

Microarray technologies

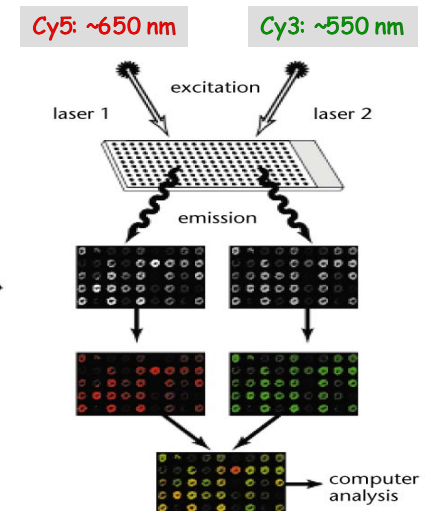
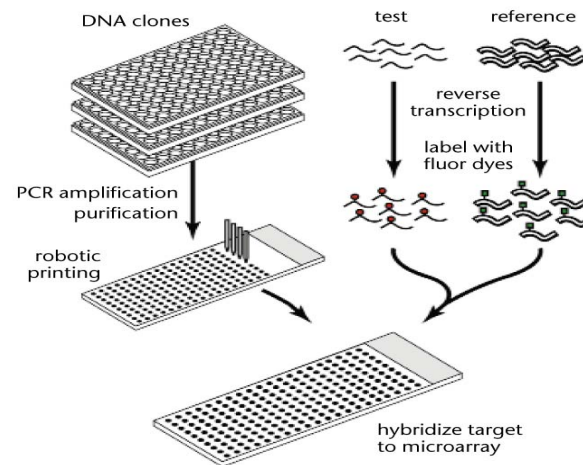
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Rita Holdhus
J-Express Analysis Course
April 2010



Two channels

- Two samples pr array
 - Test sample (Cy5) vs. control sample (Cy3)
- Competitive hybridization
- Relative signals (ratio)
- Uneven degradation of Cy-dyes
- Allows more variability in spotting the microarray
- Need dye-swaps (doubles the cost)

