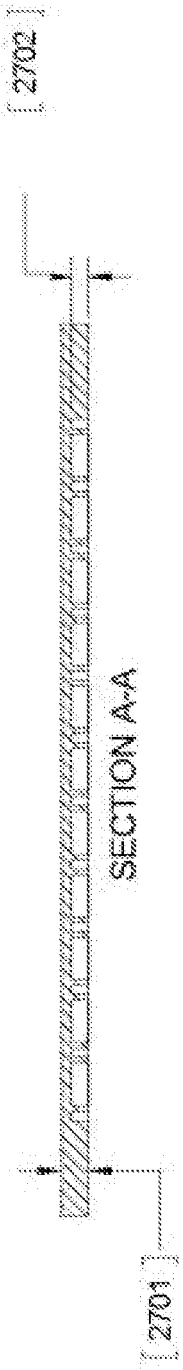


Figure 26B

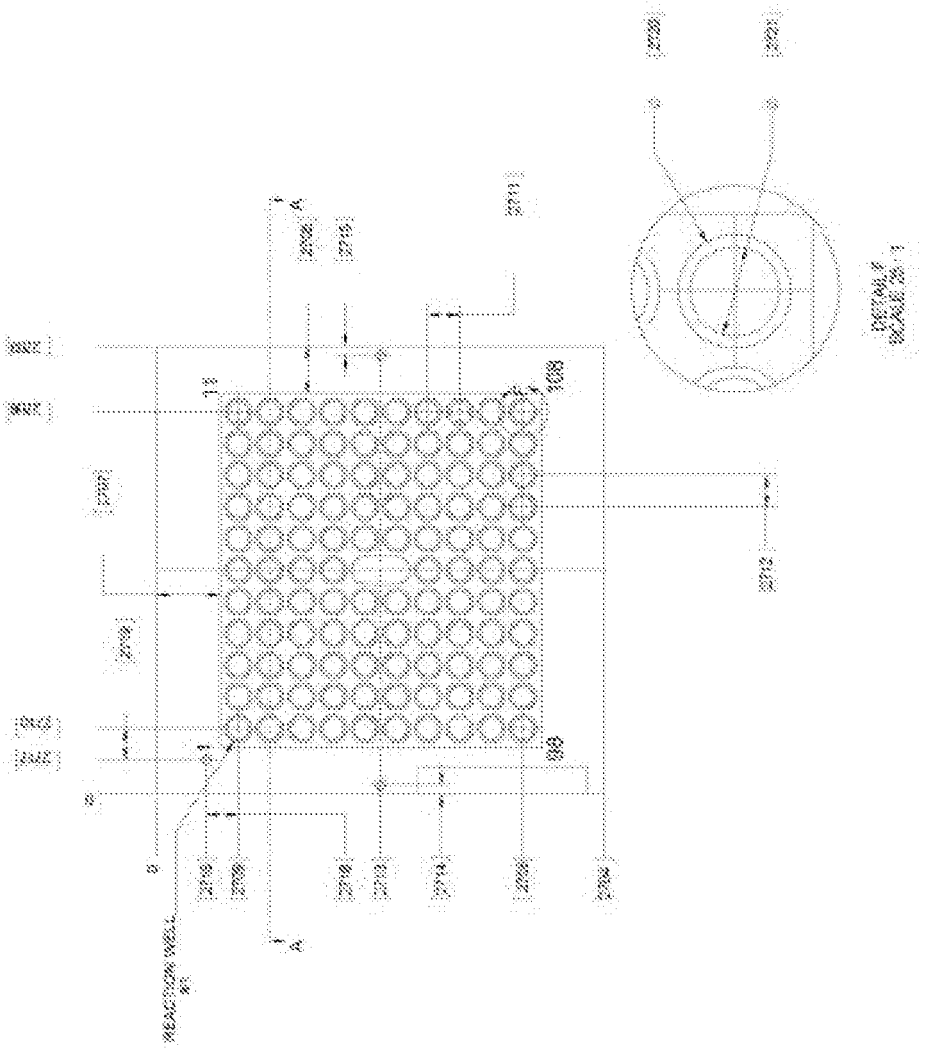


Figure 26C

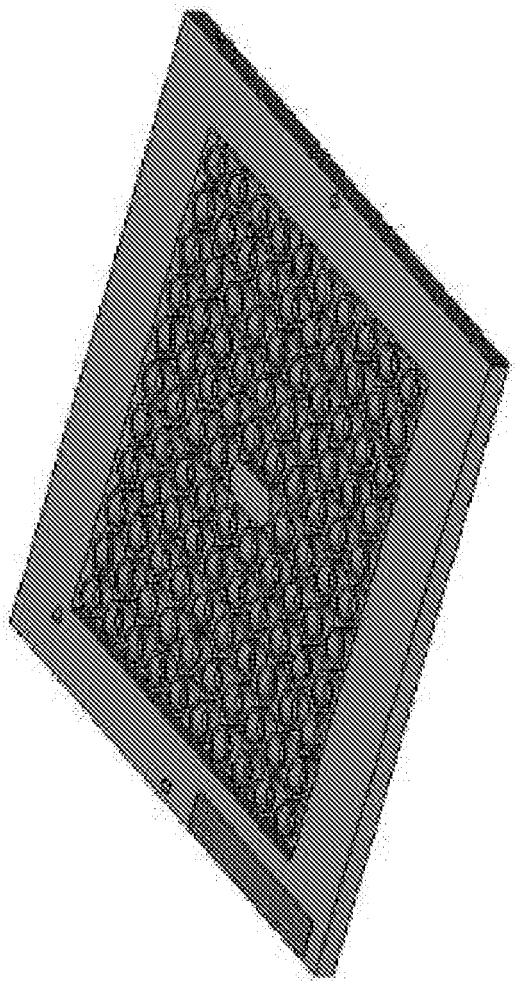


Figure 26D

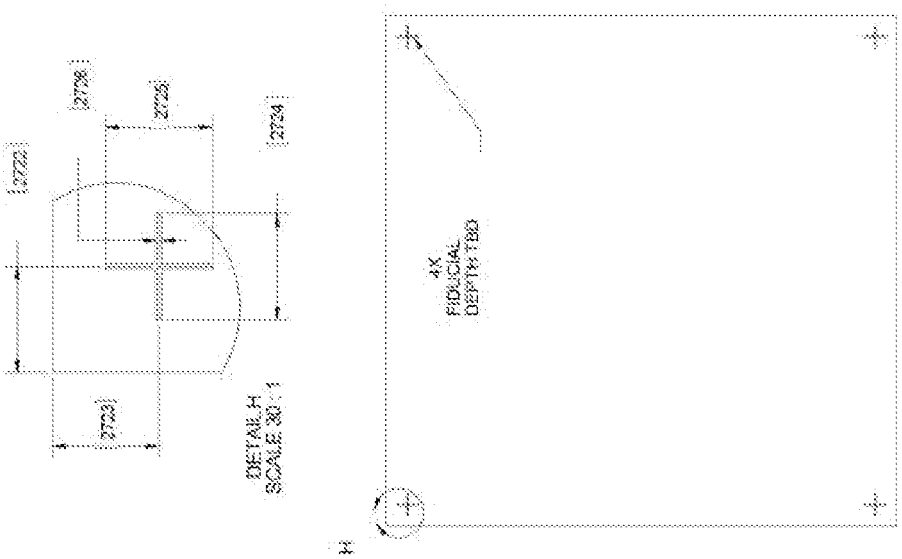

2726
2725
2724
2722
2723
DETAIL H
SCALE 30 : 1
4X
FIDUCIAL
DEPTH TBD
H

Figure 26E

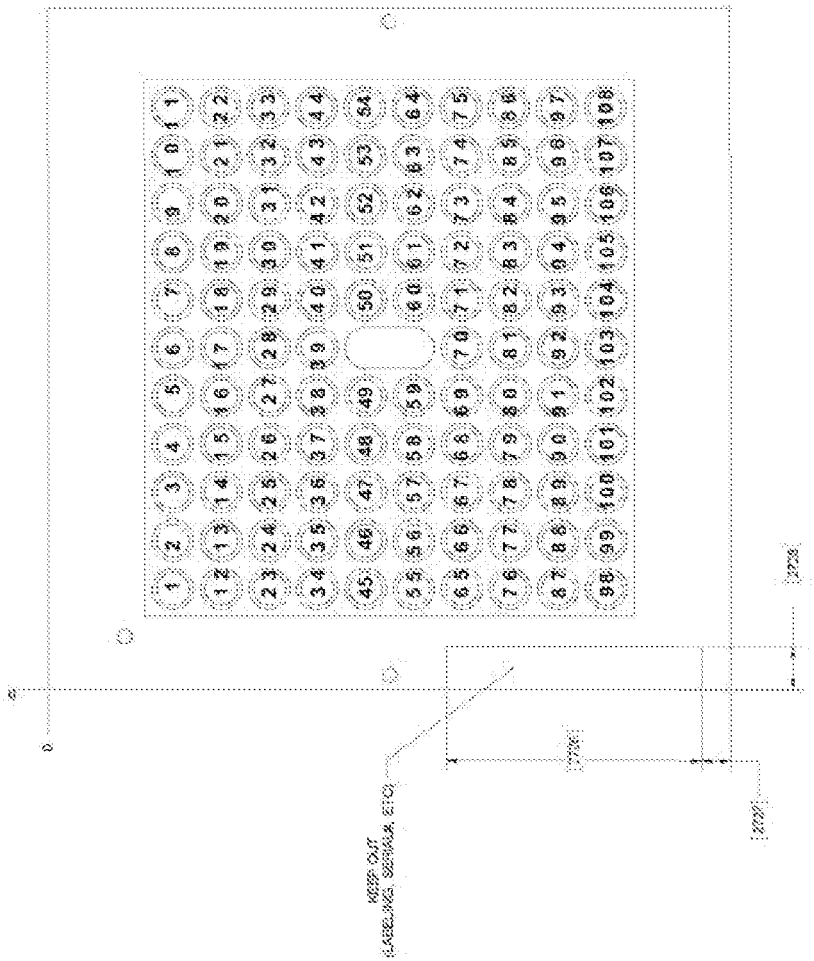


Figure 27

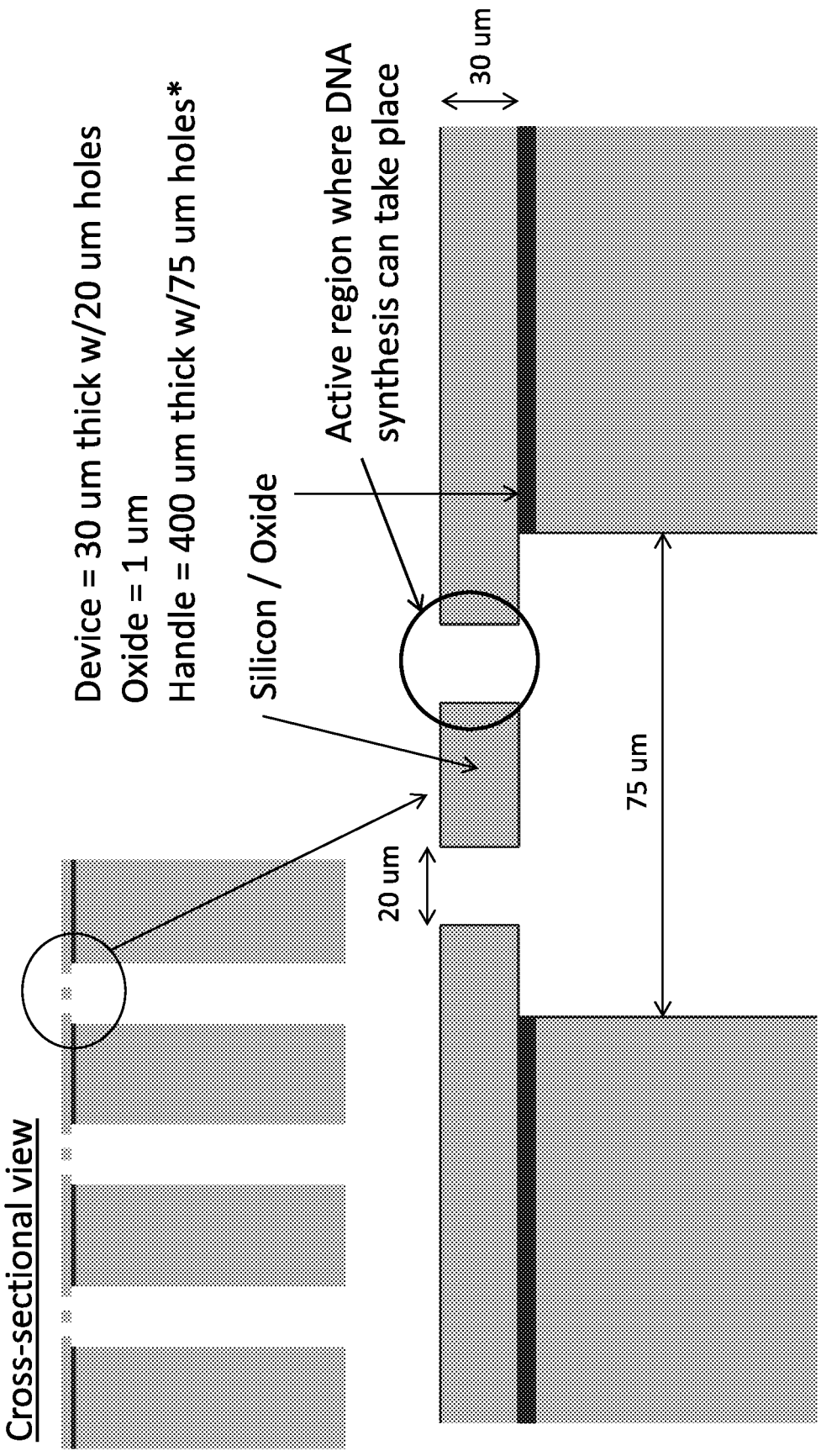

Cross-sectional view
Device = 30 um thick w/20 um holes
Oxide = 1 um
Handle = 400 um thick w/75 um holes*
Silicon / Oxide
Active region where DNA synthesis can take place
30 um
20 um
75 um

