Introduction

- **Ultimate goal:**
  Sequence the human genome
- **Intermediate goal:**
  Identify a landmark every 100 kb
- **Radiation hybrid maps can achieve this goal**
- **Lecture goals:**
  - What is a radiation hybrid (RH) map?
  - How to construct, obtain, and use an RH map
- **Bottom line:**
  - Mapping is a complex process
  - Use and generation of maps requires a thorough understanding

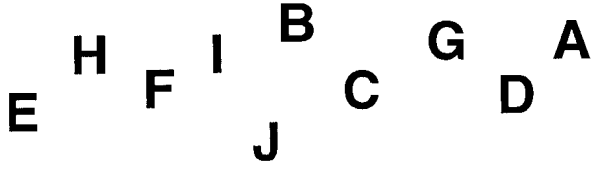
What is a map?

- **A map simply shows the order of a set of landmarks, with distances between neighbors and an indication of level of support for order.**

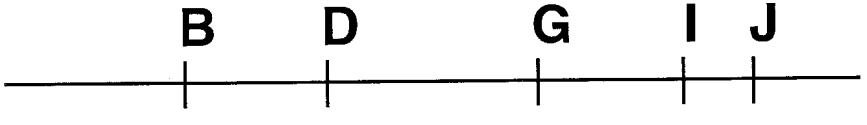
Why do we need maps?

- Positional cloning --> gene identification
  - Disease->map->gene->function
- Genetic counseling - risk prediction
- Sequence analysis - fragment ordering and display

Construction of a Linkage or RH Map

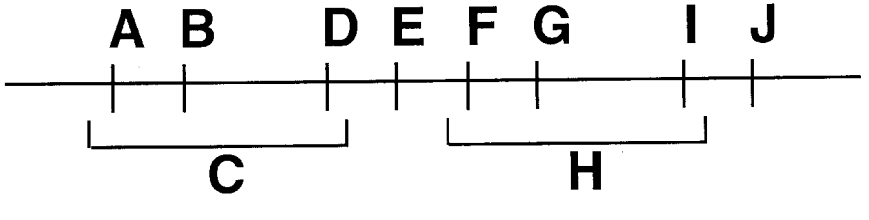


UNORDERED



FRAMEWORK MAP

Evenly spaced intervals
high support for order
most useful markers
(heterozygosity > 70%)



COMPREHENSIVE MAP

2-5 cM intervals
all markers positioned
with odds > 1000:1

RH Maps

- **RH maps are similar to genetic linkage maps**
- **Screening for presence or absence of a locus is similar to genotyping**
- **Chromosome breaks are caused by radiation, not recombination.**
- **Level of Support**
  - For genetic and RH maps, finding the absolute best map of a a set of markers cannot be guaranteed.
  - Therefore, a statistical measure of support for any order is necessary
  - Support can be expressed by a lod score or in terms of odds. For example, one marker order may be 1000 times more likely than another.

Types of Maps

- **Framework Map**
  - evenly spaced markers with high level of support for order
- **Comprehensive Map**
  - shows position of all markers, sometimes regardless of support
- **Integrated Map**
  - contains markers of different types or from different sources
  - often indicates integration of genetic, RH and/or physical maps

RH Advantages

- **10x higher resolution can be achieved**
  - 100-500 kb as opposed to 1-3 Mb
  - Resolution can be controlled
- **Monomorphic markers can be mapped**
  - Not restricted to polymorphic markers
  - Can map ESTs and monomorphic genes
- **RH map distances are more proportional to physical distances (than on linkage maps)**
  - Chromosome breakage is random, no hot spots, interference, or gender-specific differences
- **RH maps are a bridge between linkage maps and contig maps --> sequence-ready maps**