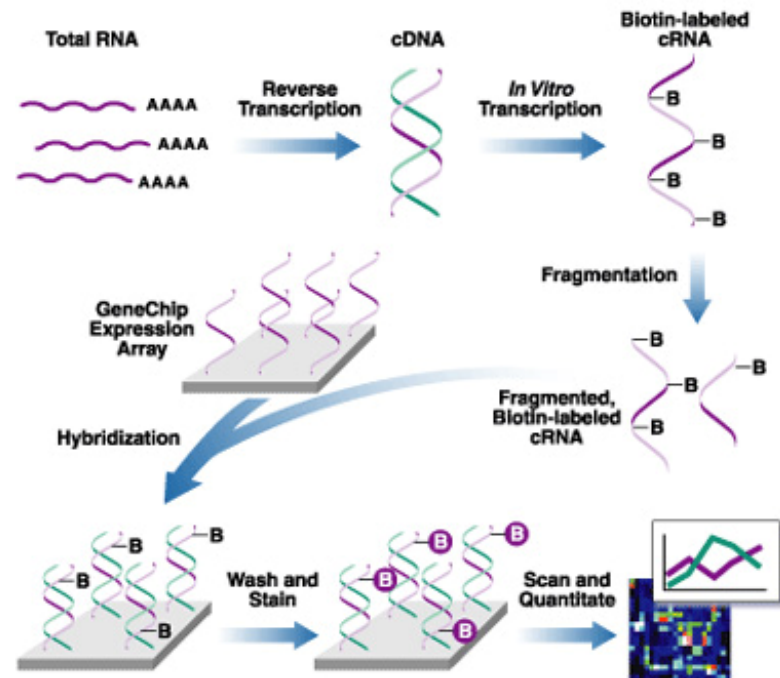


- No differential expression
- Induced
- Repressed



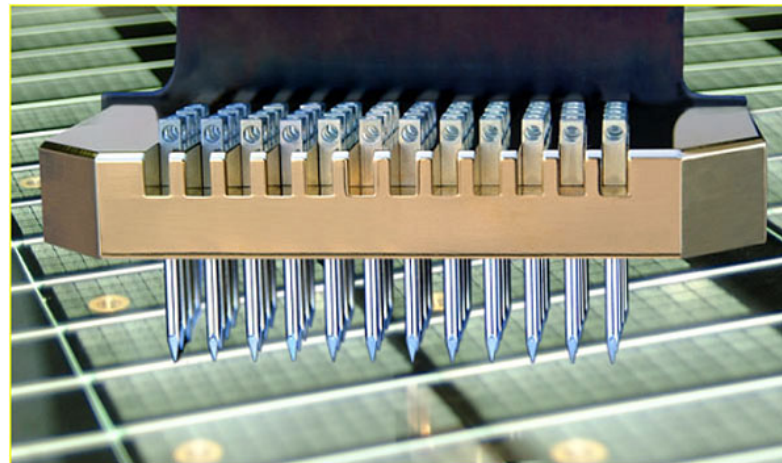
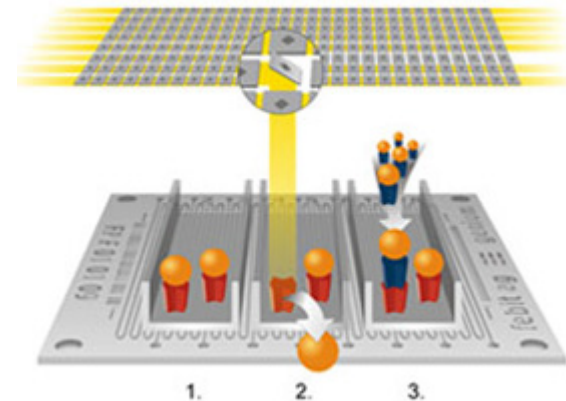
One channel

- One sample per array
 - Labeled with Cy3 or biotin
- “Absolute” intensity signals
- Degradation problem smaller issue than for two channel platforms
- Allows very small variance in printing
- Flexible research design

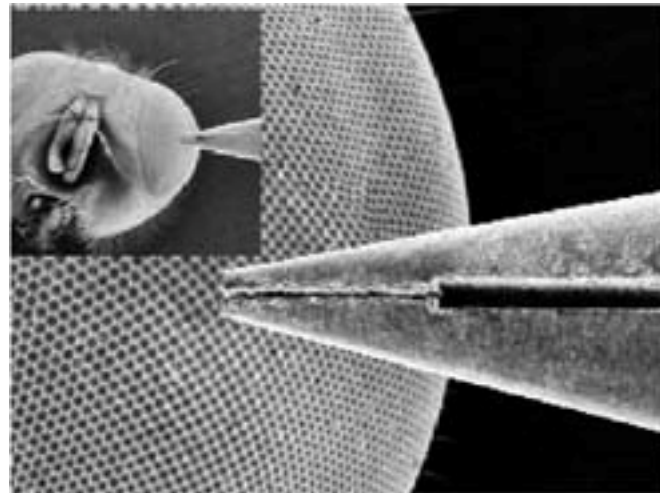
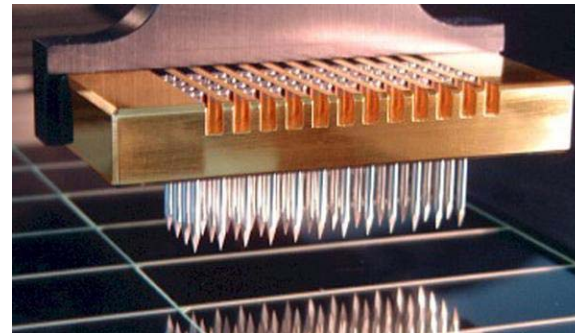
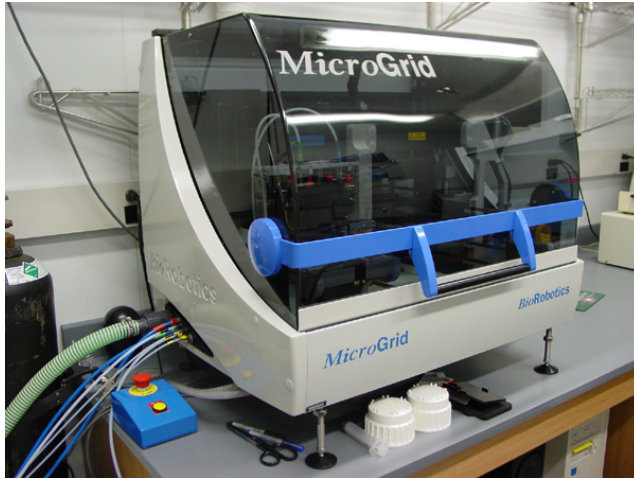


