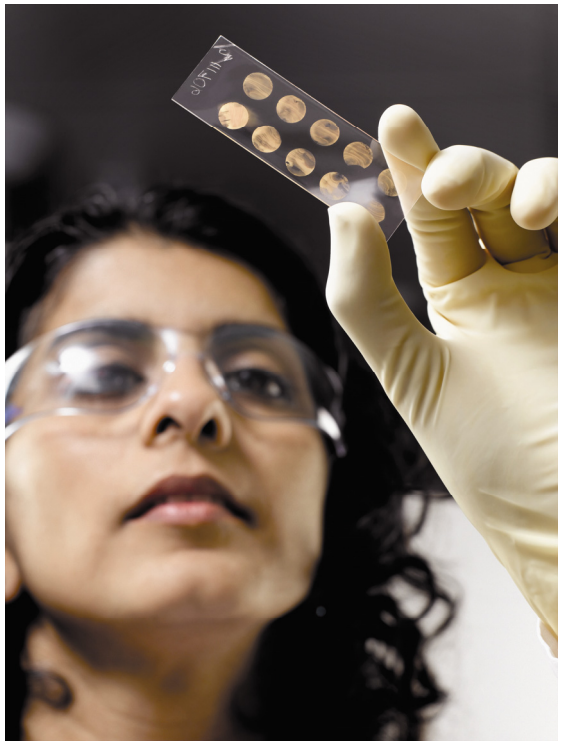
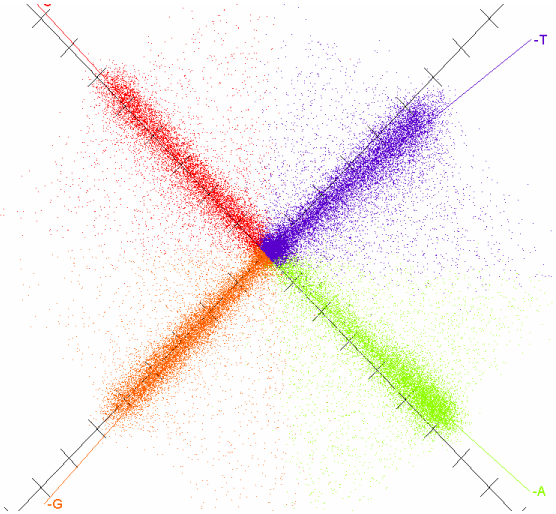
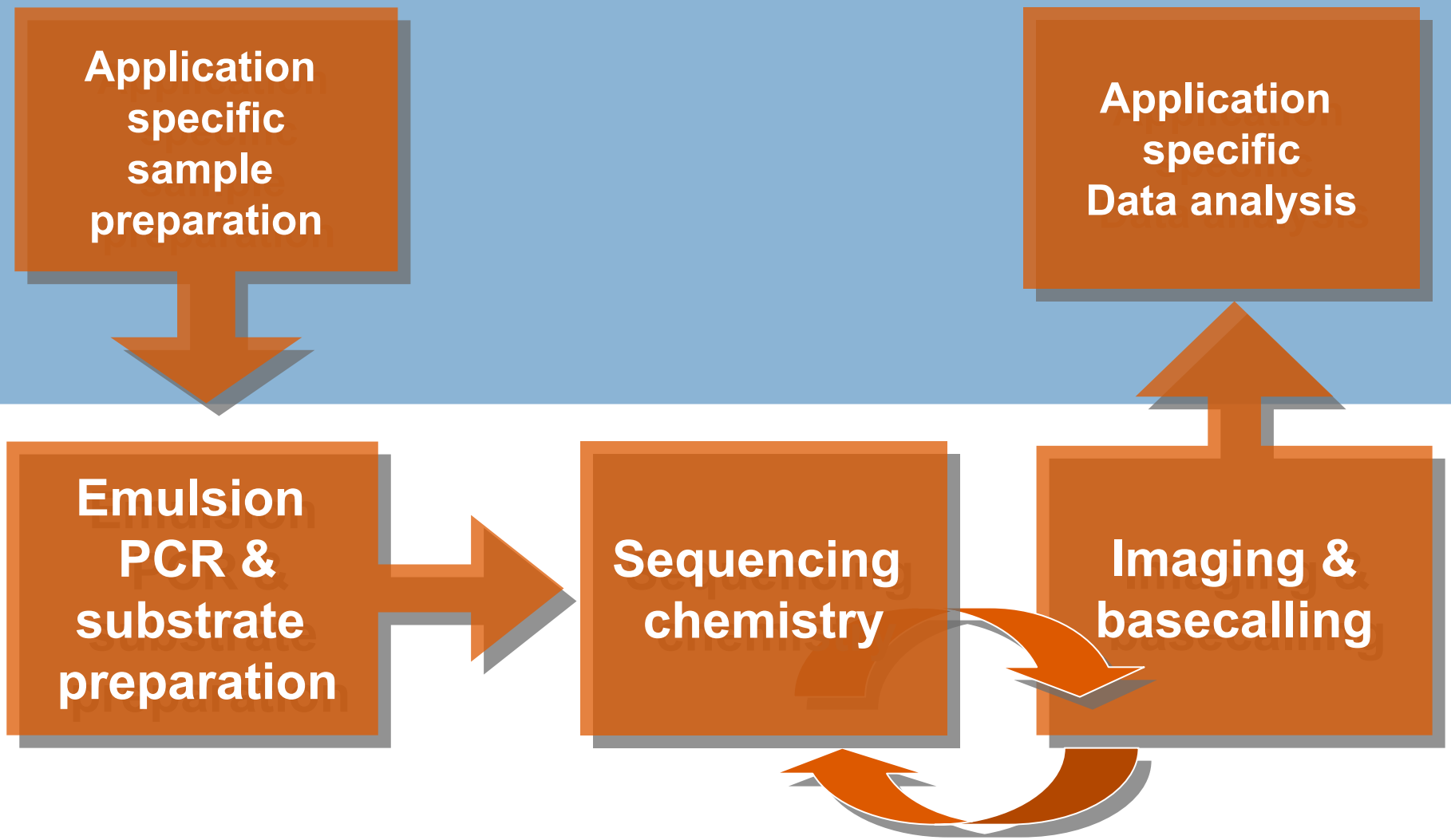




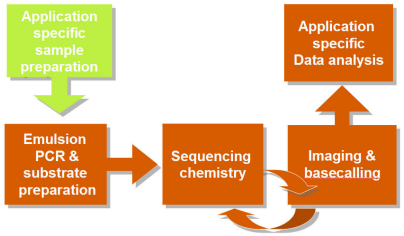

Applied Biosystems

# SOLiD™ System

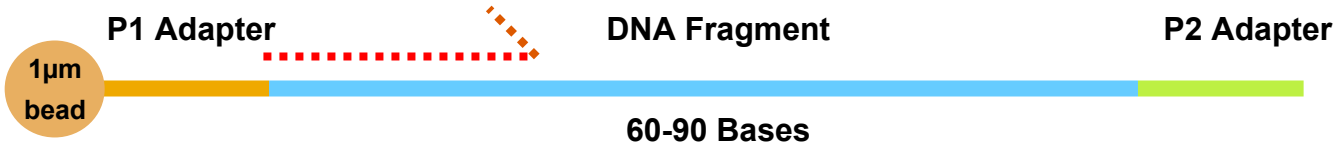
# Workflow



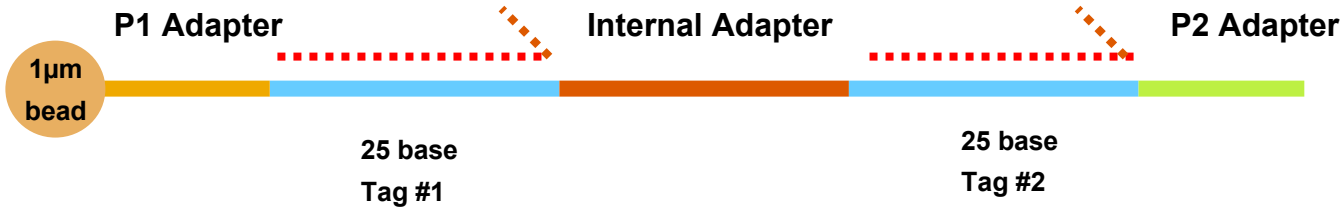
# Create library of DNA fragments: 2 methods