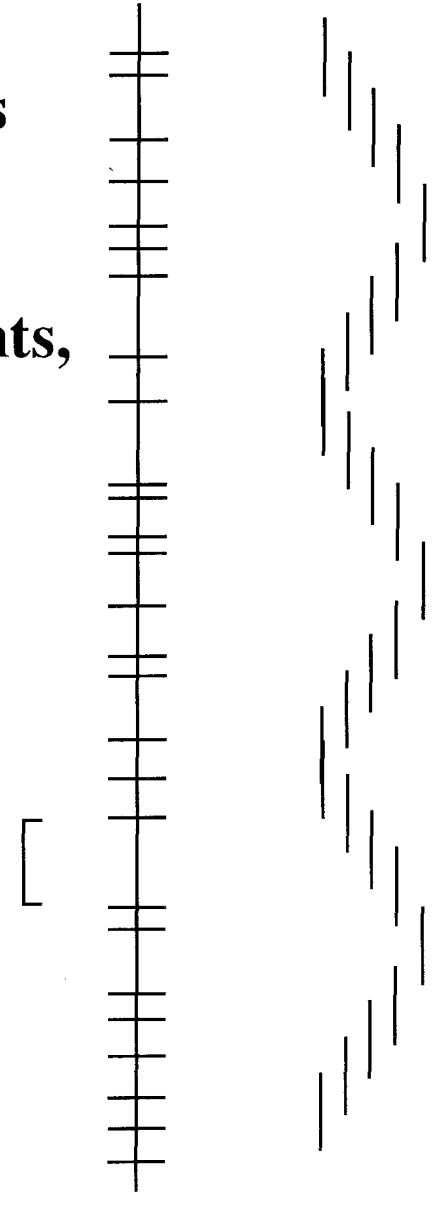
Ideal Sequence-ready Map

ordered markers

<u>polymorphic:</u>
anonymous segments,
genes

<u>monomorphic:</u>
cDNAs/ESTs

100 kb
average
resolution



Ordered
DNA
clones

RH Background

New method for mapping genes in human chromosomes.

S. Goss and H. Harris. Nature, 1975

"If human cells are subjected to large doses of ionising radiation and then fused with rodent cells, hybrid clones are obtained in which linked human genes may be segregated. By measuring the frequency with which pairs of linked genes are cotransferred after irradiation, it is possible to determine the linear order of groups of genes and to estimate the distances between them. This technique offers a new and systematic approach to the mapping problem."

1977 Goss and Harris, 4 genes mapped on X chromosome
1990 D. Cox et al: Methods and chromosome 21.
1992 Chromosomes 5, 11 and 21, and additional methods papers.

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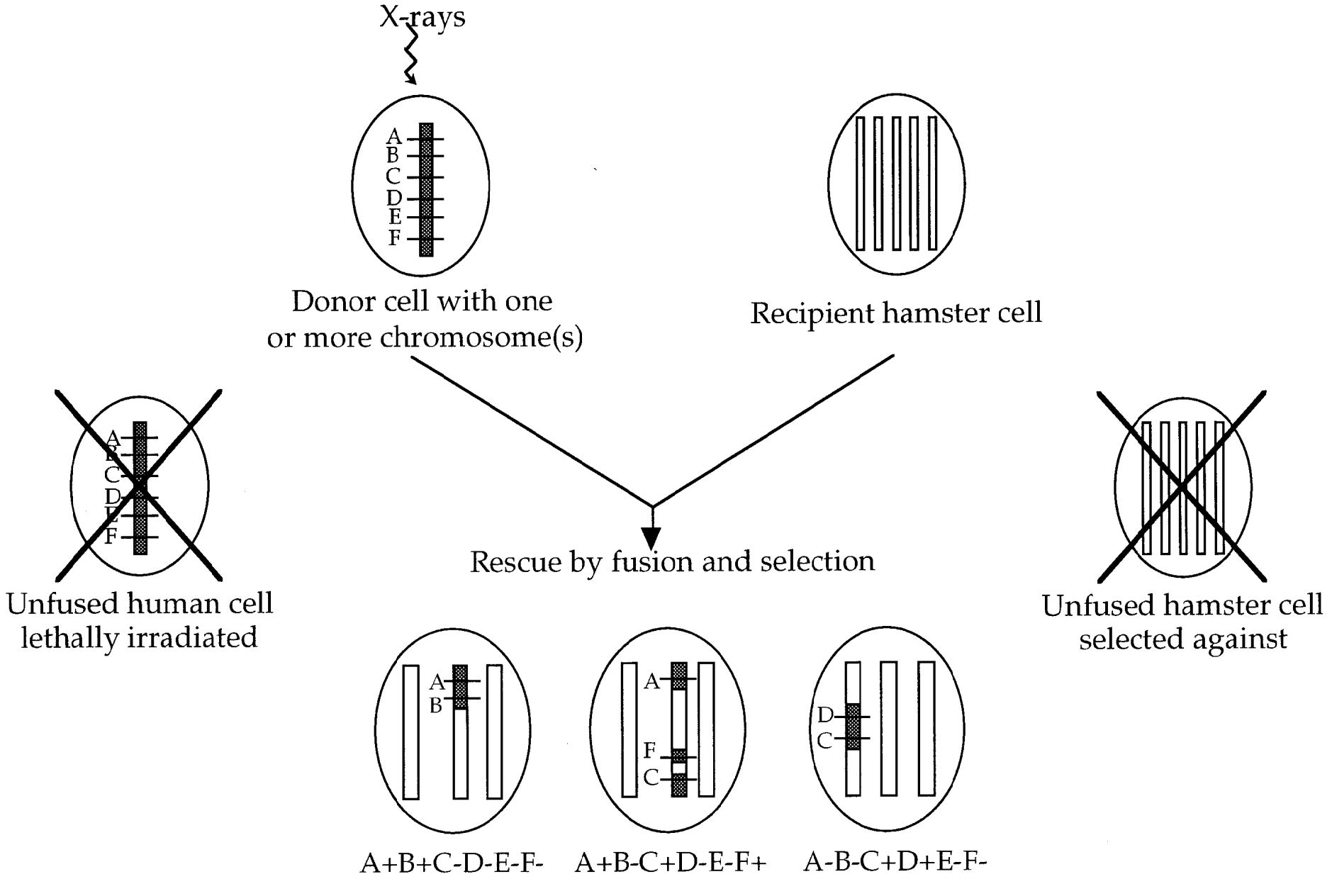
1995-97 Genome-wide radiation hybrid maps