Microarray formats

- Oligo arrays
 - Oligonucleotides (>200nt)
 - Spotted
 - Synthesized
- cDNA
 - cDNA probes (50-80nt)
 - Usually produced by PCR
 - "rare" species



Contact printing



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Commercial platforms

Platform	Technology	Organism
Affymetrix	<ul style="list-style-type: none">• Synthesized probes• One channel (biotin)	<ul style="list-style-type: none">• Several
Agilent	<ul style="list-style-type: none">• Spotted on glas slides• One/Two channel (fluorescence)	<ul style="list-style-type: none">• Several
Illumina	<ul style="list-style-type: none">• Beads• One channel (fluorescence)	<ul style="list-style-type: none">• Human• Mouse• Rat
Nimblegen	<ul style="list-style-type: none">• Synthesized probes• One channel (fluorescence)	<ul style="list-style-type: none">• Several



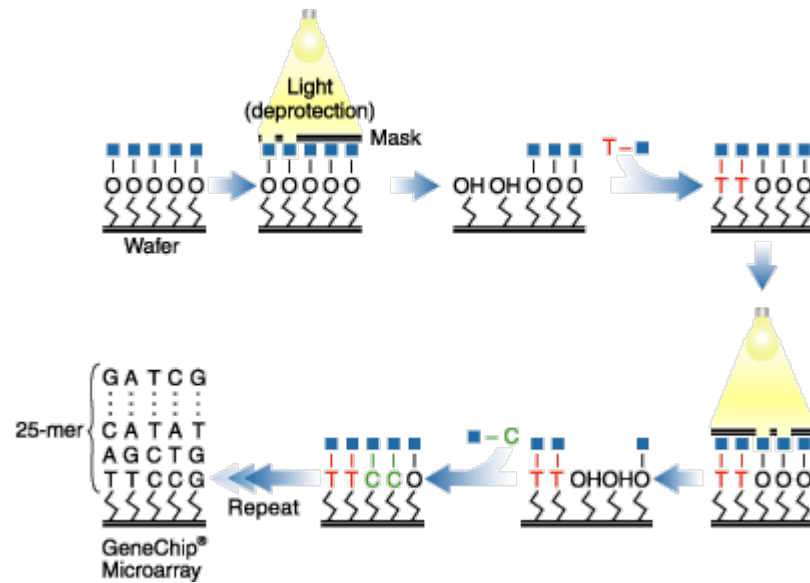
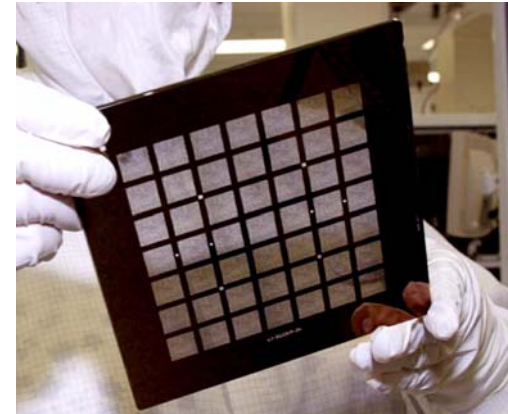
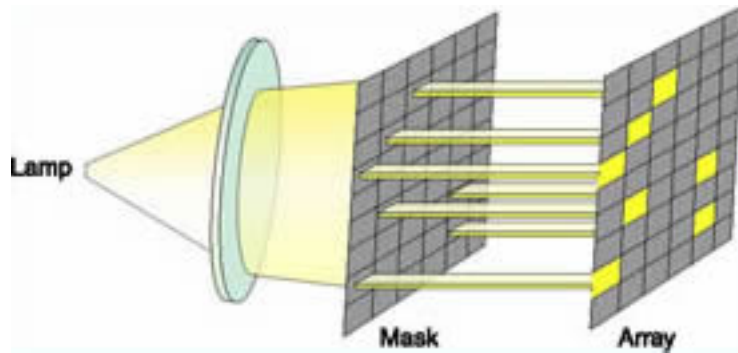
Affymetrix

