

Probing DNA

DNA Hybridization

- Single-stranded DNA will naturally bind to complementary strands.
- Hybridization is used to locate genes, measure the level of gene expression, and determine the degree of similarity between DNA from different sources.
- Hybridization is also referred to as annealing or re-naturation.

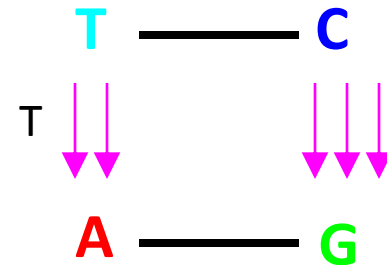
Molecular Biology Laboratory Techniques

:Hybridization

- Given a short (8- to 30-nucleotides or oligonucleotides) synthetic fragment DNA, called *probe*, a target single stranded DNA molecule (produced by denaturing) will *hybridize* or bind to the probe if there is a substring in the target sequence that is complementary to the probe.
- For example, a target DNA sequence *CCCTGGCACCTA* will hybridize to a probe *ACCGTGGA* since the complementary sequence *TGGCACCT* is present in the target.
- In the mix of a DNA, the presence of a particular DNA can be tested by making the probe fluorescent or radioactive. This idea has led to the development of DNA chips or *microarrays* that allows rapid DNA sequencing.

Create a Hybridization Reaction

1. Hybridization is binding two genetic sequences. The binding occurs because of the hydrogen bonds [pink] between base pairs.

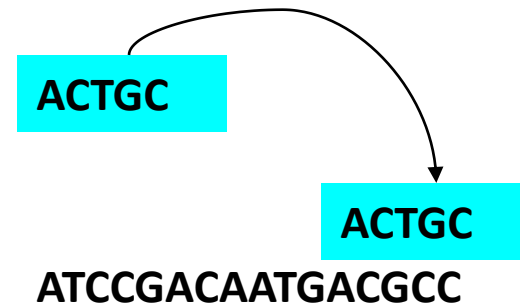


2. When using hybridization, DNA must first be denatured, usually by using heat or chemical. To study the level of gene expressions for a large number of genes in one experiment, mRNA's are used . So, this step is skipped.



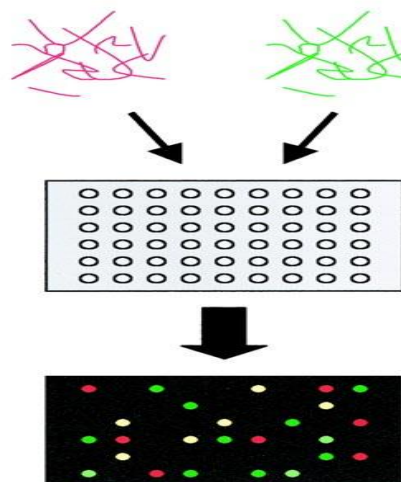
Create a Hybridization Reaction Cont.

- Once DNA has been denatured, a single-stranded radioactive probe [light blue] can be used to see if the denatured DNA contains a sequence complementary to probe.
- Sequences of varying similarity stick to the DNA even if the fit is poor.

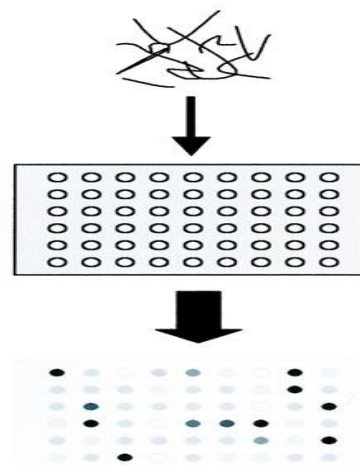


Labeling technique for DNA arrays

Two-color fluorescence



Radiolabeling



RNA samples are labeled using fluorescent nucleotides (**left**) or radioactive nucleotides (**right**), and hybridized to arrays. For fluorescent labeling, two or more samples labeled with differently colored fluorescent markers are hybridized to an array. Level of RNA for each gene in the sample is measured as intensity of fluorescence or radioactivity binding to the specific spot. With fluorescence labeling, relative levels of expressed genes in two samples can be directly compared with a single array.

DNA Arrays--Technical Foundations

- An array works by exploiting the ability of a given mRNA molecule to hybridize to the DNA template.
- Using an array containing many DNA samples in an experiment, the expression levels of hundreds or thousands genes within a cell by measuring the amount of mRNA bound to each site on the array.
- With the aid of a computer, the amount of mRNA bound to the spots on the microarray is precisely measured, generating a profile of gene expression in the cell.