RH Basics

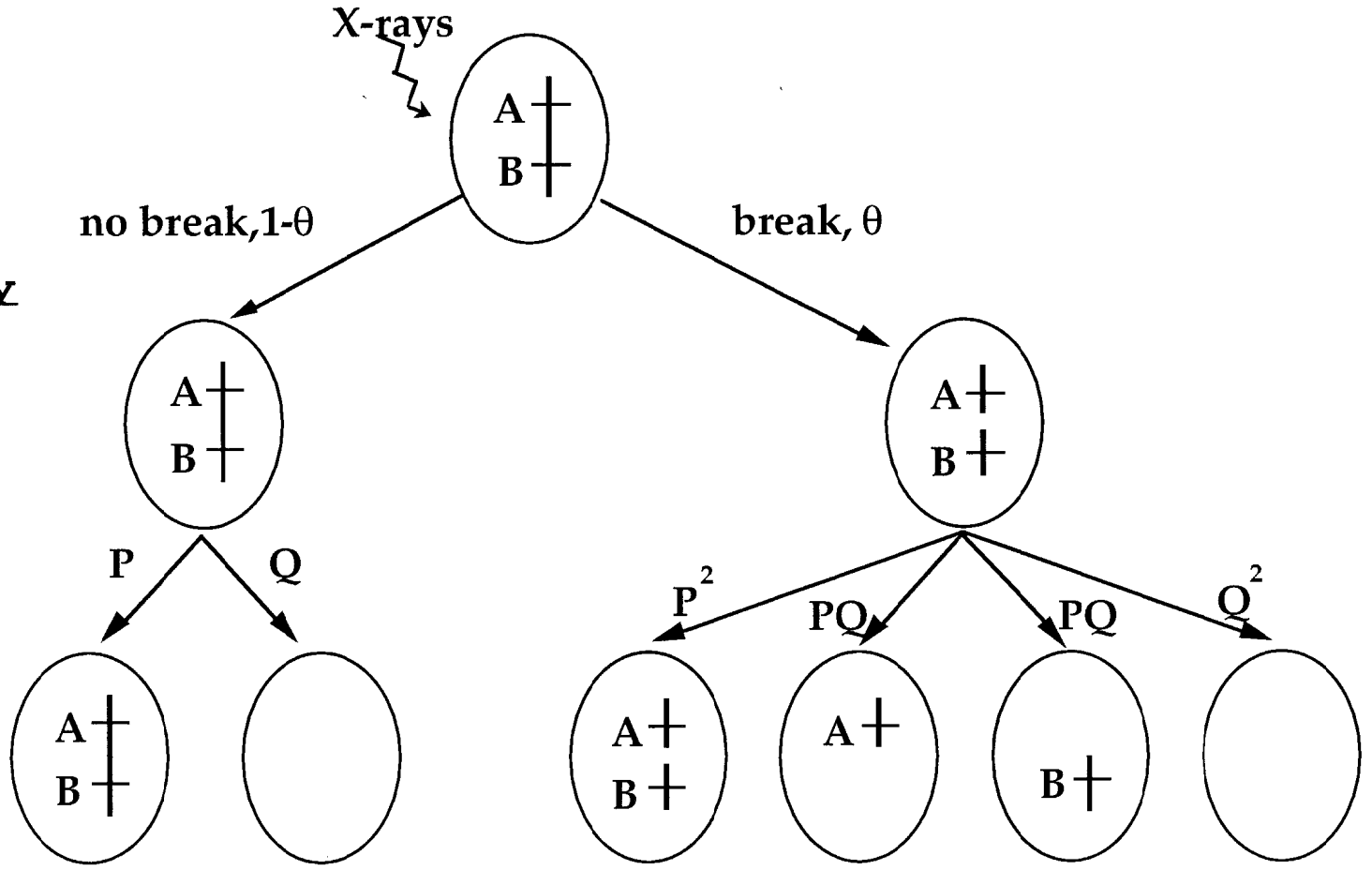
- **Donor chromosomes are exposed to radiation**
- **Each chromosome is broken into multiple fragments**
- **Human cells are fused with hamster cells**
  - each human-hamster fusion cell (radiation hybrid) will contain a random selection of human chromosome fragments
- **Neighboring markers**
  - are less likely to be separated by a chromosomal break
  - likely to be retained or lost together on a single fragment, i.e. linked
- **Distant markers**
  - are likely to be separated by at least one break
  - will be retained or lost independent of one another, i.e. unlinked