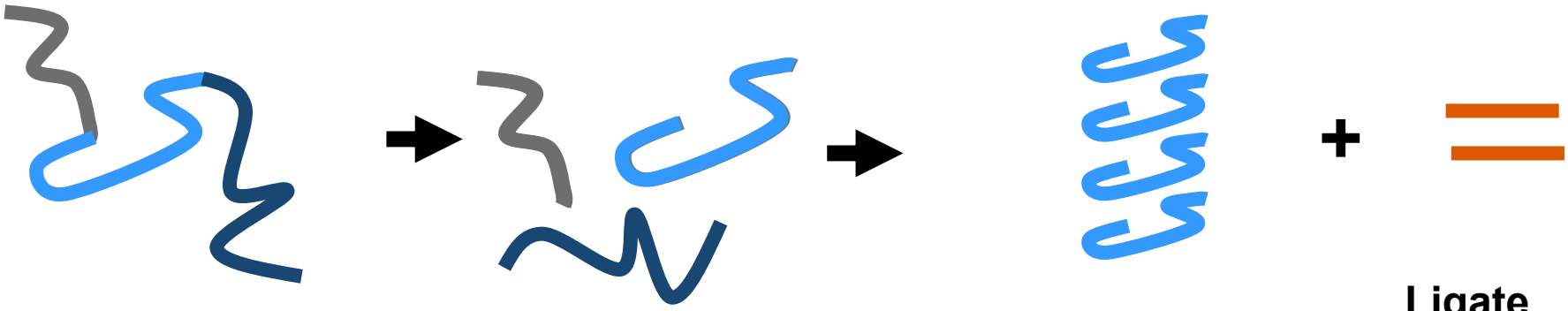

## Fragment Library (directed resequencing)




## Mate Pair Library (whole genome sequencing)



# Creating a mate-paired library (i)

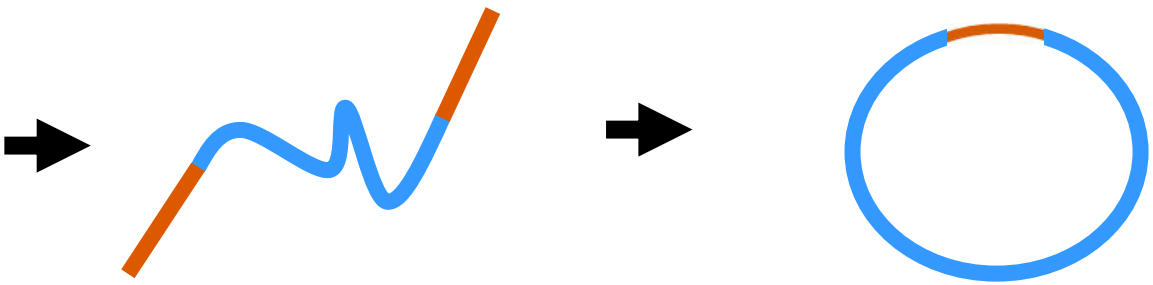


Complex sample

Randomly Fragment sample

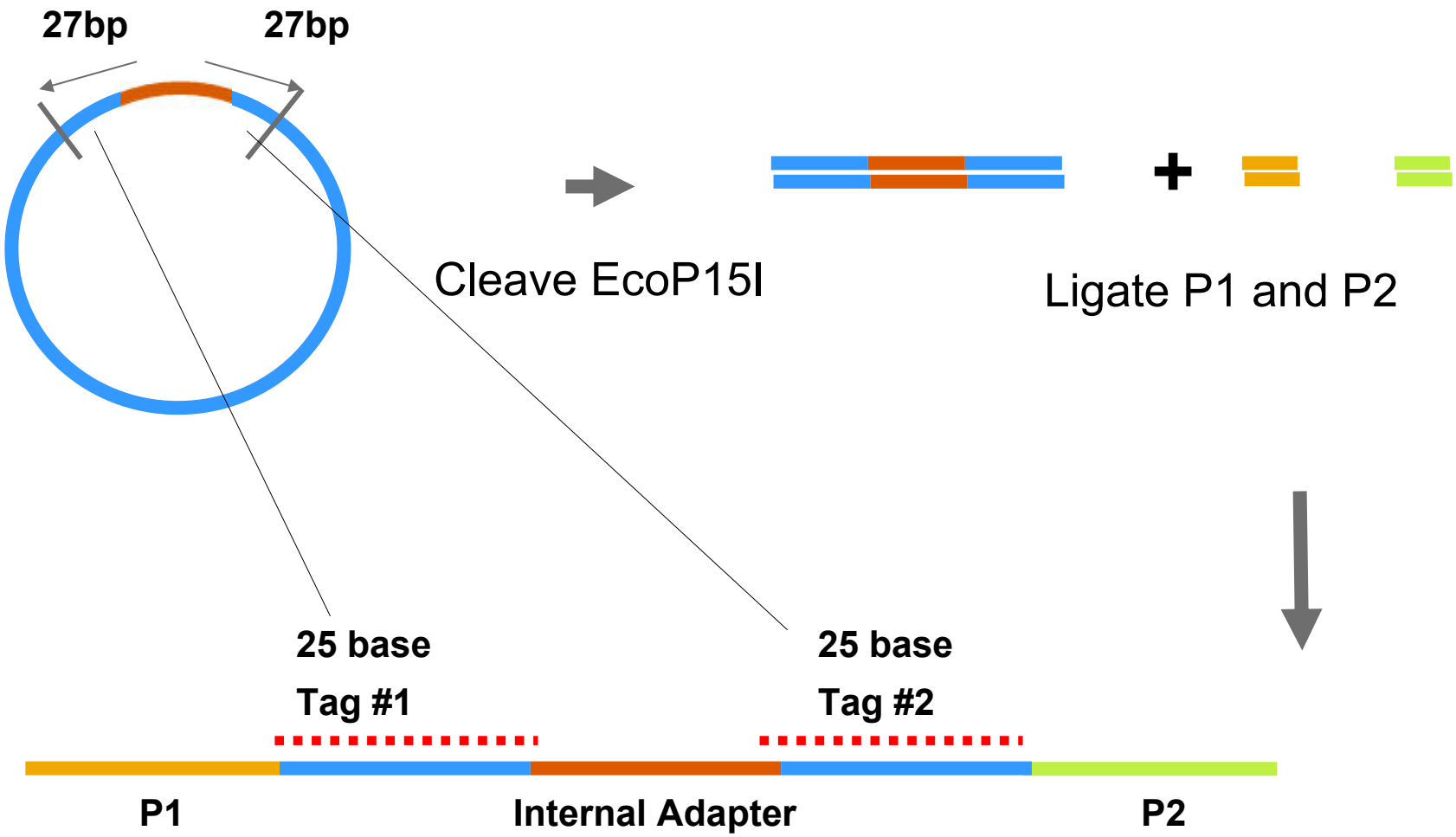
size select (eg:1, 2, 3, 5, 10 KB)

Ligate Internal adapters

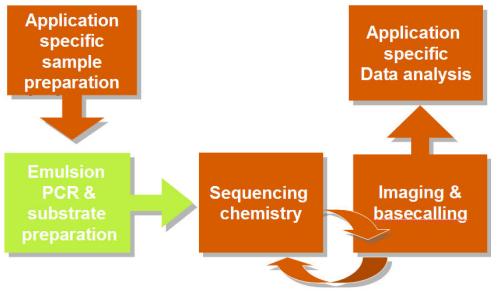


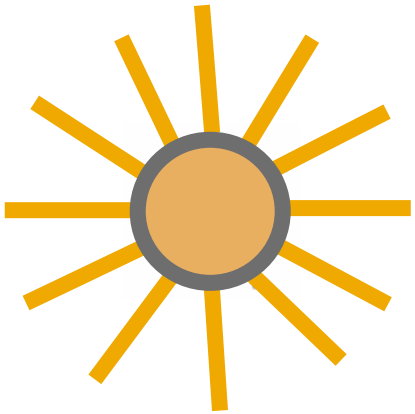
Circularize

# Creating a mate-paired library (ii)



# Emulsion PCR (i)



+

Templates

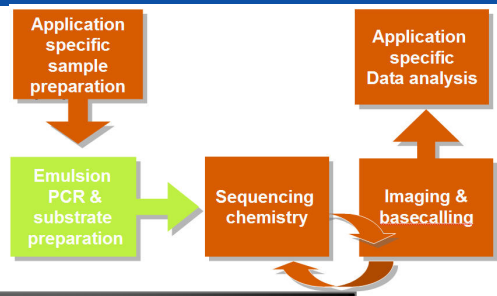
Primers P1<<P2

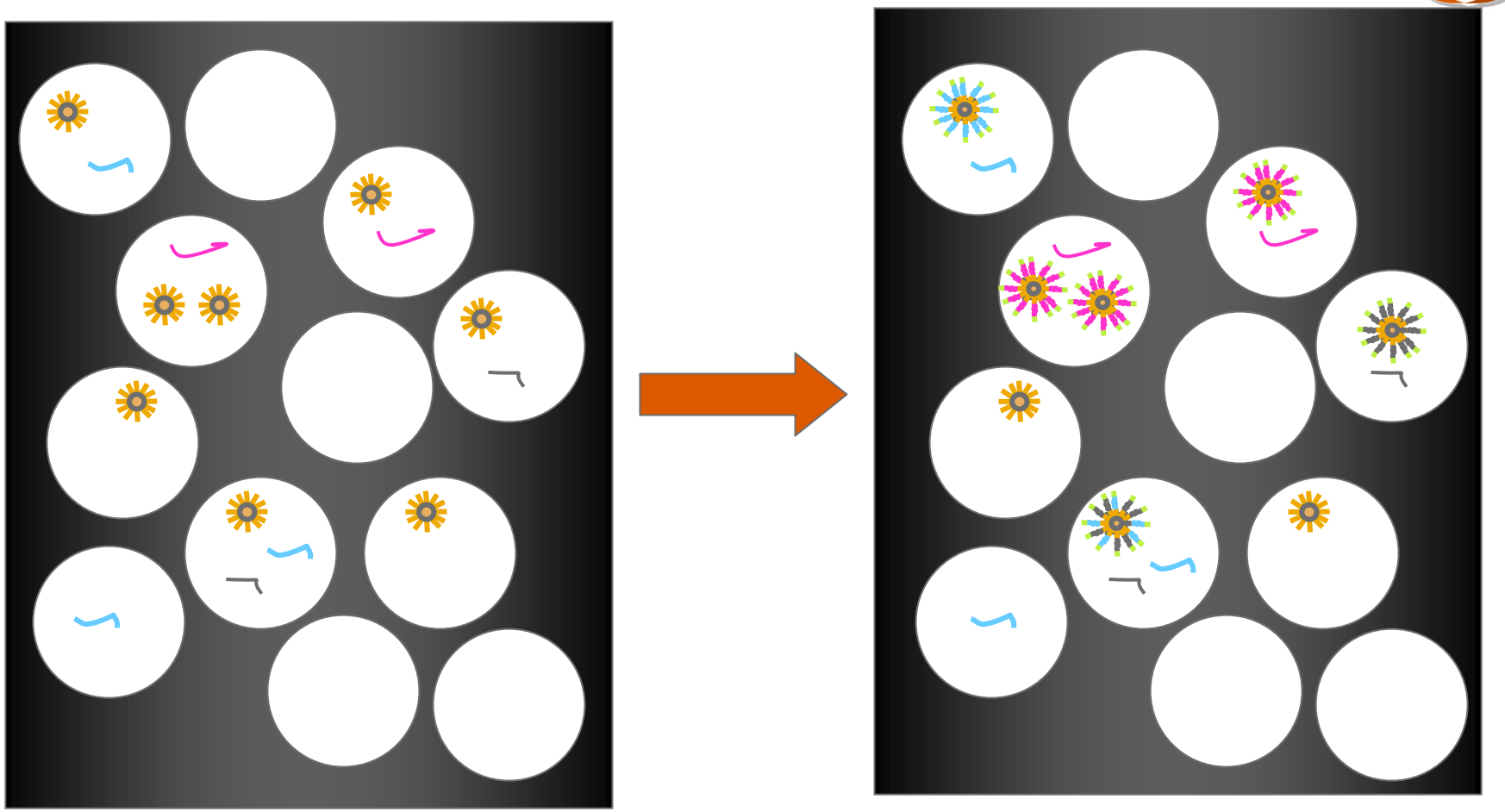
P1-coupled beads


Enzyme

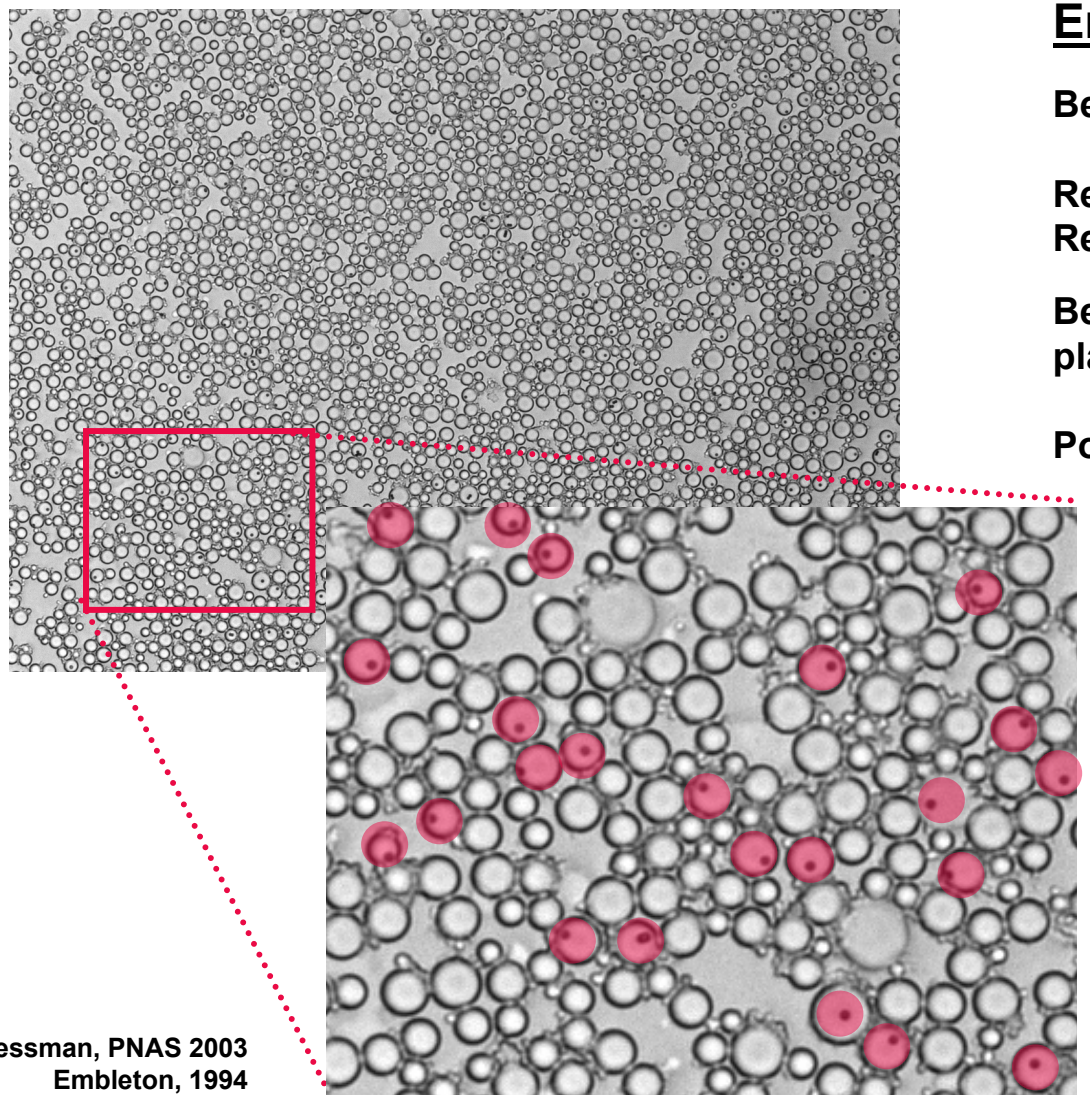
# Emulsion PCR (ii)

Mix PCR aqueous phase into a water-in-oil (w/o) emulsion and carry out emulsion PCR



# SOLiD system: Clonal Amplification

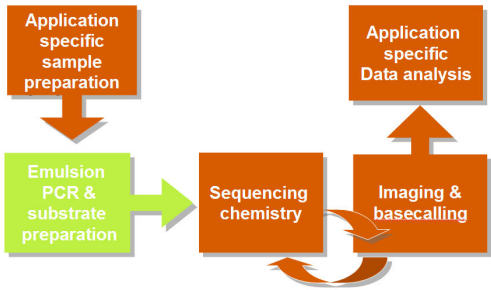


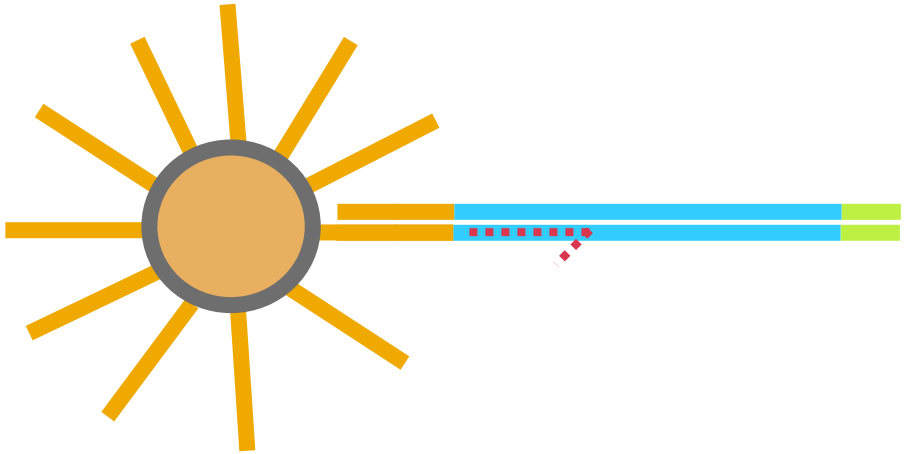
Dressman, PNAS 2003
Embleton, 1994

## Emulsion Metrics

| | |
|---|---|
| **Bead size:** | **1 μm** |
| **Reactor size:** | **4 μm** |
| **Reactor volume:** | **34 fL** |
| **Beads / emulsion plate (96-well):** | **2-4 x $10^9$** |
| **Post Enrichment:** | **~500M / plate** |

