Johns Hopkins Engineering

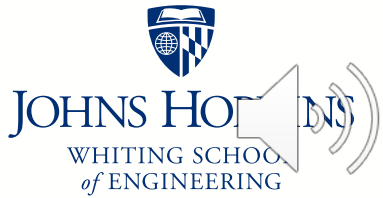
Molecular Biology 585.607

Instructor

Richard S. Potember, Ph.D.

Potember@jhu.edu

Module 10 / Lecture 1



DNA Technology

The PCR Revolution

The ability to work with minuscule amounts of DNA is proving valuable in a wide range of endeavors, from **paleontology** to **criminology**.

DNA fingerprinting analysis can be used to identify and characterize particular sequences contained in as little as 1 microgram of DNA, the amount in a small drop of blood.

But sometimes even that amount of DNA may not be available. In such cases another method, called the **polymerase chain reaction** (PCR), can come to the rescue. With PCR, it is possible to rapidly replicate, or amplify, selected DNA segments that are initially present in extremely small amounts.

In only a few hours, PCR can make millions or even billions of copies of a particular DNA sequence, thereby producing enough material for DNA fingerprinting, DNA sequencing, or other uses.

Like DNA fingerprinting, PCR is often in the news in connection with the solving of violent crimes.

PCR

The keys to the simplicity of PCR are an **unusual DNA polymerase** and the fact that synthetic primers can set up a chain reaction that produces an exponentially growing population of specific DNA molecules.

For this insight, biochemist Kary Mullis received a Nobel Prize. https://en.wikipedia.org/wiki/Kary_Mullis
https://www.ted.com/talks/kary_mullis_on_what_scientists_do

To carry out PCR, it is usually necessary to know part of the base sequence of the DNA segment that one wishes to amplify. Based on this information, short single-stranded DNA primers are chemically synthesized; these primers are generally 15-20 nucleotides long and consist of sequences that are complementary to sequences located at the two ends of the DNA segment being amplified.

DNA polymerase is then added to catalyze the synthesis of complementary DNA strands using the two primers as starting points.

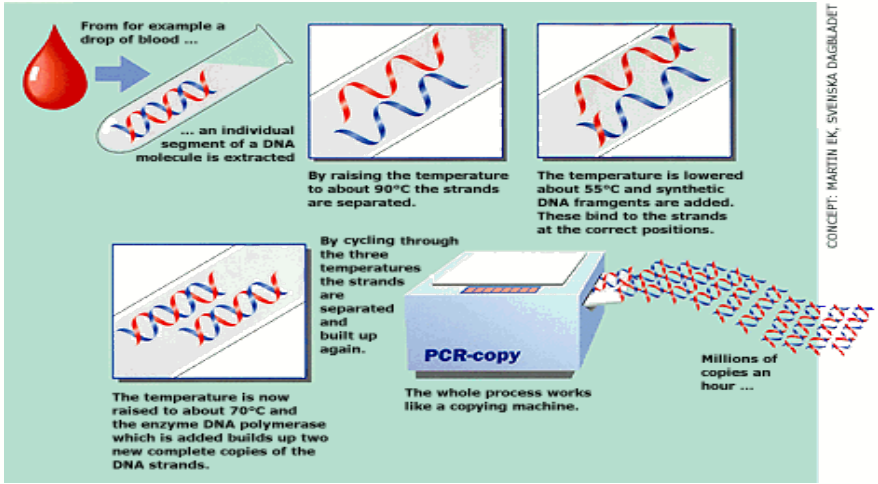
The DNA polymerase used for this purpose was first isolated from the bacterium Thermus aquaticus, an inhabitant of thermal hot springs where the waters are normally 70-80°C. The optimal temperature for this enzyme, called Taq polymerase, is 72°C, and it is stable at even higher temperatures - a property that made possible the automation of PCR.

DNA Extraction

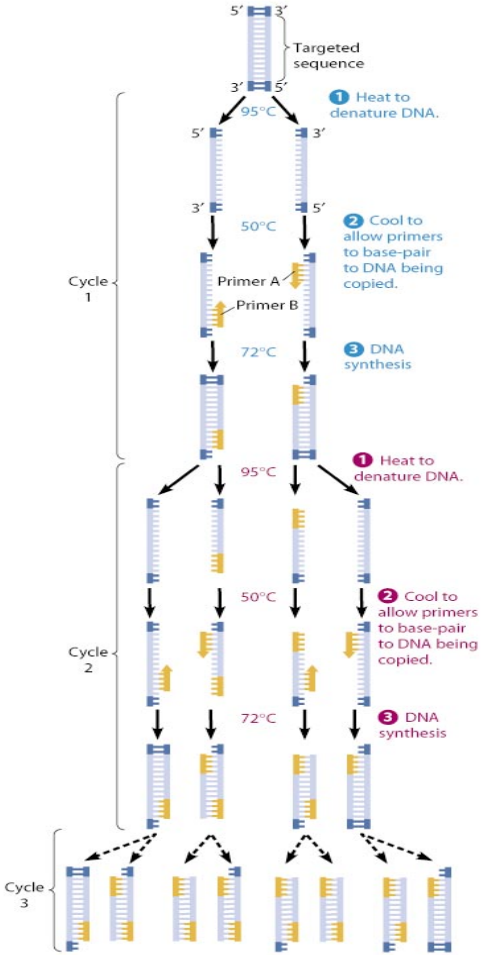
- Chemical treatments cause cells and nuclei to burst
- The DNA is inherently sticky, and can be pulled out of the mixture
- This is called “spooling” DNA