Radiation Hybrid Construction



Radiation Hybrid Outcomes

A	B	Probability
+	+	$P(1-\theta)+P^2\theta$
+	-	$PQ\theta$
-	+	$PQ\theta$
-	-	$Q(1-\theta)+Q^2\theta$



RH Basics

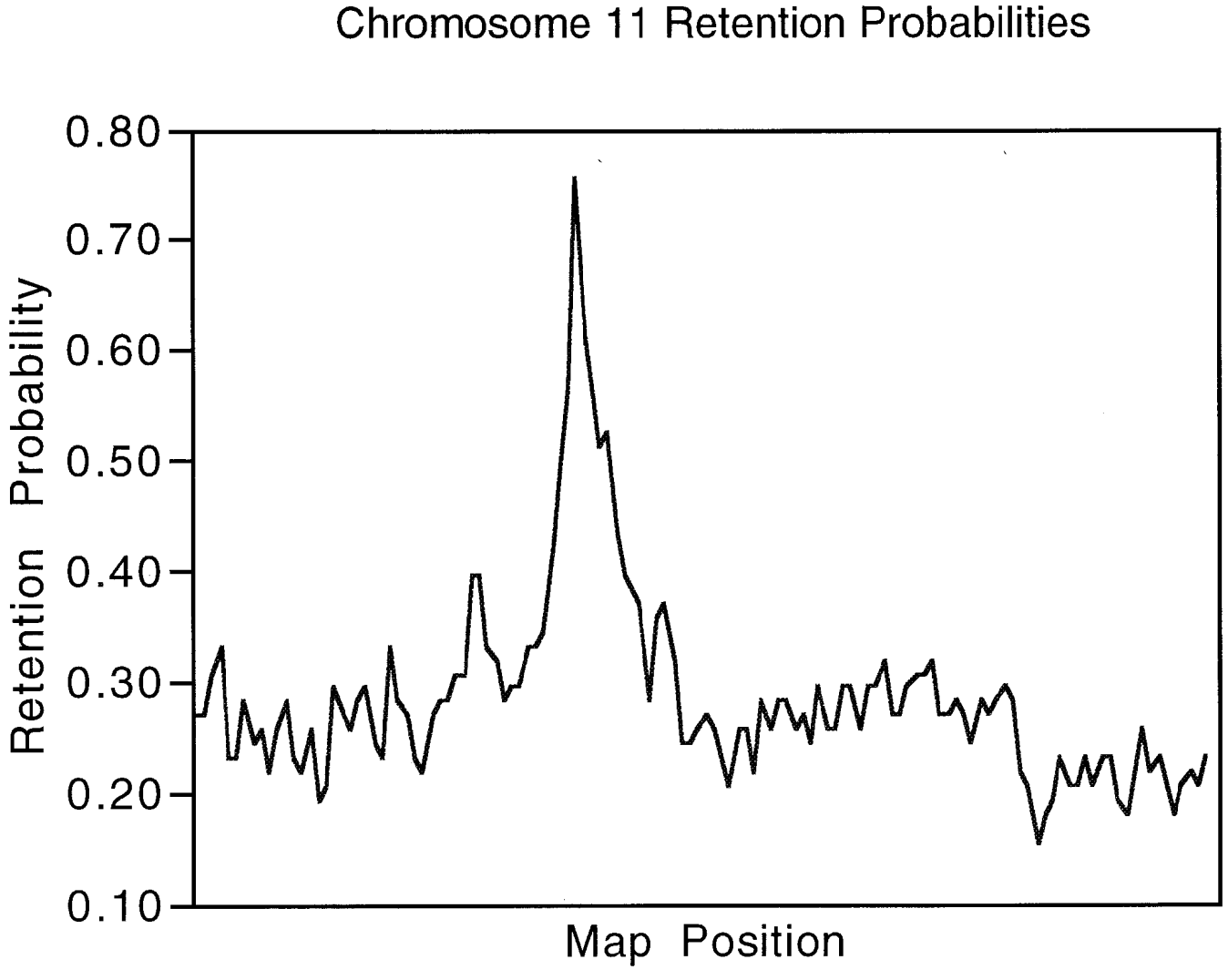
- **The RHs are screened using PCR for presence or absence of markers**
- **Patterns of co-retention are analyzed**
  - used for marker ordering and distance estimation

	A	B	C	D	E	F	G	H	I
hyb1	+	+	+	−	−	−	−	−	−
hyb2	+	+	+	−	−	−	−	+	−
hyb3	−	+	+	+	+	+	+	+	+
hyb4	+	+	+	+	−	−	−	−	−
hyb5	+	+	+	−	−	+	+	+	+
hyb6	−	−	−	−	−	−	−	+	+
hyb7	−	−	−	−	−	−	+	+	+
hyb8	−	−	−	+	+	+	+	+	+
hyb9	+	+	−	−	−	−	−	−	−
hy10	−	−	+	+	+	+	+	+	+