- Short oligos, 25mer
- Synthesized by photolithography
- One channel (biotin)
- Several organisms

- Gene Expression
- Array-CGH
- SNP analysis
- Copy number analysis
- Exon/tiling arrays



Photolithography



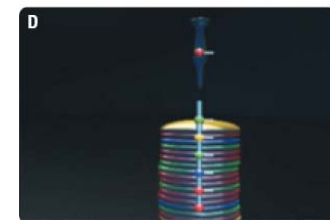
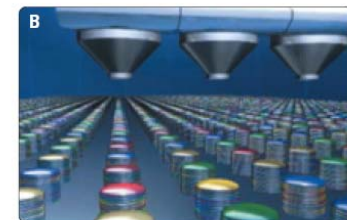
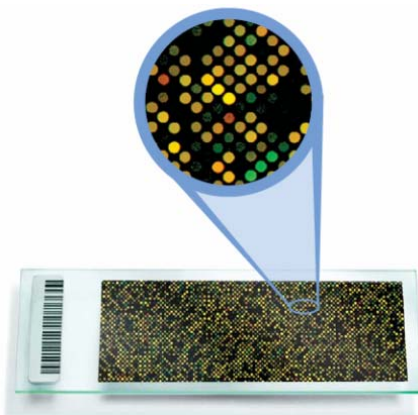
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Agilent technologies

- Inkjet in situ synthesis
- Many different organisms and array formats
- Custom printed arrays (at no extra cost)
- 60 mer oligos
- One or two channel
- Variable number of replicates per probe

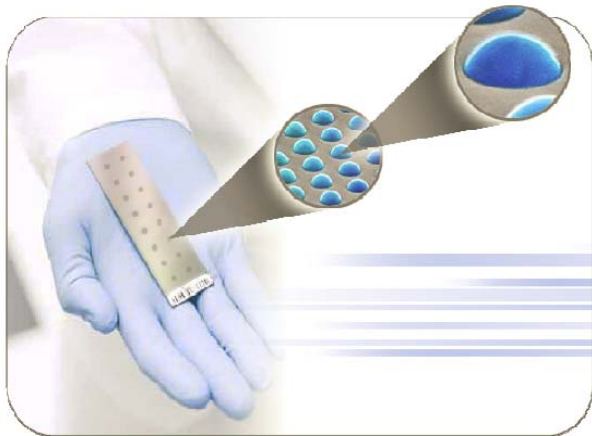
- Gene Expression
- Array-CGH
- Copy number analysis
- Exon/tiling arrays
- ChIP-on-chip