Copying DNA

- Polymerase Chain Reaction
- Also called PCR
- A method of making many copies of a piece of DNA



DNA amplification using PCR



Additional notes on PCR can be found in the Appendix

Technologies and utility

Historical perspective

DNA hybridization (1960s)

Detection of hybrids (methods)
- hydroxyapatite
- radioactive labelling
- enzyme-linked detection
- fluorescent labelling

Fixing sample on solid support
- Southern blots (1970s)
- Northern blots
- Dot blots

Basic principles

Main novelty DNA chips is **scale**

- hundreds or thousands of probes rather than tens
- it cannot be achieved manually

Probes are attached to solid supports

Robotics are used extensively

Informatics is a central component at all stages

Major technologies

- cDNA probes usually produced by PCR, attached to either nylon or glass supports
- Oligonucleotides attached to glass support
- Oligonucleotides synthesized in situ on silica wafers (Affymetrix method)
- Probes attached to tagged beads

Principal uses of chips

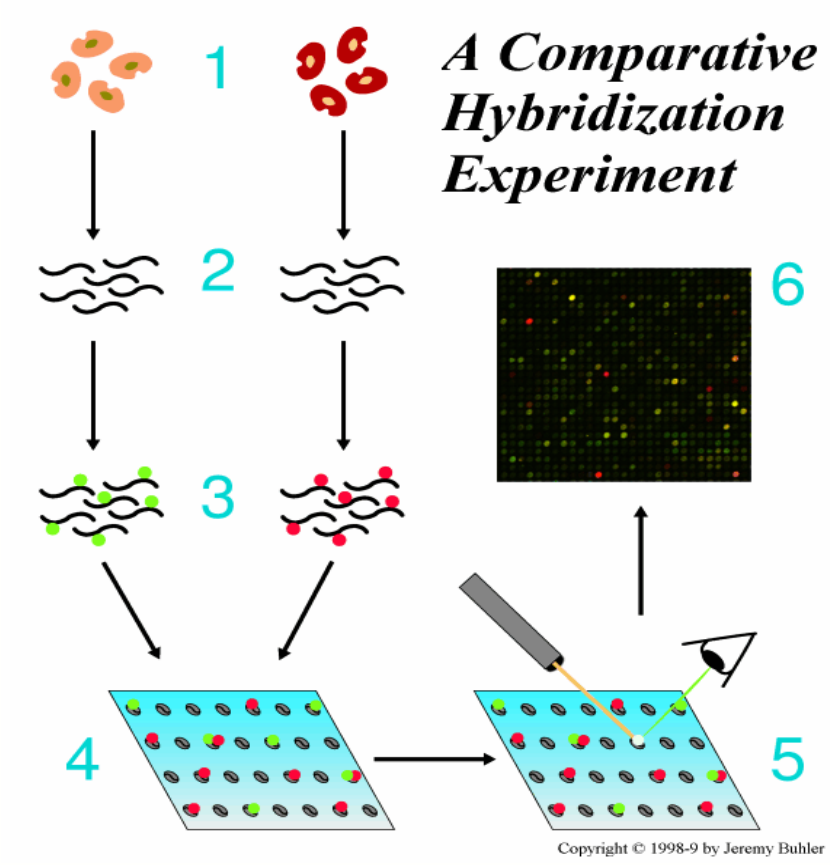
- Genome-scale gene expression analysis
  - Differentiation
  - Responses to environmental factors
  - Disease processes
  - Effects of drugs

- Detection of sequence variation
  - Genetic typing
  - Detection of somatic mutations (e.g. in oncogenes)
  - Direct sequencing

DNA chips

- Probes are DNA fragments, usually amplified by PCR
- Probes are deposited on a solid support, either positively charged nylon or glass slide
- Samples are labeled using fluorescent dyes
- At least two samples are hybridized to chip
- Fluorescence at different wavelengths measured by a scanner

Standard protocol for comparative hybridization



DNA chip design

- Probe selection
  - Non-redundant set of probes
  - Includes genes of interest to project
  - Corresponds to physically available clones