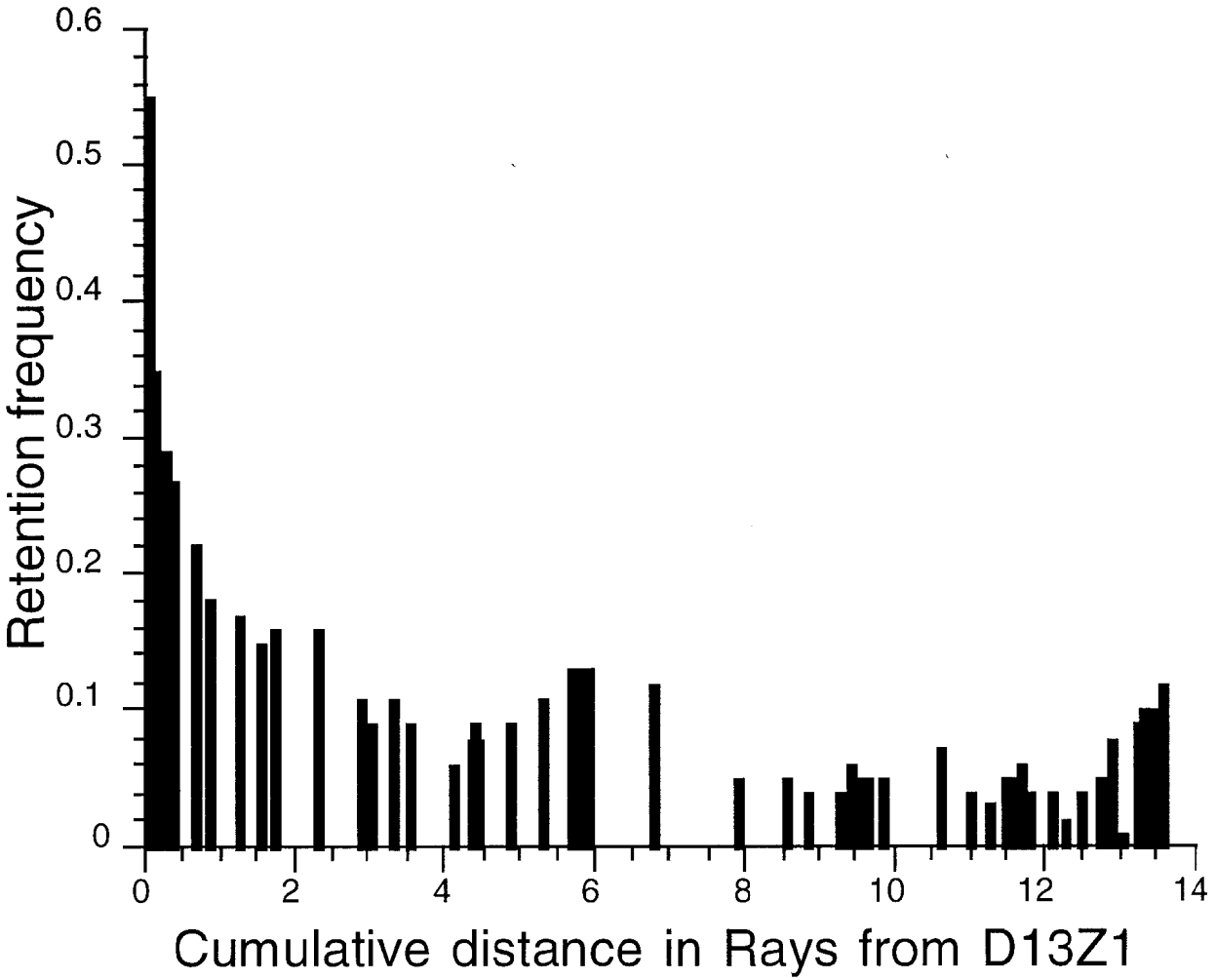
RH Mapping Theory

- **Probability of retention (P)**
  - probability that a DNA segment is present in an RH clone
  - proportion of clones containing a specific DNA segment
  - average proportion of target chromosome present in any RH clone
  - function of the radiation dose and cell lines used
  - usually ranges from 10-50%, 100% near selectable marker
  - maximum mapping power occurs when P = 50%
  - assume P is constant over small regions or entire chromosome
  - assume segments are lost or retained independently of one another