Figure 28

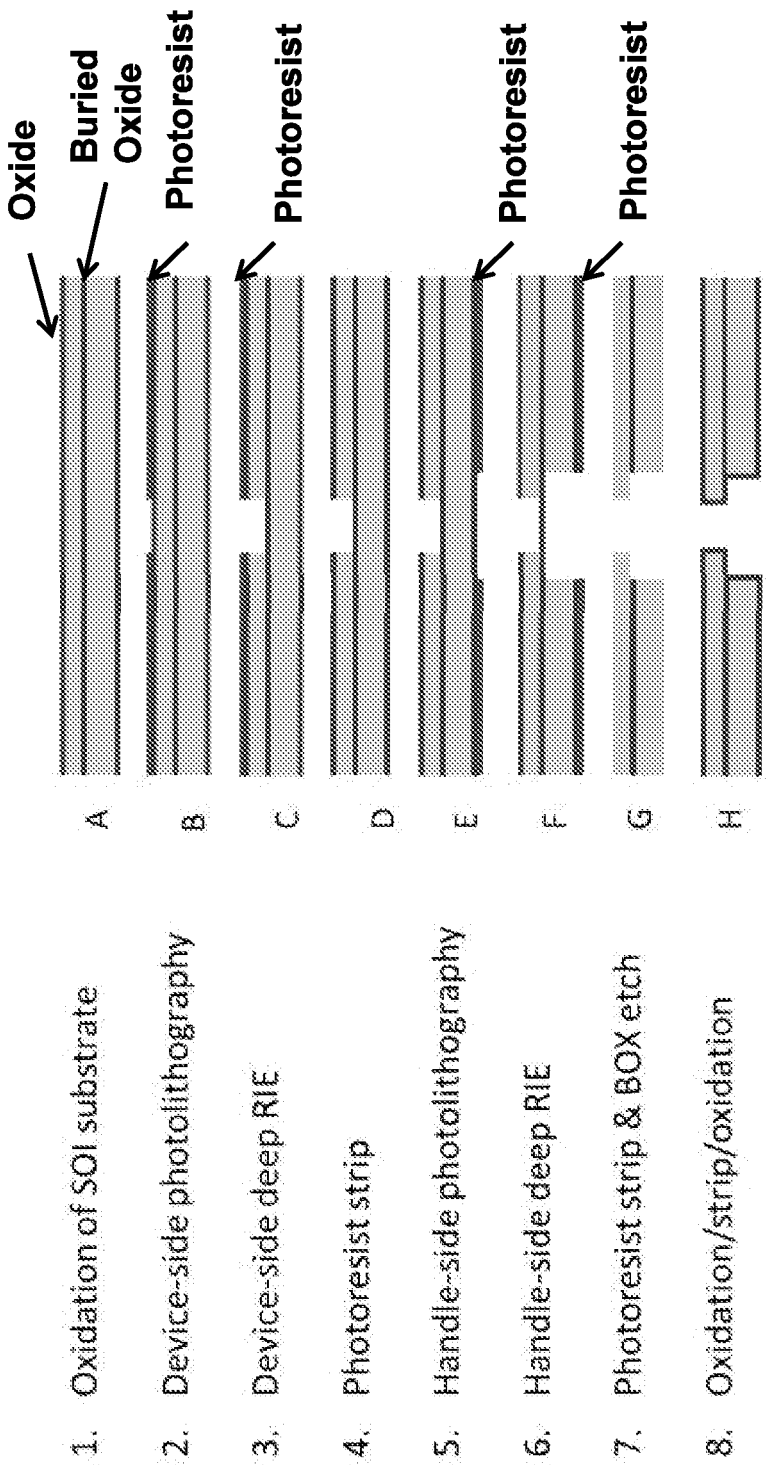
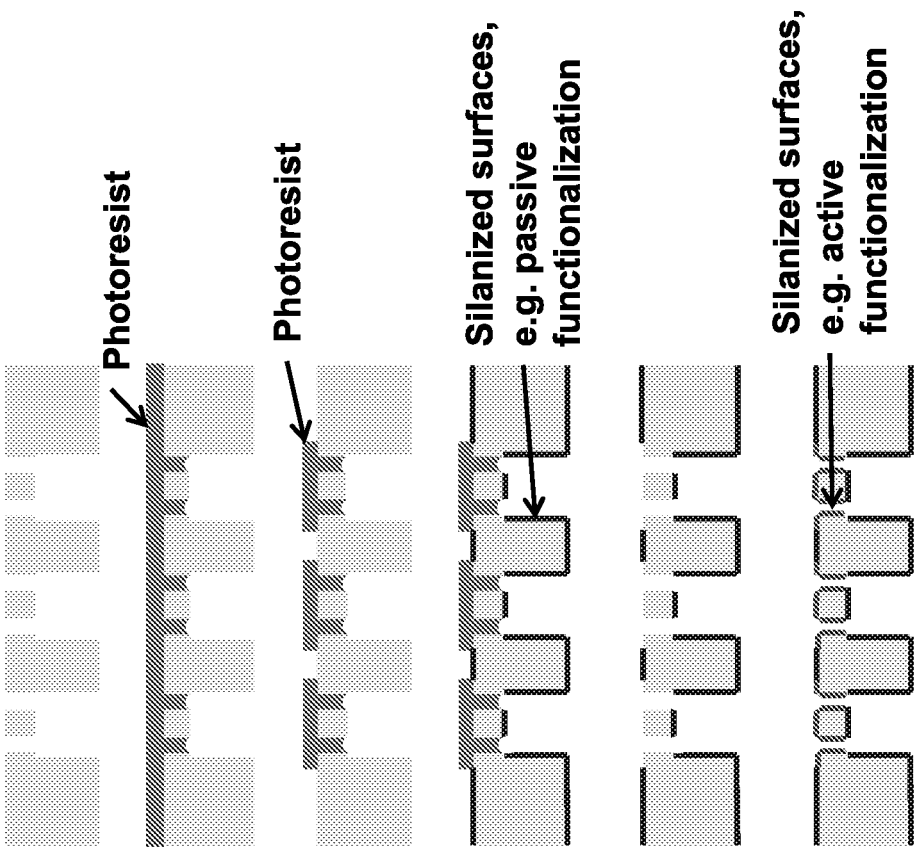

Oxide
Buried Oxide
Photoresist
Photoresist
Photoresist
Photoresist
A
B
C
D
E
F
G
H
1. Oxidation of SOI substrate
2. Device-side photolithography
3. Device-side deep RIE
4. Photoresist strip
5. Handle-side photolithography
6. Handle-side deep RIE
7. Photoresist strip & BOX etch
8. Oxidation/strip/oxidation

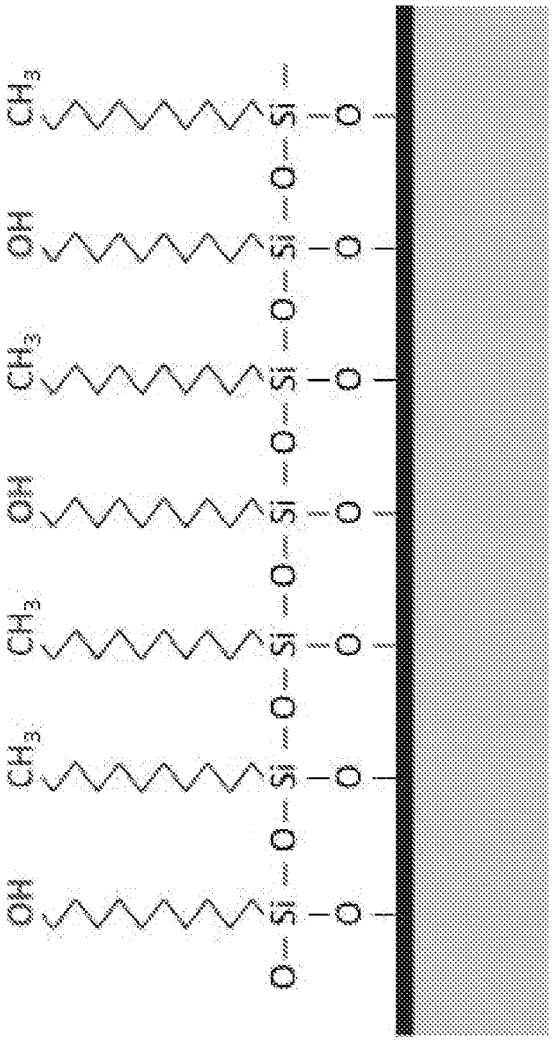
Figure 29



1. Clean chip (piranha + O_2 plasma)
2. Coat with photoresist
3. Photolithography + descum
4. Functionalization, e.g. with fluorosilane CVD
5. Resist strip
6. Active functionalization

Figure 30

Active Functionalization



- Dilute concentration of hydroxyl groups in a sea of passive groups by applying mixed silanes

Figure 31

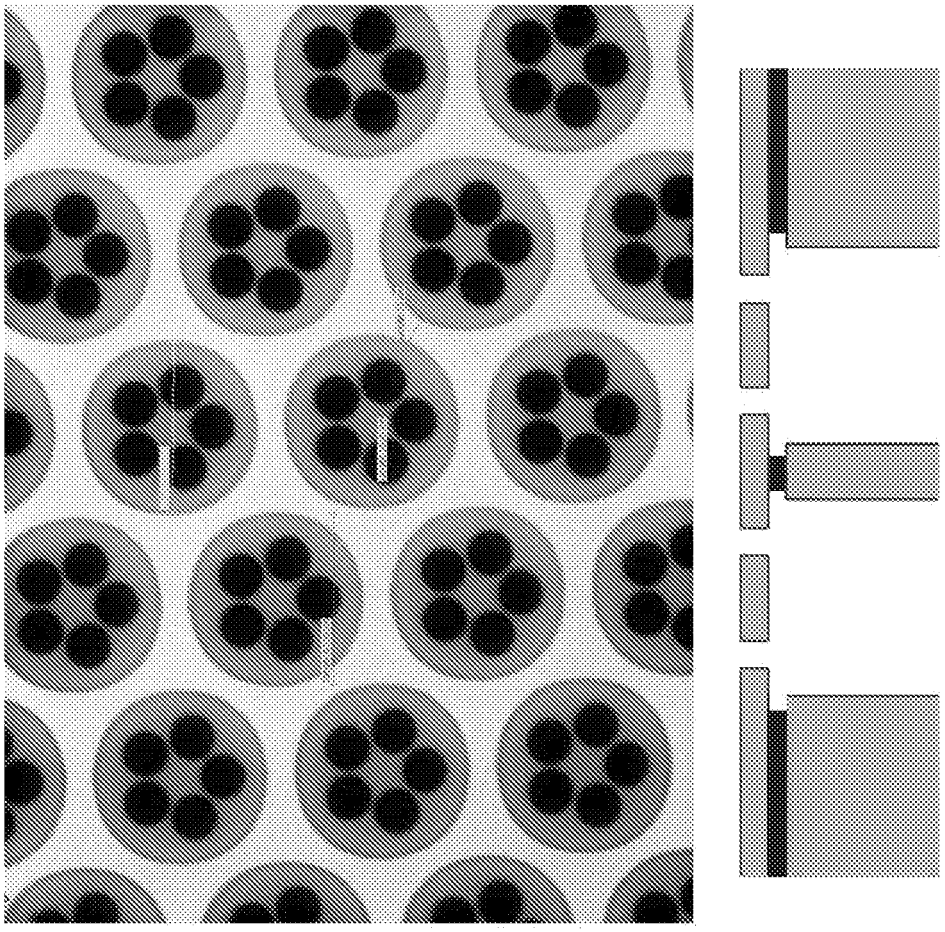


Figure 32

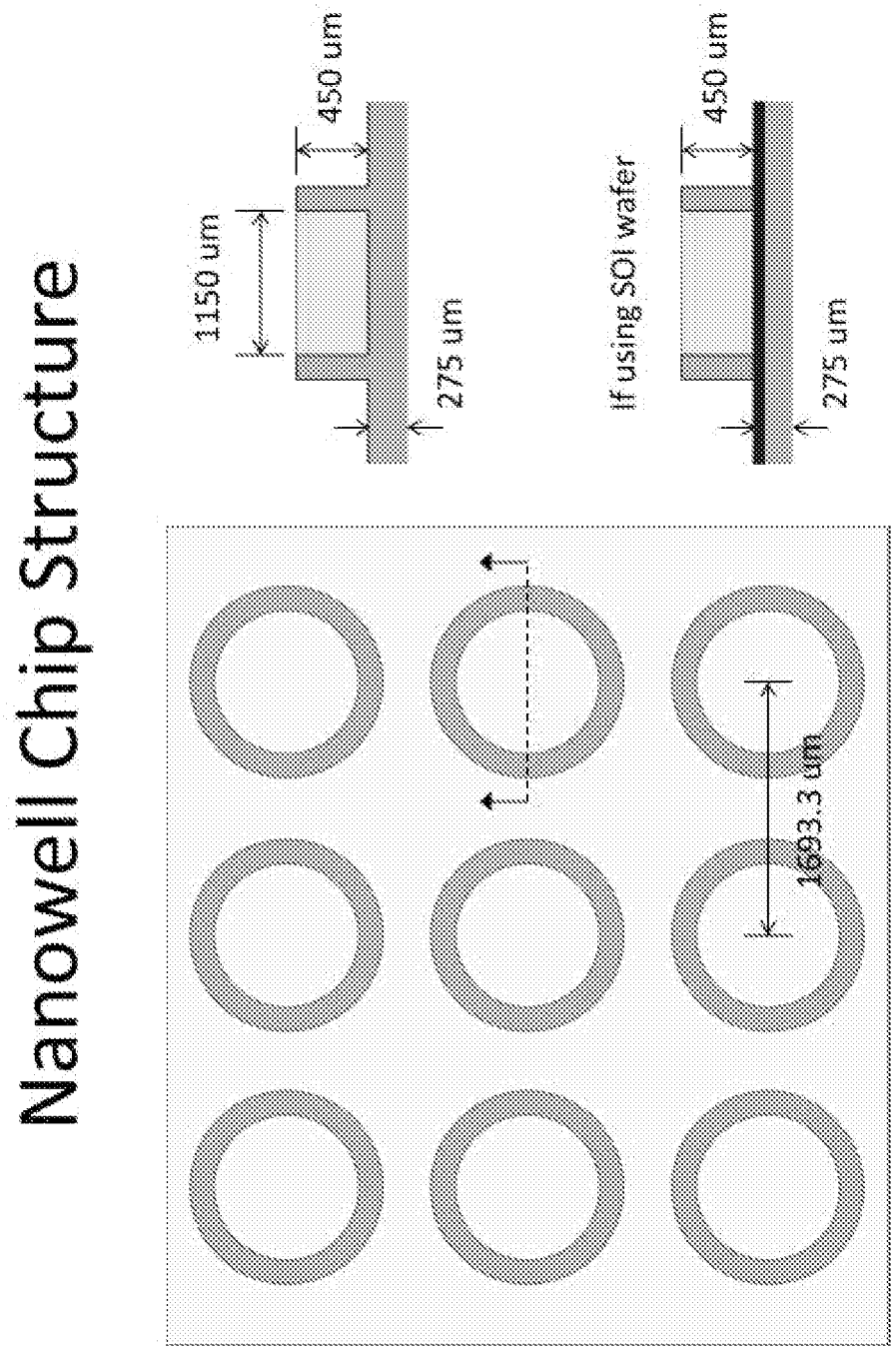

Nanowell Chip Structure
1150 um
450 um
275 um
If using SOI wafer
450 um
275 um
1693.3 um

Figure 33

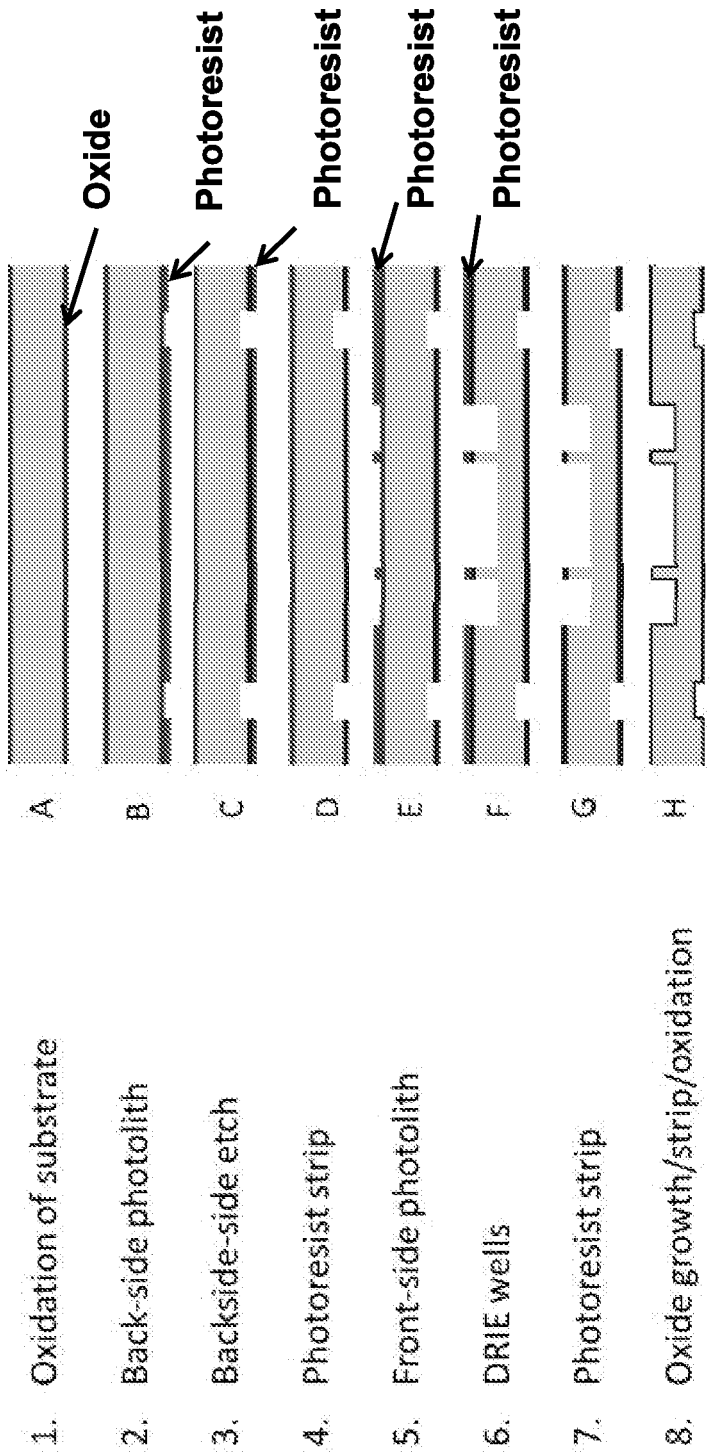

Oxide
Photoresist
Photoresist
Photoresist
Photoresist
A
B
C
D
E
F
G
H
1. Oxidation of substrate
2. Back-side photolith
3. Backside-side etch
4. Photoresist strip
5. Front-side photolith
6. DRIE wells
7. Photoresist strip
8. Oxide growth/strip/oxidation

