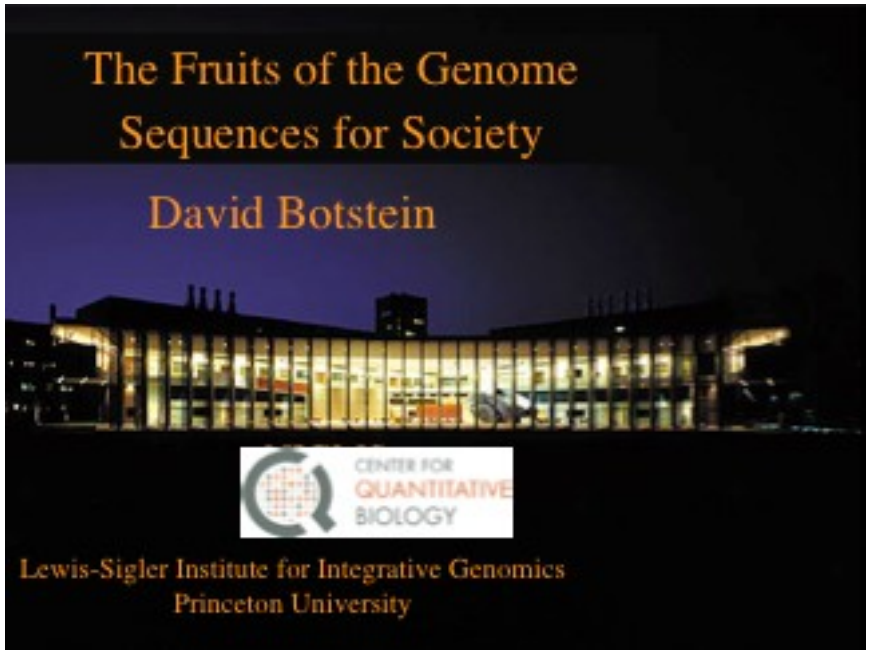
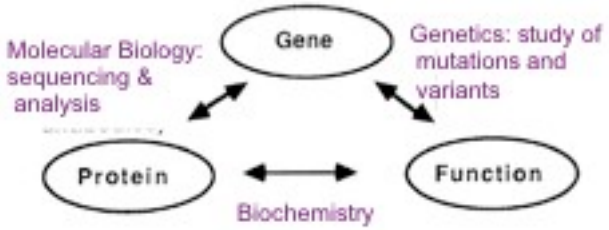


Genome Sizes and Gene Numbers

Organism	Genome Size	Genes (for Proteins)
Yeast	12 megabases	5,800
Worm	100 megabases	19,400
Fly	120 megabases	13,400
Plant	115 megabases	25,500
Human/Mouse	3300 megabases	22,000

The basic cellular functions of all eukaryotes are carried out by proteins (and RNAs) whose **structure and function** are conserved.

Associating Biological Information with DNA Sequence



[Botstein & Fink (1988) Yeast: An Experimental Organism for Modern Biology, **Science** 240: 1439-1443].

The Amino Acid Sequence of a Protein

```
MDSEVAALVIDNGSGMCKAGFAGDDAPRAVFPSIV
GRPRHQGIMVGMGQKDSYVGDEAQSKRGILTLRYP
IEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLL
TEAPMNPKSNREKMTQIMFETFNVPAFYVSIQAVL
SLYSSGRTTGIVLDSGDGVTHVVPIYAGFSLPHAI
LRIDLAGRDLTDYLMKILSERGYSFSTTAEREIVR
DIKEKLCYVALDFEQEMQTAAQSSSIEKSYELPDG
QVITIGNERFRAPEALFHPSVLGLESAGIDQTTYN
SIMKCDVDVRKELYGNIVMSGGTTMFPGIAERMQK
EITALAPSSMKVKIIAPPERKYSVWIGGSILASLT
TFQQMWISKQEYDESGPSIVHHKCF*
```