Chromosome 11 Retention



Chromosome 13 Retention



RH Mapping Theory

- **Breakage Probability (θ)**
  - probability that 2 markers are separated by 1 or more breaks
  - $0 \leq \theta \leq 1$, completely linked to unlinked
  - non-additive measure of physical distance
    - can't determine the actual number of breaks
  - function of radiation dose and cell lines used
- **Map function**
  - map distance $D = - \log_e(1- \theta)$ Rays
  - assumes the number of breaks is determined by a Poisson distribution with mean D
  - analogous to Haldane's map function for genetic mapping
- **Map distance (Ray, centiRay)**
  - 1 cR = 1% frequency of breakage
  - e.g. $\theta = 0.1$ = 11 cR, 0.3 = 36 cR, 0.6 = 92 cR

RH Mapping Theory

- **Conversion to physical distance**
  - use the map function to convert to additive cR units
  - determine relationship between cR and kb:
    - compare cR distance between markers of known physical distance, scale appropriately
    - eg.: chr11q, 1618 cR over 86 mb, 1cR=53 kb
  - dependent on radiation level
  - examples (rough estimates):
    - $1cR_{3000} \approx$ 300 kb
    - $1cR_{9000} \approx$ 50-70 kb
    - $1cR_{10000} \approx$ 25 kb
    - $1cR_{50000} \approx$ 4 kb
    - 1 cM $\approx$ 1 mb
- **Map resolution**
  - average distance between markers
  - function of radiation level, cell lines, mapping approach

Analysis of Co-retention

- Distance between markers is used to determine order
- Simple example: 3 markers, A, B and C
- A - B = 500 kb, B - C = 300 kb, A - C 800 kb
- Probable order: A-B-C
- In RHs, distance is estimated by the frequency of breakage between 2 markers.
- Simplest case: 2 markers
- Can't determine distance directly, so estimate distance, likelihood and lod score.

Likelihood and Lod Score

- Estimate of theta is most accurate for a large sample size
- This estimate of theta does not indicate the sample size
- Need a statistic to indicate the level of support
- For two-point analysis, we compare the likelihood of the data at the estimated theta to the likelihood of being completely unlinked, theta = 1. This is measured by a lod score.

Likelihood

"Within the framework of a statistical model, a particular set of data supports one statistical hypothesis better than another if the likelihood of the first hypothesis, on the data, exceeds the likelihood of the second hypothesis."

From the book "Likelihood"- A.W.F. Edwards

$$L = \prod_{i=1}^{k} p_i^{a_i}$$

- L=likelihood, of *k* categories, each with *a* observations and probability *p*

$$LL = \log L = \sum_{i=k}^{k} a_i \log p_i$$

- LL=log likelihood

$$\frac{dLL}{d\theta} = 0, \text{solve for} \theta = \hat{\theta}$$

- Maximum likelihood estimate of θ: find that value of θ that maximizes the LL.

$$Z(\theta) = LL(\hat{\theta}) - LL(1.0)$$

- $Z(\theta)$= lod score = relative difference in likelihood of H_1 (linked) vs H_0 (unlinked)

Notation

Observation	Obs. number	Probability
A+B+	a	$P(1-\theta)+P^2\theta$
A+B-	b	$PQ\theta$
A-B+	c	$PQ\theta$
A-B-	d	$Q(1-\theta)+Q^2\theta$

- n = number of RHs = a+b+c+d
- P = retention frequency = average of P_A (A+/n) and P_B (B+/n)
- Q =1-P

Likelihood and Distance Estimation

$$L = \left[P\left(1-\theta\right)+P^{2}\theta \right]^{a} \bullet \left(PQ\theta\right)^{b} \bullet \left(PQ\theta\right)^{c} \bullet \left[Q\left(1-\theta\right)+Q^{2}\theta \right]^{d}$$

$$LL(\theta) = a\log\{P(1-Q\theta)\} + (b+c)\log\{PQ\theta\} + d\log\{Q(1-P\theta)\}$$

$$\hat{\theta} = \frac{\left(n-aP-dQ\right) - \sqrt{\left(n-aP-dQ\right)^{2} - 4nPQ\left(b+c\right)}}{2nPQ}$$

Likelihood Examples

- **Example**
  - 94 hybrids scored for markers A and B
  - a=#(A+B+)=34
  - b=#(A+B-)=2
  - c=#(A-B+)=1
  - d=#(A-B-)=57
  - P_A =36/94 = 0.38, P_B =35/94 = 0.37, P(avg)=0.38

$\hat{\theta} = 0.068$

LL(0.07) = -32.8,
LL(1.0) = -54.1
Z(0.07) = LL(0.07) - LL(1.0) = 21.3

- **Lod score > 3 is significant in genetic linkage analysis**
- **Interpretation:** A and B are linked with $\theta = 0.07$ and a lod score of 21.3

Multipoint Likelihoods

- The θ and likelihood computations for a map of 3 or more markers are more complicated.
- Must compare the likelihoods of all possible orders to have best chance of finding correct order
- Shortcuts are used to reduce the # of comparisons
- Example: B - C - D are ordered, add A