Figure 34

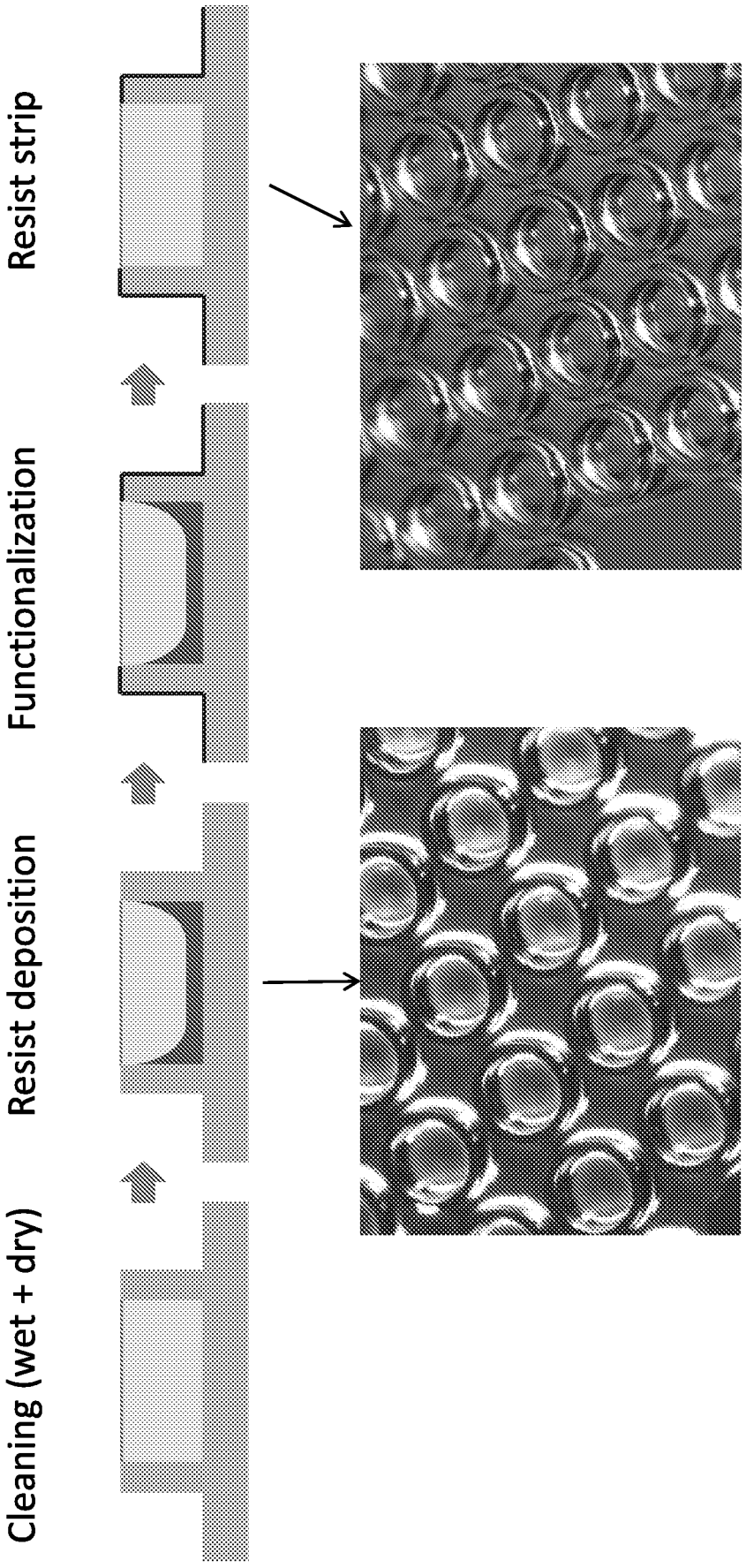

A
Cleaning (wet + dry)
B
Resist deposition
C
Functionalization
D
Resist strip

Figure 35

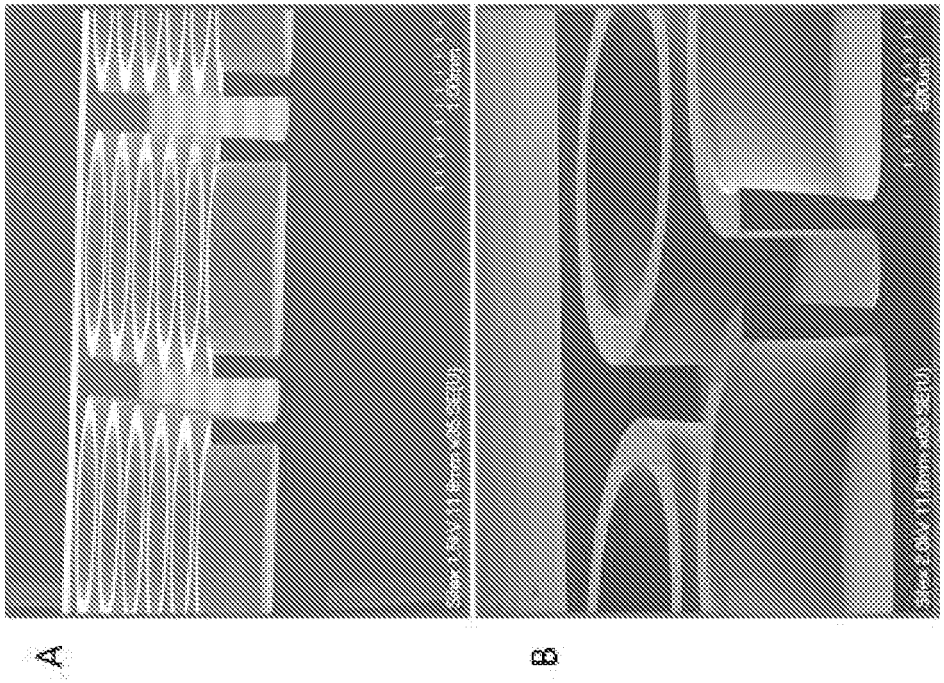


Figure 36

Various functional configurations
Active/
Light/
Yellow
Passive/
Dark/
Blue
1. Uniformly functionalized
A
2. Sharp top/bottom revolver edges, passive via
B
3. Topside revolver boarder, passive via
C
4. Topside cluster boarder, passive via
D
5. Sharp top revolver edge, active via
E
6. Sharp top revolver edge, active via + bottom
F

Figure 37

Process Flow A (for config 2 – 4)

1. Clean chip (piranha + O_2 plasma) A
2. Application of photoresist B
3. Photolithography + descum C
4. Fluorosilane deposition D
5. Resist strip in solvents E
6. Active functionalization F

Figure 38

Resist wicking control process

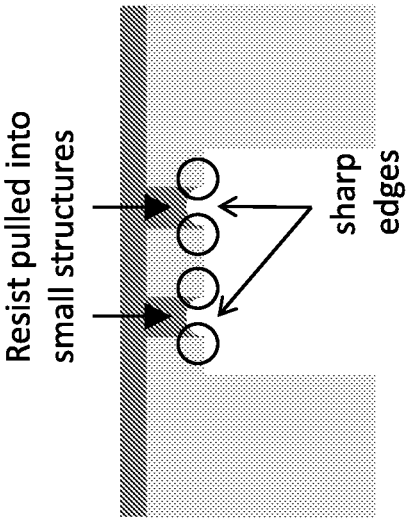


Figure 39

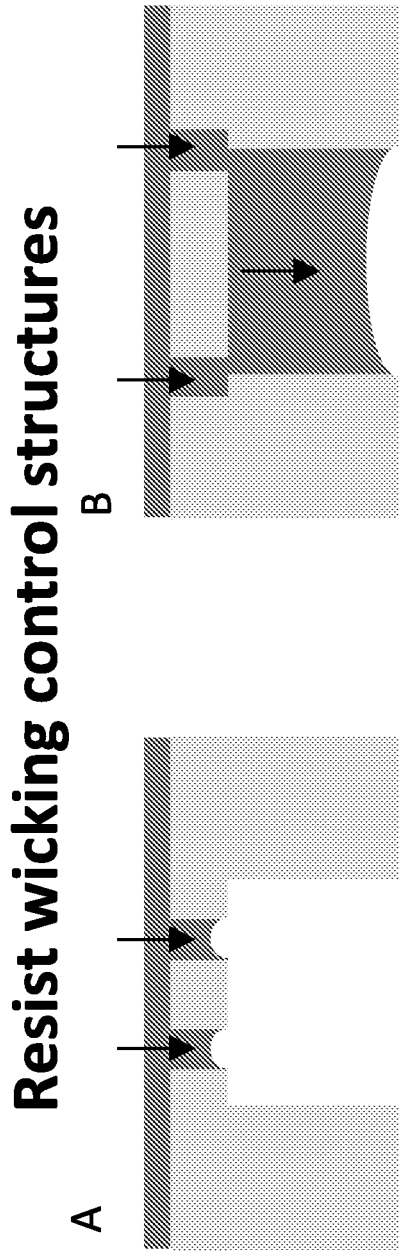


Figure 40

Post-litho resist patterns

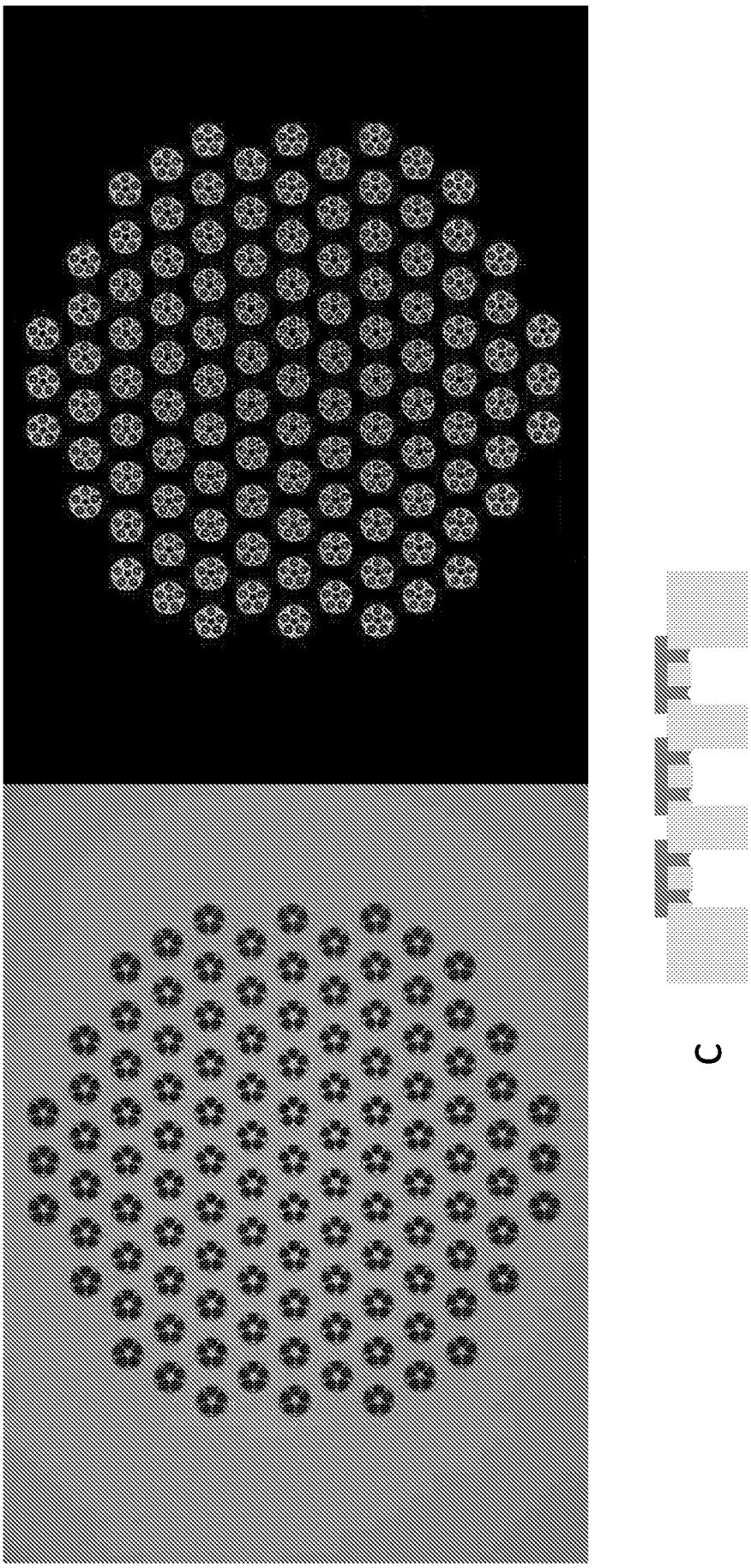


Figure 41

Post-litho resist patterns

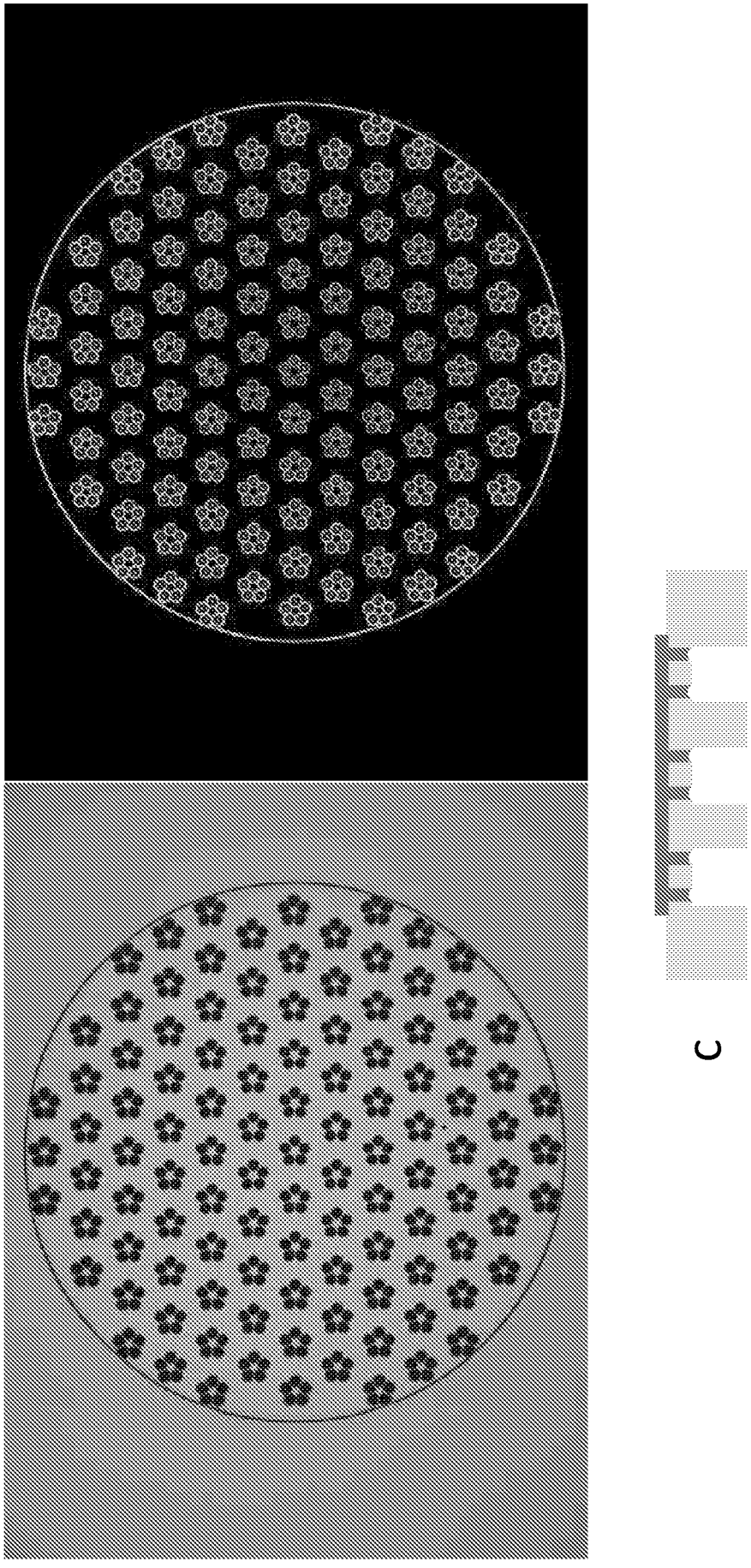


Figure 42

Post resist strip (after fluorination)