An experiment on a microarray



In this schematic:

GREEN represents **Control DNA**

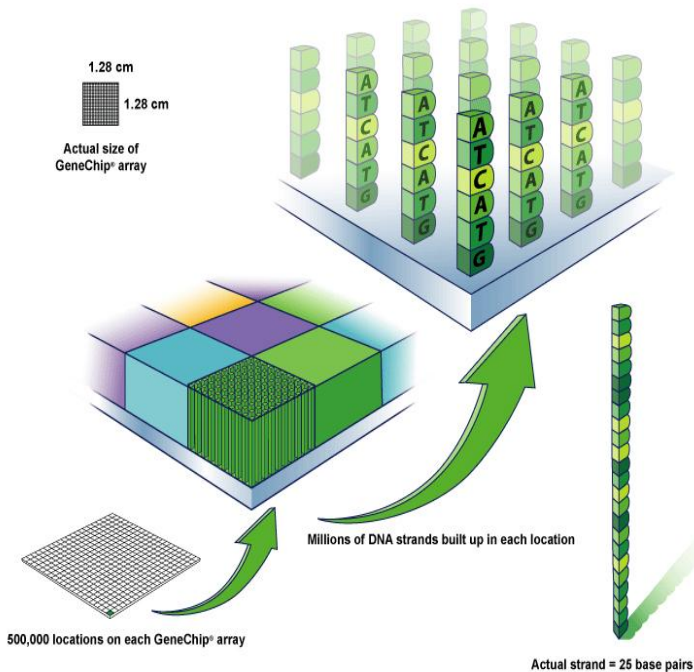
RED represents **Sample DNA**

YELLOW represents **a combination of Control and Sample DNA**

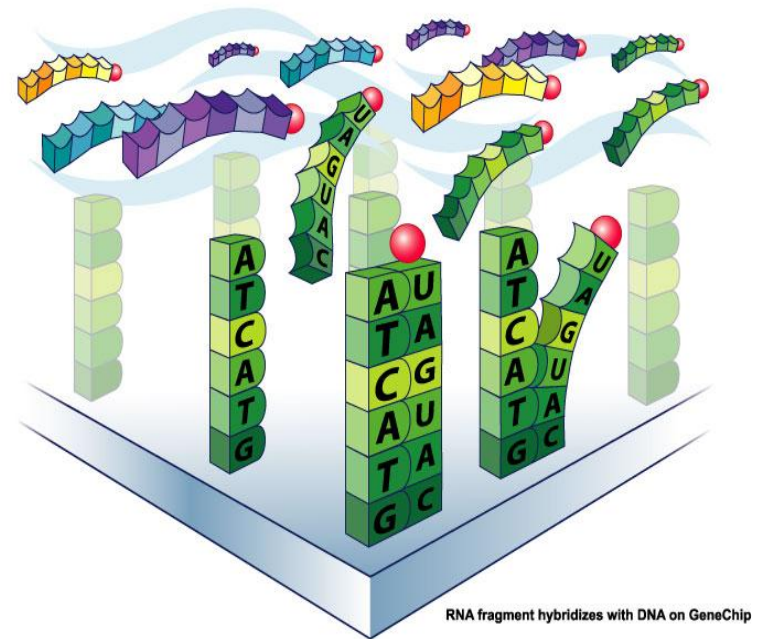
BLACK represents areas where **neither the Control nor Sample DNA**

Each color in an array represents either healthy (control) or diseased (sample) tissue. The location and intensity of a color tell us whether the gene, or mutation, is present in the control and/or sample DNA.

DNA Microarray



RNA fragments with fluorescent tags from sample to be tested



Millions of DNA strands build up on each location.

Tagged probes become hybridized to the DNA chip's microarray.

DNA Microarray



Affymetrix

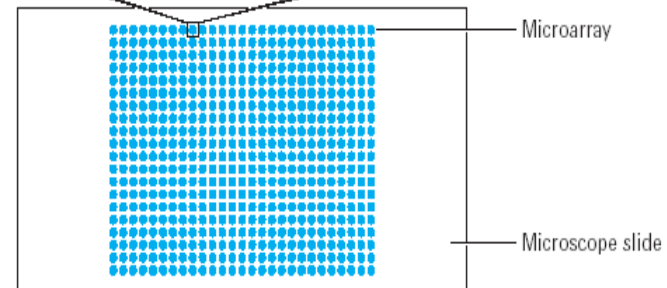
Microarray is a tool for analyzing gene expression that consists of a glass slide.

