Library Preparation

1.The SOLiD System can use two types of libraries—fragment or mate-paired. Your c
of library depends on what type of application you're performing and what sort of
information you want from your experiment.

Emulsion PCR/Bead Enrichment

2.Prepare clonal bead populations in microreactors containing template, PCR reactic
components, beads, and primers.
3.After PCR, denature the templates and perform bead enrichment to separate beac
with extended templates from undesired beads. The template on the selected beads
undergoes a 3' modification to allow covalent bonding to the slide.

Bead Deposition

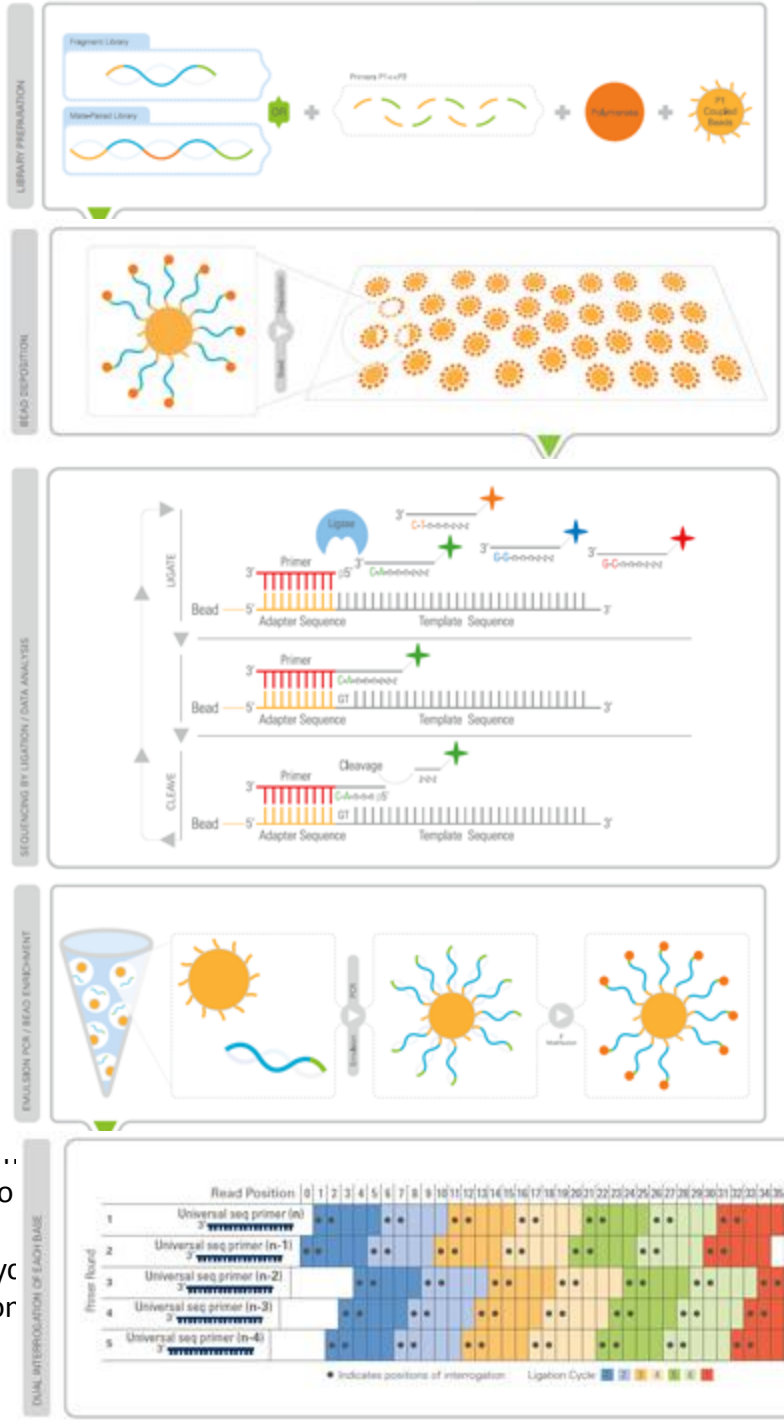
4.Deposit 3' modified beads onto a glass slide. During bead loading, deposition
chambers enable you to segment a slide into one, four, or eight chambers. A key
advantage of the system is the ability to accommodate increasing densities of beads
slide, which will give you a higher level of throughput from the same system.