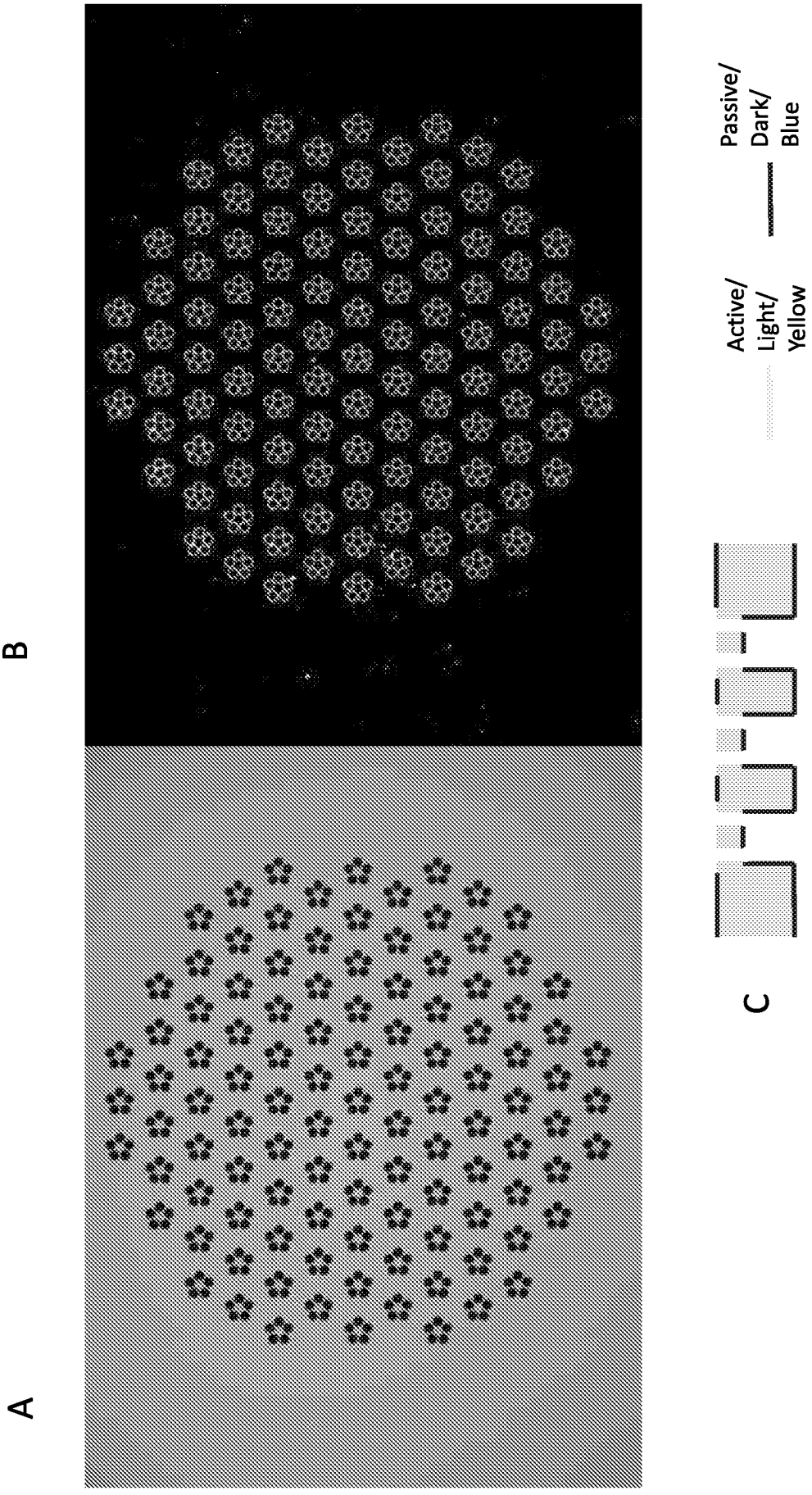


Figure 43

Keratin chip fully loaded with DMSO

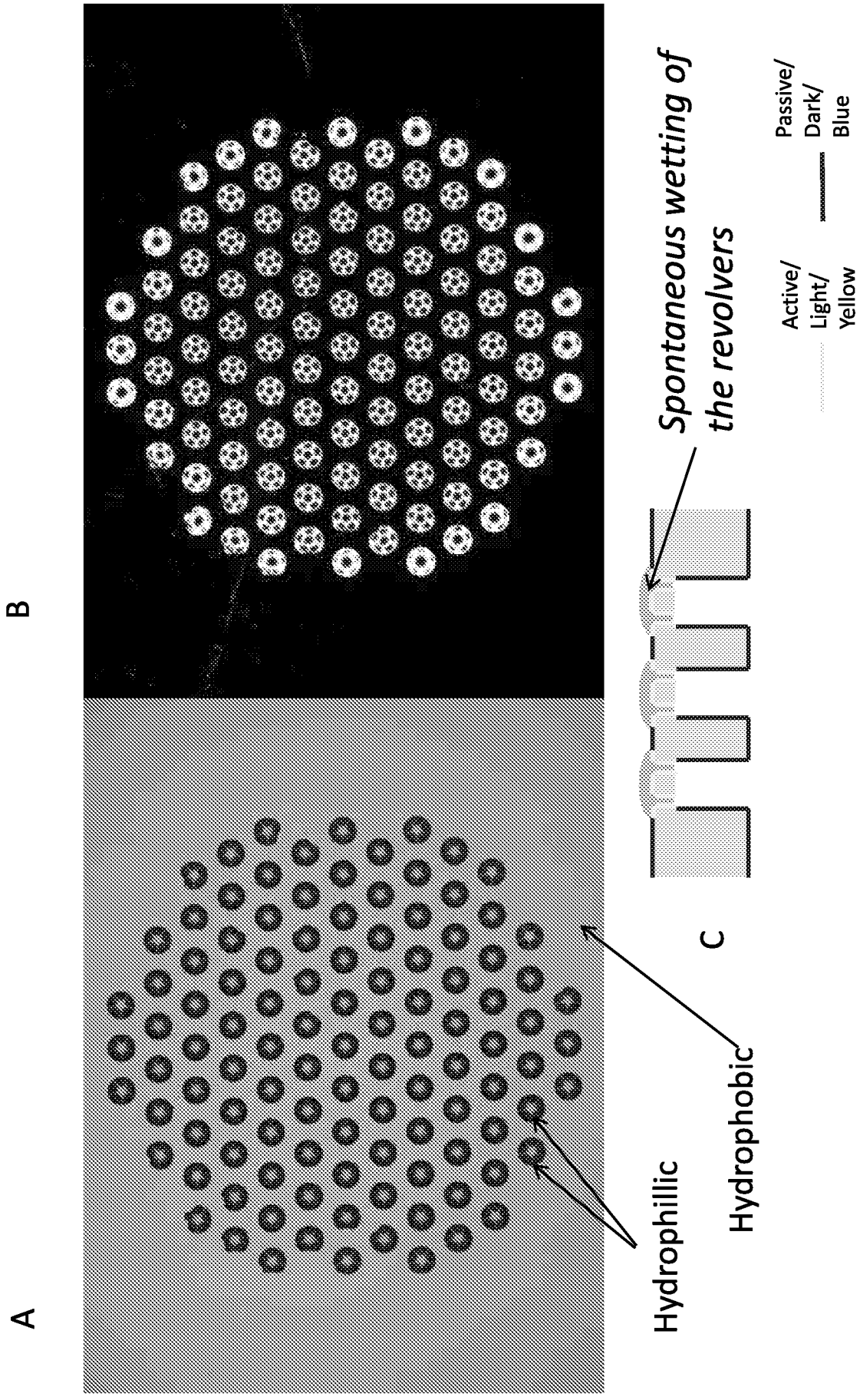
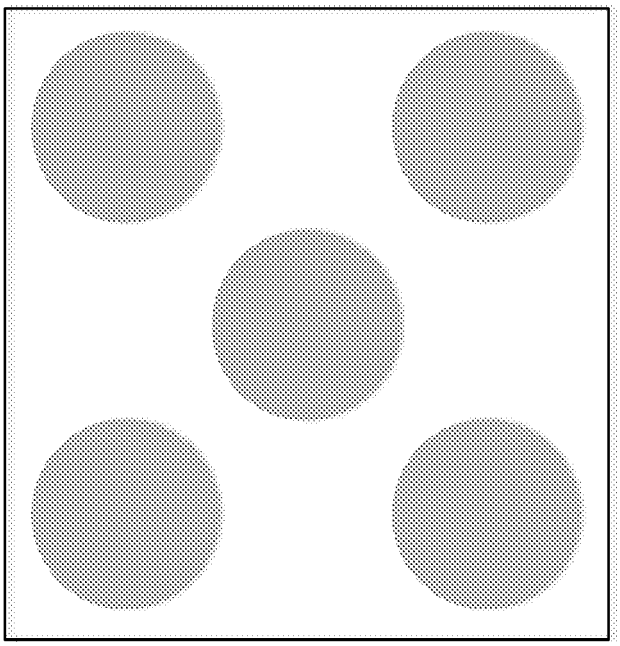


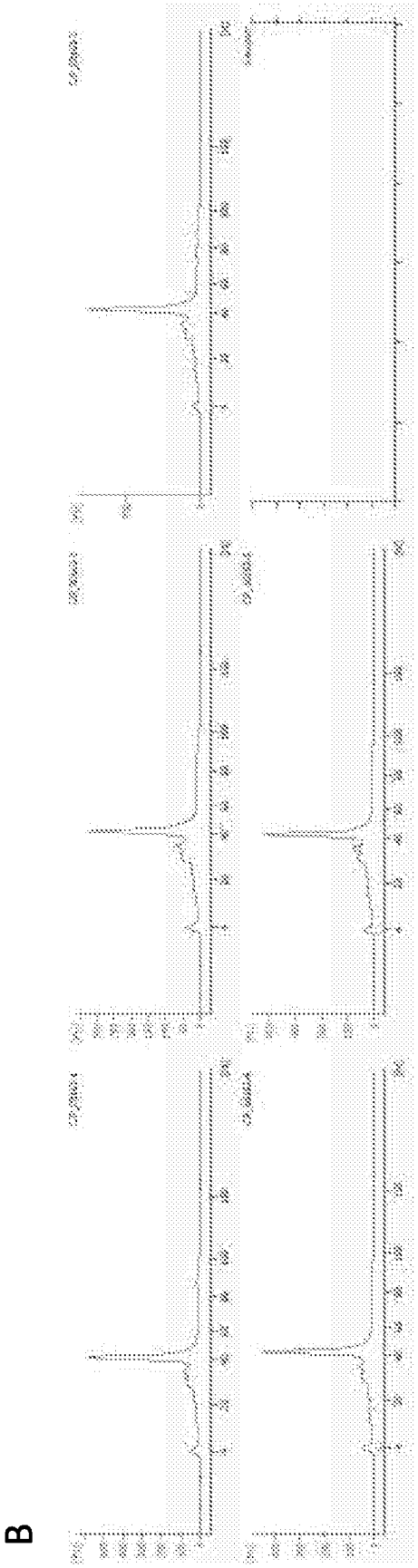
Figure 44

Process Flow B (for config 6)