# emulsion PCR Individual Bead



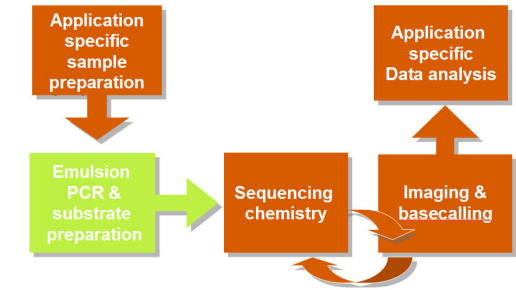
1) Template Anneals to P1

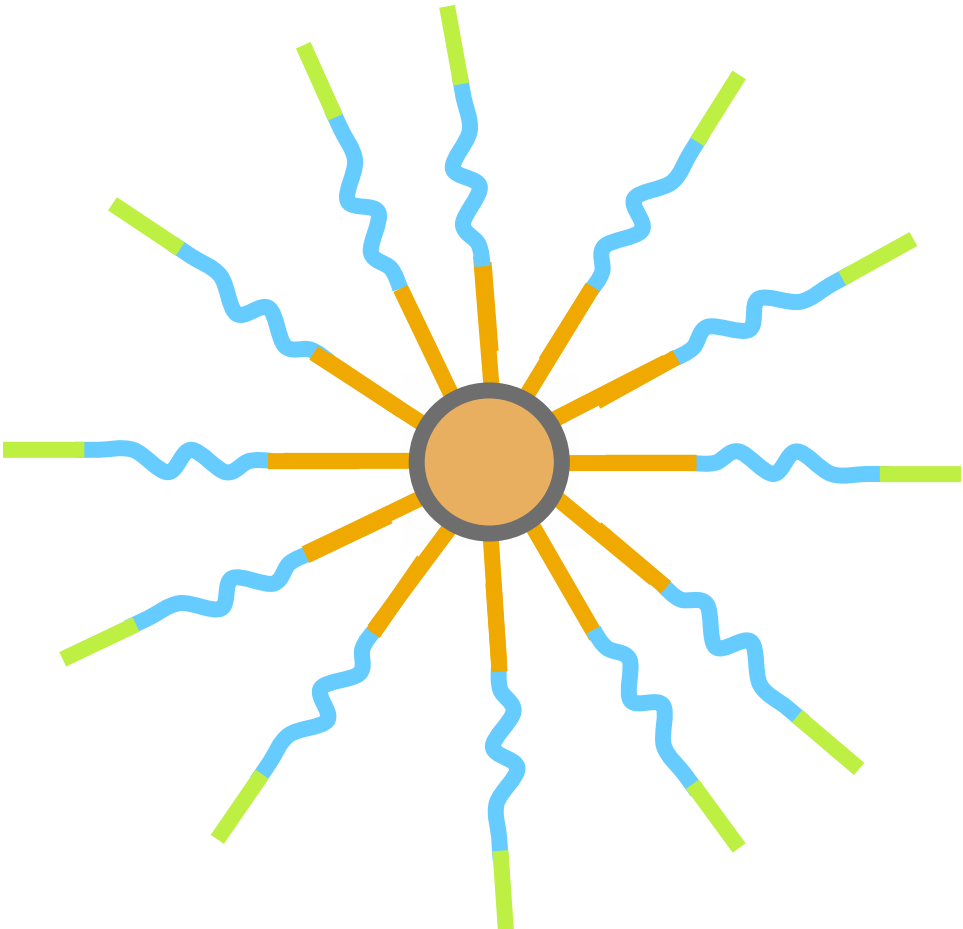
2) Polymerase extends from P1

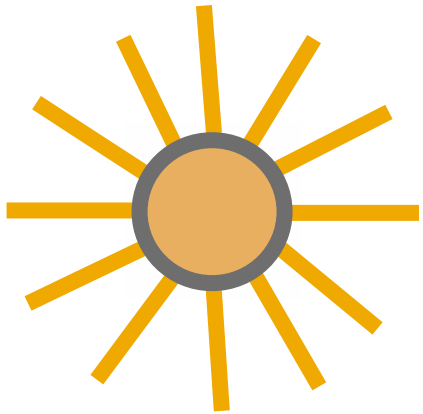
3) Complementary sequence is extended off bead surface

4) Template disassociates

# Results of emulsion PCR, after breaking emulsion



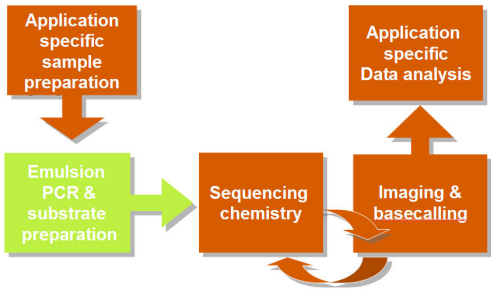



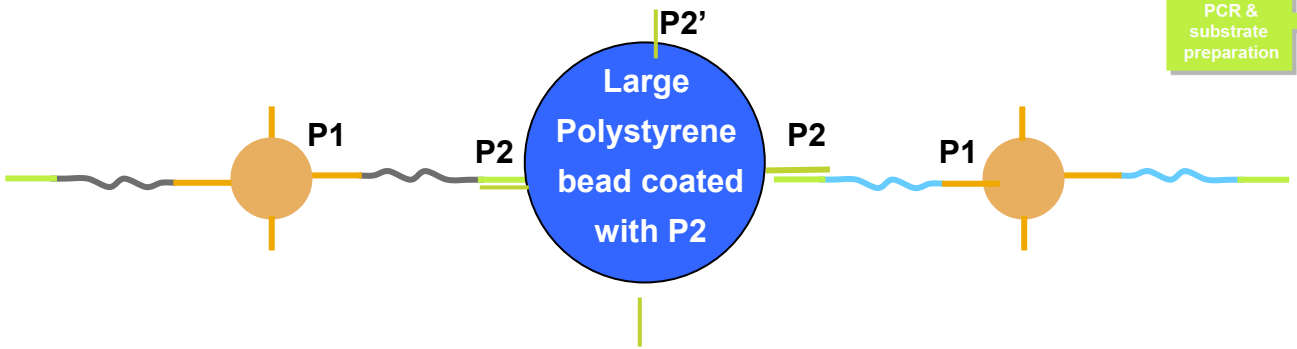


Bead contains ~20K amplified products from original single strand molecule

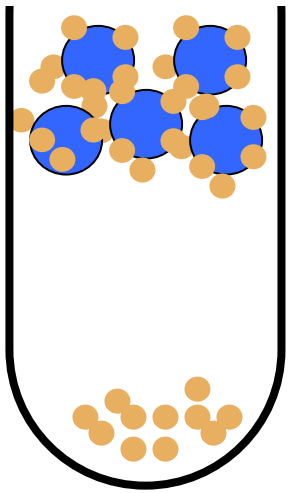
Beads with no product

# Enrichment



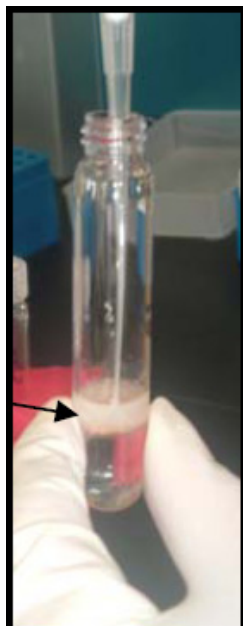




**Centrifuge in glycerol gradient**



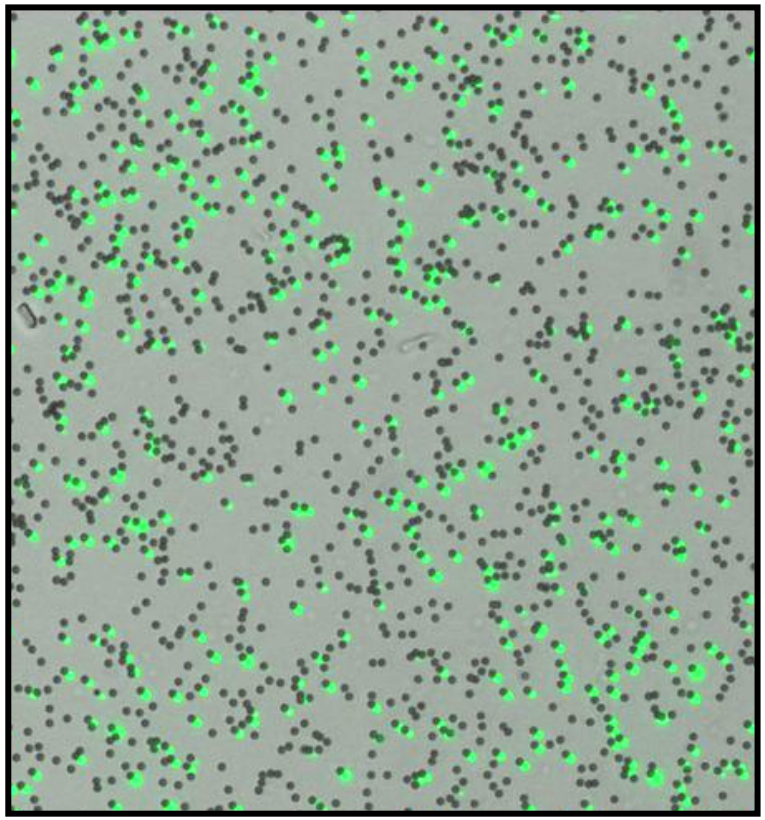
**Supernatant**
***Captured beads with templates***

**Pellet**
***Beads with no template***

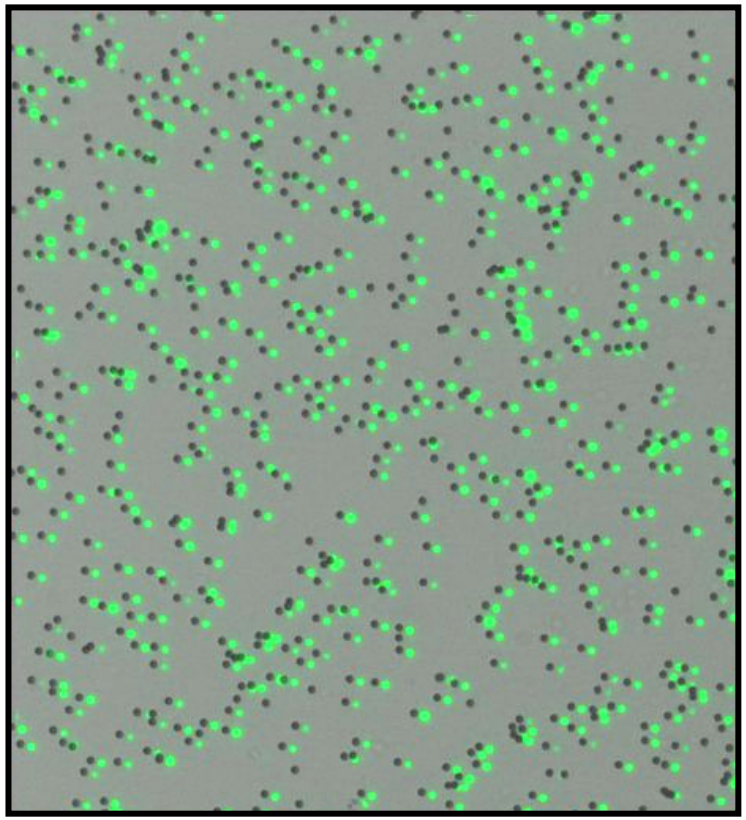


# P2-hybridization, Pre- and post-enrichment

Pre-enrichment
(**30%**)

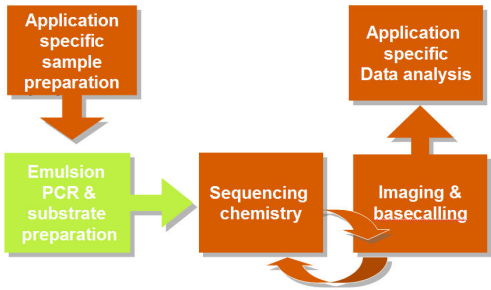


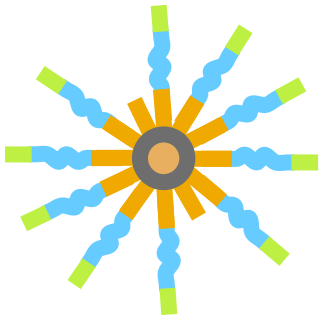
Post-enrichment
(**80%**)



