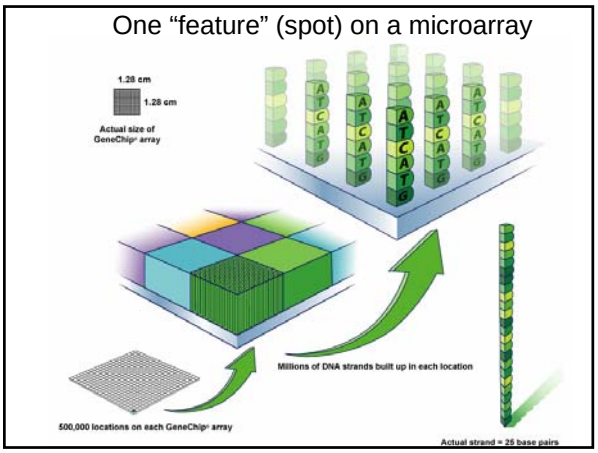
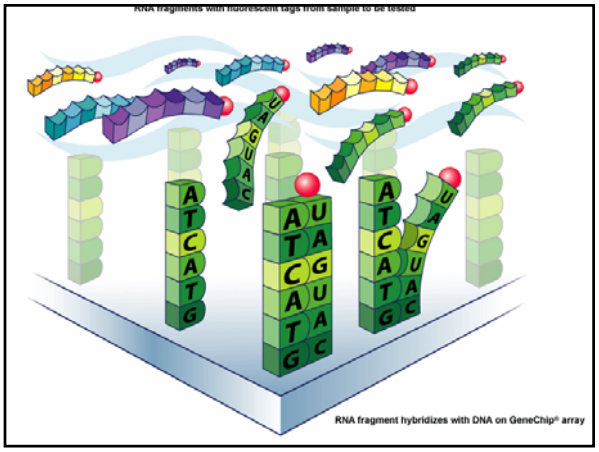
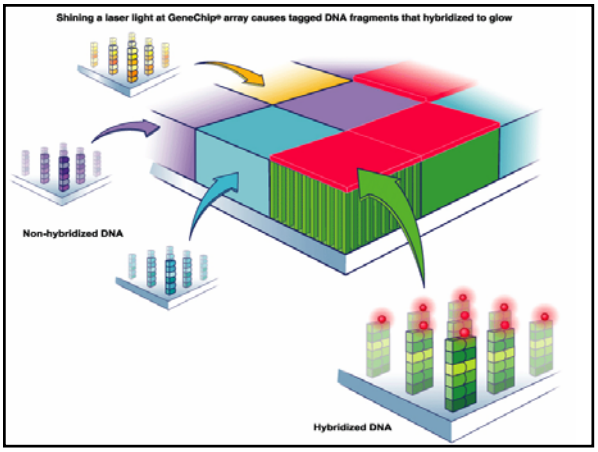
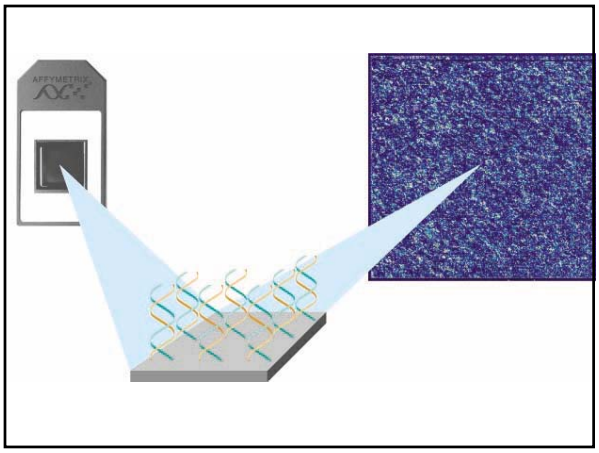
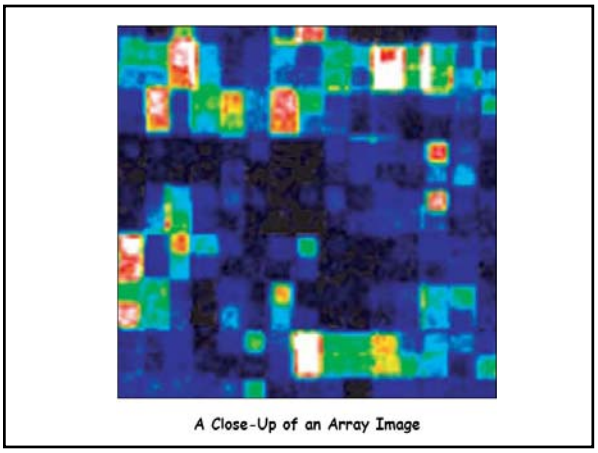
One “feature” (spot) on a microarray











A Close-Up of an Array Image

Factory in West Sacramento

How are (Affymetrix) DNA microarrays made?