Illumina – Bead technology



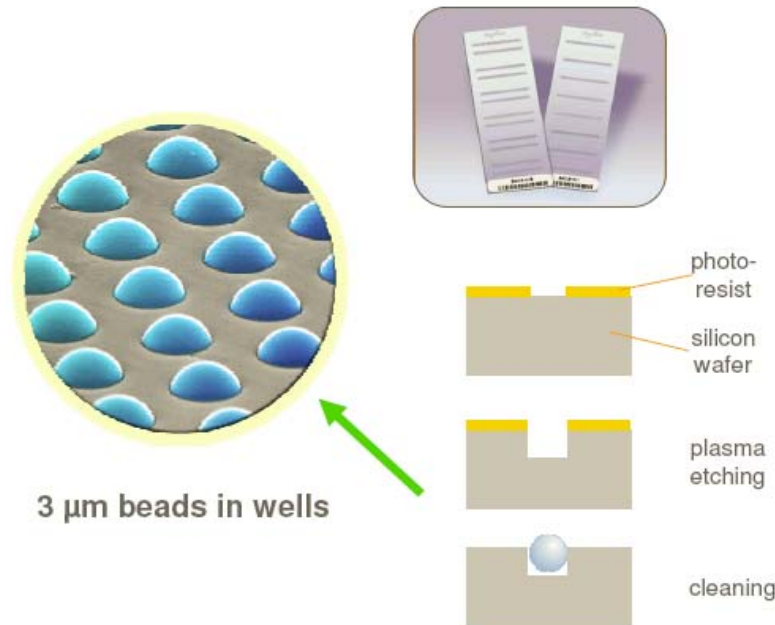
- Gene expression
- Whole Genome Genotyping (infinium)
- DNA copy number
- Custom genotyping (GoldenGate)