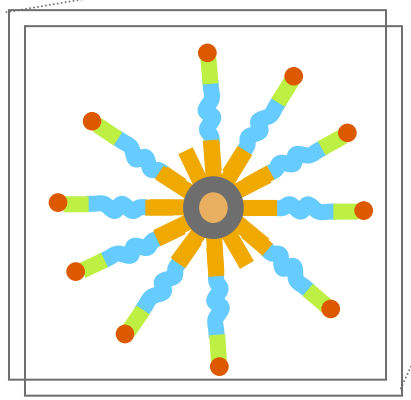
em6395

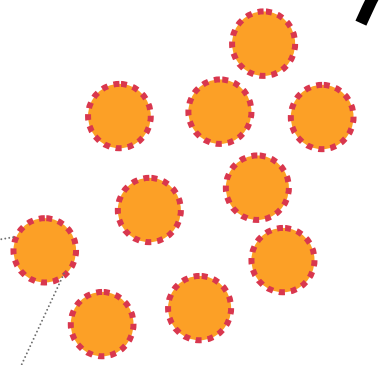
# Bead Deposition

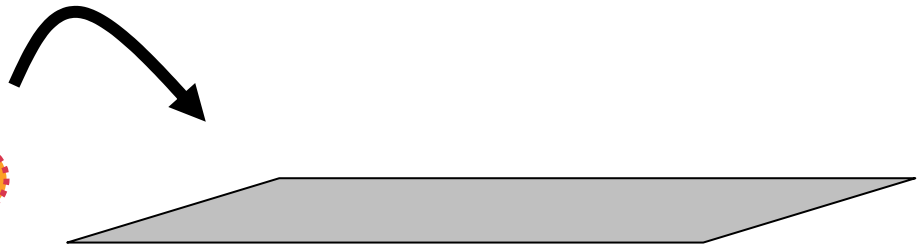


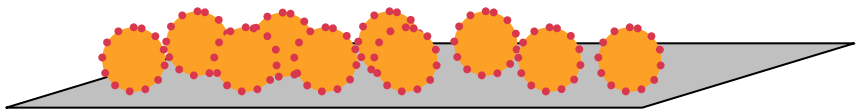



3'-end modification





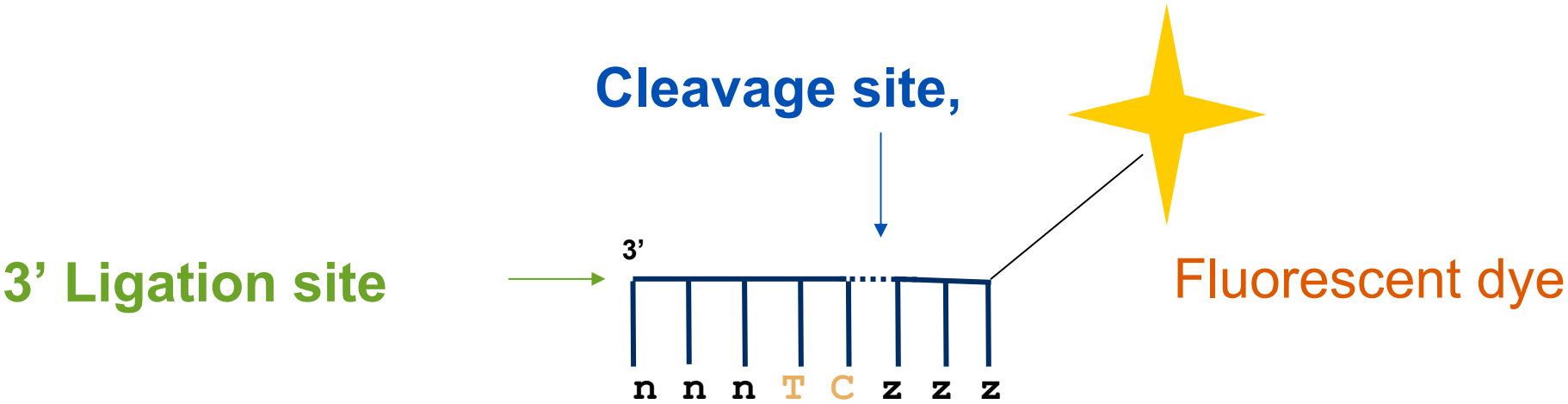




Beads attached to glass surface in a **random** array

# Properties of the Probes

## Spatial separation among dye, ligation & cleavage sites

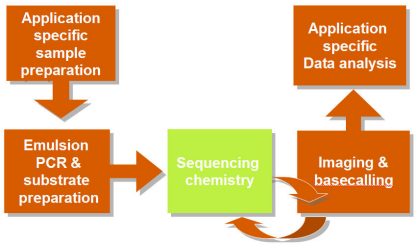


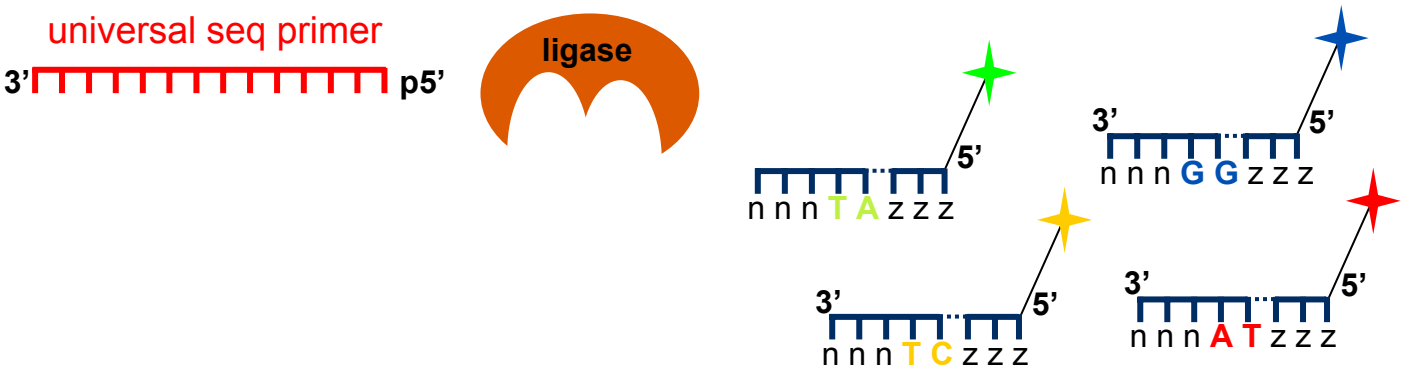

1,024 Octamer Probes ($4^5$)

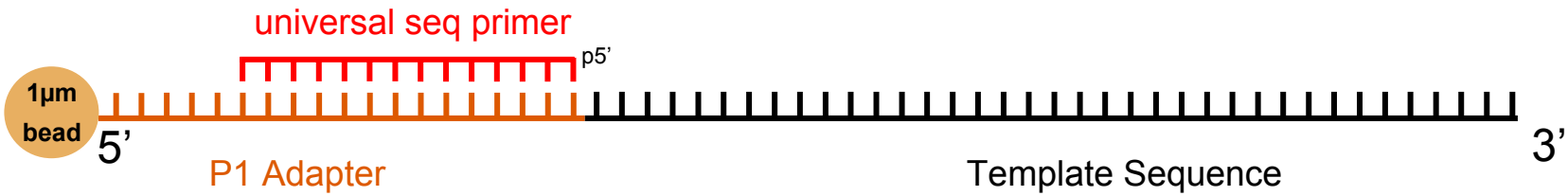
4 Dyes, 4 dinucleotides, 256 probes per dye

N= degenerate bases Z= Universal bases

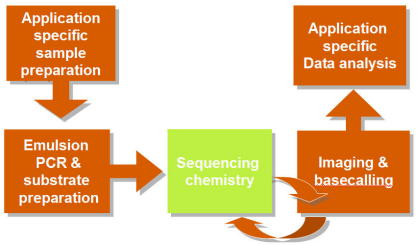
# SOLiD Chemistry System 4-color ligation
# Ligation reaction

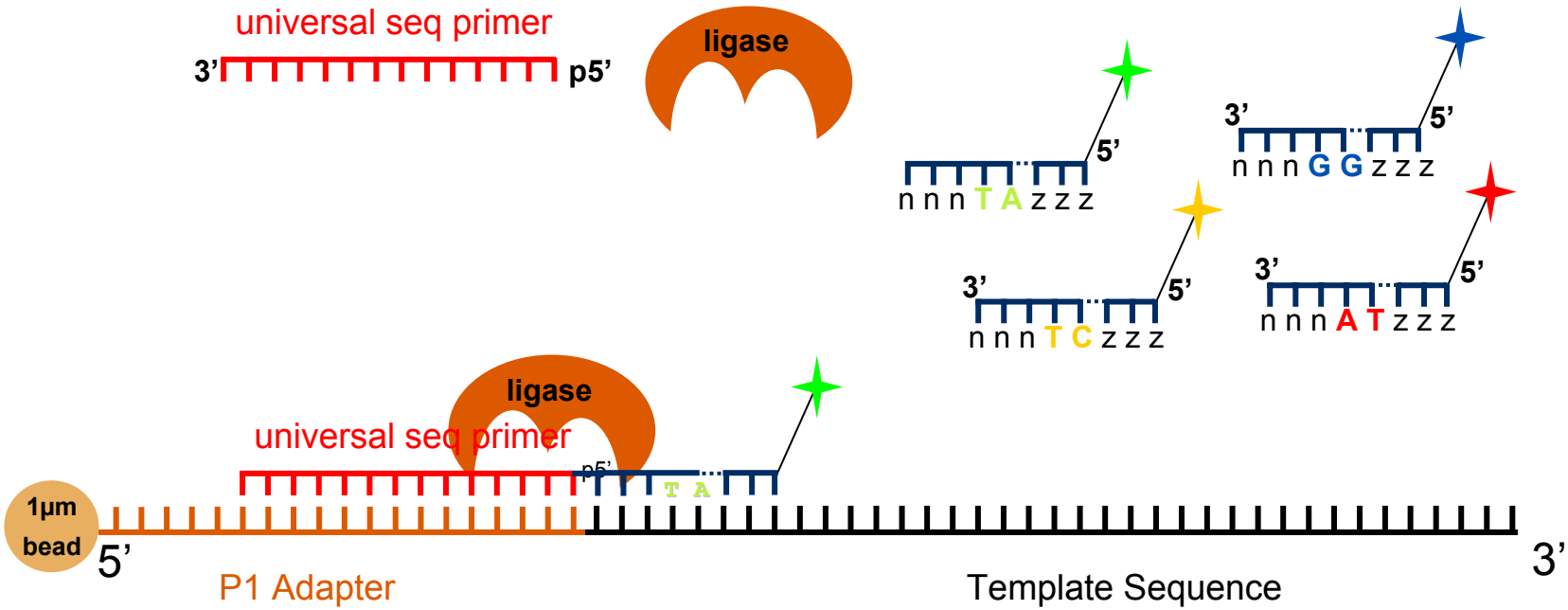


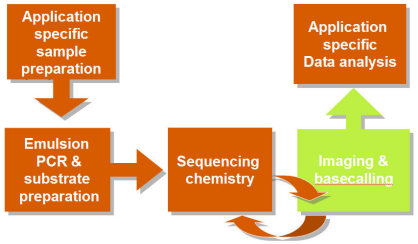



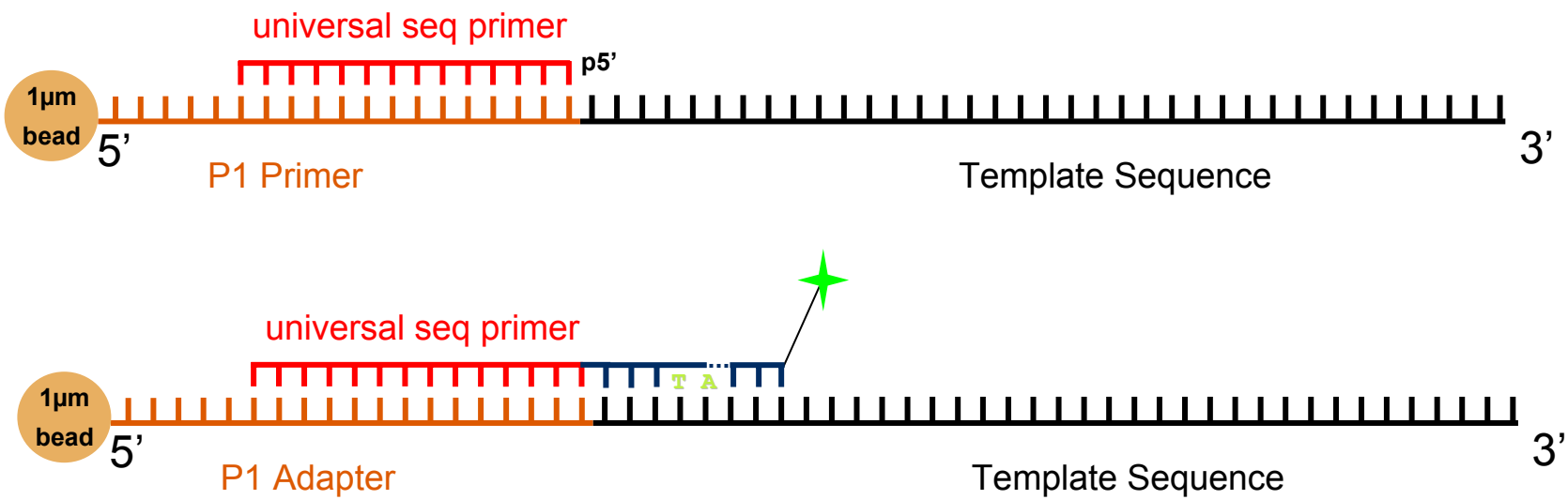
# SOLiD Chemistry System 4-color ligation
# Ligation reaction