- Photolithography technique for building up specific DNA sequences in each feature
- *dnachip animation*

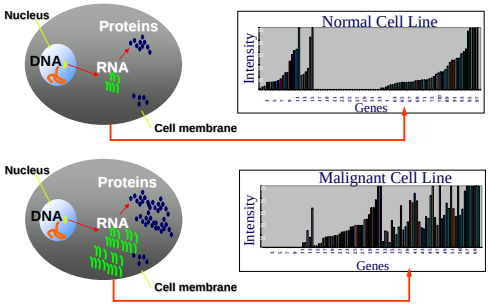
Microarrays can be used to find the genetic basis for phenotypic variation between individuals

- **Single Nucleotide Polymorphism (SNP)**
- **Polymorphism**
- **Gene Expression**

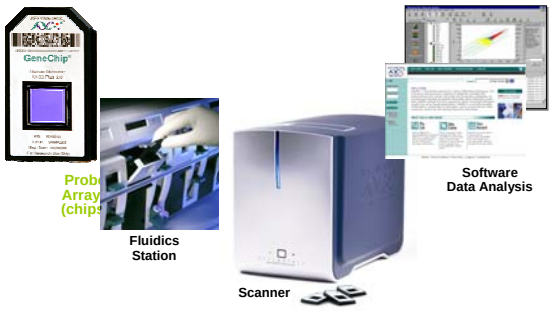
Applications

- Gene expression studies
  - Absolute level of expression of *all* genes in a genome
    - With northern blotting, you could only study 1 or 2 at a time!
  - Relative level of expression, comparing mRNAs in two cell types (same cell, different conditions; or different cells)
- Resequencing
- *Do expression chip animation*

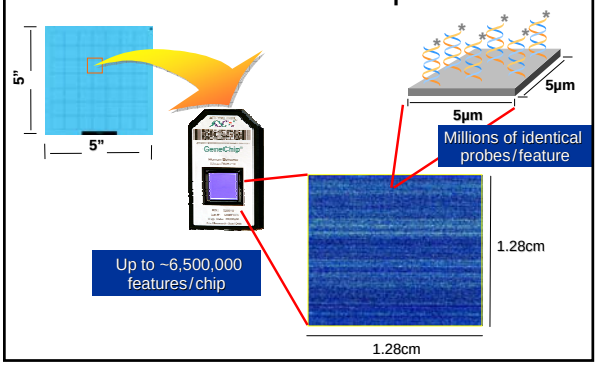
Gene expression = RNA "volume"