- 50mer probes
- Bead technology
- ~30 copies of each bead type per array

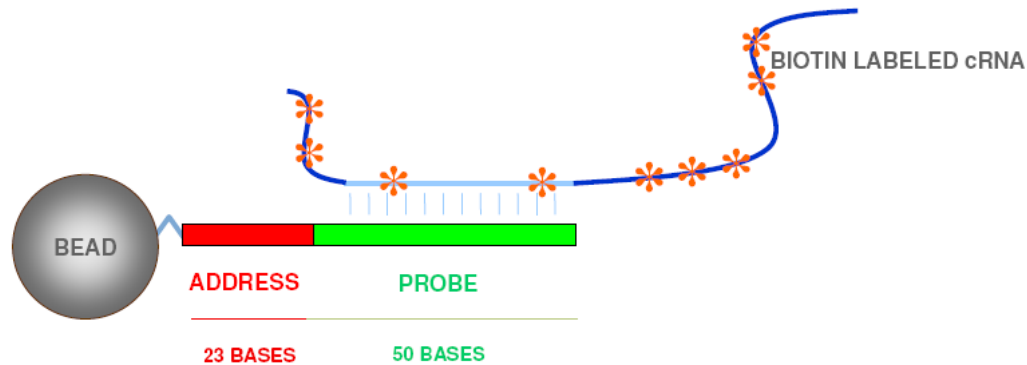


Oligo coated beads in wells



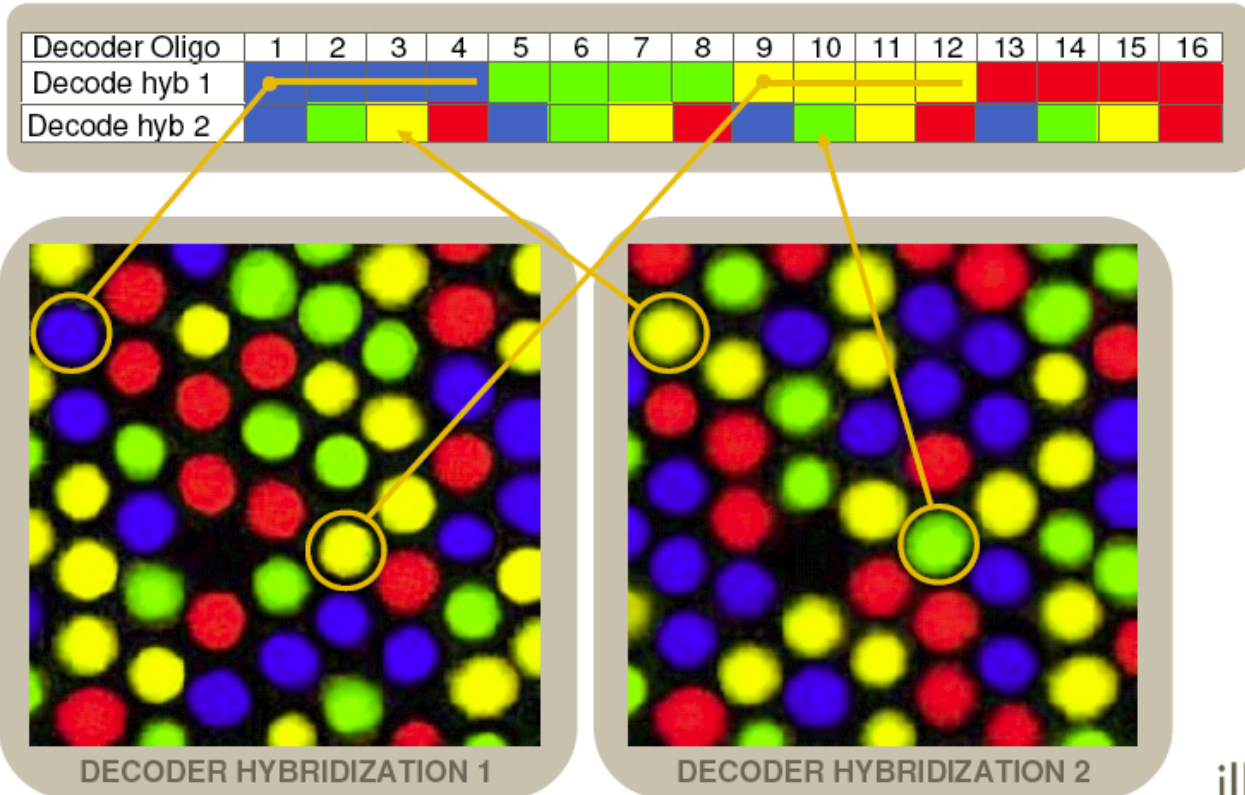
Illumina – Gene expression

- 50mer probes
- Bead technology
- ~30 copies of each bead type per array
- Input requirements: 50 – 500 ng total RNA



Bead decoding

EXAMPLE: 16 BEAD TYPES



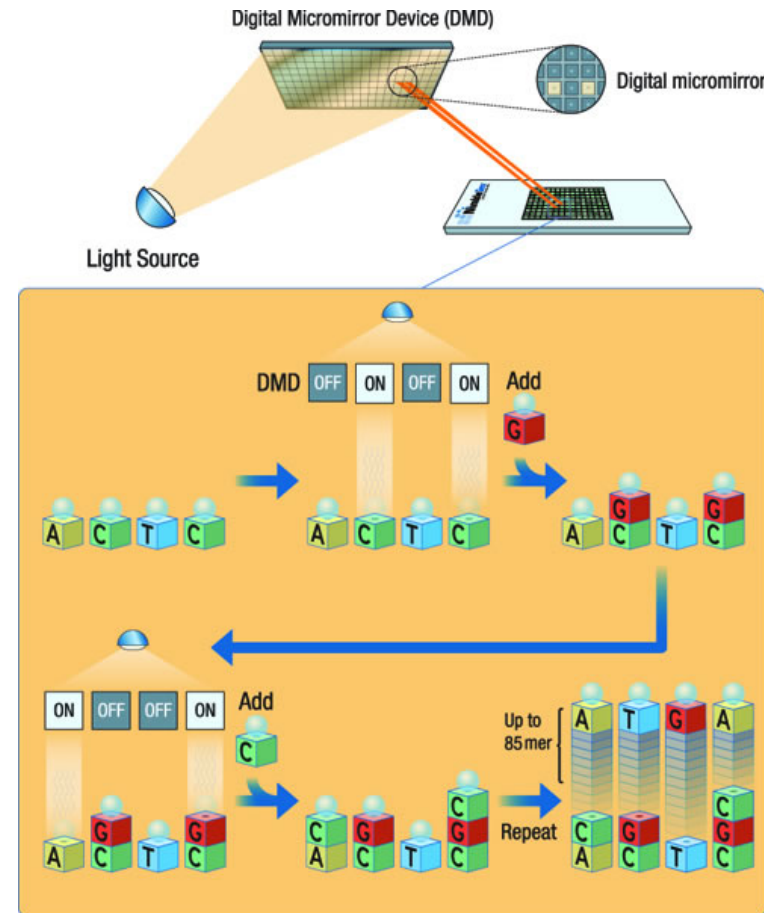
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Nimblegen

- 60 mer probes
- Synthesized on glass slides by photolithography (using mirrors)
- Sample requirement: 1µg cDNA



● = photolabile protecting group



Which platform to use?