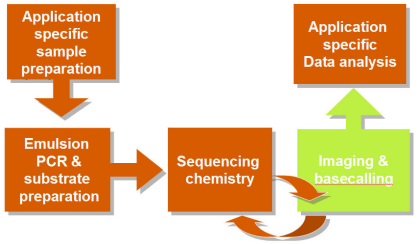



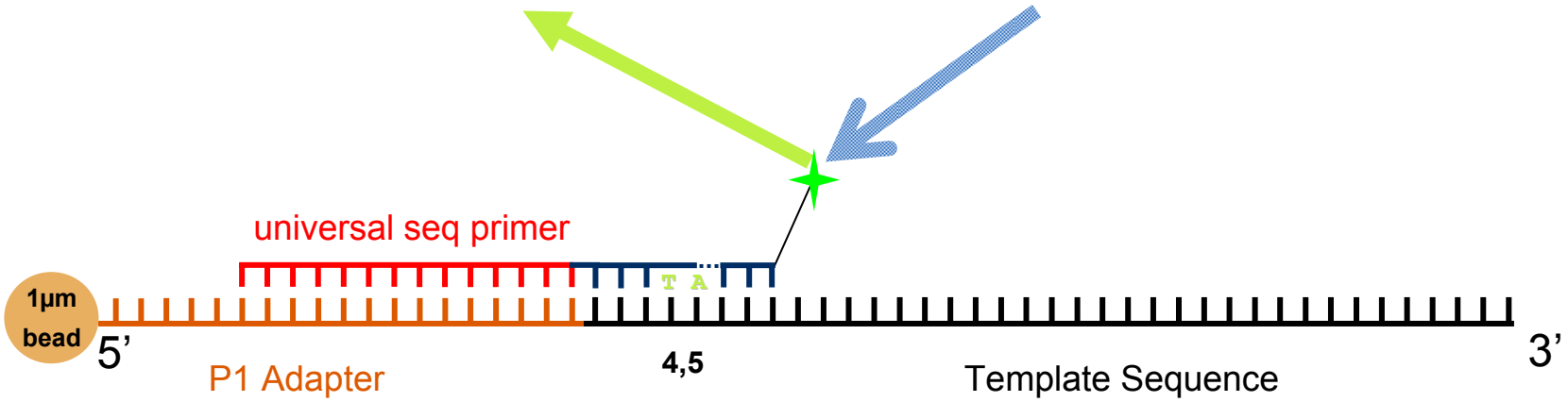
# SOLiD Chemistry System 4-color ligation De-Phosphorylation



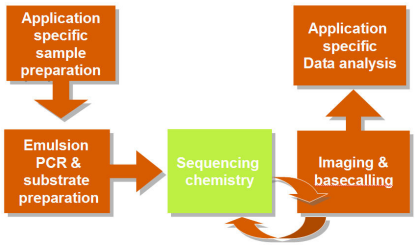



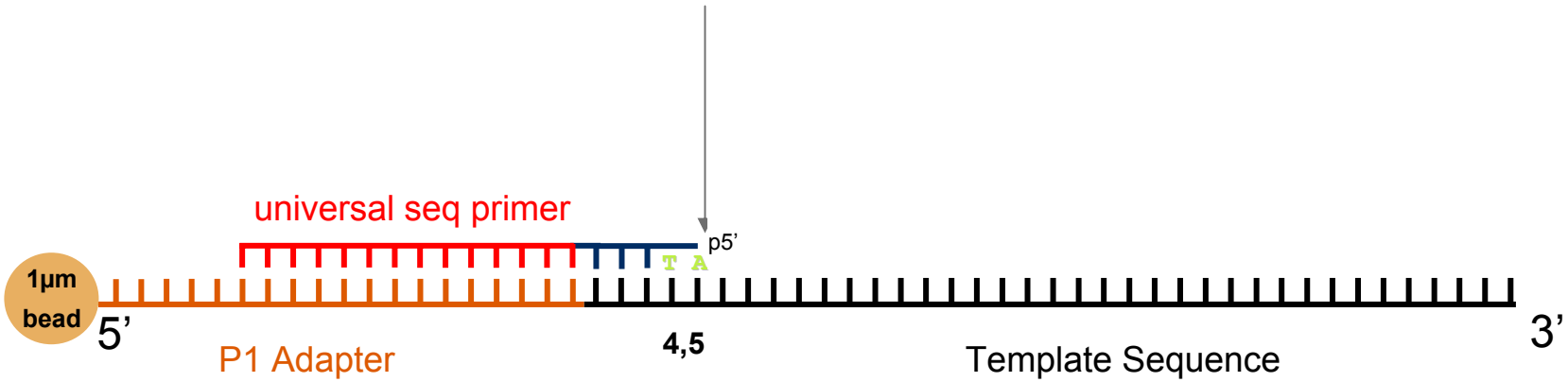
# SOLiD Chemistry System 4-color ligation Visualization



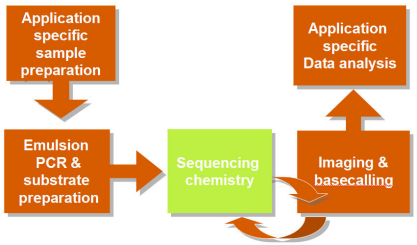



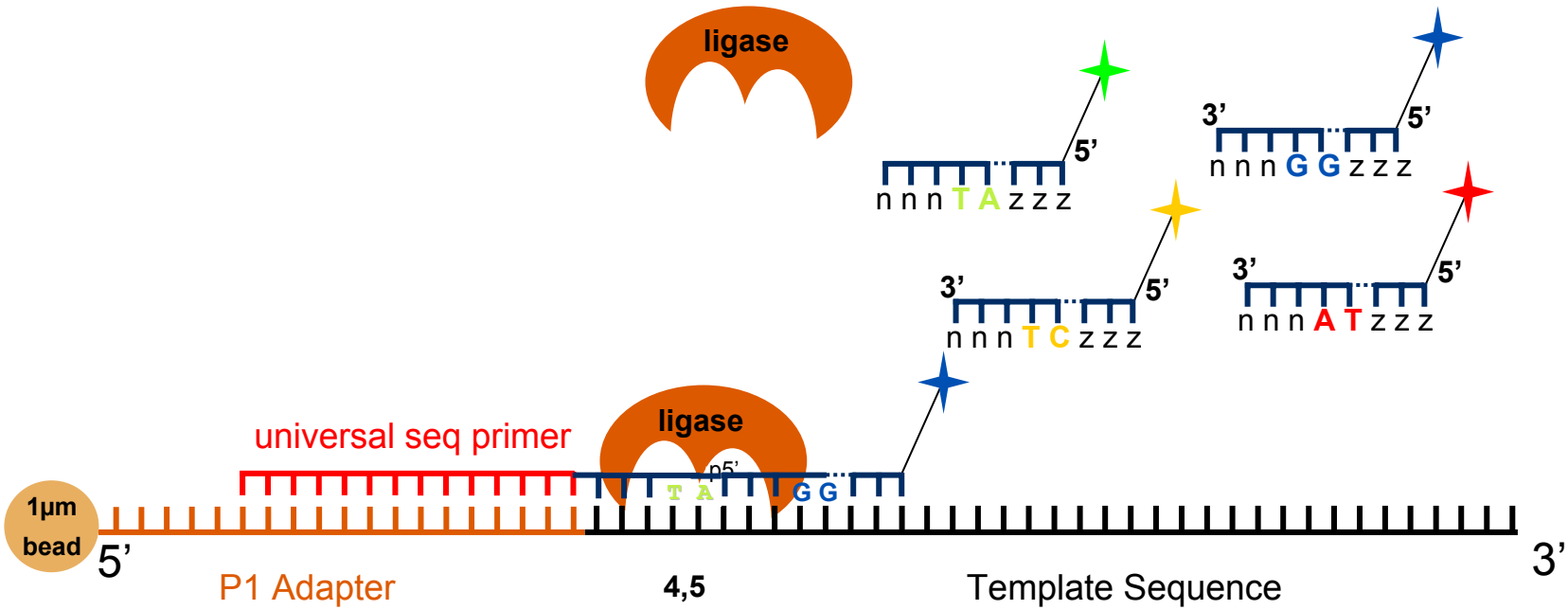
# SOLiD Chemistry System 4-color ligation Cleavage



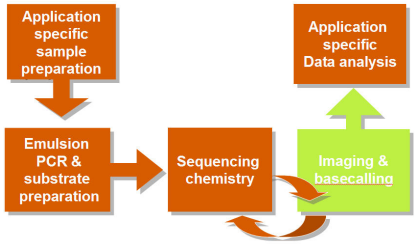



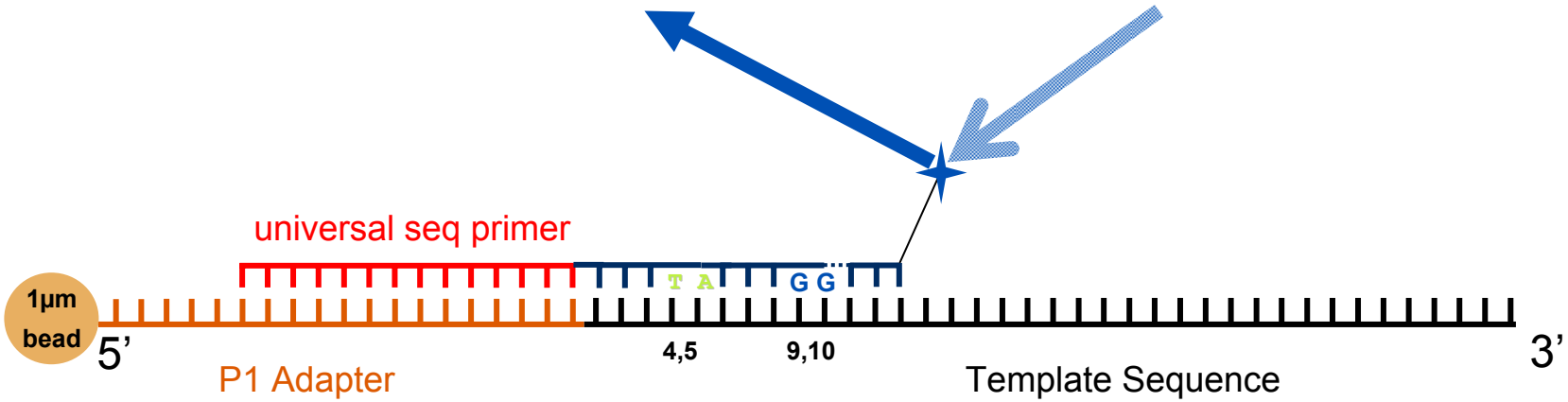
# SOLiD Chemistry System 4-color ligation Ligation (2nd cycle)