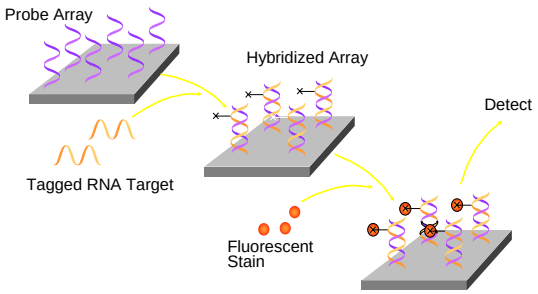
GeneChip® System



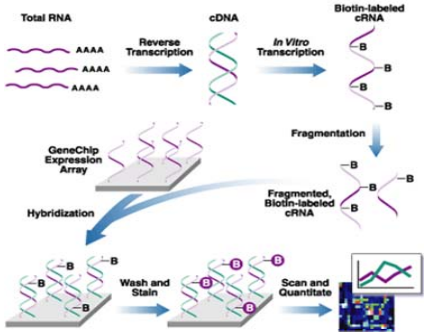
The Dimensions of a GeneChip

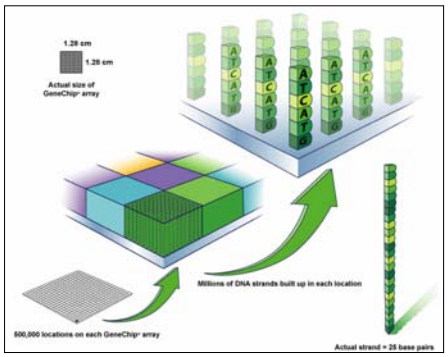


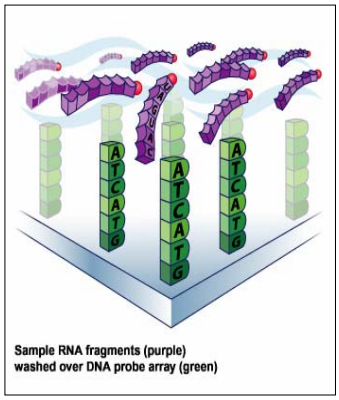
Hybridization

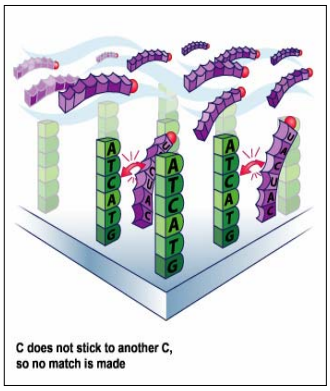


Steps of the Expression Assay

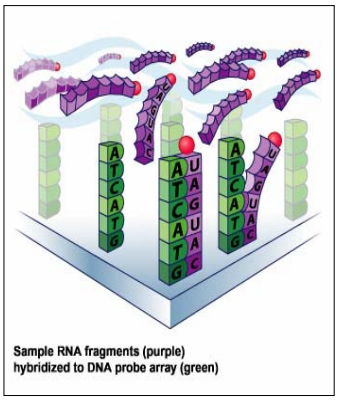




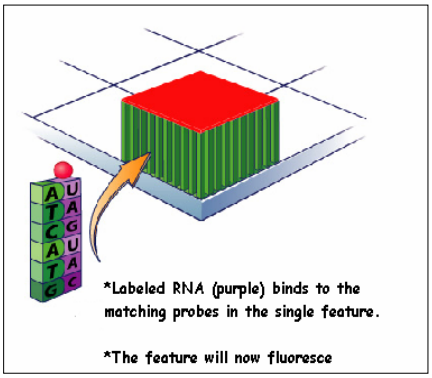




C does not stick to another C,
so no match is made

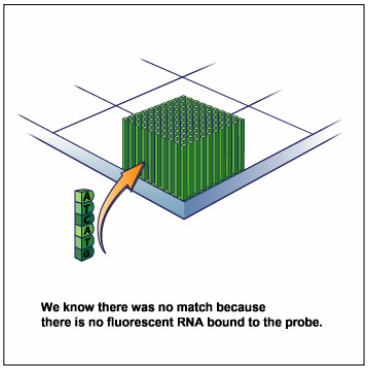


Sample RNA fragments (purple)
hybridized to DNA probe array (green)



*Labeled RNA (purple) binds to the matching probes in the single feature.

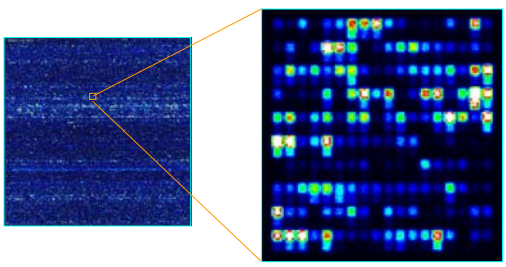
*The feature will now fluoresce

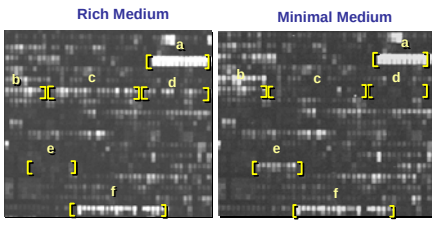


We know there was no match because
there is no fluorescent RNA bound to the probe.