Sequence Similarity Between Yeast and Human Actin

Score = 720 bits (1858), Expect = 0.0 Identities = 334/375 (89%), Positives = 360/375 (95%)

```
Yeast: 27  MDGEVAALVIDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGIMVGMGQKDSYVGDEAQS 86
           M+ E+AALVIDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQG+MVGMGQKDSYVGDEAQS
Human: 1   MEEEIAALVIDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGVMVGMGQKDSYVGDEAQS 60

Yeast: 87  KRGILTLRYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPMNPKSNREKMT 146
           KRGILTL+YPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAP+NPK+NREKMT
Human: 61  KRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKMT 120

Yeast: 147 QIMFETFNVPAFYVSIQAVLSLYSSGRTTGIVLDSGDGVTHVVPIYAGFSLPHAILRIDL 206
           QIMFETFN PA YV+IQAVLSLY+SGRTTGIV+DSGDGVTH VPIY G++LPHAILR+DL
Human: 121 QIMFETFNTPAMYVAIQAVLSLYASGRTTGIVMDSGDGVTHTVPIYEGYALPHAILRLDL 180

Yeast: 207 AGRDLTDYLMKILSERGYSFSTTAEREIVRDIKEKLCYVALDFEQEMQTAAQSSSIEKSY 266
           AGRDLTDYLMKIL+ERGYSF+TTAEREIVRDIKEKLCYVALDFEQEM TAA SSS+EKSY
Human: 181 AGRDLTDYLMKILTERGYSFTTTAEREIVRDIKEKLCYVALDFEQEMATAASSSSLEKSY 240

Yeast: 267 ELPDGQVITIGNERFRAPEALFHPSVLGLESAGIDQTTYNSIMKCDVDVRKELYGNIVMS 326
           ELPDGQVITIGNERFR PEALF PS LG+ES GI +TT+NSIMKCDVD+RK+LY N V+S
Human: 241 ELPDGQVITIGNERFRCPEALFQPSFLGMESCGIHETTFNSIMKCDVDIRKDLYANTVLS 300

Yeast: 327 GGTTMFPGIAERMQKEITALAPSSMKVKIIAPPERKYSVWIGGSILASLTTFQQMWISKQ 386
           GGTTM+PGIA+RMQKEITALAPS+MK+KIIAPPERKYSVWIGGSILASL+TFQQMWISKQ
Human: 301 GGTTMYPGIADRMQKEITALAPSTMKIKIIAPPERKYSVWIGGSILASLSTFQQMWISKQ 360
```

Yeast/Mammalian Protein	Sequence Identity (%)	Function
Ubiquitin	96	yes
Actin	89	yes
ADP-Ribosylation Factor	77	yes
Beta-tubulin	75	partial
Alpha-tubulin	74	partial
Heat Shock HSP70	73	
YPT1/Rab1	71	yes
HMG-CoA Reductase	67	yes
Transcription Init. Factor IID	65	yes
Cytochrome C	63	
KAR2/BiP	62	yes
Calmodulin	60	yes
RAS1/N-ras; RAS2/K-ras	60	yes
CDC28/CDC2	59	yes
SEC18/NSF	46	yes
Cu-metallothionein	30	
Dihydrofolate Reductase	32	yes
Profilin	28	yes
P-glycoprotein/MDR	26	yes
Glucose Transporter	25	yes

[Hoffstein and Fink, 1988 (updated)]

The Intellectual Impact of the Genomic View

• The "grand unification" of biology: the functional parts of all living things are related by lineage.

"Once we understand the biology of E. coli, we will understand the biology of the elephant"
---Jacques Monod, ca.1960

• The challenge for the future is to understand not just mechanisms at the individual process level, but also the interactions among all the processes and their mechanisms.

• Genomics makes possible experiments and analysis at the "systems" level. This requires highly parallel experimental methods and computation-intensive analysis.

Fruits of the Genome

• Quantitative understanding of evolution from sequence.

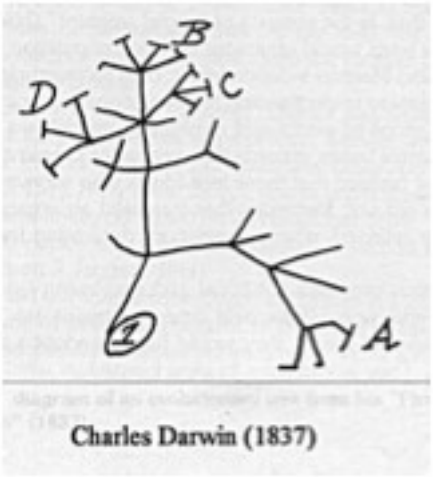
• Comparative Genomics:"grand unification" of biology.

• The many uses of DNA sequence polymorphism: from forensics to disease gene identification.

• Functional Genomics: defining diseases through gene identities and genome-scale patterns of gene expression.

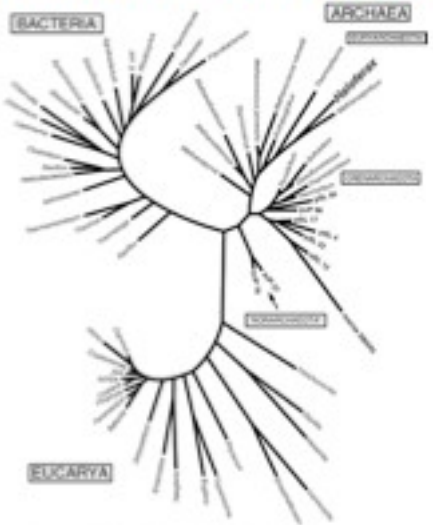
• DNA Diagnostics: detecting disease, disease progression and predisposition to disease.