Sequencing by Ligation

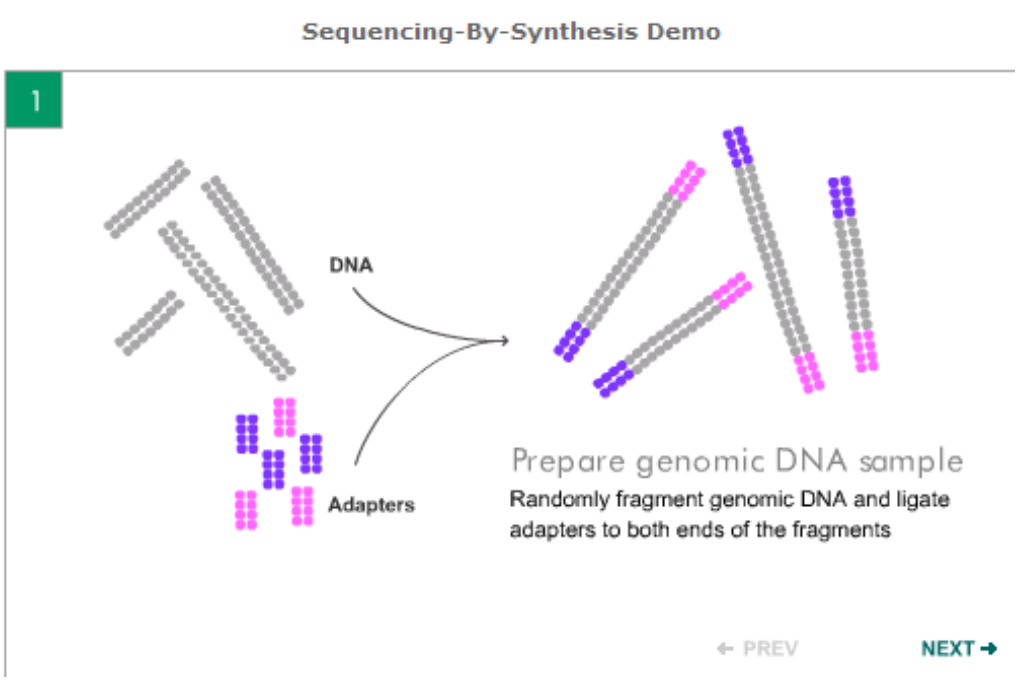
5.Primers hybridize to the P1 adapter sequence within the library template.
6.A set of four fluorescently labeled di-base probes compete for ligation to the
sequencing primer. Specificity of the di-base probe is achieved by interrogating ever
and 2nd base in each ligation reaction.
7.Multiple cycles of ligation, detection and cleavage are performed with the number
cycles determining the eventual read length.
8.Following a series of ligation cycles, the extension product is removed and the tem
is reset with a primer complementary to the n-1 position for a second round of ligati
cycles.