Affymetrix GeneChip® Array

Black = no signal
Bright color = intense signal

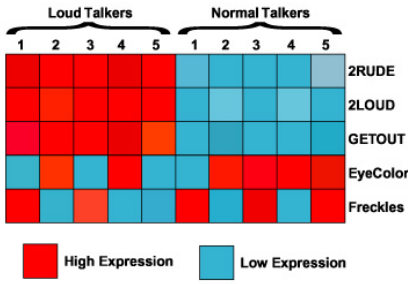




Wodicka, L., et al. 1997.
Nature Biotechnology 15:1359-1367

Genes
a = RPL2A
b = TIP1
c = BAP2
d = VAP1
e = YBR147W
f = SUP46

Use microarrays to study gene expression and correlate with phenotype or disease

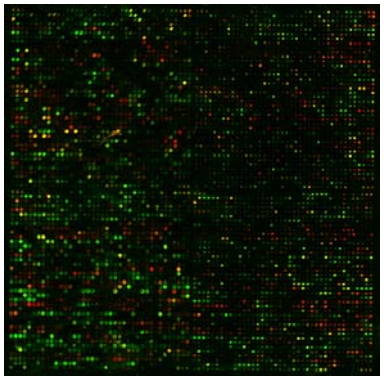


Yeast genome microarray

- http://www.bio.davidson.edu/Courses/genomics/chip/chip.html
- Yeast cells grown under aerobic vs anaerobic conditions: Ask:

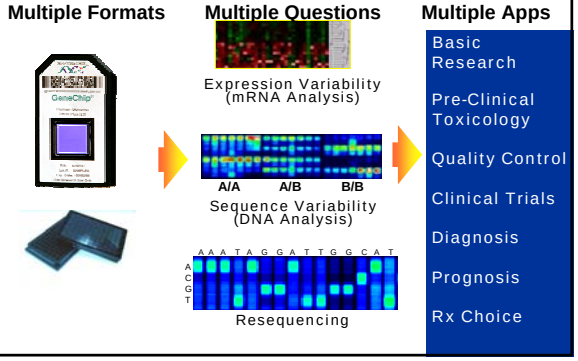
How does their gene expression differ?

Comparing gene expression: red & yellow samples from 2 cell types, added to one microarray

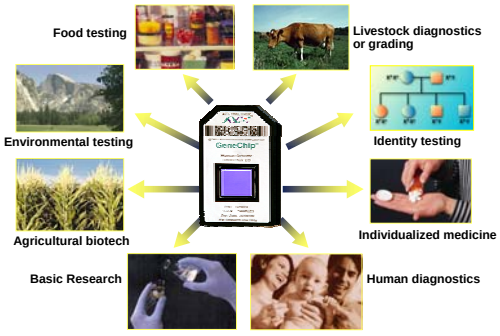


Red = gene expressed in cell A; Green = gene expressed in cell B;
Yellow = gene expressed in cell A and cell B; Black = gene not expressed in either

Three Arrays – Multiple Uses



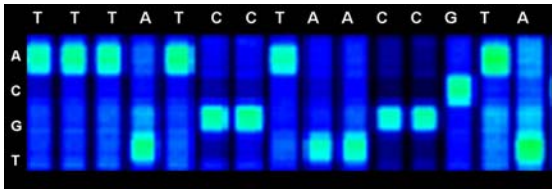
The Multiple Uses of the GeneChip® Microarray



"Resequencing" microarray

- To identify DNA sequences in a sample
- Sequence you are looking for must be known and included in the array
- All permutations of the sequence you're looking for are present
  - for each nucleotide position, there are 4 features on the array (one for A/T/G/C)
- Single base mismatches do not hybridize
  - can detect single nucleotide polymorphisms
- Example applications:
  - Detect microbial or viral strains
  - Detect contaminants (such as unwanted plant DNA in a crop sample)

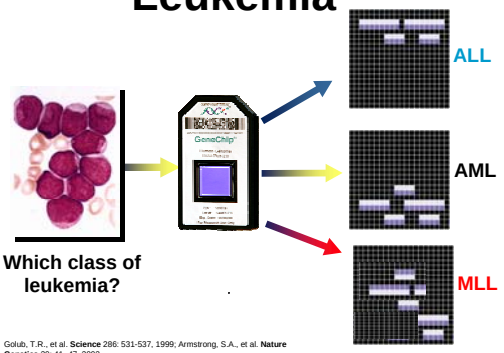
Resequencing array data



Each column represents a set of probes (4 features) which differ at a single nucleotide position.
(There is an A in probe in the top row; C in next row; etc.)

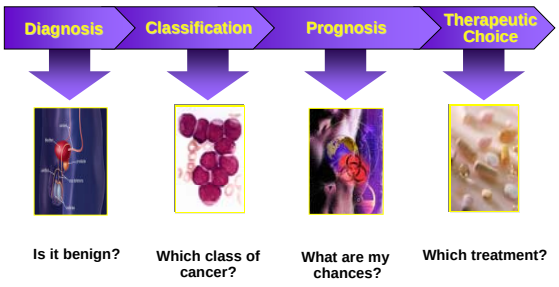
The letters at top are the actual sequence detected in the sample.