Figure 45



A



B

Figure 46

Synthesis of a 100mer sequence on 2D oligonucleotide synthesis device

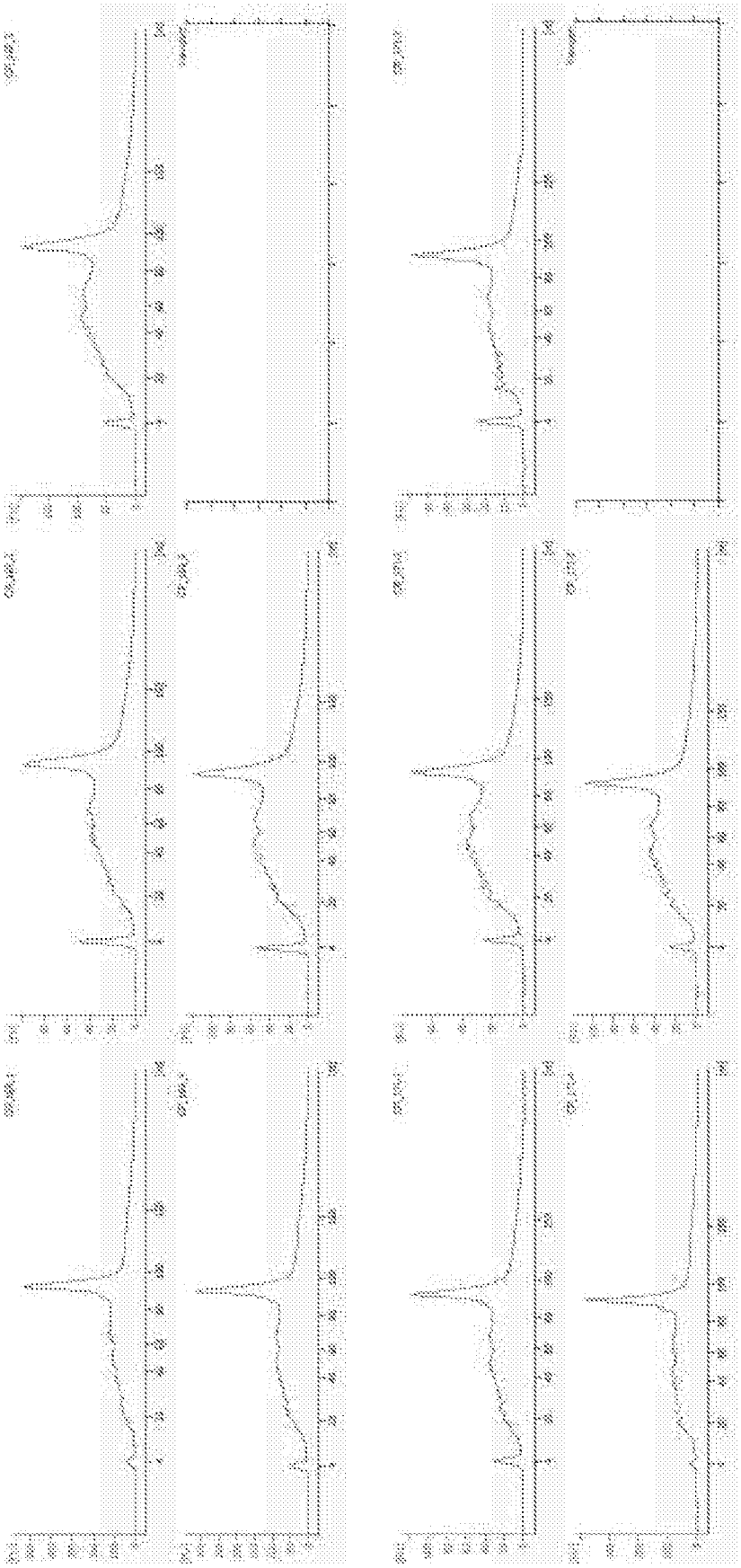


Figure 47

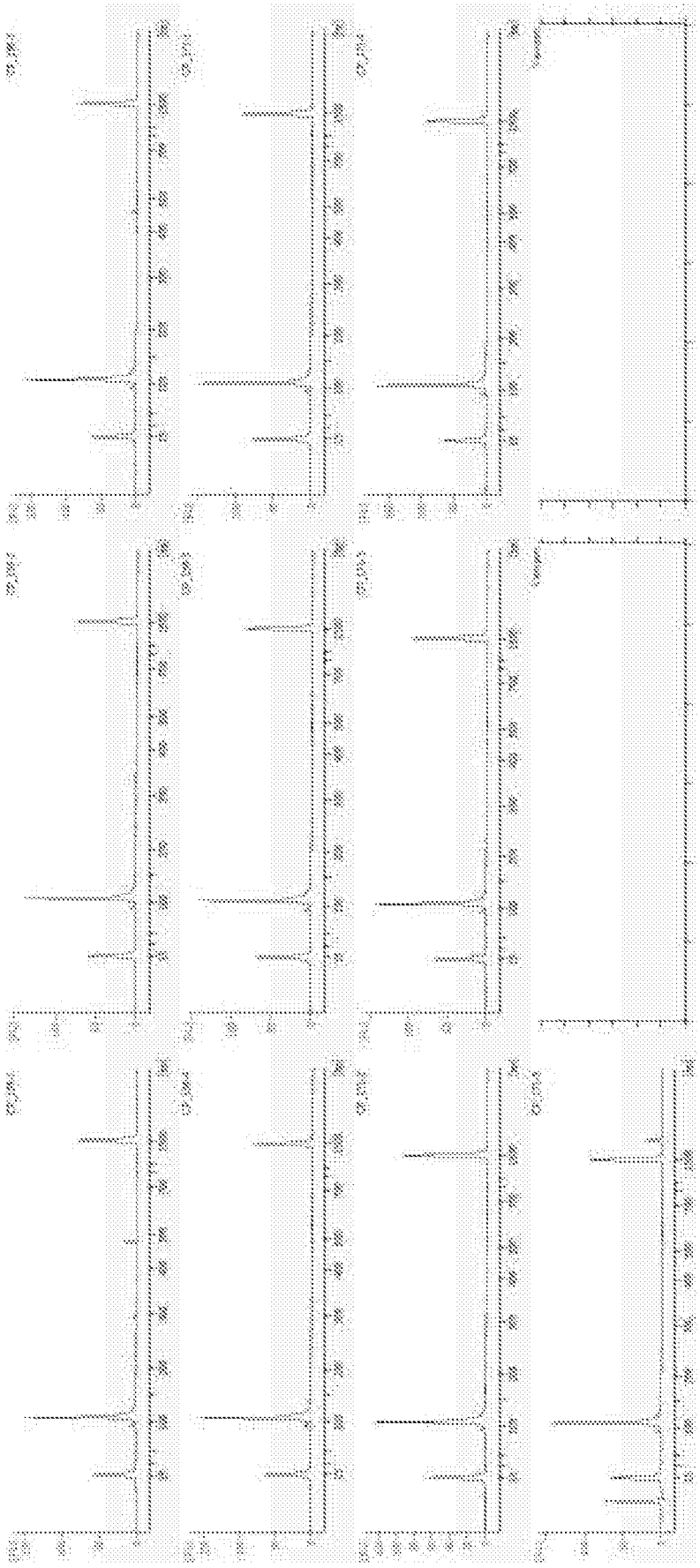


Figure 48

Alignment Map

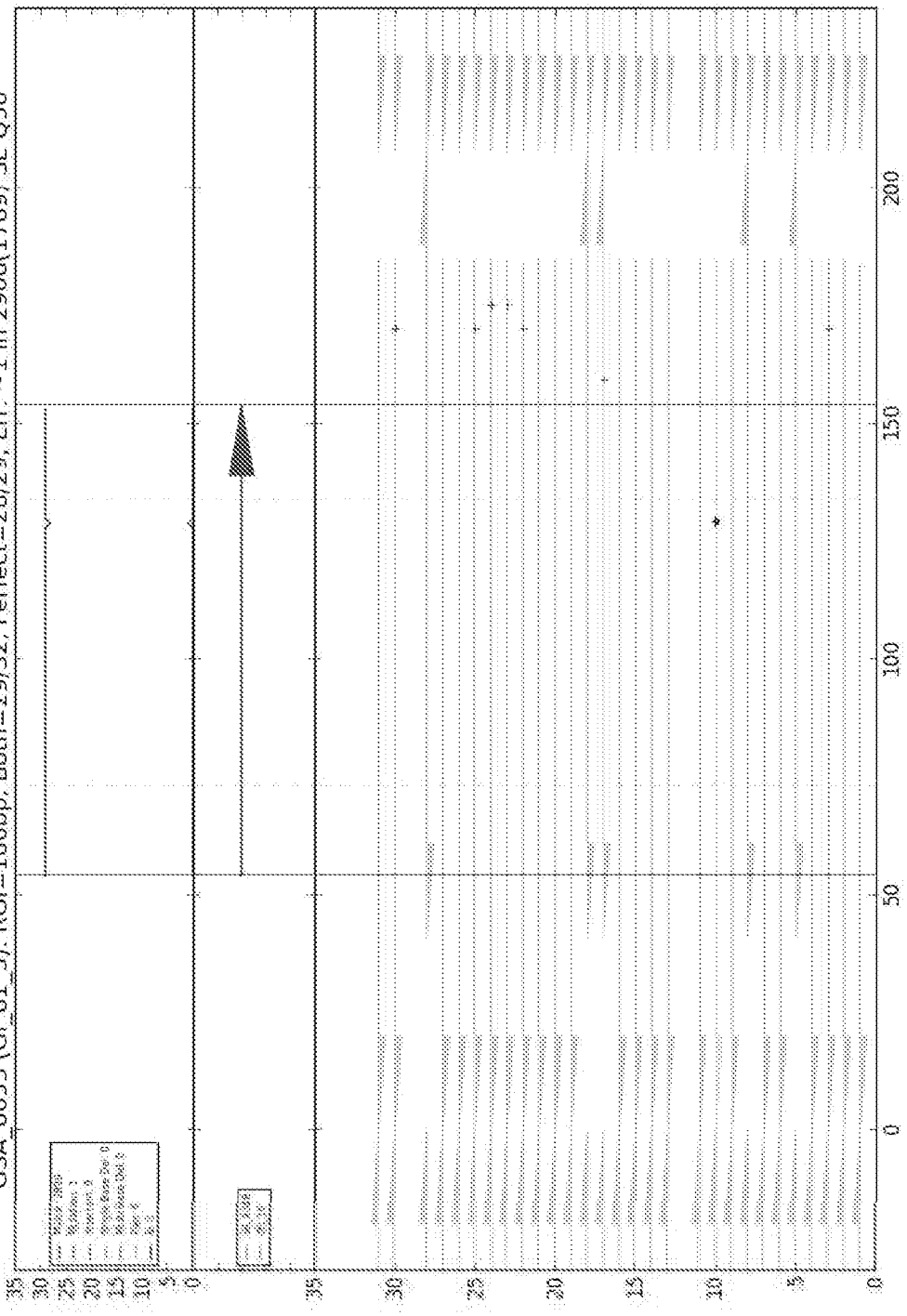
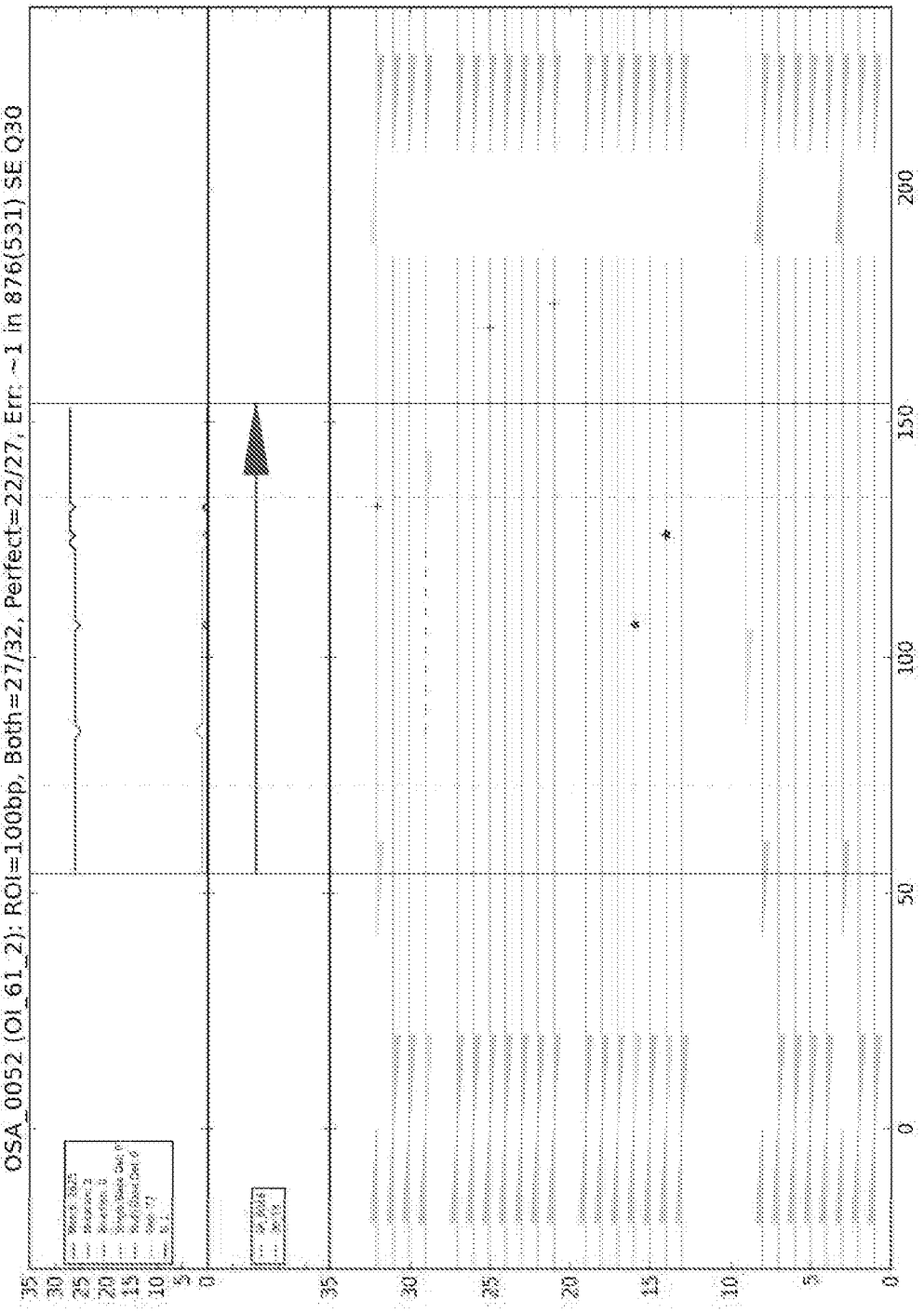


Figure 49

Alignment Map



8/5/2014

Figure 50

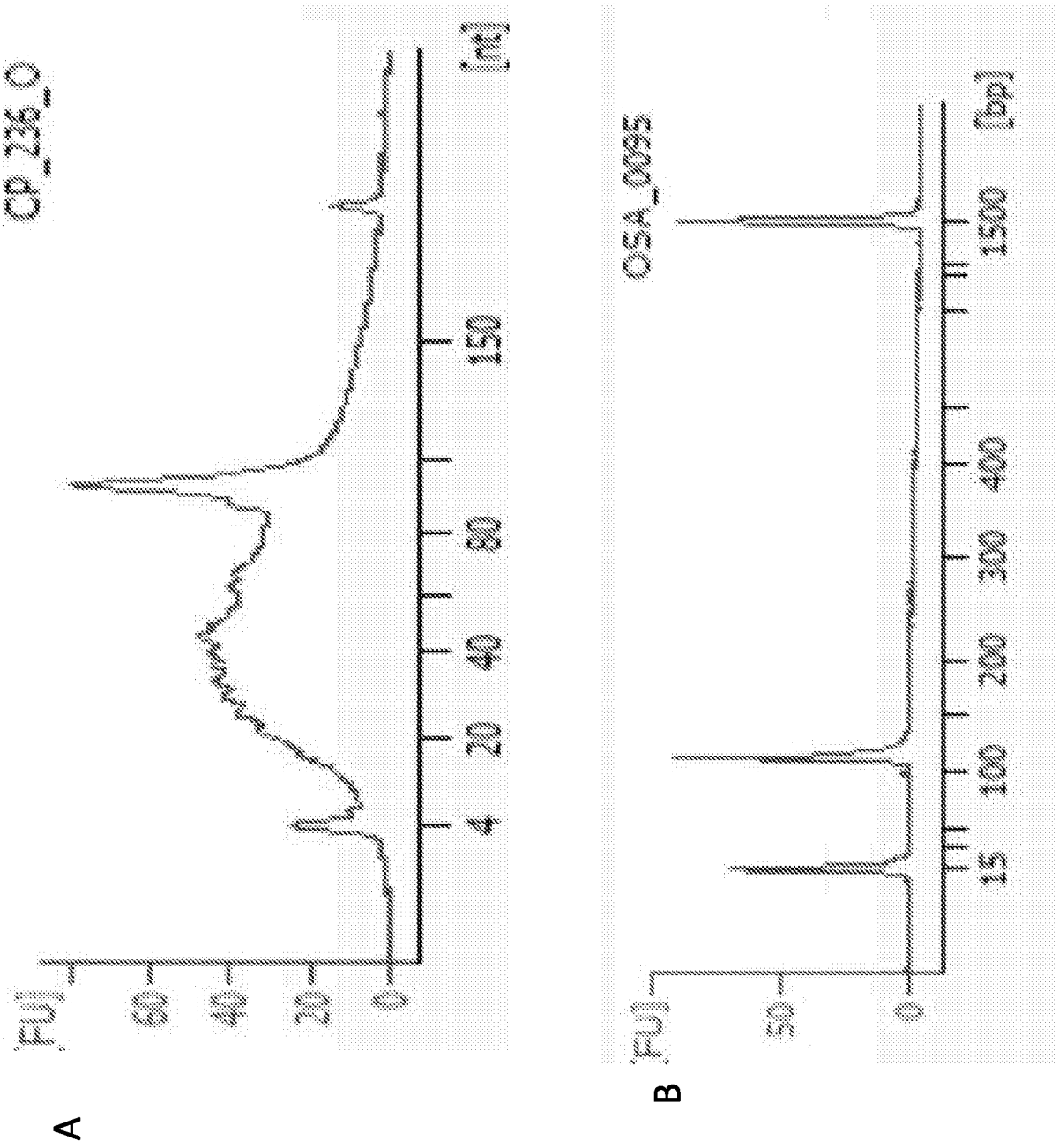

A
CP_235_O
FU
80
40
20
0
4
20
40
80
150
[nt]
B
OSA_0055
FU
50
0
15
100
200
300
400
1500
[bp]