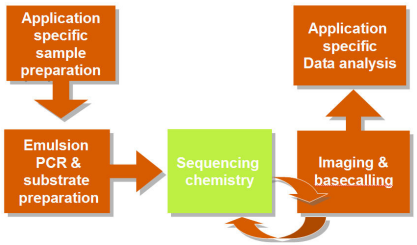



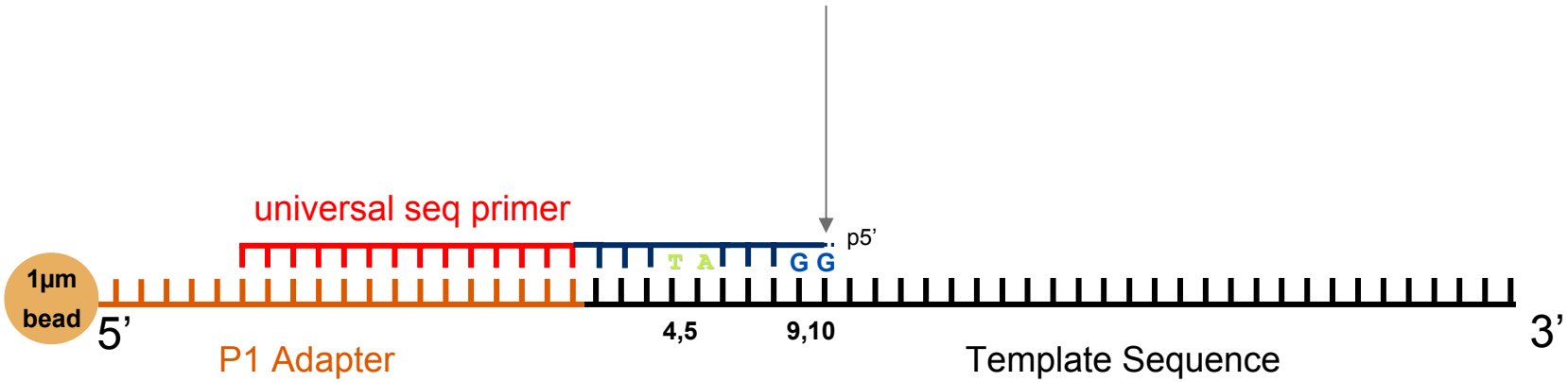
# SOLiD Chemistry System 4-color ligation Visualization (2nd cycle)



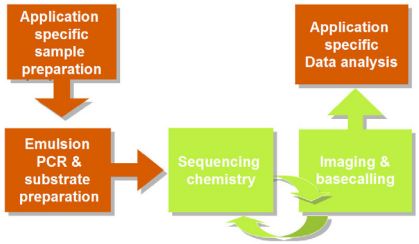



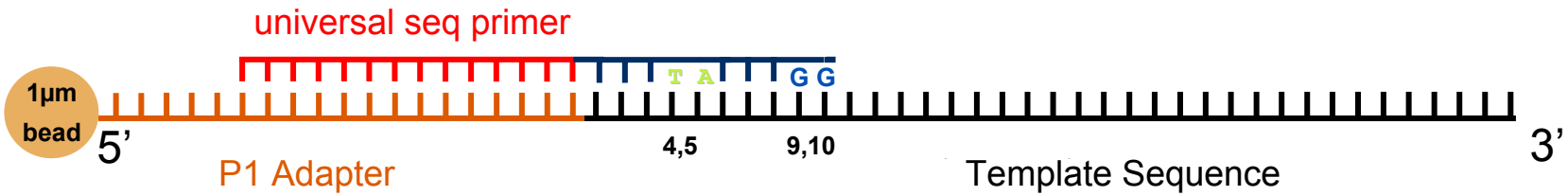
# SOLiD Chemistry System 4-color ligation Cleavage (2nd cycle)



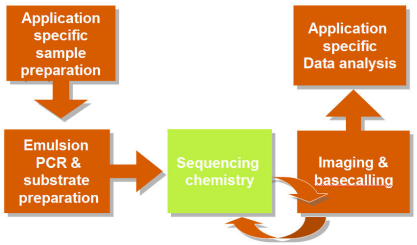



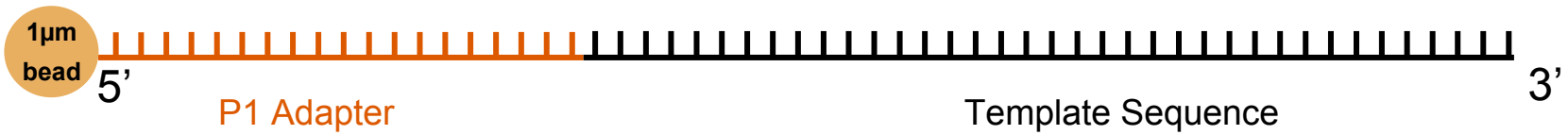
# SOLiD Chemistry System 4-color ligation interrogates every 5th base



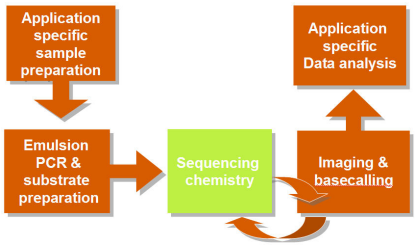



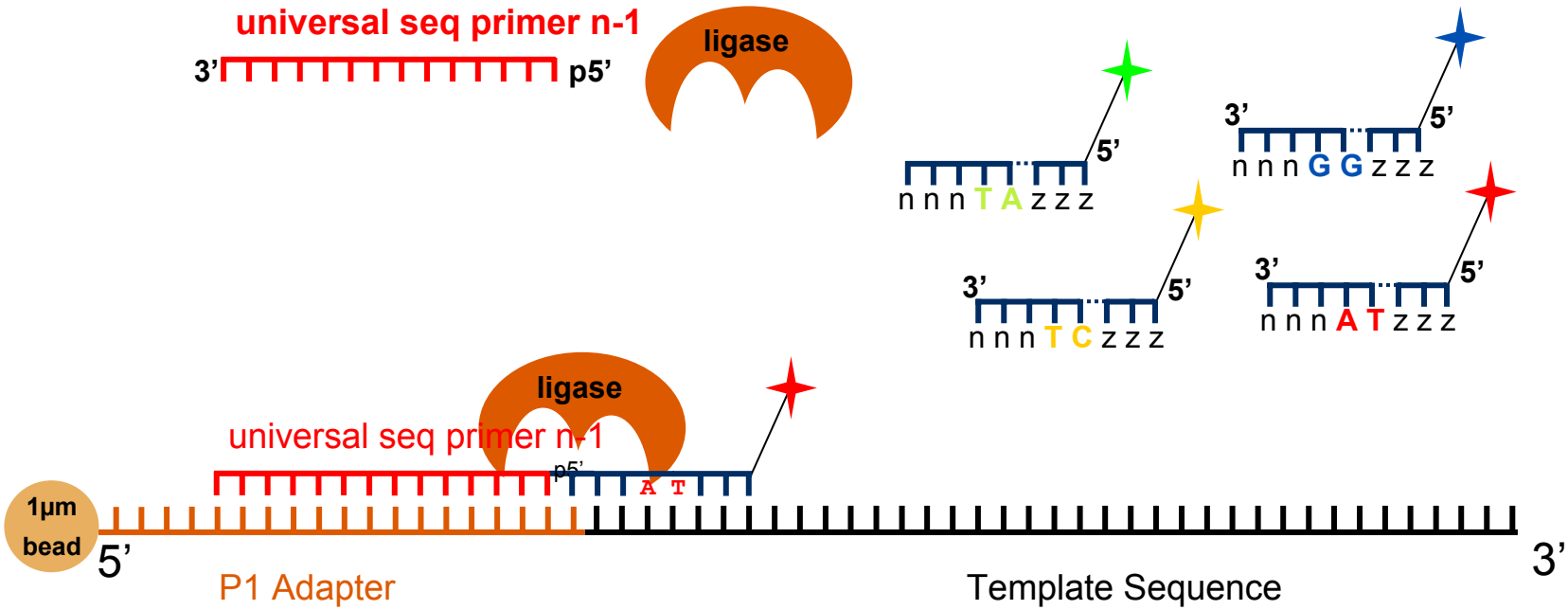
# SOLiD Chemistry System 4-color ligation Reset



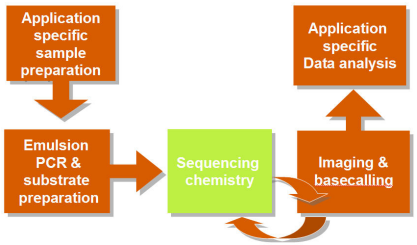



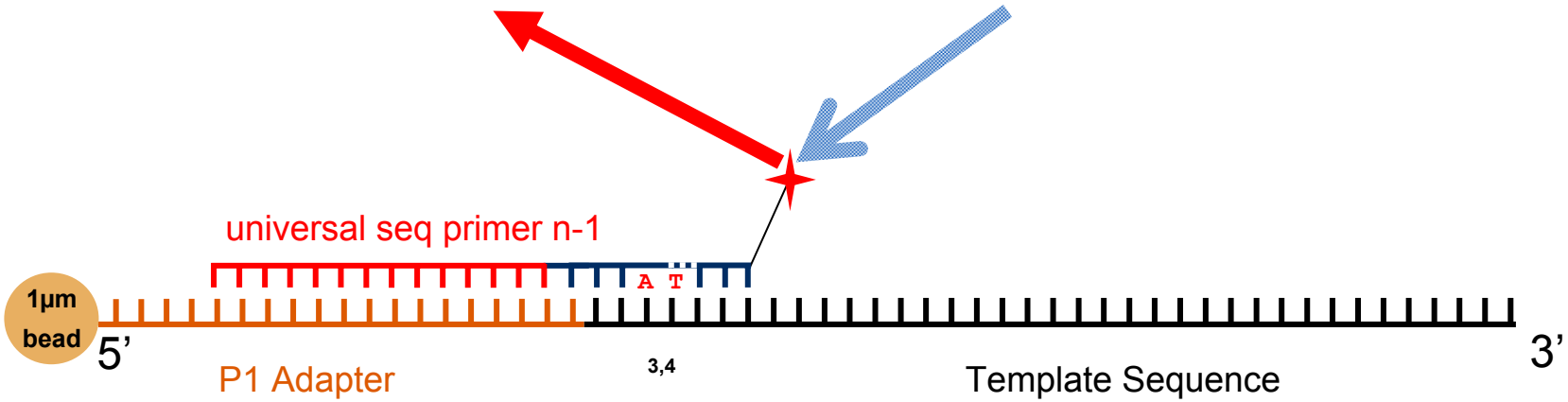
# SOLiD Chemistry System 4-color ligation (1st cycle after reset)



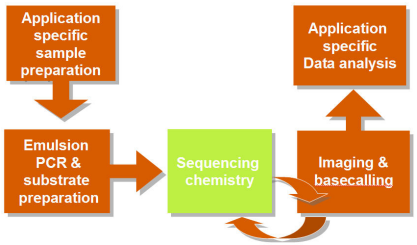



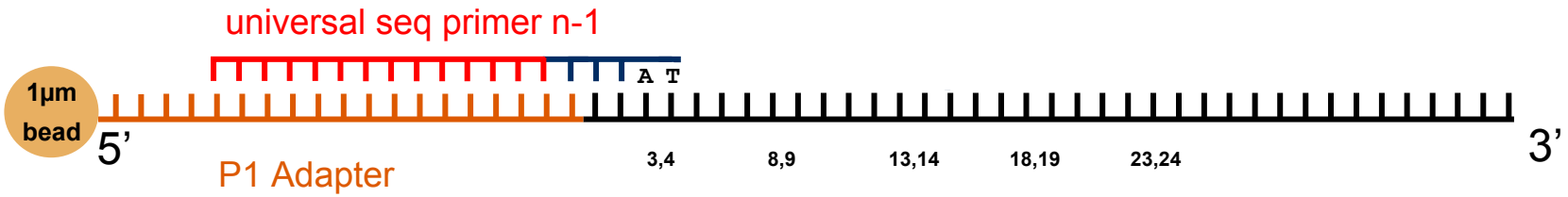
# SOLiD Chemistry System 4-color ligation (1st cycle after reset)



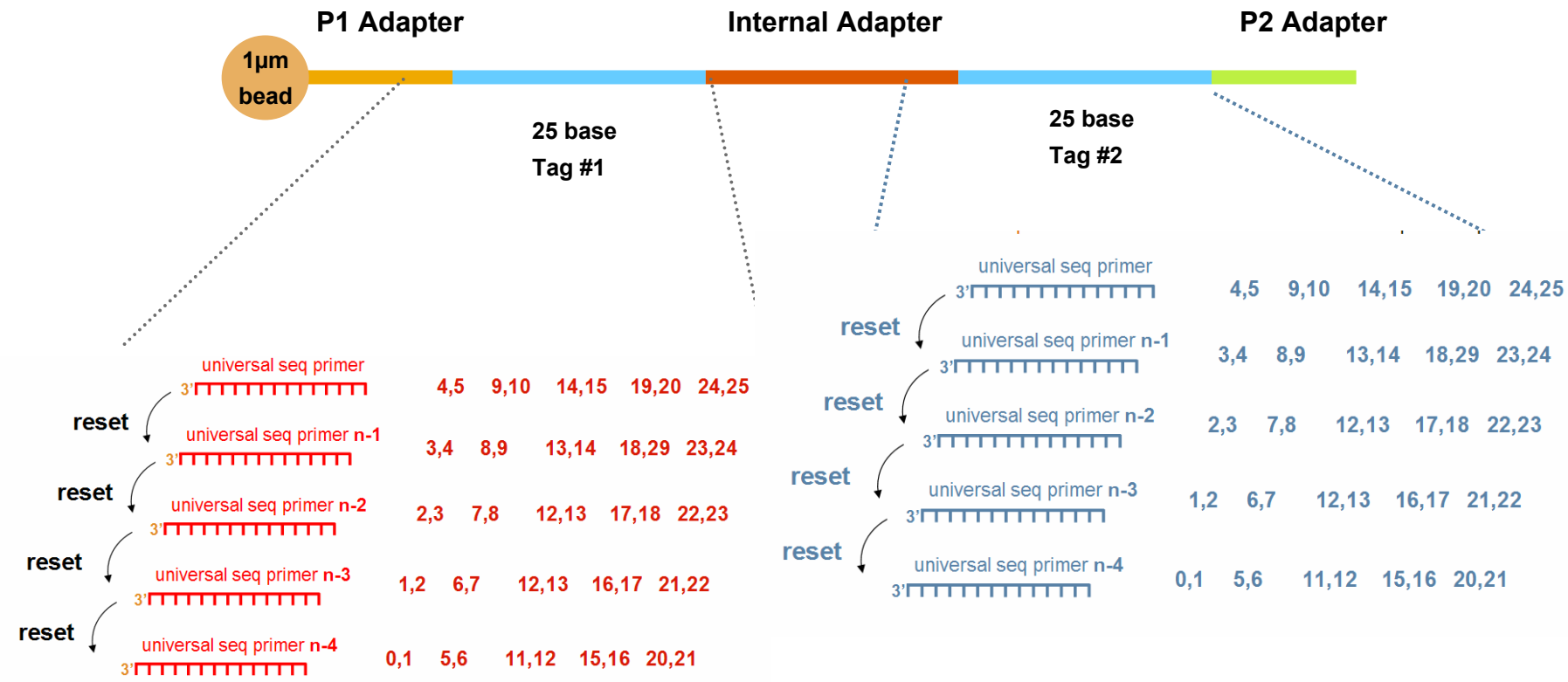
# SOLiD Chemistry System 4-color ligation (2nd Round)