Darwin's Great Intuitive Insight

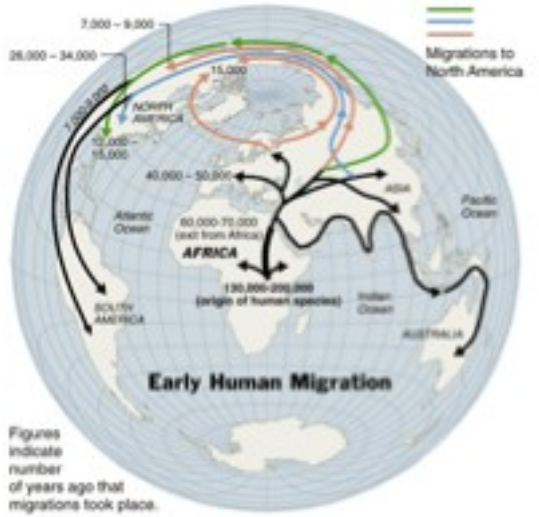


Charles Darwin (1837)

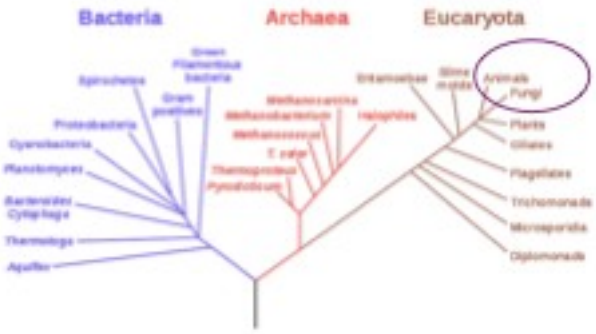
"Universal" Unrooted Phylogenetic Tree of Life



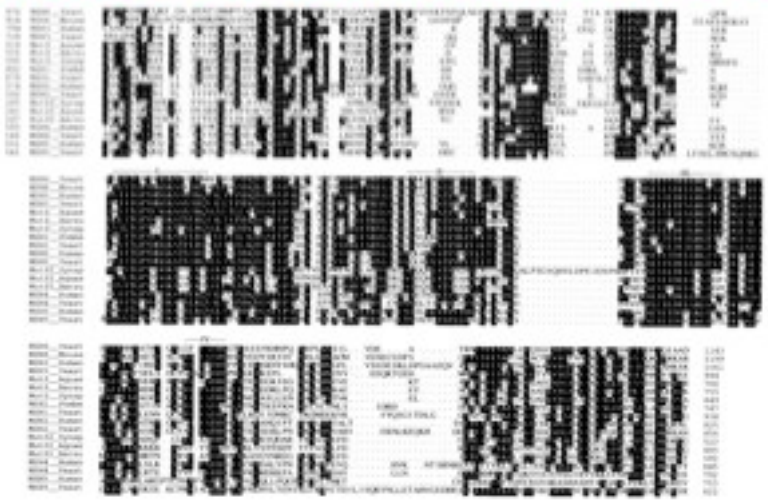
Out of Africa: The evolutionary path of the human species



Rooted Phylogenetic Tree of Life

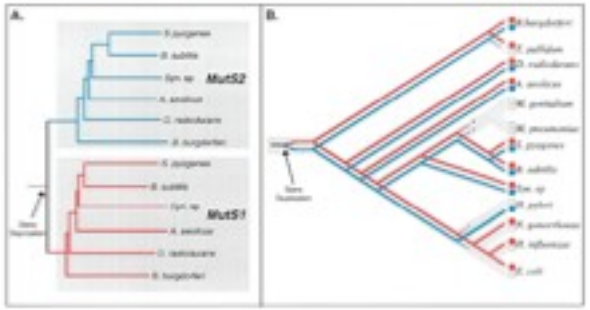


Multiple Sequence Alignment of *mutS* Homologs



[J.A. Eisen *Nucleic Acids Research, 1998, Vol. 26, No. 18*]

Distinguishing Orthologs and Paralogs from a Gene Family by Parsimonious Assignment of Gene Duplications and Losses



[J.A. Eisen *Nucleic Acids Research, 1998, Vol. 26, No. 18*]

MutS Homologs Evolve Diverged Functions

[J.A. Eisen *Nucleic Acids Research, 1998, Vol. 26, No. 18*]

Extracting Functional Information from the Human Genome Sequence

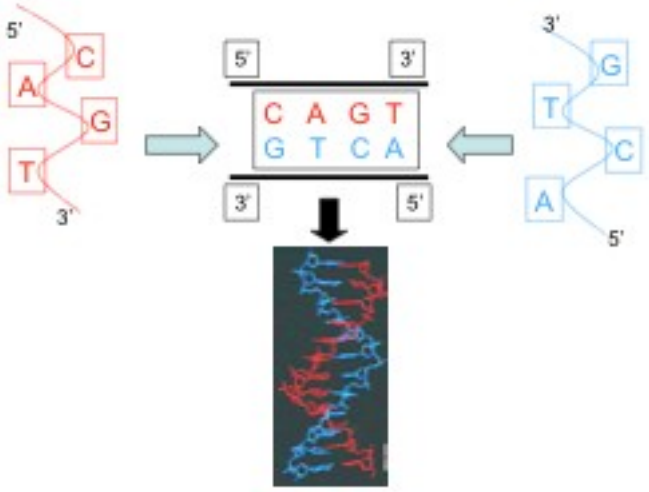
- Finding and Characterizing Human Disease Genes
  - DNA polymorphisms (SNPs & haplotypes)
  - Simple mendelian (ca. 3000) & complex (very few)
  - Complex disorders (a handful, maybe)
- Comparative Genomics: associating human genes with their functional equivalents in experimental model systems
  - Using the evolutionary information:
    - orthologs and paralogs
  - Genetic alterations, RNAi and other gene-based interventions