Checklist:

- Organism
- Application
- Budget
- Amount of extracted RNA
- Number of samples (for some arrays there is a minimum order of 2 or 5 arrays)



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Deep sequencing

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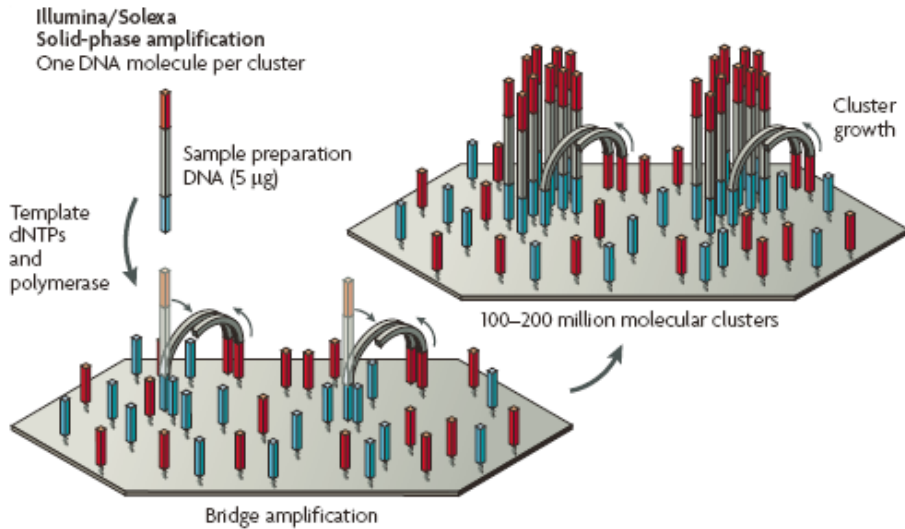


Sanger vs. Deep sequencing

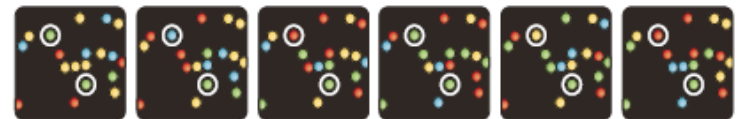
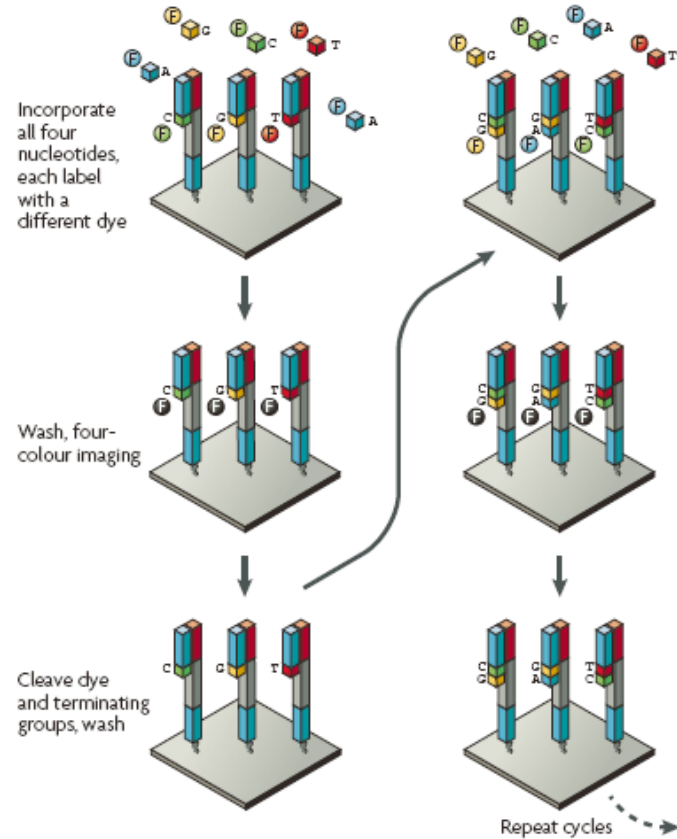
Sanger	Deep Sequencing
<ul style="list-style-type: none">• Read length: ~750 bp	<ul style="list-style-type: none">• Read length: 25 – 500 bp
<ul style="list-style-type: none">• Microliter volumes	<ul style="list-style-type: none">• Picoliter volumes
<ul style="list-style-type: none">• Capacity: 96- capillar	<ul style="list-style-type: none">• Higly parallellized
<ul style="list-style-type: none">• Expensive per base	<ul style="list-style-type: none">• Cheap per base
<ul style="list-style-type: none">• Less errors	<ul style="list-style-type: none">• More error prone
<ul style="list-style-type: none">• Some bias in amplification	<ul style="list-style-type: none">• Bias free amplification