Figure 51

Alignment Map

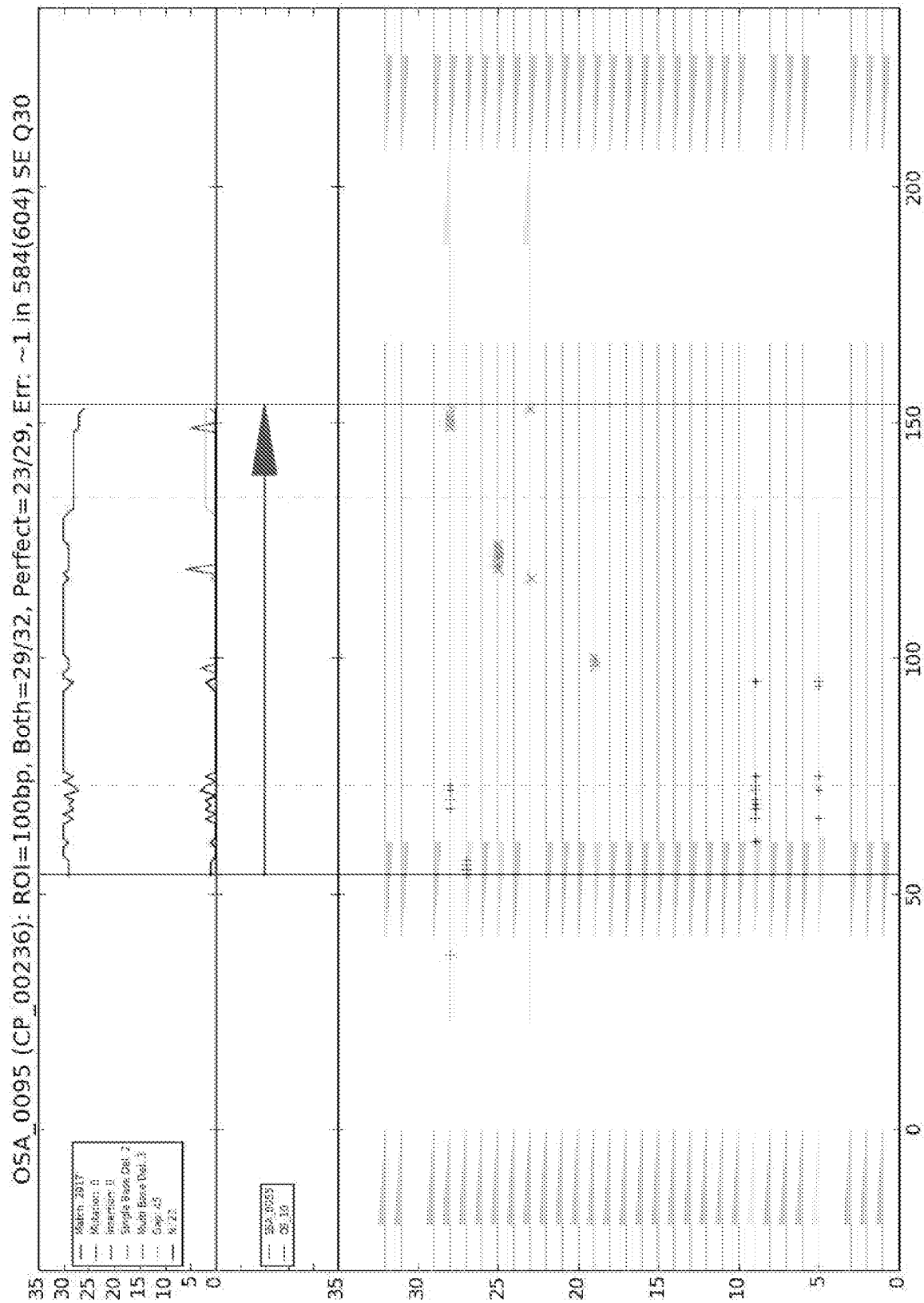


Figure 52

Error Correction
performance achieved with CorrectASE - error rate lower than 1 error in 5kb
CorrectASE treatment of a 1kb
gene synthesized from low quality oligos
Errors result in broad peak and tails
Before error correction, 1 error in 789bp
After 1st error correction, 1 error in 5,190bp
After 2nd error correction, 1 error in 6,315bp