Sequence of one gene

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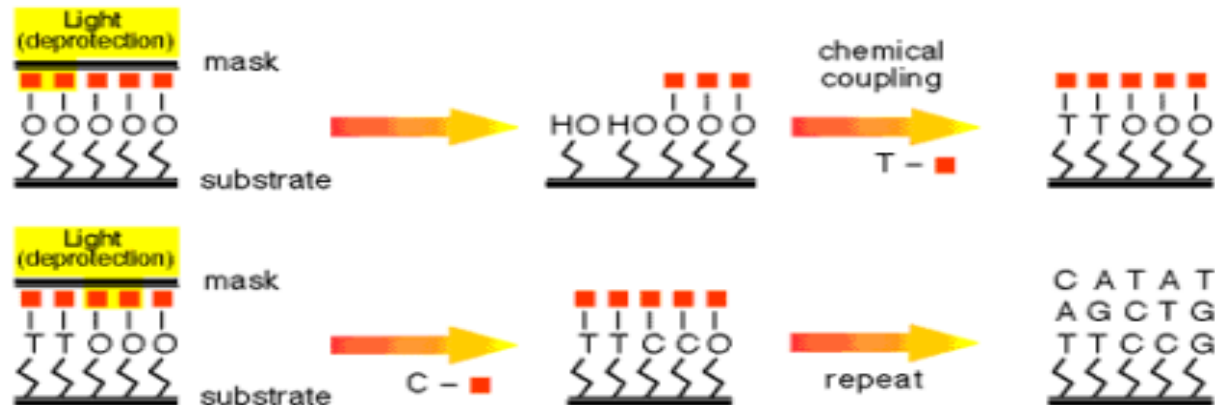
TCCTTTCCGG AACGGTTGGC GTCTGCGCAC GCGGGTGTGG GGCATGACAT
GCCGCCCCAG GAACAACCCC GACACGGCTT TAAGCCTCTC AAATCGCTGT
AGACATCATC TTTACGTGCT TGCCACCATT TGCCACCATT AGGGCTGTTC
CCGCGACGAC TCGCATTCA ACCTCAGTCC TTCGGTTGA GCGAGTGGGT
CGCGCGCAAG GTGCGAATGG GTCGCGCGCA AAGTGTTCG CTGGCTGTAT
TATATGCTGC CTATAGCGAG ACTAACGACC CACACTTTCA CACAAGGATT
TCCCGCTAAT GGGTACCTCG CGTCAGGACC TTGACGCAAG CGCGCCTTCG
GTTGGCCCCA AGCTTGCTAG GACTACTTAT CTTGAGCTCA TTTAACATCC
CGGCGCCTCT CCGGGAGCGG TCGTCGCGAA GAAGTCAAAC CCGGAACGGC
GTTGACAAAG CGTGGAGACA TCGATACCTC TGTGTCAGCG GCCACAAATC

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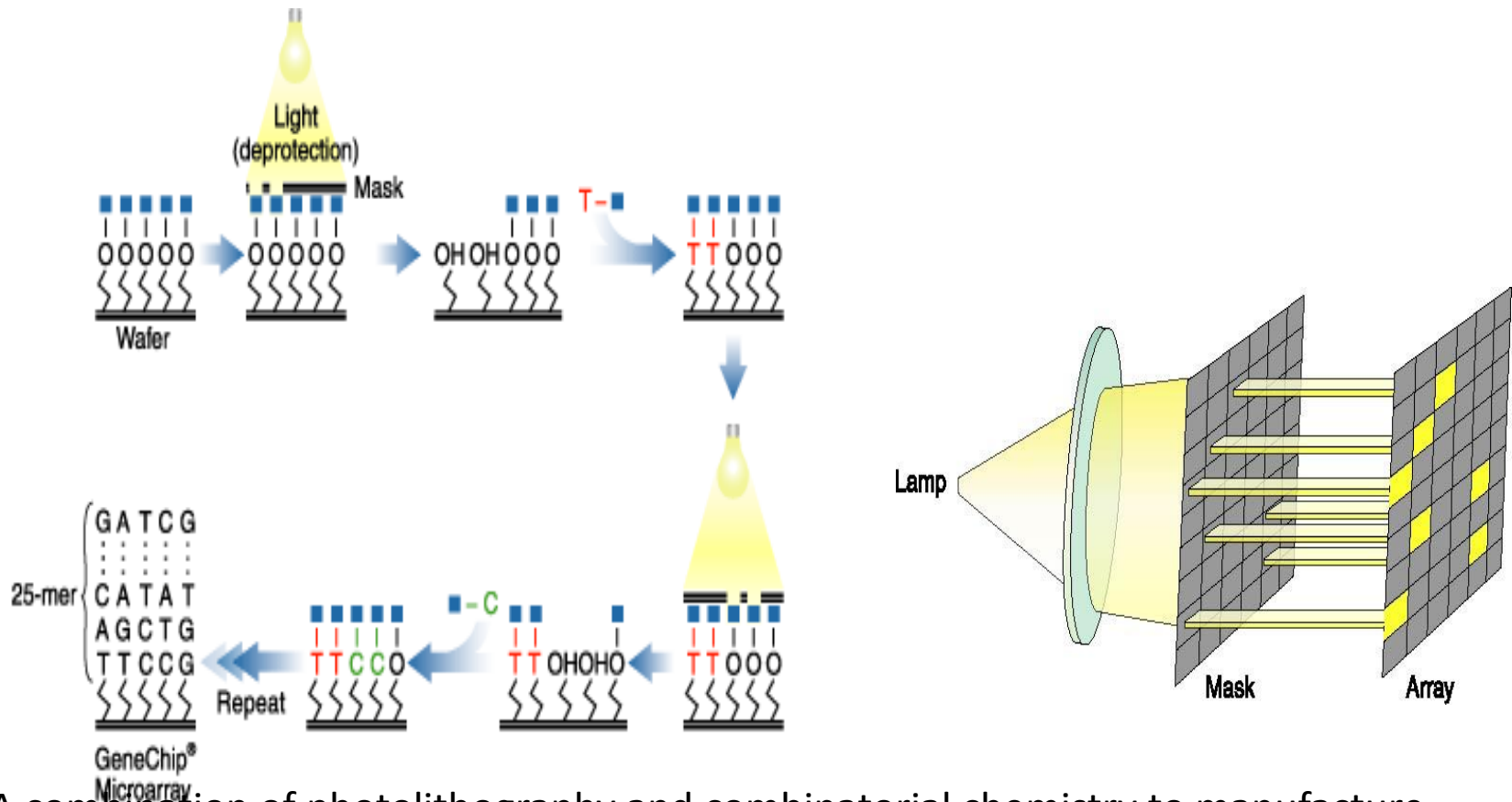
Each blue spot indicates the location of a PCR product. On a real microarray, each spot is about 100um in diameter.