Extracting Functional Information from the Human Genome Sequence

- Patterns of Gene Expression
  - DNA microarrays & Quantitative PCR
  - Immediately useful for diagnosis
  - (e.g. cancer subtypes)

- Systems Biology: understanding at a different level?
  - Signal transduction, pathways, interactions

DNA Hybridization: Complementary Sequences Find Each Other to form Double Helices



Mapping Human Genes using DNA Polymorphisms

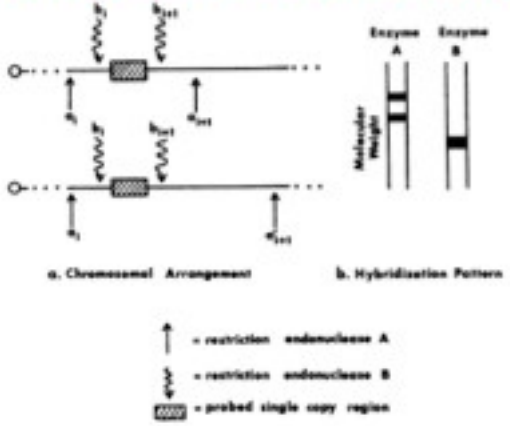
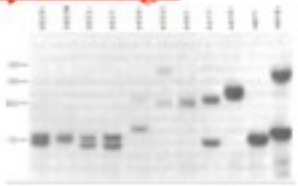


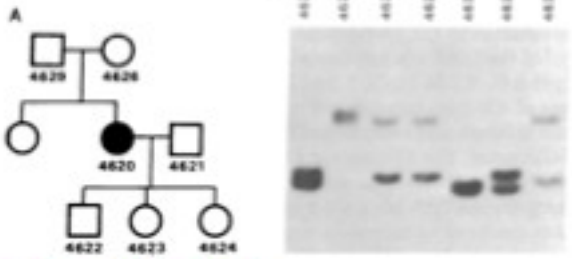
FIG. 1.—a, Cuts made in pair of homologous chromosomes by enzyme A and enzyme B; b, hybridization pattern of enzymes A and B gives cuts of *a*.

[Botstein, White, Skolnick & Davis, 1980]

DNA Polymorphisms Can Map Human Disease Genes by Linkage

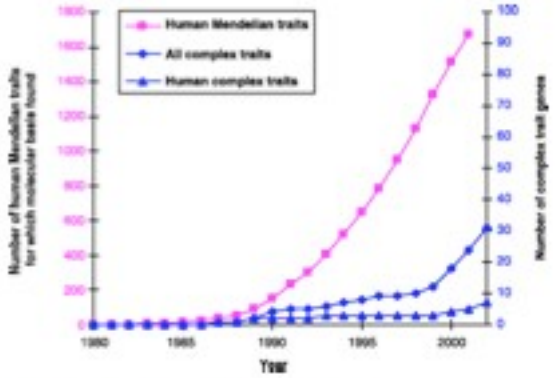
The original RFLP





[Wyman and White, 1980]

Thousands of Inherited Disease Genes have been Found



[Glazier Nadeau & Aikman, 2006]

Today, OMIM lists 2,799 of a total of 4,466 Mendelian phenotypes (mostly inherited diseases) have been associated with specific genes.

Gene Identification through Linkage Mapping Provides Basic Mechanistic Information for Inherited Diseases

Huntington's Disease ---> class of amplification of trinucleotide repeat diseases (myotonic dystrophy, fragile X, spinocerebellar ataxia, etc.)

Amyotrophic Lateral Sclerosis ---> understanding of the critical issues around reactive oxygen species in the brain.

Ataxia-telangiectasia and BRCA1---> implication of cell cycle checkpoints and DNA repair in the etiology of cancer.

Retinoblastoma ---> realization that cancer can be caused by loss of function as easily as by inappropriate gain of function.

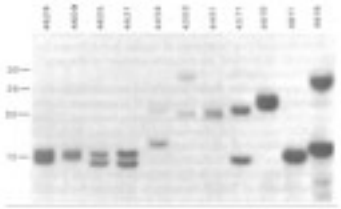
DNA Evidence is Ubiquitous in Crime Fiction

Watching these shows, it becomes clear that most (if not quite all) plots involve DNA evidence.

