

The Basics of Oligonucleotide Microarray Technology

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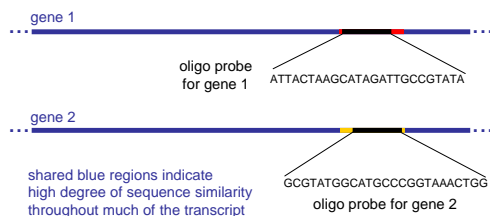
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Oligonucleotides

- An oligonucleotide is a short sequence of nucleotides. (oligonucleotide=oligo for short)
- An oligonucleotide microarray is a microarray whose probes consist of synthetically created DNA oligonucleotides.
- Probes sequences are chosen to have good and relatively uniform hybridization characteristics.
- A probe is chosen to match a portion of its target mRNA transcript that is unique to that sequence.
- Oligo probes can distinguish among multiple mRNA transcripts with similar sequences.

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Simplified Example



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Oligo Microarray Fabrication

- Oligos can be synthesized and stored in solution for spotting as is done with cDNA microarrays.
- Oligo sequences can be synthesized on a slide or chip using various commercial technologies.
- In one approach, sequences are synthesized on a slide using ink-jet technology similar to that used in color printers. Separate cartridges for the four bases (A, C, G, T) are used to build nucleotides on a slide.
- The company Affymetrix uses a photolithographic approach which we will describe briefly.