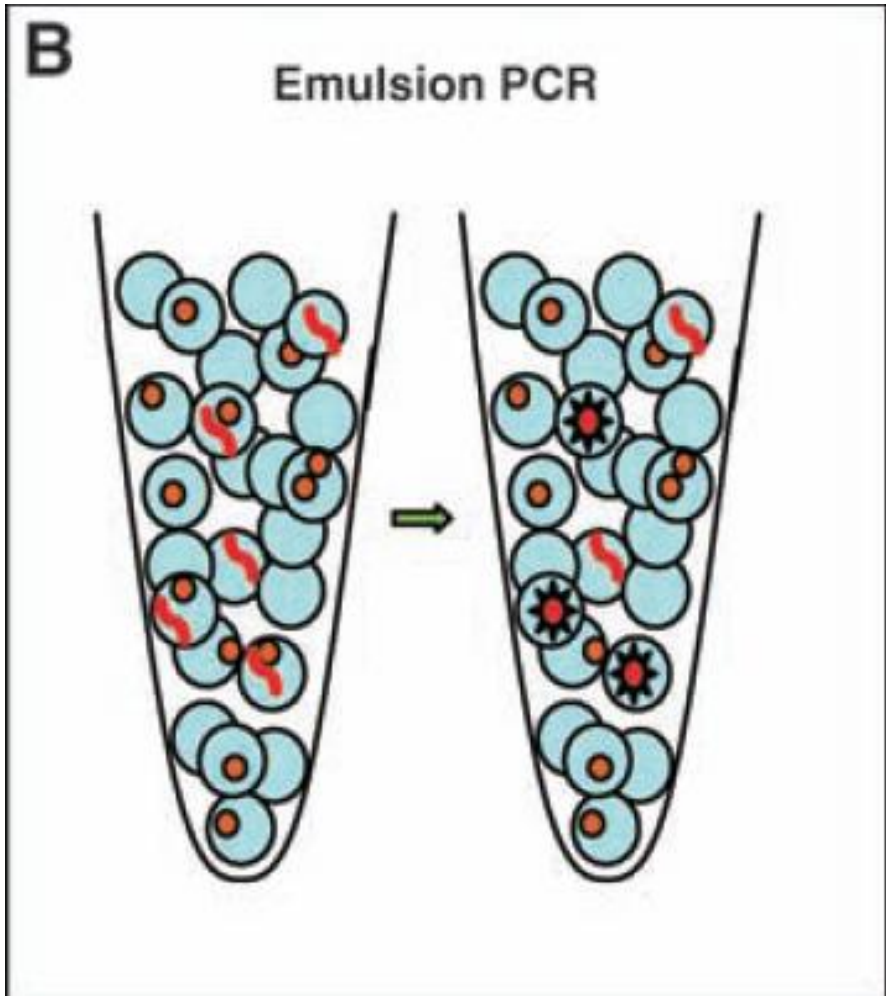
Primer Reset

9.Five rounds of primer reset are completed for each sequence tag. Through the prir
reset process, each base is interrogated in two independent ligation reactions by two
different primers.
For example, the base at read position 5 is assayed by primer number 2 in ligation cyc
and by primer number 3 in ligation cycle 1 (see figure at right). This dual interrogatior
fundamental to the unmatched accuracy characterized by the SOLiD System.

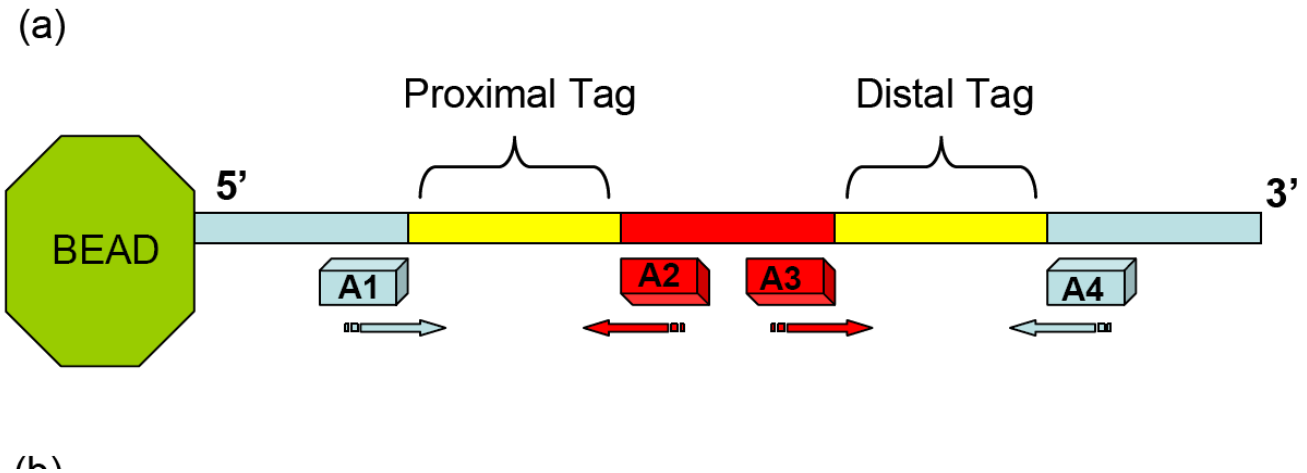


ABI Solid


Sequencing-By-Synthesis Demo
1
DNA
Adapters
Prepare genomic DNA sample
Randomly fragment genomic DNA and ligate adapters to both ends of the fragments
← PREV
NEXT →