DNA Polymorphisms are Abundant in the Human Genome

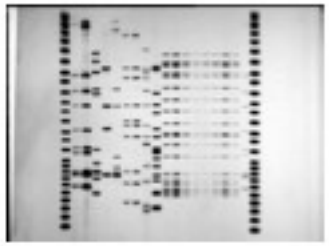
The original RFLP

[Wyman and White, 1980]

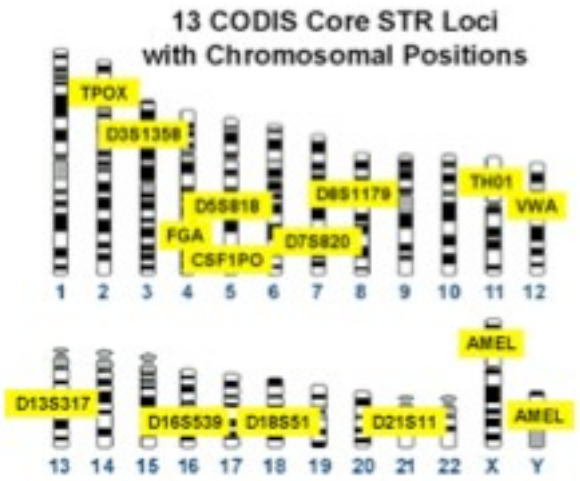


Markers from a commercial DNA Forensics laboratory

[Ryan Forensic website]



The FBI has Settled on a Standard Set of Multiallelic Markers



CODIS: Combined DNA Index System (FBI)

Non-Inherited Dinucleotide Repeat Polymorphisms Appear in Colon Tumor Cells

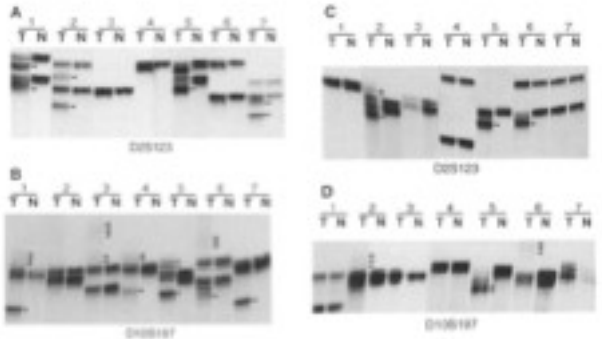


Fig. 2. (A and B) Dinucleotide repeat polymorphisms in normal and tumor tissue from HNPCC patients. The microsatellite markers D2S123 and D13S197 were used in PCR analysis (5, 20), and

[Aaltonenen et al., 1993]

Isolation of Yeast *msh2* and *mlh1* Mutations, with a Hypothesis, September 1993

Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair

Micheline Strand*, Tomas A. Prolla†§, R. Michael Liskay‡§ & Thomas D. Petes*

Finally, we note that the phenotype of the mutation involved in one type of familial colorectal cancer (decreased stability of simple repeats)[2–4] is that predicted for a mutation affecting DNA mismatch correction. Such a mutation could represent a functional homologue of *PMS1*, *MLH1* or *MSH2* or another component of the mismatch repair system (for example, a DNA helicase or single-strand binding protein). □

Nature 365:274 (September 16, 1993)

The Human *MSH2* Ortholog Predisposes to HNPCC (Human Non-Polyposis Colon Cancer)

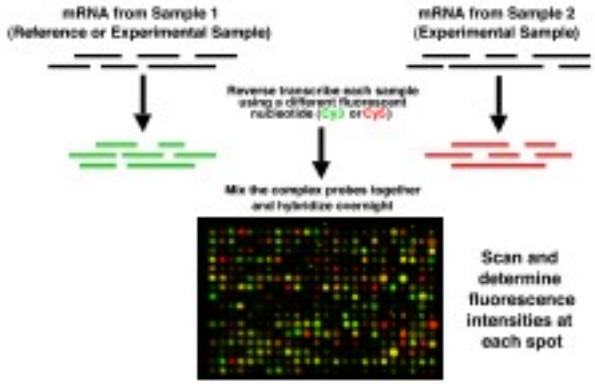
Cell, Vol. 75, 1027–1038, December 3, 1993, Copyright © 1993 by Cell Press

The Human Mutator Gene Homolog *MSH2* and Its Association with Hereditary Nonpolyposis Colon Cancer