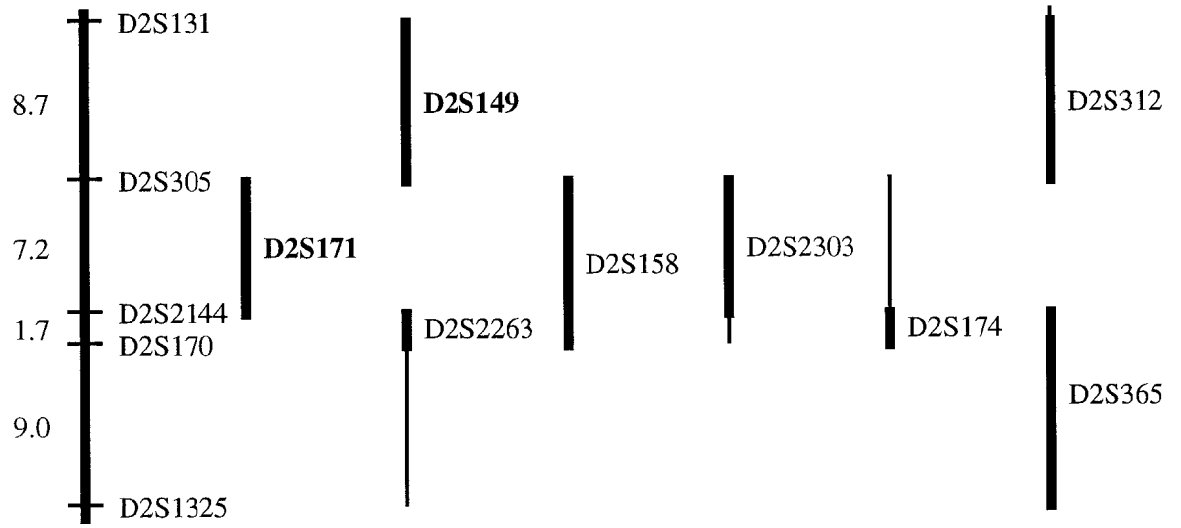
Map	$\log_{10}$Likelihood(LL)	LL diff.	odds
A-B-C-D	-45.3	-	1
B-**A**-C-D	-47.2	1.9	79
B-C-**A**-D	-48.4	3.1	1259
B-C-D-**A**	-48.6	3.3	1995

Approaches to Mapping

- **Mapping "From scratch"**
  - no prior information available on marker order
  - less efficient, more error prone
- **Expanding a framework map**
  - marker order known for a subset of markers (may have a framework or skeletal map)
  - saves mapping time, helps reduce error
- **Mapping by linkage groups**
  - small sets of markers mapped separately
  - separate maps linked by statistical analysis or according to other maps (genetic, RH framework, etc)

Expanding a Framework, A.

- The order of 5 markers is known:
  - S131-S305-S2144-S170-S1325
- Identify the relative locations of the remaining 8 markers