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Affymetrix GeneChips

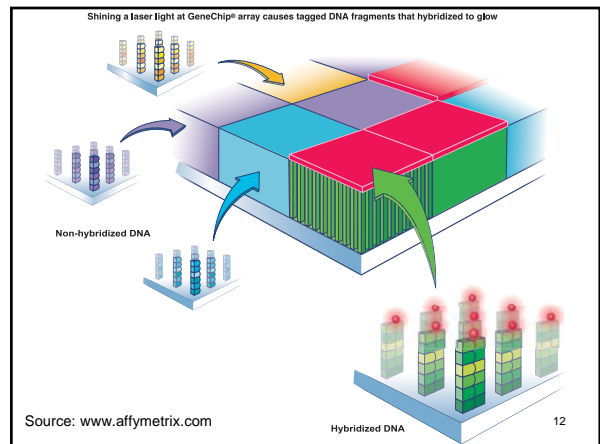
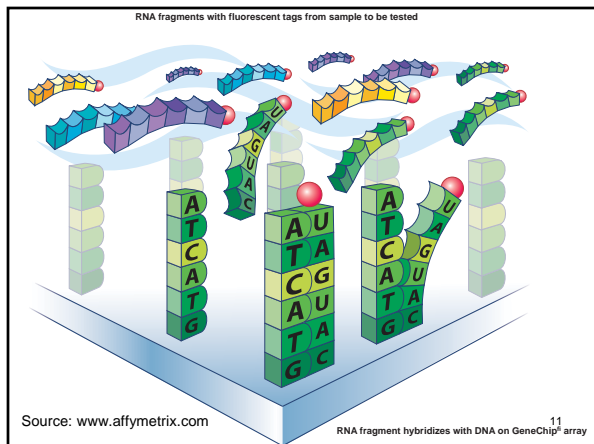
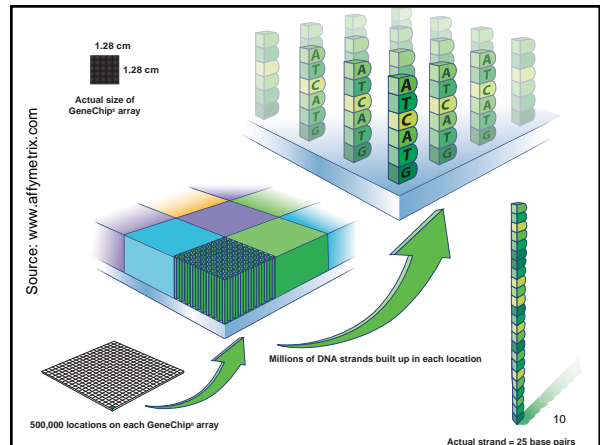
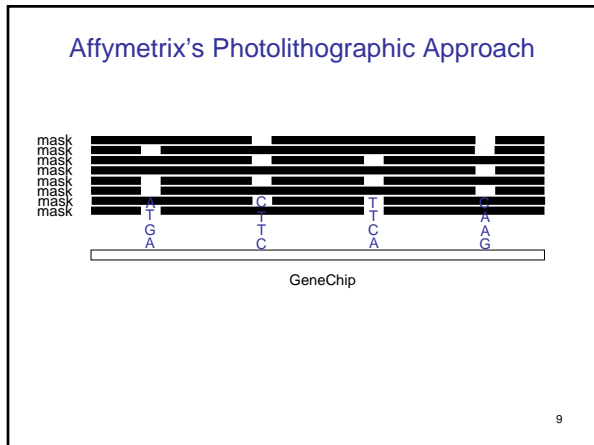
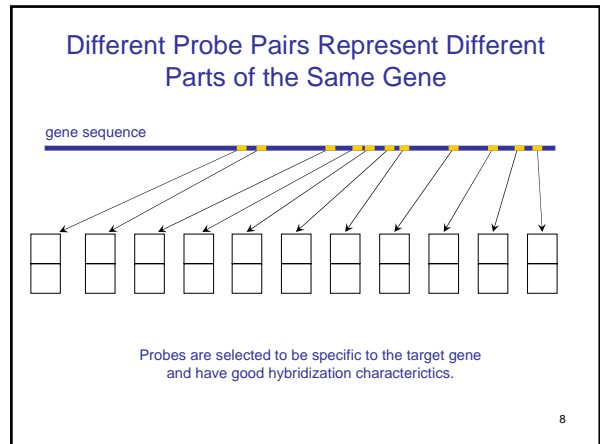
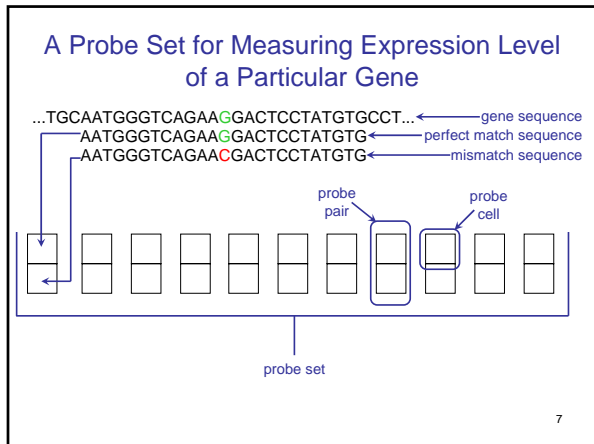
- Affymetrix (www.affymetrix.com) is a company that manufactures GeneChips.
- GeneChips are oligonucleotide arrays.
- Each gene (more accurately sequence of interest or *feature*) is represented by multiple short (25-nucleotide) oligo probes.
- Some GeneChips include probes for around 60,000 genes.
- mRNA that has been extracted from a biological sample can be labeled (dyed) and hybridized to a GeneChip in a manner similar to that described for cDNA microarrays.
- Only one sample is hybridized to each GeneChip rather than two as in the case of cDNA microarrays.

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Affymetrix Probe Sets

- A *probe set* is used to measure mRNA levels of a single gene.
- Each probe set consists of multiple *probe cells*.
- Each probe cell contains millions of copies of one oligo.
- Each oligo is intended to be 25 nucleotides in length.
- Probe cells in a probe set are arranged in *probe pairs*.
- Each probe pair contains a *perfect match* (PM) probe cell and a *mismatch* (MM) probe cell.
- A PM oligo perfectly matches part of a gene sequence.
- A MM oligo is identical to a PM oligo except that the middle nucleotide (13th of 25) is intentionally replaced by its complementary nucleotide.

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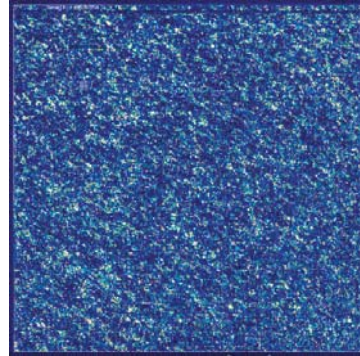
Obtaining Labeled Target

1. RNA → single strand cDNA
2. single strand cDNA → double strand cDNA
3. double strand cDNA → labeled single strand cRNA complementary to coding sequence

Number of copies of each sequence gets amplified in conversion to cRNA.

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Image from Hybridized GeneChip



Source: www.affymetrix.com

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