Solexa sequencing method



Illumina/Solexa — Reversible terminators



Top: CATCGT
Bottom: CCCCC

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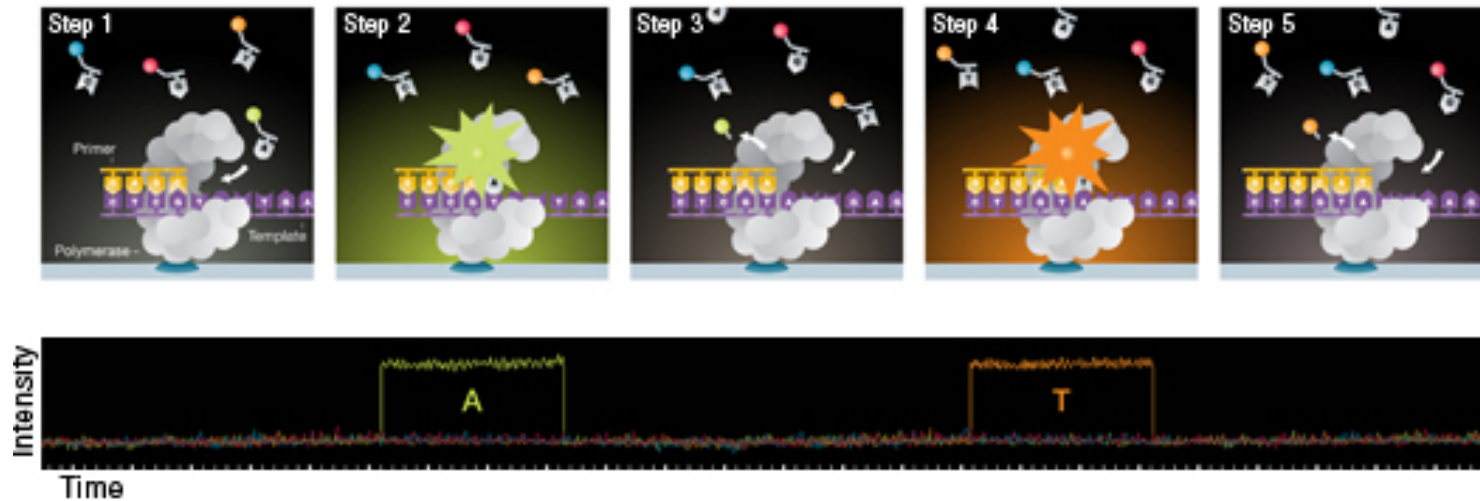
Why deep sequencing

- View the whole transcriptome
- Distinguish strand specific expression patterns
- Detect SNPs at a low coverage
- Assess globally DNA-protein binding interactions
- Discover novel transcripts and splice variations
- Characterize structural rearrangements



3. Generation technology

Pacific Biosciences – SMRT™ Technology



NanoPore Sequencing