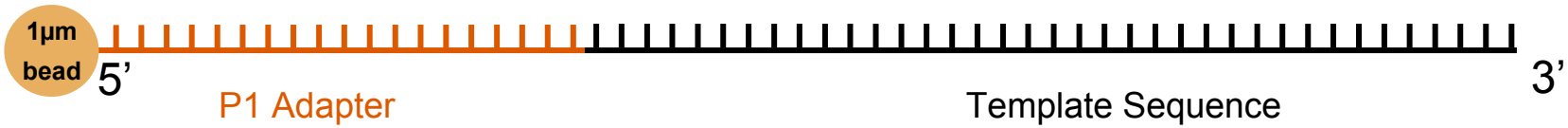



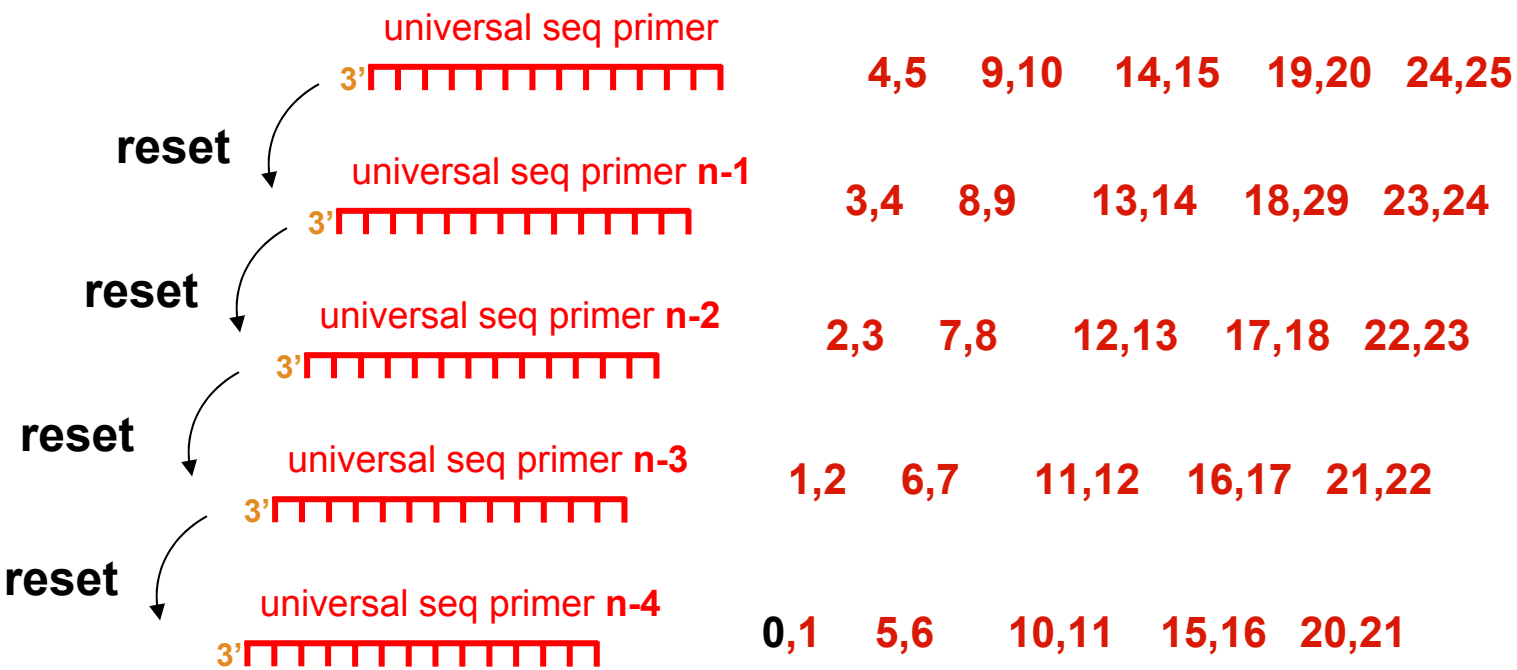
# Paired End two sequences generated

## Sequential rounds of sequencing
## Multiple cycles per round



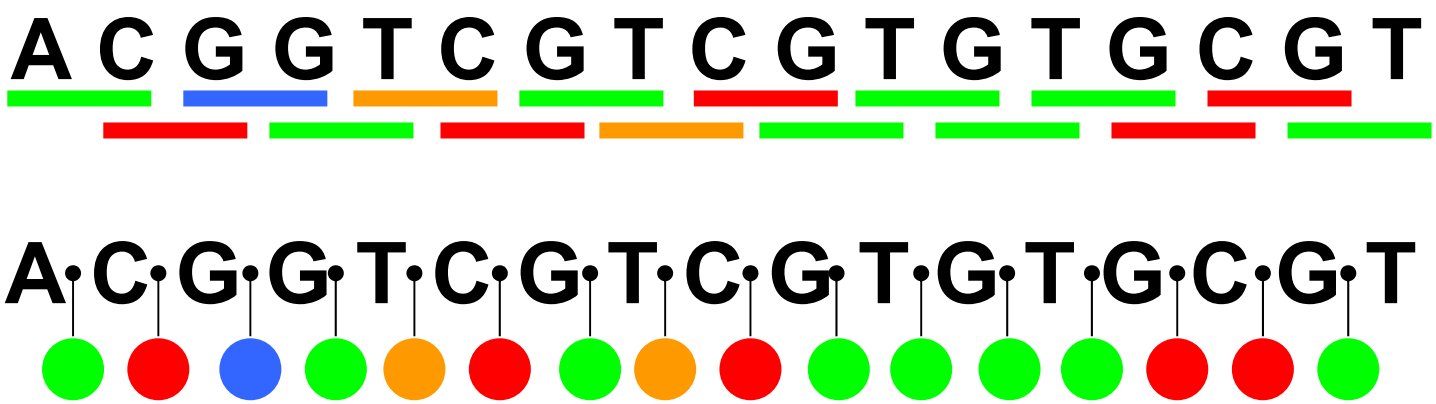
# Sequential rounds of sequencing
# Multiple cycles per round

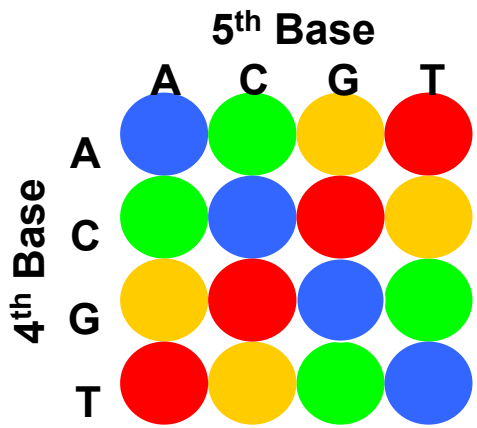


# 2 base encoding

- Double base interrogation eases the discrimination between system ***errors*** and ***true*** polymorphism



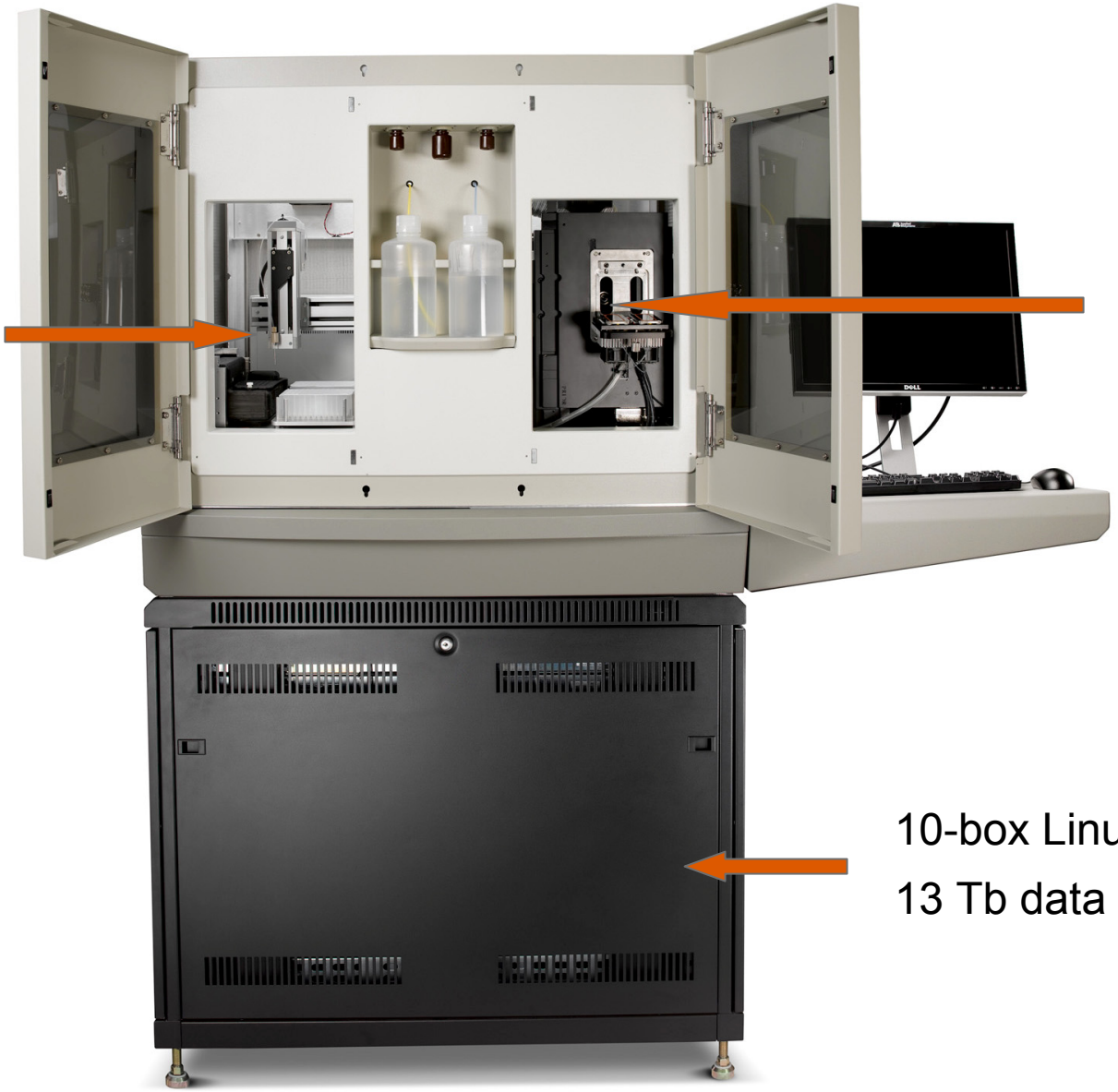



# Benefits of SOLiD Chemistry System

- **Terminating Chemistry**
  - **No issues with homopolymers**
- **Capping step eliminates dephasing**
  - **Control increase in noise with subsequent cycles**
- **Primer reset**
  - **removes accumulated out-of-phase ligations**
  - **longer reads (Signal recovers)**
- **Ligation**
  - **high fidelity**
  - **high read quality**
- **Double interrogation of each base**
  - **unique to ligation method**
  - **increases base calling accuracy**
  - **SNP results in a two-color change**