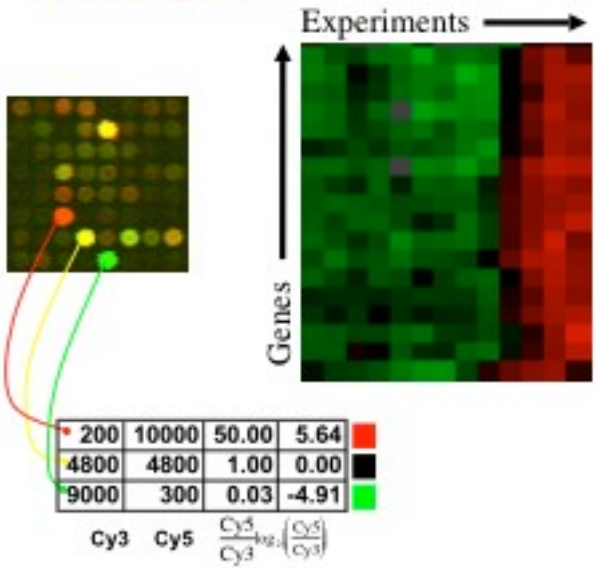
Richard Fishel,* Mary Kay Lescoe,* M. R. S. Rao,† Neal G. Copeland,† Nancy A. Jenkins,† Judy Garber,‡ Michael Kane,§ and Richard Kolodner§

Today, it is known that ca. 90% of all familial HNPCC families have mutations in either the human MSH2 or MLH1 homologs

Genome-Wide Gene Expression Patterns Determined Using Hybridization to DNA Microarrays

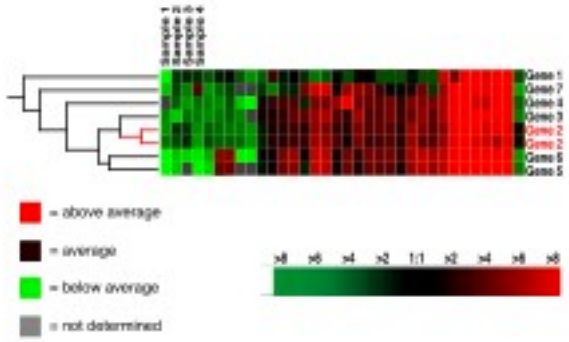


Extracting Data



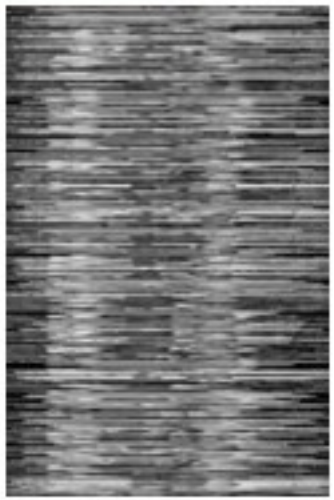
Hierarchical Clustering

Bringing Together Similar Patterns of Gene Expression

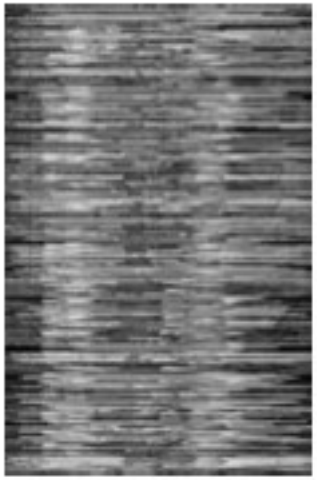


[Eisen et al., 1998]

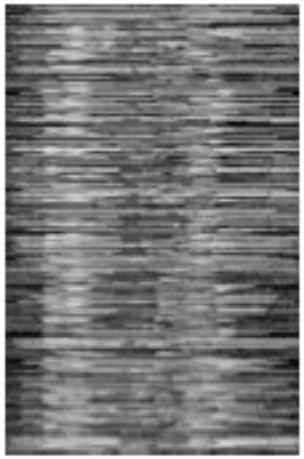
Randomized Data



Rows Ordered by Hierarchical Clustering

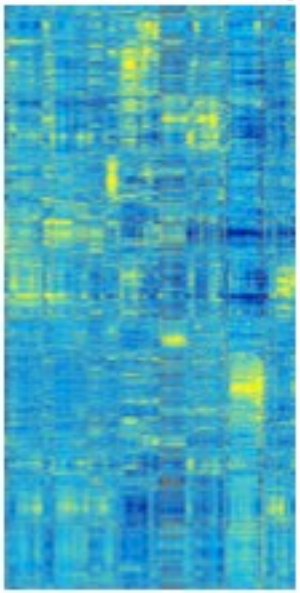


Rows Ordered by Hierarchical Clustering with Nodes Flipped to Optimize Ordering



440 human cell and tissue samples (out of more than 20,000)