Photolithography



- Light directed oligonucleotide synthesis.
- A solid support is derivatized with a covalent linker molecule terminated with a photolabile protecting group.
- Light is directed through a mask to deprotect and activate selected sites, and protected nucleotides couple to the activated sites.
- The process is repeated, activating different set of sites and coupling different based allowing arbitrary DNA probes to be constructed at each site.

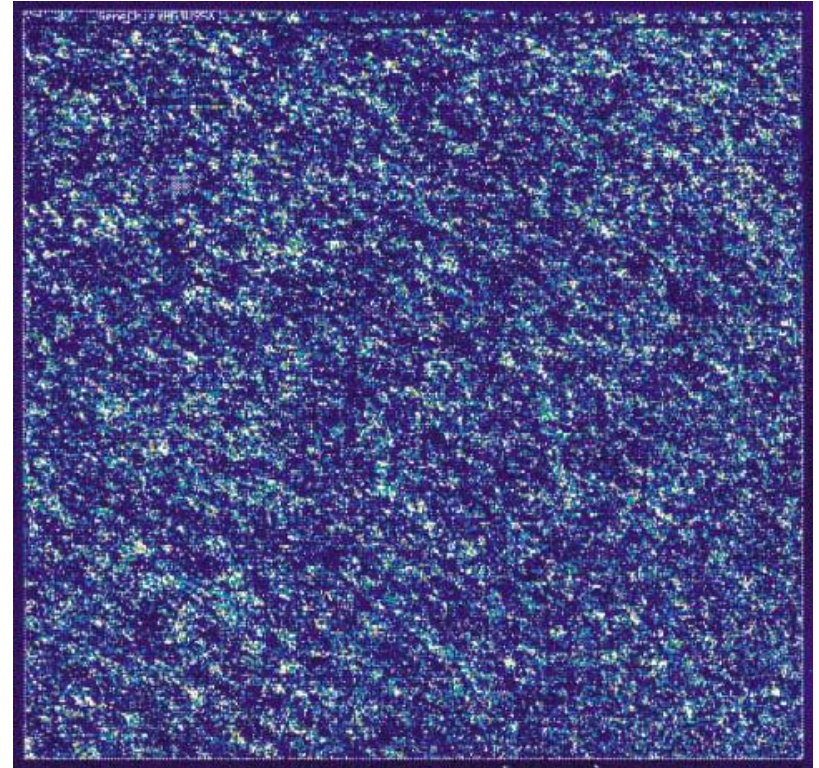
Affymetrix GeneChip® Arrays



A combination of photolithography and combinatorial chemistry to manufacture GeneChip® Arrays. With a minimum number of steps, Affymetrix produces arrays with thousands of different probes packed at extremely high density. Enable to obtain high quality, genome-wide data using small sample volumes.

Affymetrix GeneChip[®] Arrays

Data from an experiment showing the expression of thousands of genes on a single GeneChip[®] probe array.



DNA Microchip: Microarrays- A Large-scale Biological Tool

- Excellent presentations about microarray.
 - <http://www.ncbi.nlm.nih.gov/About/primer/microarrays.html>