Figure 53

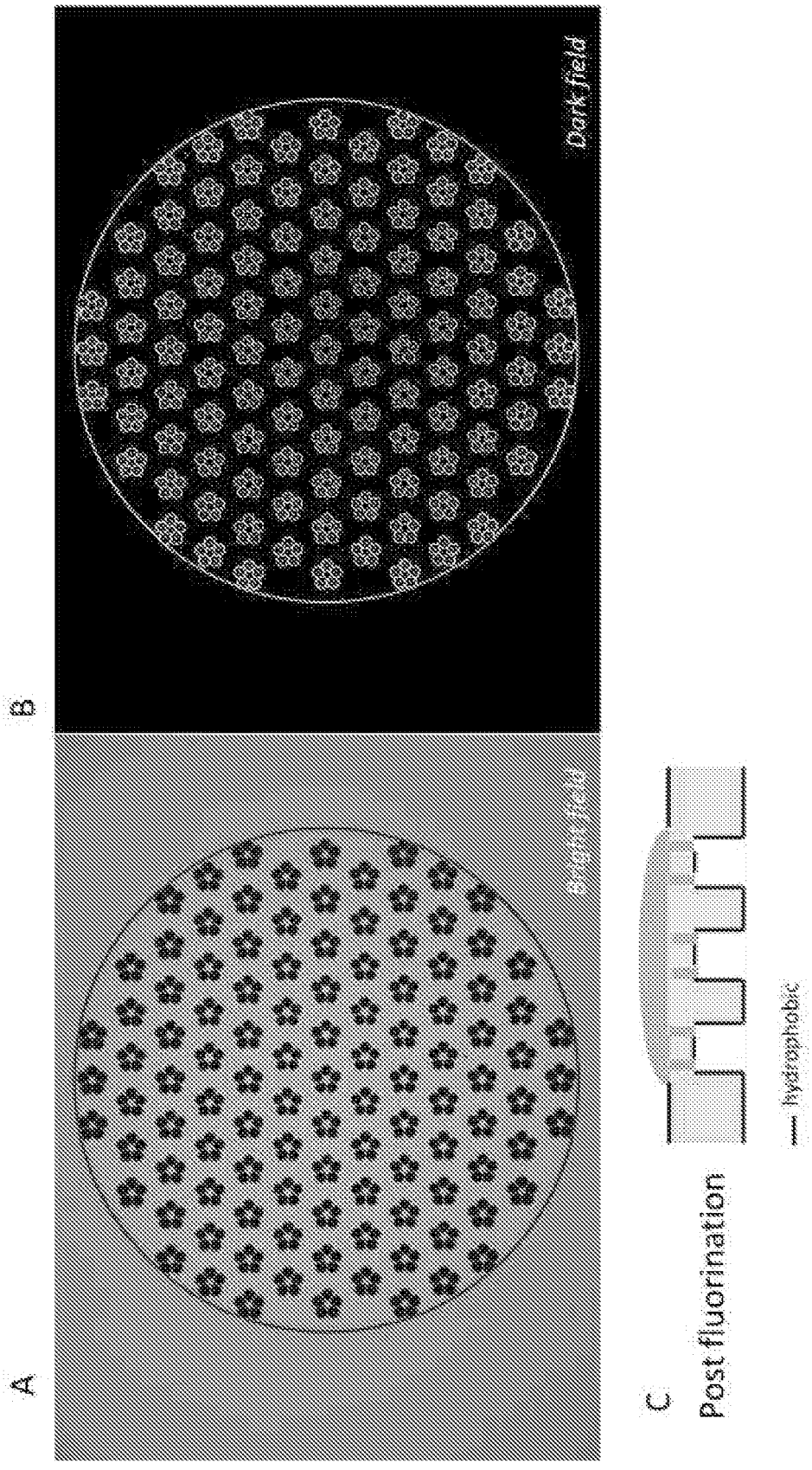

A
Bright field
B
Dark field
C
Post fluorination
hydrophobic

Figure 54

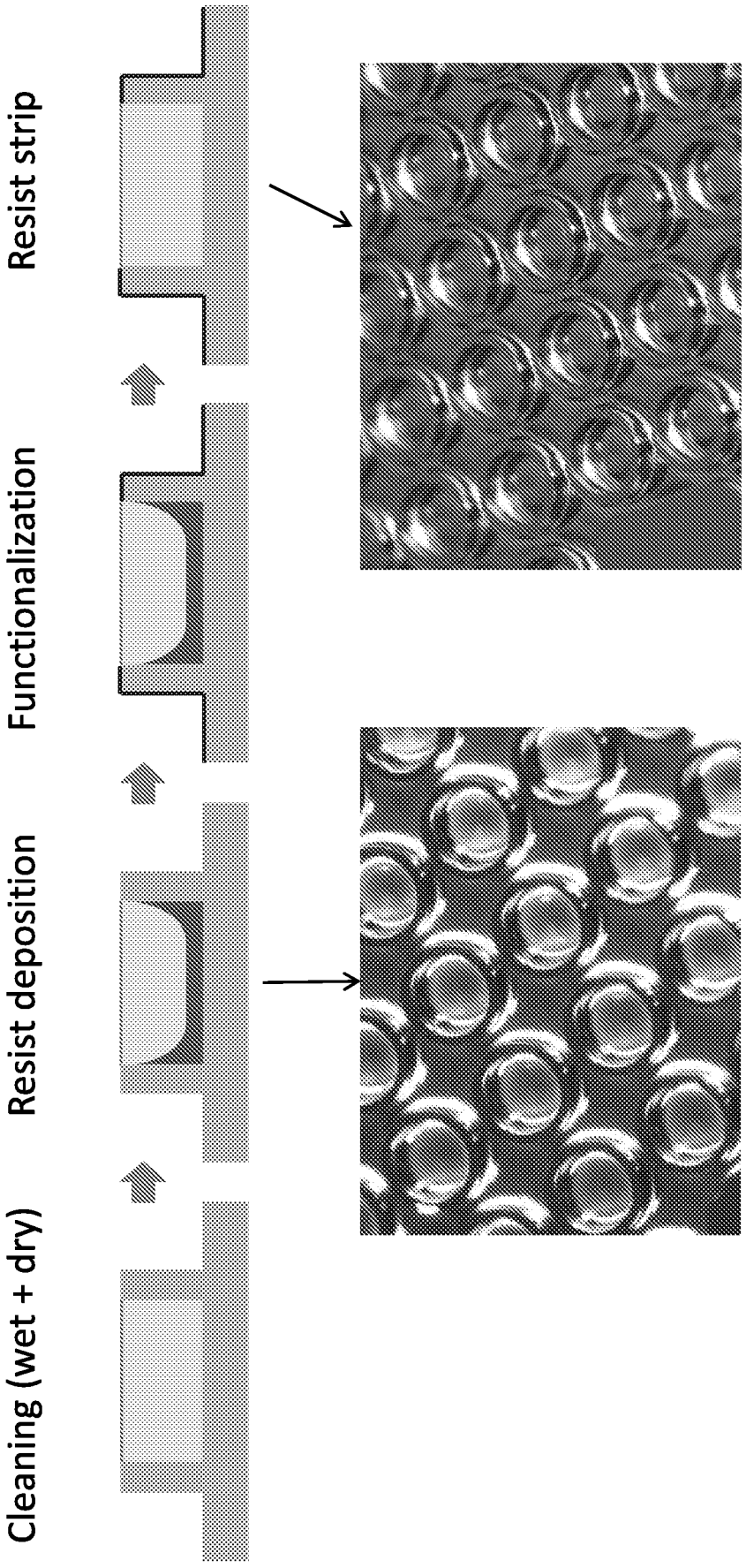

A
Cleaning (wet + dry)
B
Resist deposition
C
Functionalization
D
Resist strip

Figure 55
BioAnalyzer Results

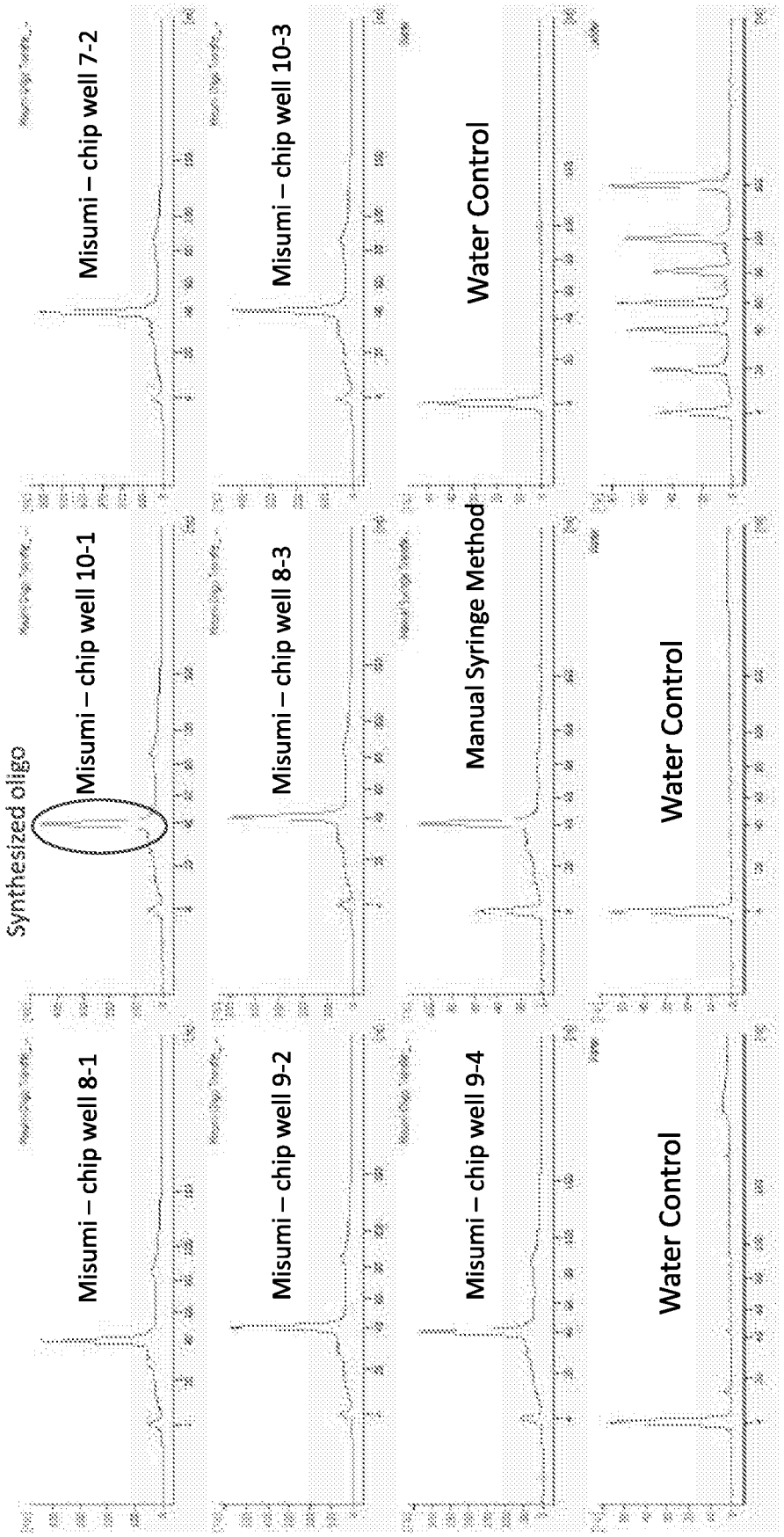


Figure 56

Point Source and Point Drain

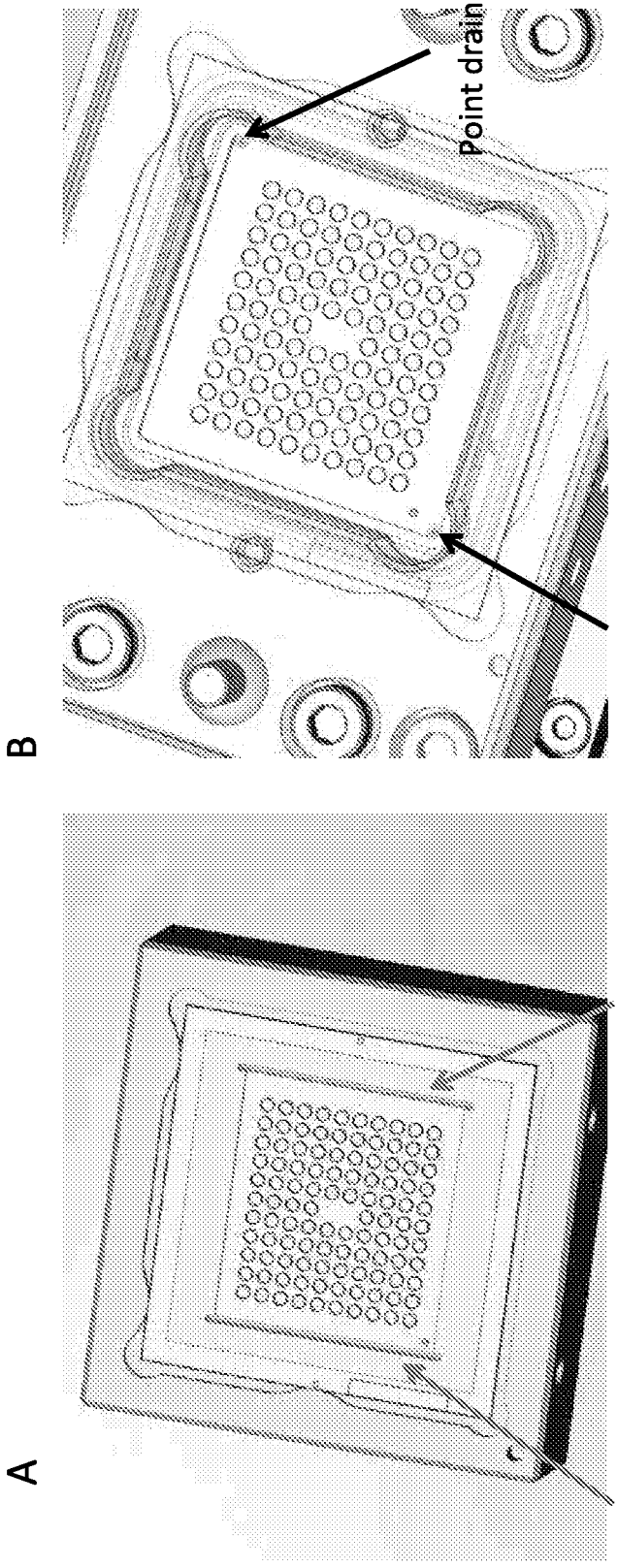
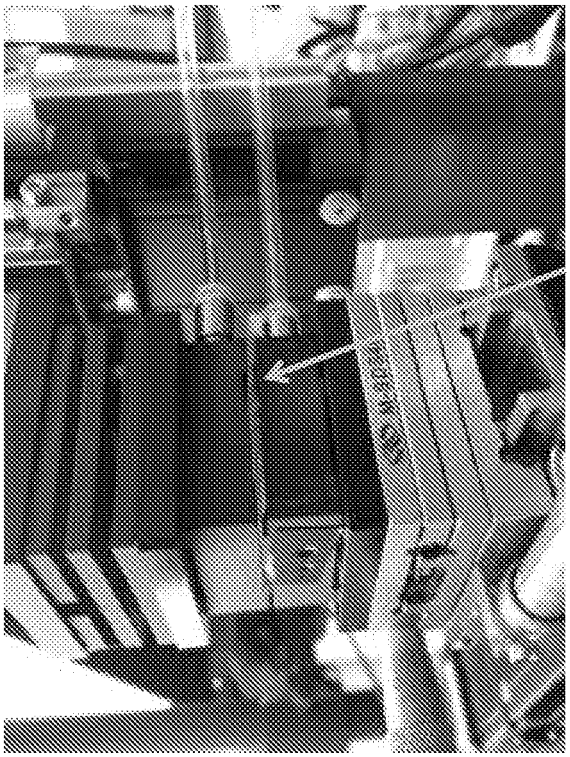


Figure 57



~ 50um gap, 10min

Figure 58

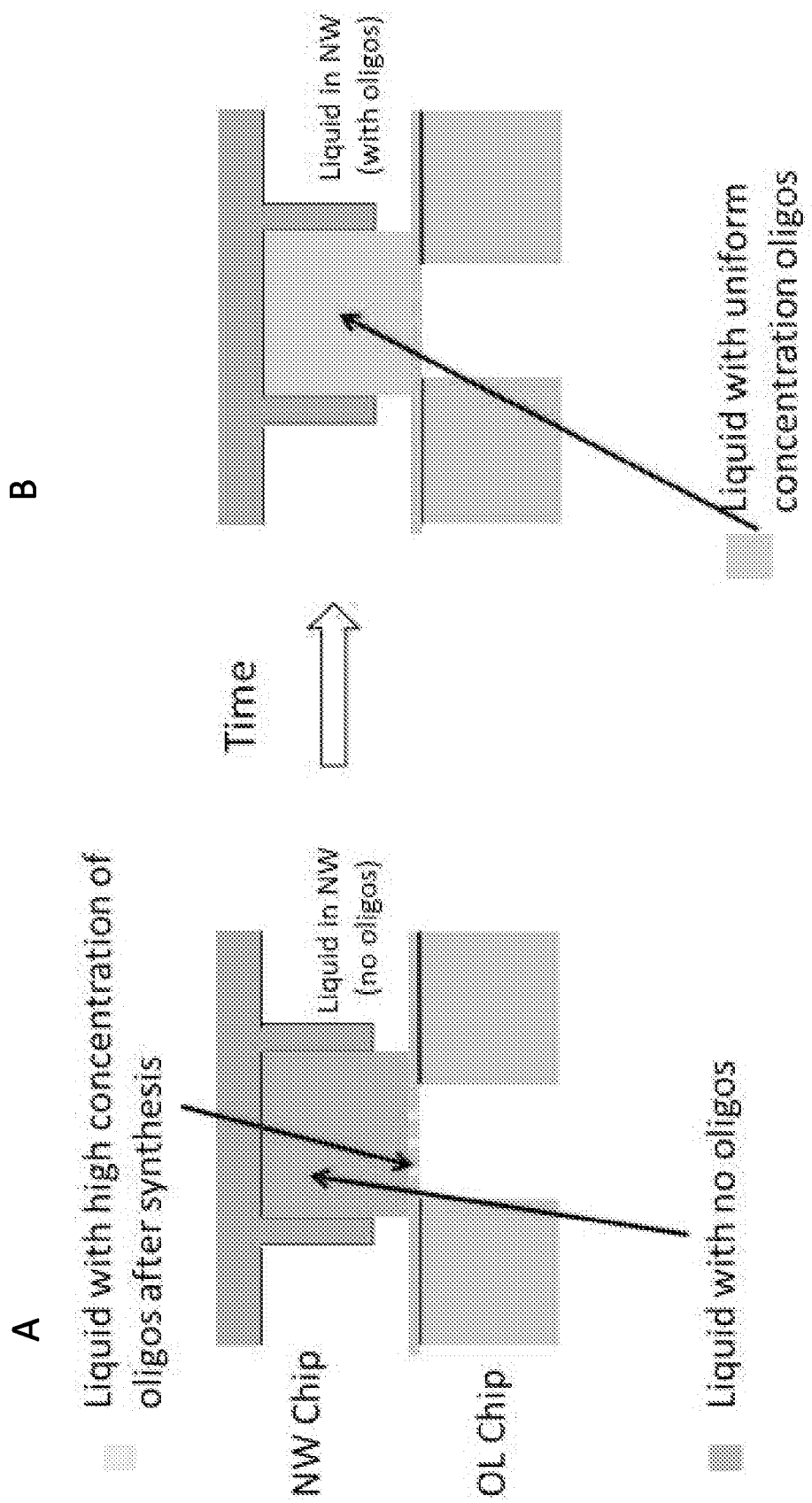
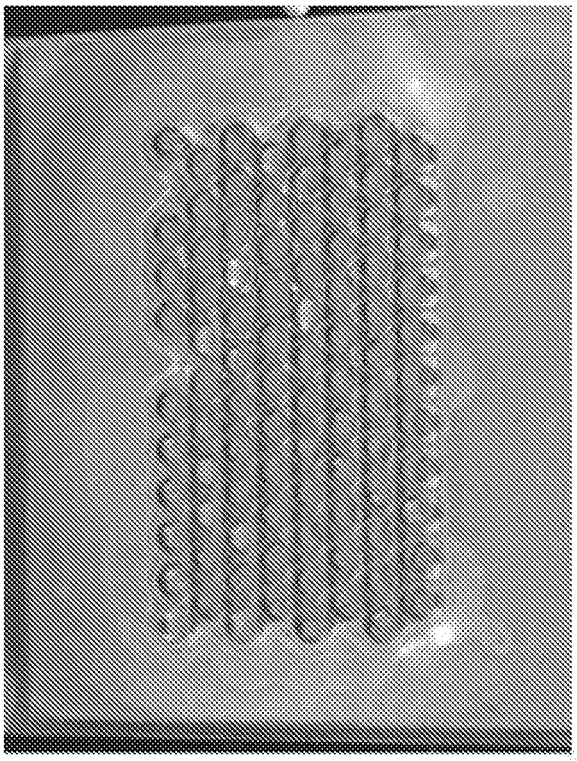

A
Liquid with high concentration of oligos after synthesis
Liquid in NW (no oligos)
NW Chip
OL Chip
Liquid with no oligos
Time
B
Liquid in NW (with oligos)
Liquid with uniform concentration oligos