B
Emulsion PCR

Sequencing By Ligation



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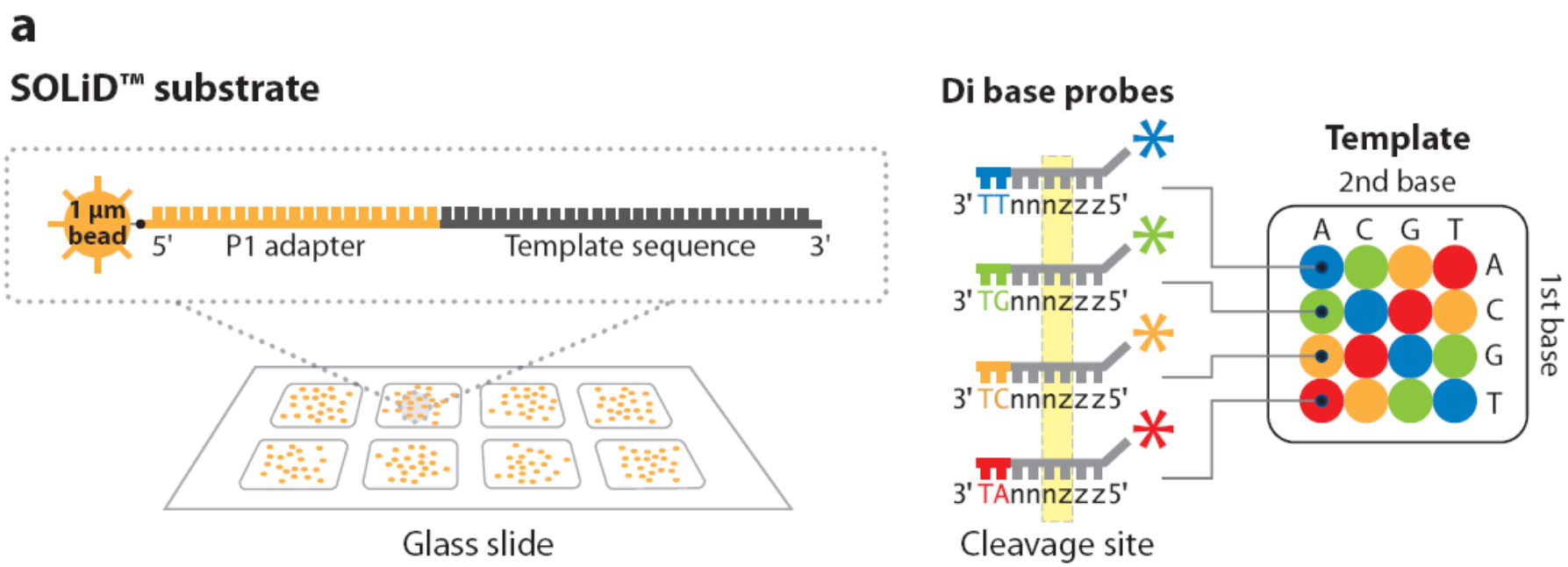
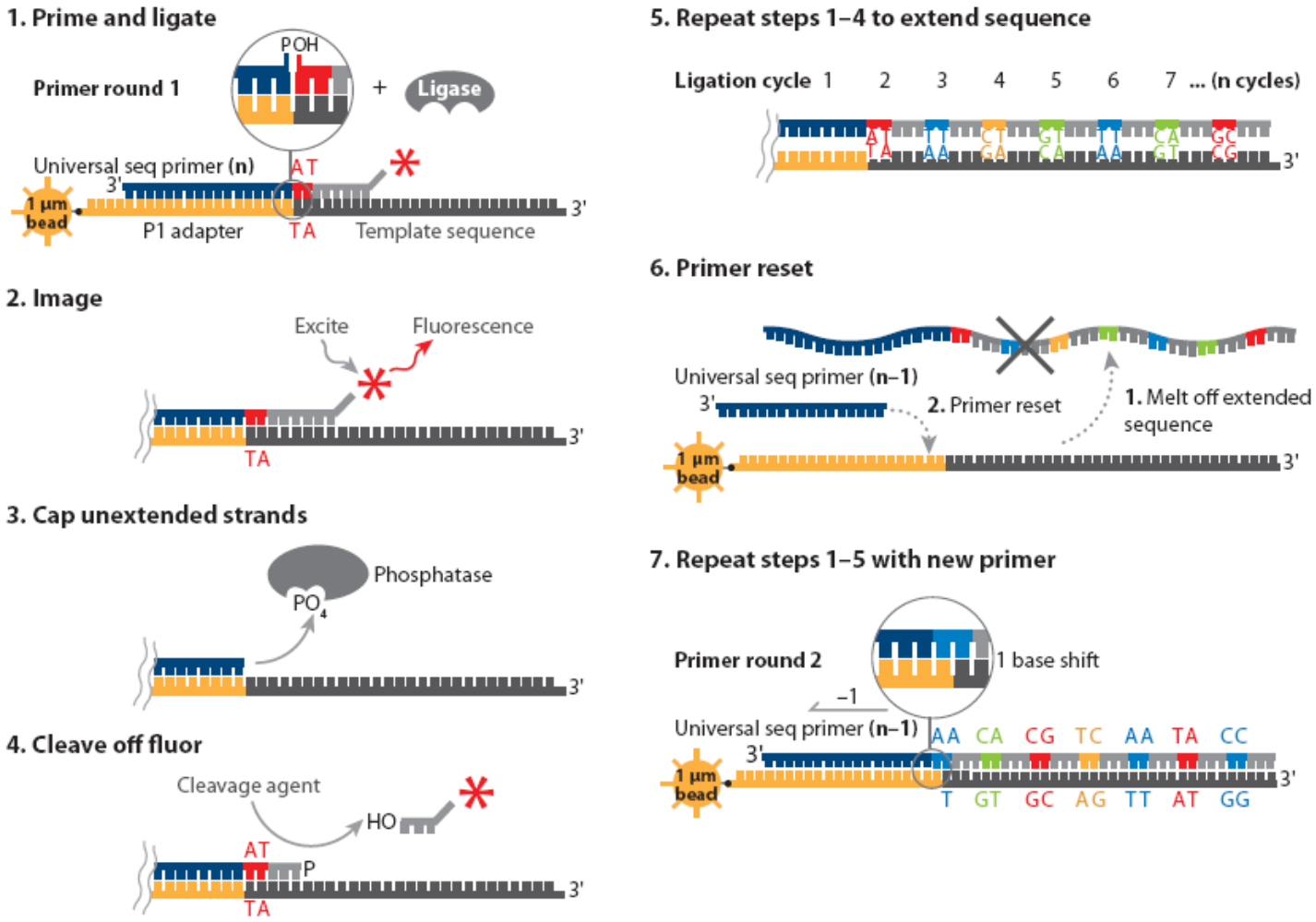
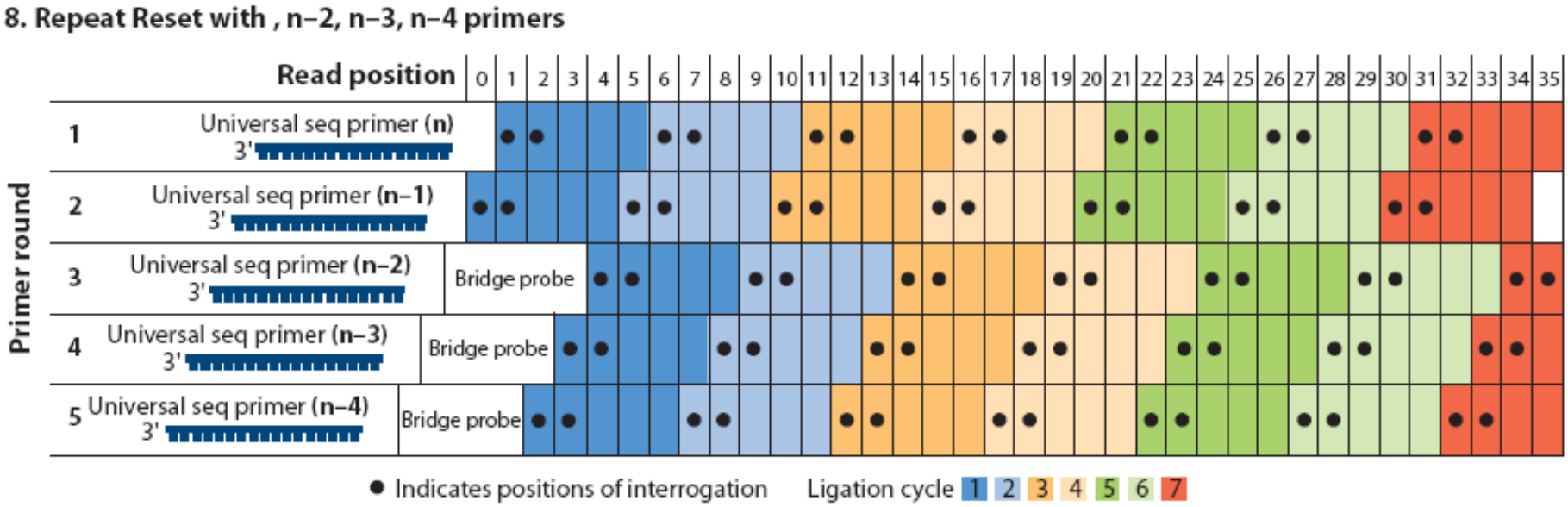