Classifying Leukemia

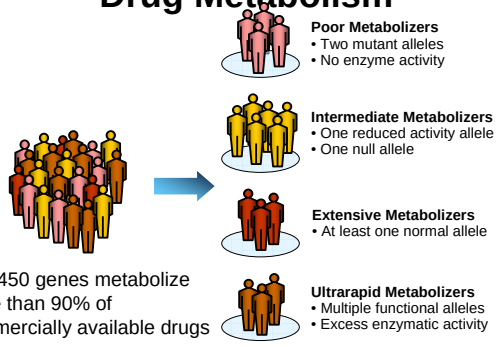


Which class of leukemia?

Golub, T.R., et al. **Science** 286: 531-537, 1999; Armstrong, S.A., et al. **Nature Genetics** 30: 41- 47, 2002



Individual Variations in Drug Metabolism



Poor Metabolizers
- Two mutant alleles
- No enzyme activity

Intermediate Metabolizers
- One reduced activity allele
- One null allele

Extensive Metabolizers
- At least one normal allele

Ultrarapid Metabolizers
- Multiple functional alleles
- Excess enzymatic activity

CYP450 genes metabolize more than 90% of commercially available drugs

Scenario A – Fish CSI?

Background:

A recent development in the food industry is the substitution of very expensive meats with a "fake" or less expensive version while still selling it at a relatively high price. Imagine you are a group of scientists hired by the FDA to go to some of the most expensive sushi restaurants in the area and randomly test their more expensive sushi for "imposter" fish. You decide to use the Fish DNA GeneChip microarray* to do the testing. This microarray contains probes representing specific gene segments of 15 different fish species. The features in the array are organized in the following way:

Arctic Char	Atlantic Bonito	Atlantic Char	Atlantic mackerel
Atlantic salmon	Brook trout	European eel	European Hake
Greenland Cod	Japanese Eel	Mozambican Eel	Rainbow Trout
Sea Trout	Skipjack Tuna	Spotted Tunny	Blue fin Tuna

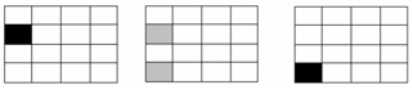
Note, on an actual array, multiple features would be used for each species. For simplicity, in this example each species is represented by a single feature

The array is simple to read. DNA is isolated from the sample (food) and then reverse transcribed into tagged RNA. The RNA is added to the array, allowed to hybridize, fluorescently tagged, and analyzed. If the DNA came from one of the species above, the RNA would hybridize to the probes in that specific feature and would fluoresce. In short, if a specific feature fluoresces, then DNA from that fish species is present.

You have collected four suspicious samples that the restaurant claimed to be the following: sake (Atlantic salmon), unagi (Japanese eel), maguro (blue finned tuna), and saba (Atlantic mackerel).

For each sample you collected enough for 20 tests.

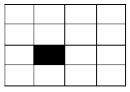
Sample #1 – samples sake (Atlantic salmon)



4 test results 13 test results 3 test results

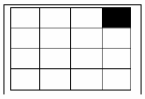
Arctic Char	Atlantic Bonito	Atlantic Char	Atlantic mackerel
Atlantic salmon	Brook trout	European eel	European Hake
Greenland Cod	Japanese Eel	Mozambican Eel	Rainbow Trout
Sea Trout	Skipjack Tuna	Spotted Tunny	Blue fin Tuna

Sample #2 – samples of unagi (Japanese eel)



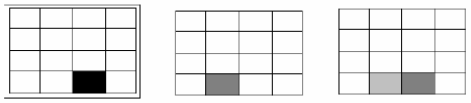
all 20 test results

Sample #3 – saba (Atlantic mackerel)



all 20 test results

Sample #4 – maguro (Blue finned tuna)



4 test results 4 test results 12 test results

Arctic Char	Atlantic Bonito	Atlantic Char	Atlantic mackerel
Atlantic salmon	Brook trout	European eel	European Hake
Greenland Cod	Japanese Eel	Mozambican Eel	Rainbow Trout
Sea Trout	Skipjack Tuna	Spotted Tunny	Blue fin Tuna