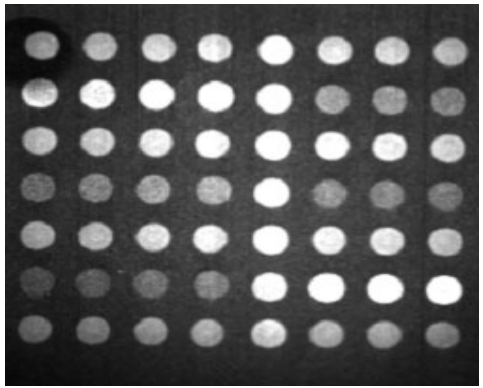
- Chip layout
  - Grouping of probes by function
  - Correspondence between wells in micro-titre plates and spots on the chip

cDNA (complementary DNA) fragments arrays on nylon and glass

- Nylon arrays
  - Up to about 1000 probes per filter
  - Use radiolabeled cDNA target
  - Can use phosphor imager or X-ray film

- Glass arrays
  - Up to about 40,000 probes per slide, or 10,000 per $2cm^2$ area (limited by arrayer's capabilities)
  - Use fluorescent targets
  - Require specialized scanner

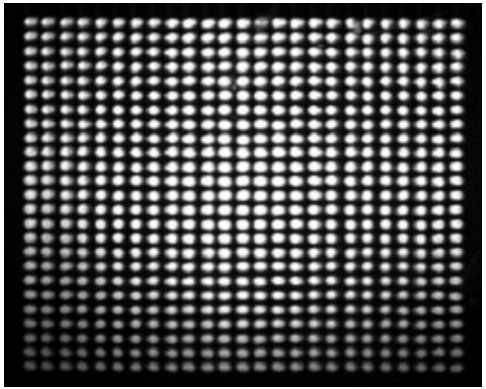
Typical ink jet spot deposition results: plastic versus glass



Volume per spot: 250 nl

Spot size: 1100 µm

Spot density: 70/cm^2



Volume per spot: 0.5 nl

Spot size: 115 µm

Spot density: 4800/cm^2

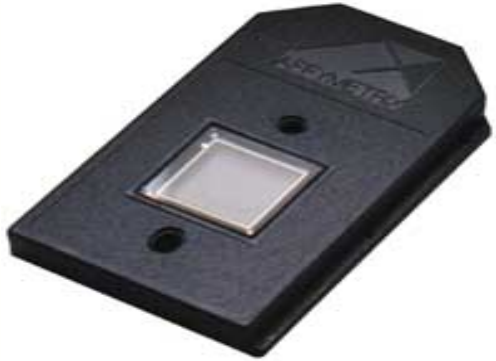
Scanning the arrays

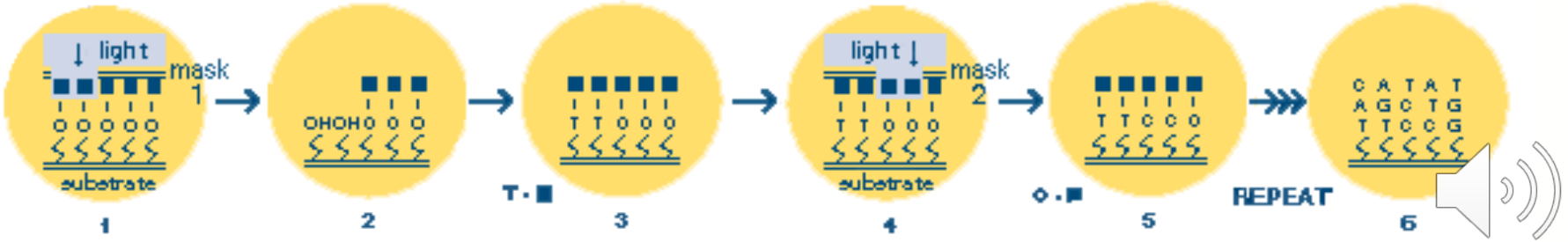
- Laser scanners
  - Excellent spatial resolution
  - Good sensitivity, but can bleach fluorochromes
  - Still rather slow
- CCD scanners
  - Spatial resolution can be a problem
  - Sensitivity easily adjustable (exposure time)
  - Faster and cheaper than lasers
- In all cases, raw data are images showing fluorescence on surface of chip

The Affymetrix approach

- Probes are oligos synthesized *in situ* using a photolithographic approach
- There are at least 5 oligos per cDNA, plus an equal number of negative controls
- The apparatus requires a fluidics station for hybridization and a special scanner
- Only a single fluorochrome is used per hybridization
- It is **very** expensive approach!

Affymetrix chip production





Examples of commercial chips

- Clontech, Incyte, Research Genetics - filter-based arrays with up to about 8,000 clones
- Incyte / Synteni – 10,000 probe chips, not distributed (have to send them target RNA)
- Affymetrix - oligo-based chips with 12,000 genes of known function (16 oligos/gene) and 4 x 10,000 genes