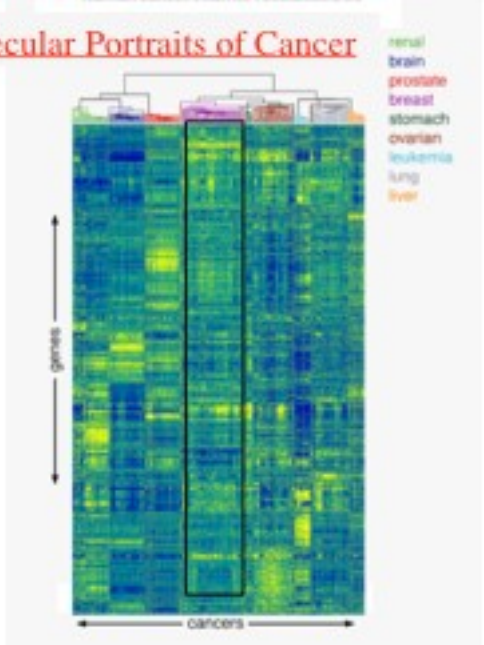
~6000 most variably-expressed genes



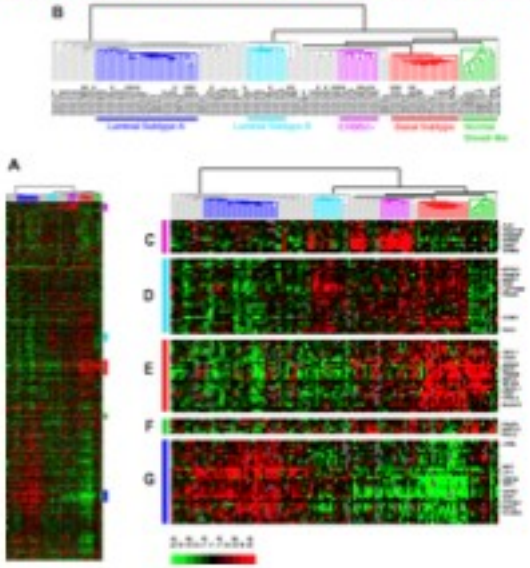
A new kind of map of the human genome…

Pat Brown
Mike Eisen
Max Diehn
Xin Chen
Jon Pollack
Chuck Perou
Therese Sorlie
Mitch Garber
Marci Schaner
Matt van de Rijn
Gavin Sherlock
Mike Fero

Molecular Portraits of Cancer

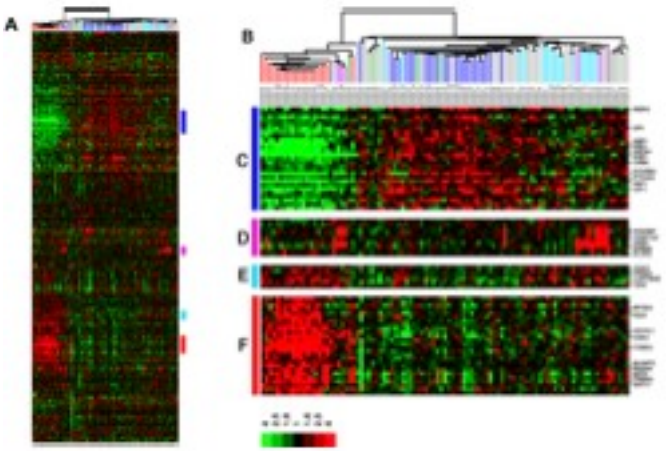


Molecular Portraits of Breast Tumors: Norway/Stanford Cohort

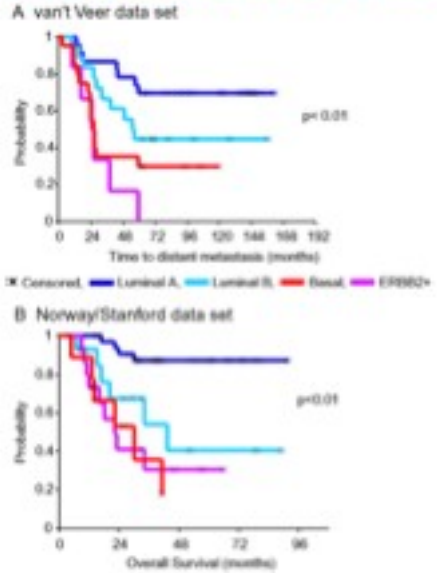


Molecular Portraits of Breast Tumors: Dutch Cohort

(Data from van t'Veer et al, 2002)



Correlation of Subtype with Outcome in Different Cohorts



A genomic hypothesis test

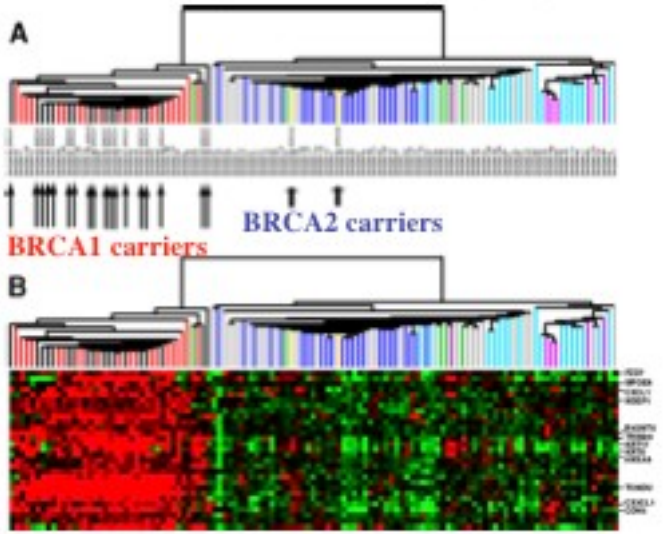
Hypothesis: the four breast cancer subtypes represent fundamentally different diseases arising from different cell types and/or by different pathways of oncogenesis.