# SOLiD System Instrumentation
# High speed automated bead imaging

Applied Biosystems



Reagent handling

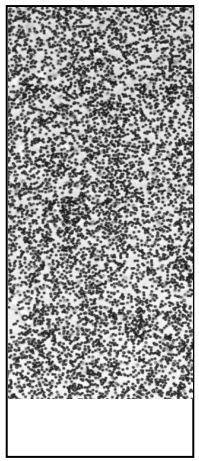
Dual Flow Cell

10-box Linux Cluster

13 Tb data storage

# SOLiD System Arrays: 4 Mega pixel Camera

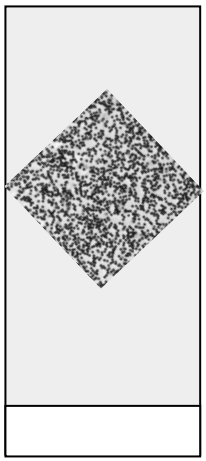
**Full Array**



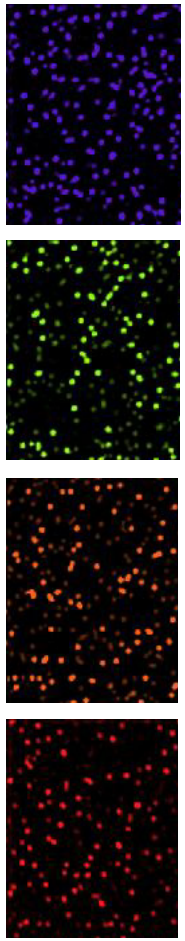
**1800 panels**

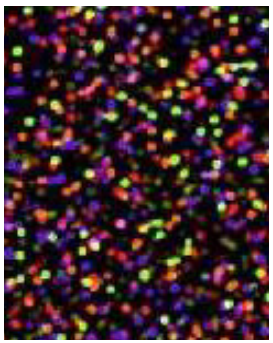
**1/6th**

**Small Array**

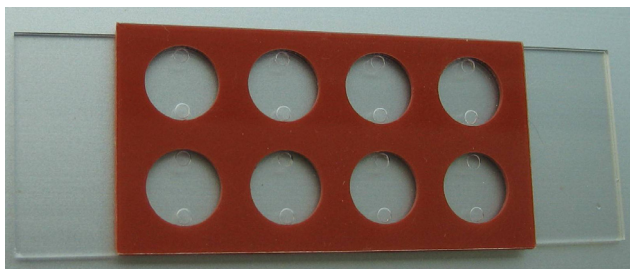


**300 panels**
**~20,000 beads/panel**

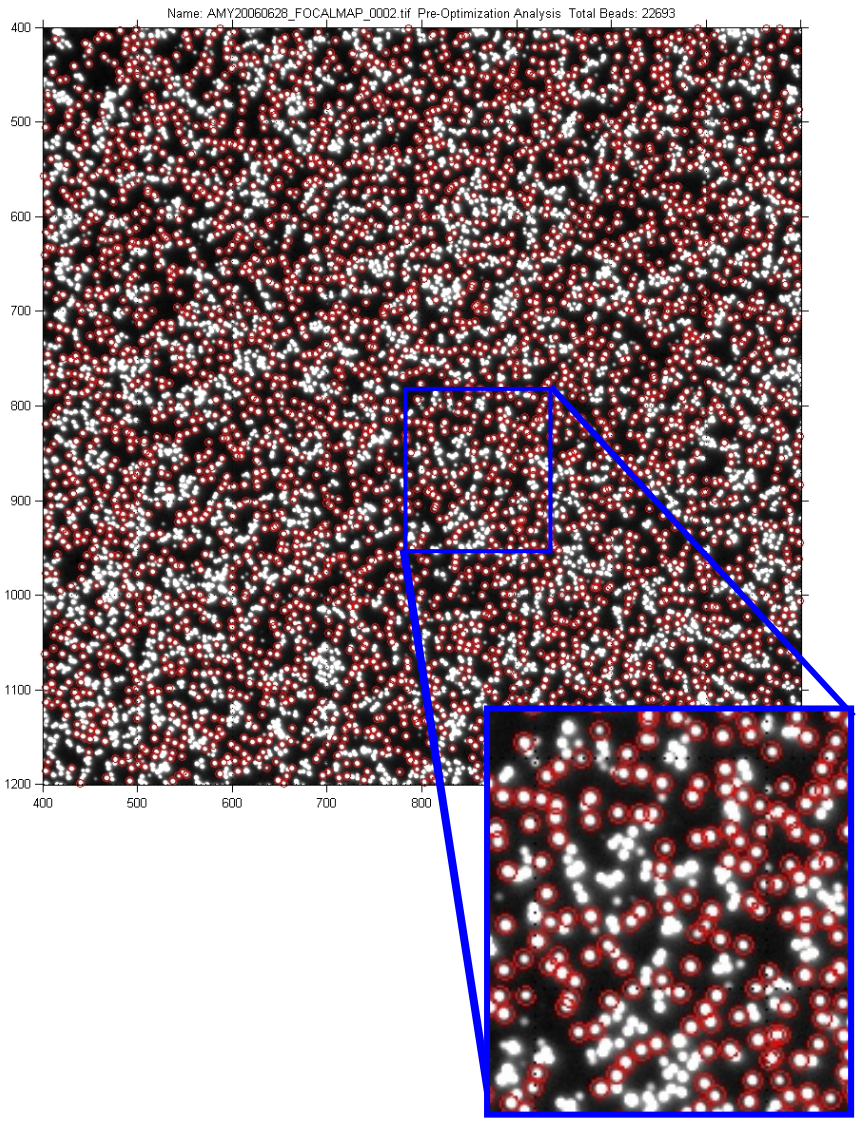


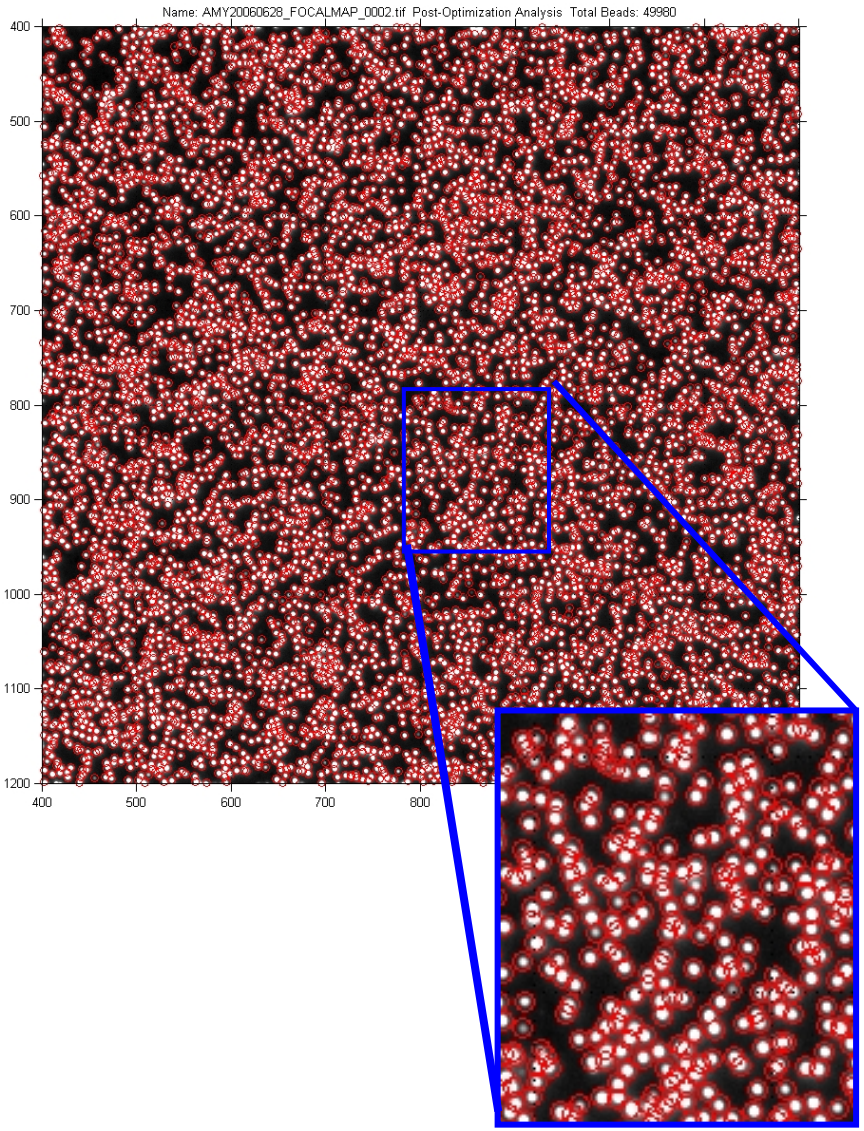


**4-Color Overlay**



# Improved feature finding

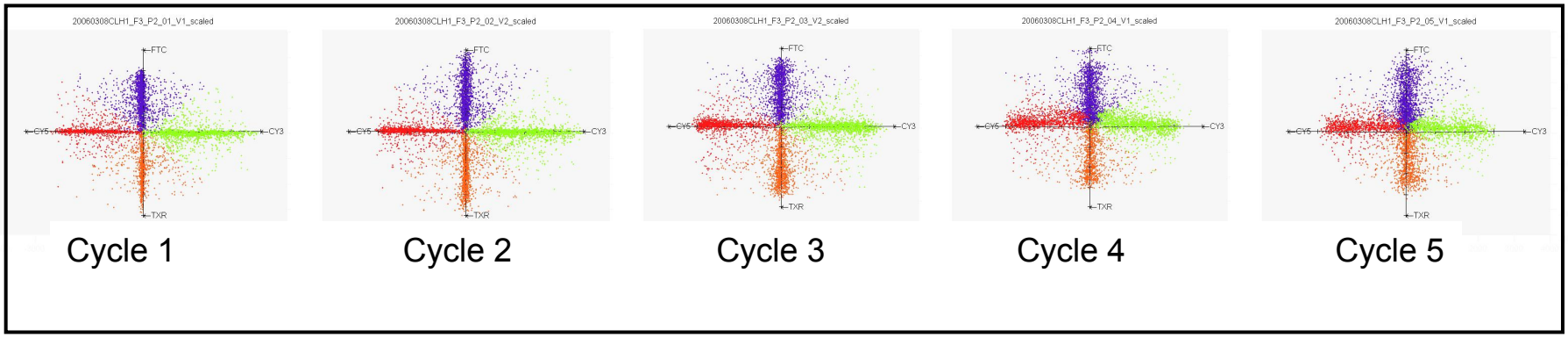


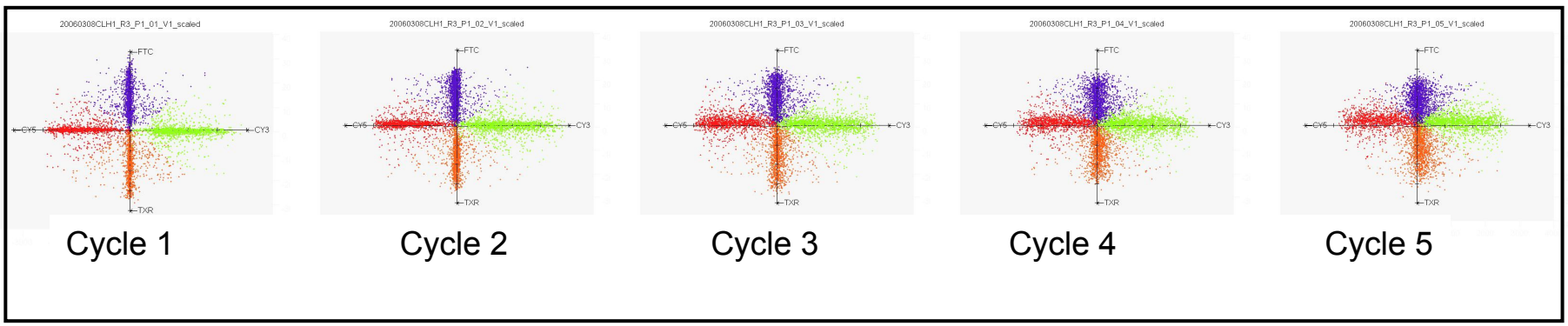

**Beads called per panel:** **22,693** **49,980**

# Reset prevents excessive noise accumulation

## Round 1




## Round 2 after Reset



# SOLiD System: Development Status

- Dedicated teams at Beverly and Foster City with Instruments up and running
- Anticipated Performance
  - Read length: 25x2 mate pair
  - Raw data output rate: anticipate 100-500 Mb per day
  - Raw accuracy: ≥98% - after 2 base encoding 99.8%
  - Consensus accuracy: >99.99% (@ 20x coverage)
  - Quality metrics: Compatible with *KB*™ Basecaller Software
  - Cost per run: *Competitive*
  - Libraries: Mate pair or Fragment
    - as required by application
  - Application Support
    - Whole genome resequencing
    - Directed (Medical) resequencing
    - Gene expression
    - Tag Counting

- For Research Use Only. Not for use in diagnostic procedures.

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