a
SOLiD™ substrate
1 µm bead
5'
P1 adapter
Template sequence
3'
Glass slide
Di base probes
3' TTnnnzzz5'
3' TGnnnzzz5'
3' TCnnnzzz5'
3' TAnnnzzz5'
Cleavage site
Template
2nd base
A C G T
A
C
G
T
1st base

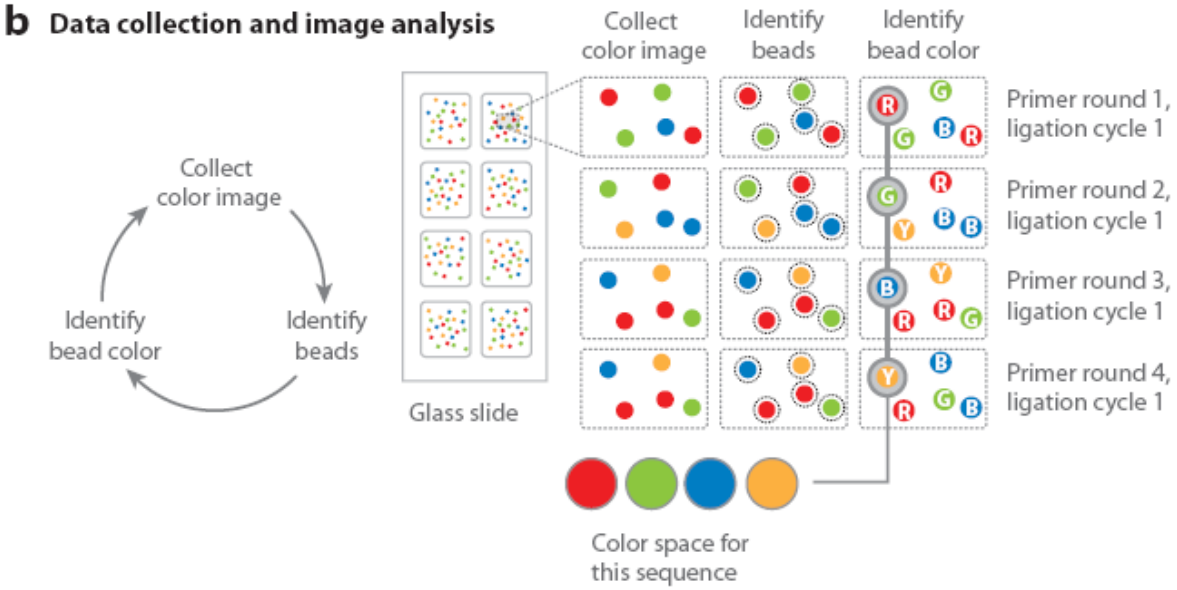
ABI SOLID

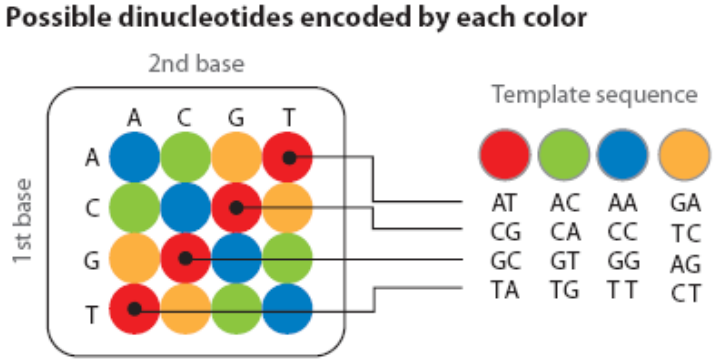

1. Prime and ligate
POH
Primer round 1
+
Ligase
Universal seq primer (n)
AT
3'
1 µm bead
P1 adapter
TA
Template sequence
3'
2. Image
Excite
Fluorescence
TA
3'
3. Cap unextended strands
Phosphatase
PO4
3'
4. Cleave off fluor
Cleavage agent
HO
AT
P
TA
3'
5. Repeat steps 1–4 to extend sequence
Ligation cycle 1 2 3 4 5 6 7 ... (n cycles)
3'
6. Primer reset
Universal seq primer (n–1)
3'
2. Primer reset
1. Melt off extended sequence
1 µm bead
3'
7. Repeat steps 1–5 with new primer
Primer round 2
1 base shift
–1
Universal seq primer (n–1)
AA CA CG TC AA TA CC
3'
1 µm bead
T GT GC AG TT AT GG
3'

ABI SOLID

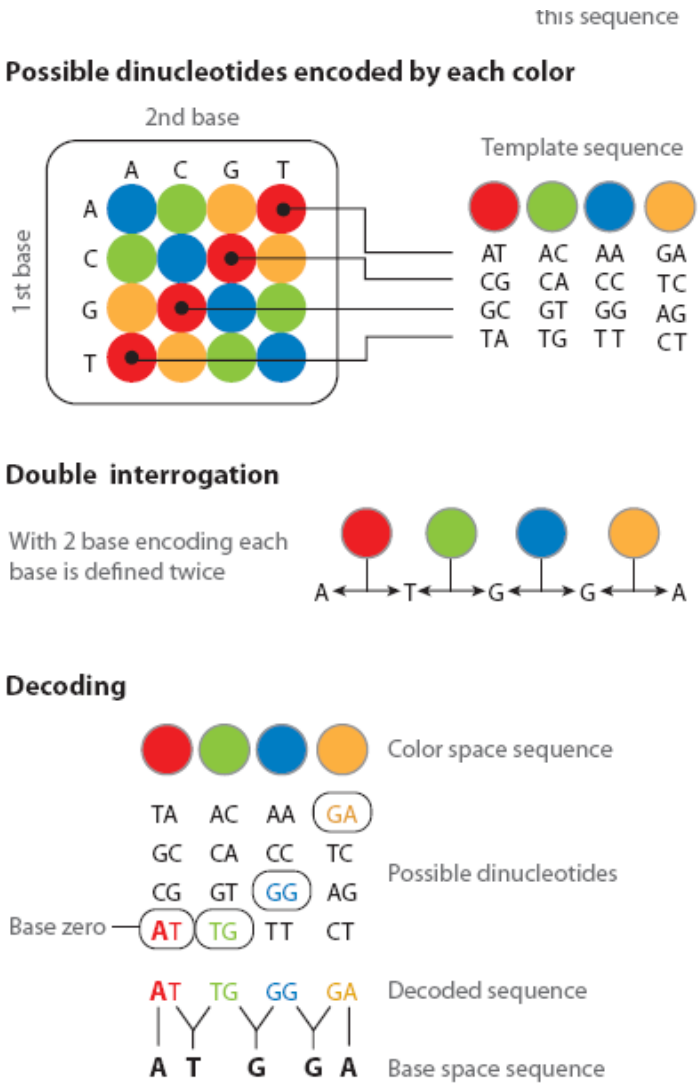

8. Repeat Reset with , n–2, n–3, n–4 primers
Read position
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35
Primer round
1 Universal seq primer (n) 3'
2 Universal seq primer (n–1) 3'
3 Universal seq primer (n–2) 3'
Bridge probe
4 Universal seq primer (n–3) 3'
Bridge probe
5 Universal seq primer (n–4) 3'
Bridge probe
● Indicates positions of interrogation
Ligation cycle 1 2 3 4 5 6 7

ABI SOLID


b Data collection and image analysis
Collect color image
Identify beads
Identify bead color
Collect color image
Identify beads
Identify bead color
Glass slide
Primer round 1, ligation cycle 1
Primer round 2, ligation cycle 1
Primer round 3, ligation cycle 1
Primer round 4, ligation cycle 1
Color space for this sequence


Possible dinucleotides encoded by each color
2nd base
A C G T
1st base
A
C
G
T
Template sequence
AT AC AA GA
CG CA CC TC
GC GT GG AG
TA TG TT CT

ABI SOLID



This allows for error correction:

See board

Raw error rate = ~3%
Corrected error rate = ~0.1%

Paired End Reads are Important!