If so, then women who inherit genes predisposing to breast cancer, and who thereby have a many- fold increased risk, should all have the same tumor subtype.

Test: Assess the patterns of gene expression of breast tumors in BRCA1 or BRCA2 carriers.

BRCA1 mutations predispose to tumors of the "Basal" subtype

(Data from van t'Veer et al, 2002)



Clinical Applications of Microarray Information

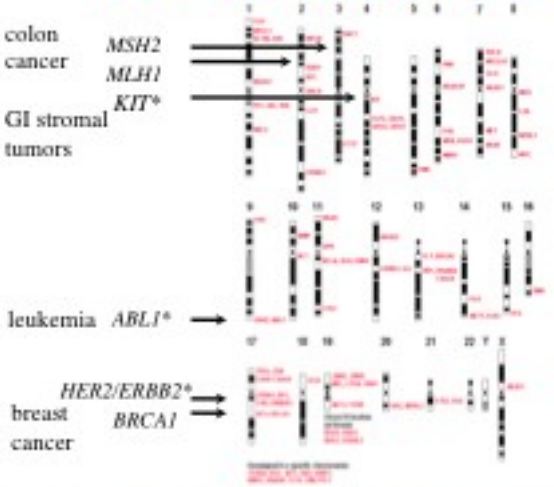
- *Better diagnosis*: definition of more biologically and clinically homogeneous cancer subtypes. Greater power to test efficacy in trials.

- *Earlier detection*: identification of secreted molecules that can be detected in blood tests

Clinical Applications of Microarray Information

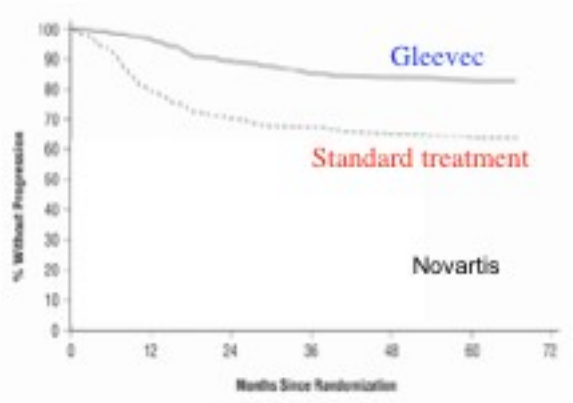
- *New therapeutic targets*: identification of molecules expressed in tumors that can be aimed at
  - membrane proteins as antibody therapy targets e.g. Her2/ERBB2 (Herceptin)
  - receptor tyrosine kinases as small molecule targets e.g. specific antagonists of Abl or Kit (Gleevec)
- *Monitoring and predicting response*: finding the appropriate therapy, old or new, for each individual tumor

Examples of Human Cancer-Causing Genes

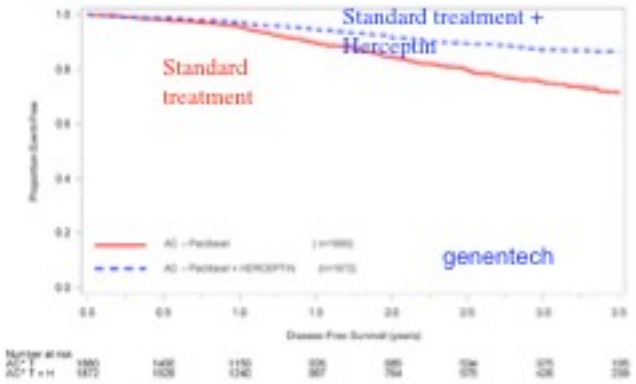


These genes have been implicated in cancer
(*) targets of successful drugs.

Chronic Myelogenous Leukemia Patients Treated with Specific Antagonist (Gleevec) Directed Against the Product of the *ABL* Gene



Breast Cancer Patients Treated with an Antibody Drug (Herceptin) Directed Against the Product of the HER2 Gene



Results of a randomized trial in which women were treated after removal of the primary tumor: the effect is about 2-fold improvement in survival, and highly significant statistically

Issues for the Future

- Personal genome as predictor of health: confronting the reality that we have no robust theory or understanding of the relationship between genotype and complex diseases (as opposed to single-gene Mendelian ones).

Issues for the Future

• How to reconcile interpretation of DNA sequence by doctors and patients (or somebody else– a statistical geneticist?) with the probabilistic nature of the connections between sequence and disease:
-- The case of Huntington's (no therapeutic options today)
-- The case of HNPCC (heightened surveillance, by colonoscopy, of obvious survival value)
-- The case of HER2 amplification in breast tumors (an effective drug, trastuzumab (Herceptin) available)