Figure 59



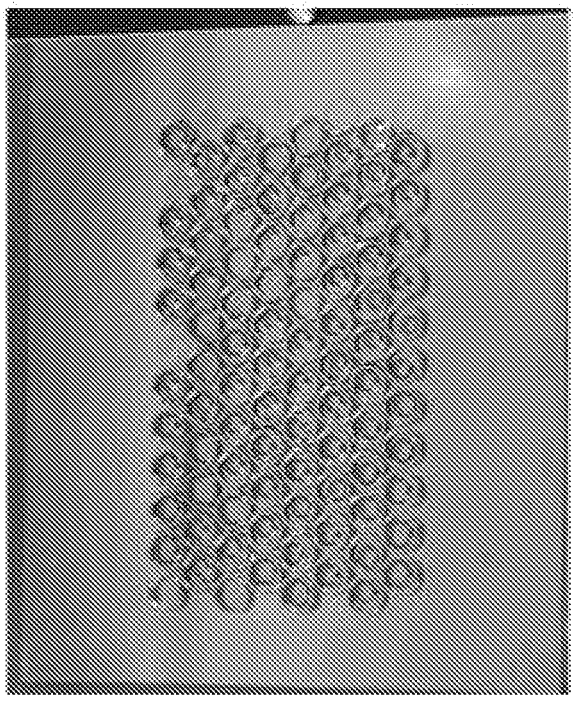


Figure 60

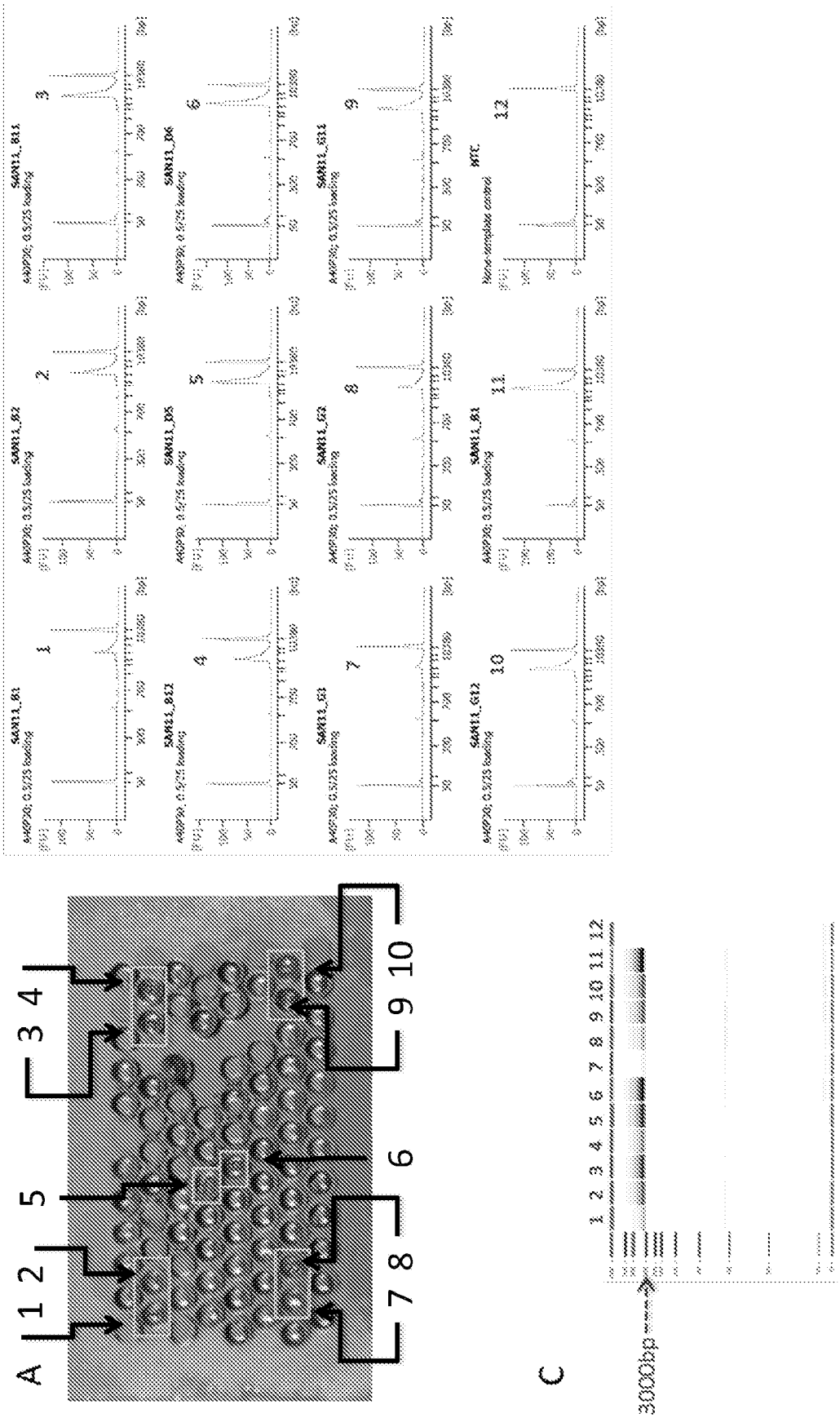


Figure 61

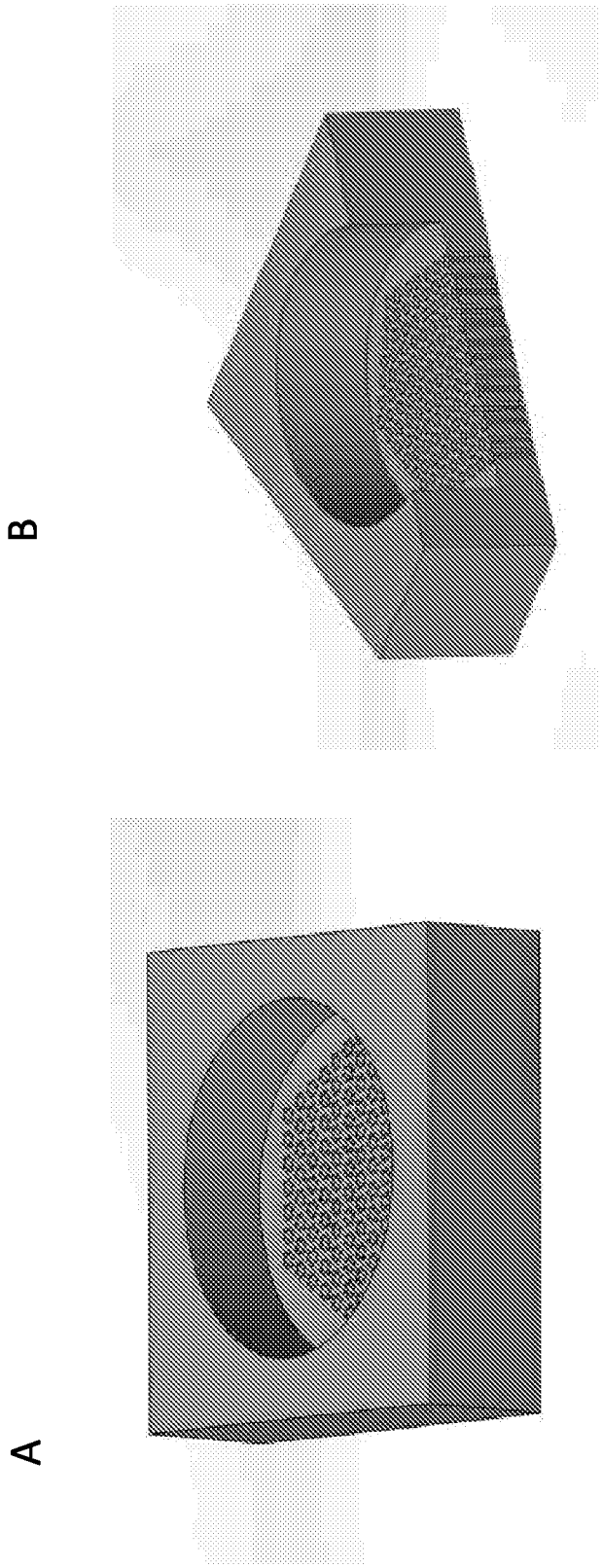


Figure 62

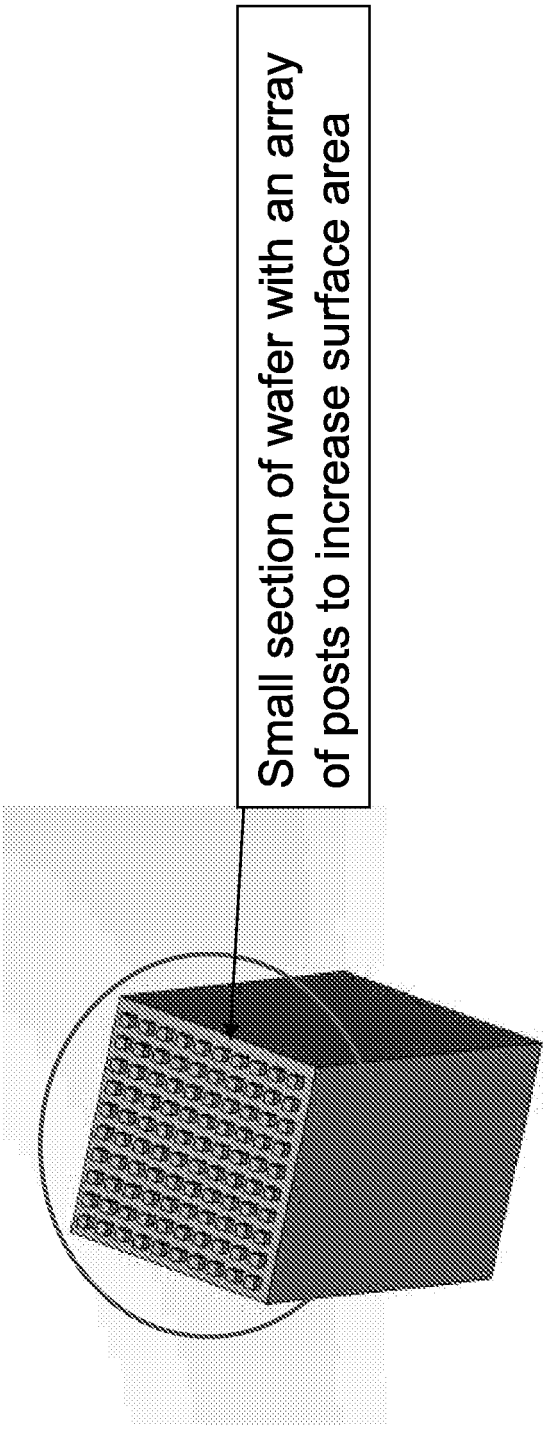

Small section of wafer with an array of posts to increase surface area

Figure 63

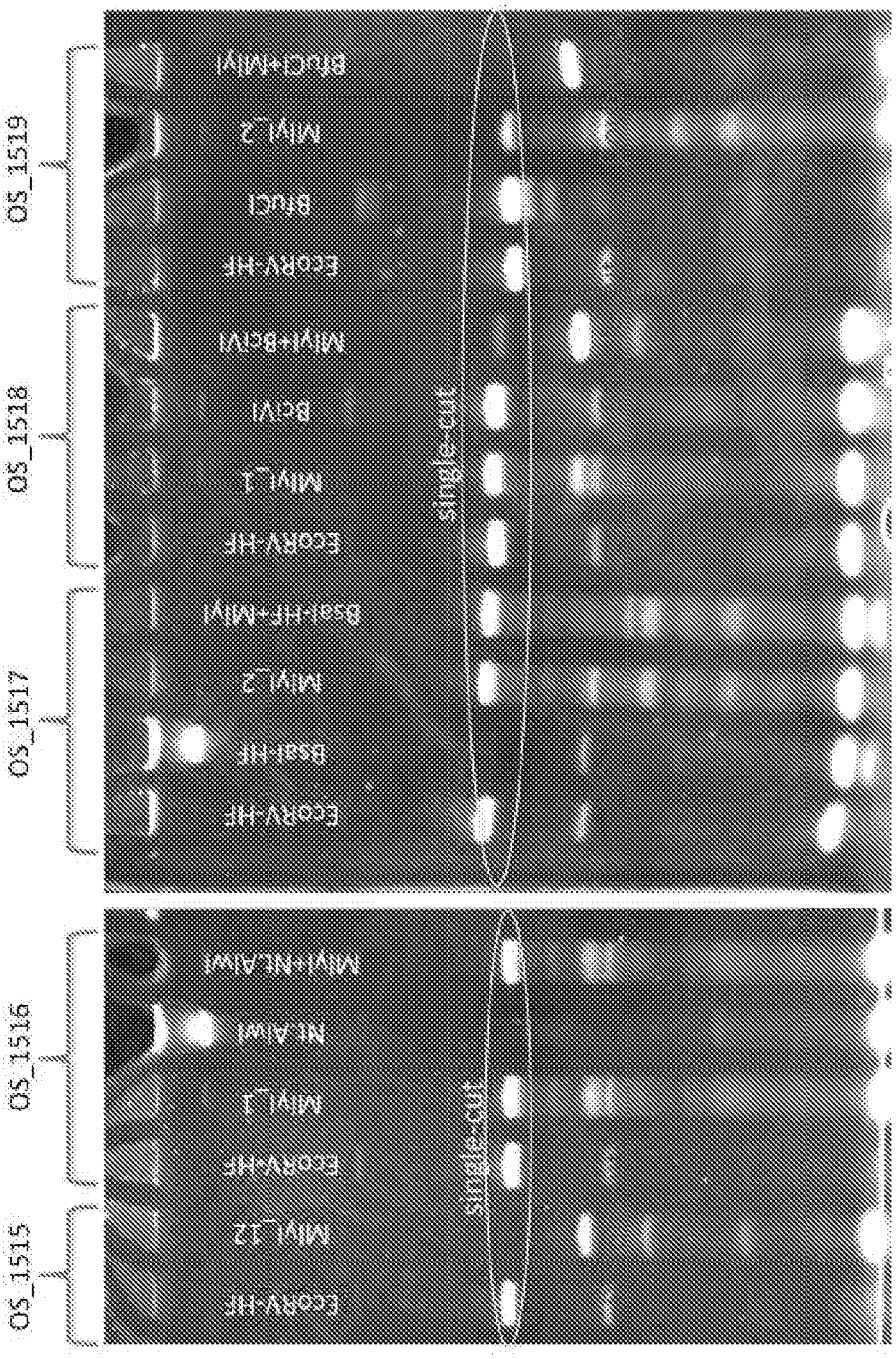

OS_1515
OS_1516
OS_1517
OS_1518
OS_1519
EcoRV-HF
MlyI_12
EcoRV-HF
MlyI_1
Nt.AlwI
MlyI+Nt.AlwI
single-cut
EcoRV-HF
BsaI-HF
MlyI_2
BsaI-HF+MlyI
EcoRV-HF
MlyI_1
BclVI
MlyI+BclVI
EcoRV-HF
BfuCI
MlyI_2
BfuCI+MlyI
single-cut

Figure 64

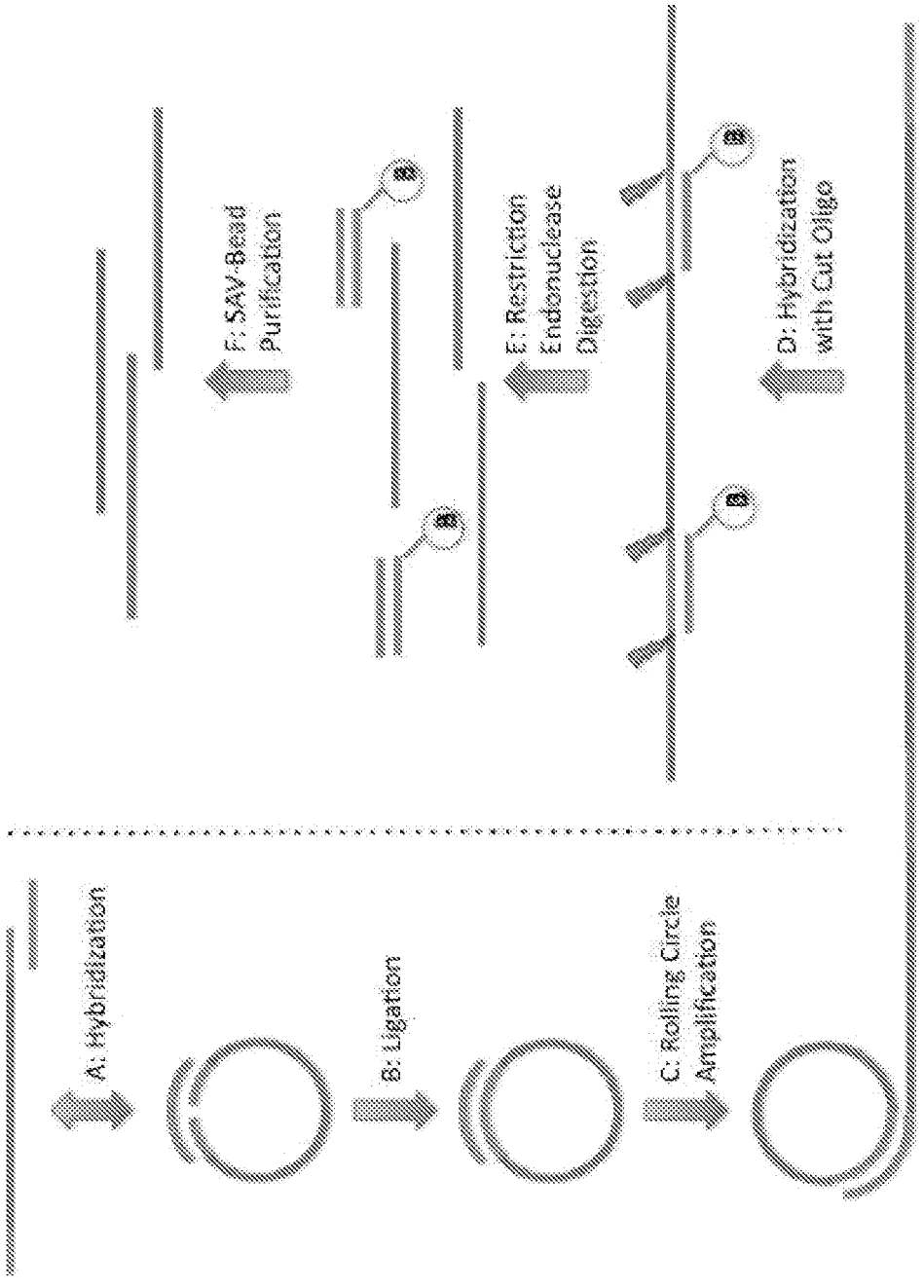
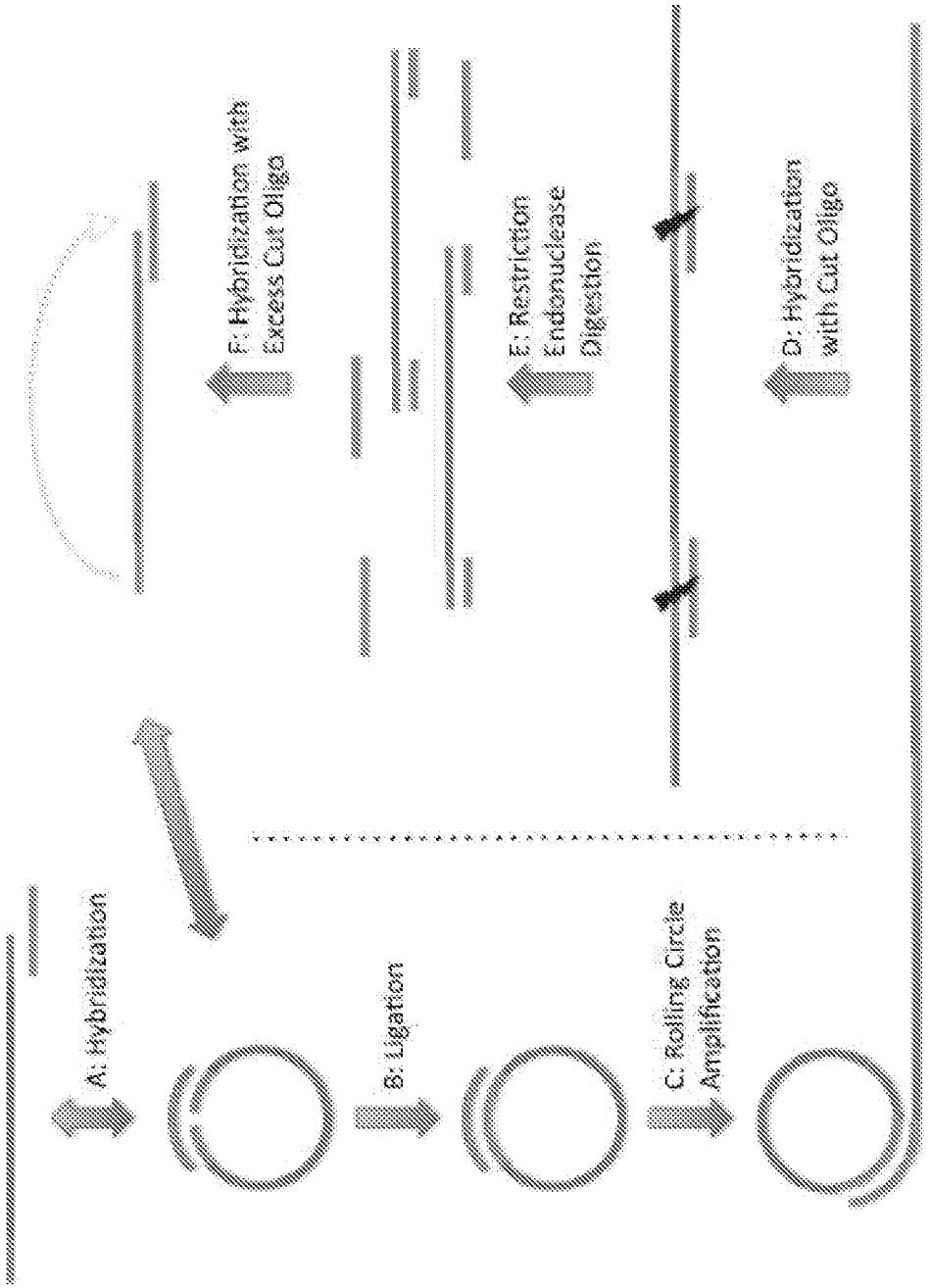

A: Hybridization
B: Ligation
C: Rolling Circle Amplification
D: Hybridization with Cut Oligo
E: Restriction Endonuclease Digestion
F: SAV-Bead Purification

Figure 65


A: Hybridization
B: Ligation
C: Rolling Circle Amplification
D: Hybridization with Cut Oligo
E: Restriction Endonuclease Digestion
F: Hybridization with Excess Cut Oligo