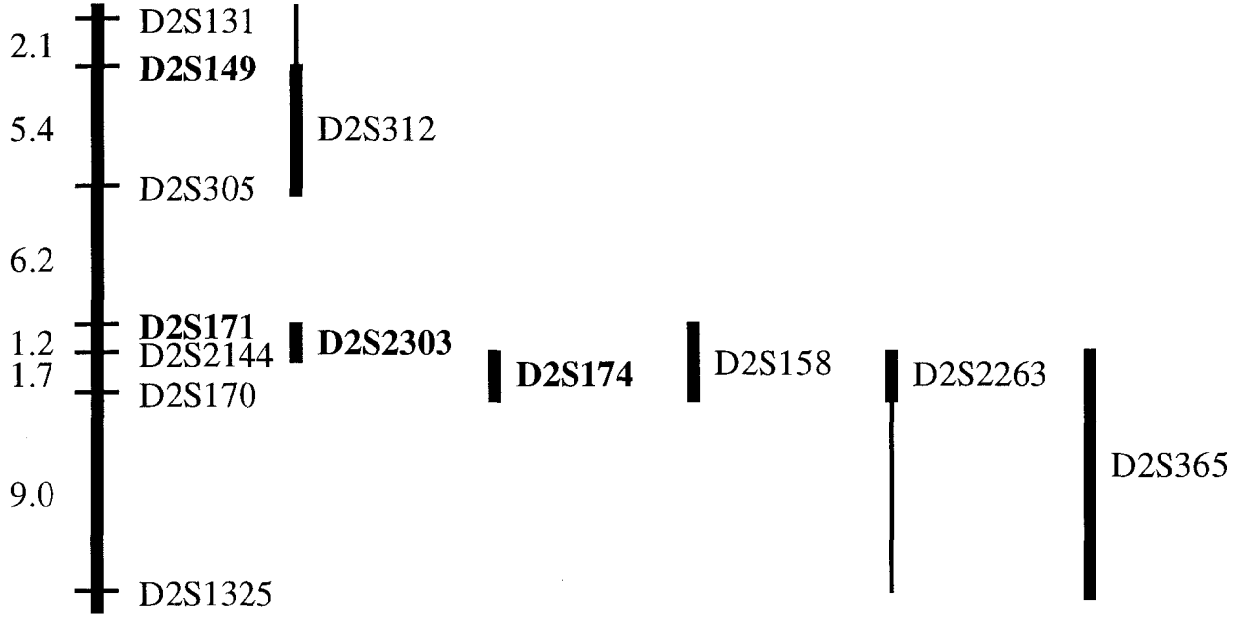


Legend: The backbone of known order is shown on the left. The bars next to the remaining markers spans their respective 1000:1 odds support intervals. The thicker part of the support bars spans the more likely map interval(s) for each marker.

- 2 markers, D2S149 and D2S171, each map to a single backbone interval with odds > 1000:1 (lod > 3).
- The "lod 3 " positions of the remaining markers span more than one backbone interval.

Expanding a Framework, B.

- **D2S149 and D2S171 can be added to the backbone map. This change could affect the map, so the remaining markers are again localized relative to the backbone.**



- **Now, 2 more markers, D2S2303 and D2S174, map to a single backbone interval with odds > 1000:1 (lod > 3).**

Expanding a Framework, C.

- **D2S2303 and D2S174 can be added to the backbone map. Again, this change could affect the map, so the remaining markers are localized relative to the backbone.**

- **The 1000:1 odds or lod 3 map is now complete, since no additional markers can be added to the backbone map.**
- **D2S365 is equally likely to lie on either side of D2S170. They are probably very tightly linked or have no breaks or recombination between them.**

Protocol for Mapping by Linkage Groups

- **identify linkage groups**
  - each marker in a linkage group is linked to at least one other marker in the same group.
- **map each linkage group**
  - do not allow any intervals with $\theta > 0.4$ or 0.5
- **validate order with local permutation of markers**
  - A-B-C-D vs. B-A-C-D vs. A-C-B-D vs. A-B-D-C
- **merge individual maps**
  - compare likelihoods of all possible orders and orientations, or use order information from other sources
- **localize any remaining markers on the map**

Haploid vs. Diploid RHs

- **Monochromosomal RHs**
  - donor is a haploid somatic cell hybrid containing a single human chromosome
  - requires about 100 hybrids to map one chromosome
  - relatively expensive (per chromosome)
- **Whole genome (WG-RHs)**
  - donor is a diploid human fibroblast
  - requires about 100 hybrids to map all chromosomes
  - relatively inexpensive
  - can be analyzed by conventional haploid methods
    - should obtain correct order
    - may increase distance estimates if not accounted for
    - faster if analysed by haploid methods
- **Polyploid RHs**
  - Lunetta et al, 1995, haploid or diploid panels are pooled to increase retention prob.

Available RH Mapping Programs

- **Several are publicly available**
- **Learning curve varies**
- **User-friendliness varies**
- **None particularly easy to use**
- **Best program depends on:**
  - how many markers
  - region of chromosome being mapped
  - desired level of "accuracy"
  - level of computer expertise
- **Consider a mapping server**
  - appropriate if you have only a few markers and/or only need global positioning

RHMAP

- **M. Boehnke, K. Lange, K. Lunetta - U. of Michigan, 91-96**
- **Two analysis approaches:**
  - minimization of breaks, maximum likelihood
- **Three mapping approaches:**
  - stepwise, simulated annealing, branch-and-bound
- **multiple retention models:**
  - equal, centromeric, general, selected-locus
- **efficient for small numbers of markers**
- **includes error detection routines**
  - errors can complicate ordering, increase distance estimates
  - recommend typing in duplicate, calling discrepancies "unknown"
- **haploid, diploid and polyploid (pooled) models**
- **machines: UNIX, DOS, VMS - requires FORTRAN 77**
- **http://www.sph.umich.edu/group/statgen/software/**
- **excellent documentation**

MultiMap

- T. Matise, A. Chakravarti, C. Kashuk - U. Pittsburgh, 91-96
- highly automated RH and linkage map construction
- maximum likelihood approach
- retention model: equal
- efficient for many markers
- full likelihood analysis of missing data
- machines: UNIX based - uses LISP, C++
- documentation for RH mapping available but still under development
- detailed documentation for linkage mapping available
- http://linkage.rockefeller.edu/multimap

RHMAPPER

- D. Slonim, L. Stein, unpublished, Whitehead Institute, 95
- maximum likelihood approach
- user writes PERL scripts to aid automation
- retention model: equal
- accommodates many markers
- detection of and allowance for typing errors
- detailed documentation available
- machines:UNIX-based - uses PERL, C
- http://www-genome.wi.mit.edu/ftp/distribution/software/rhmapper/

MAP

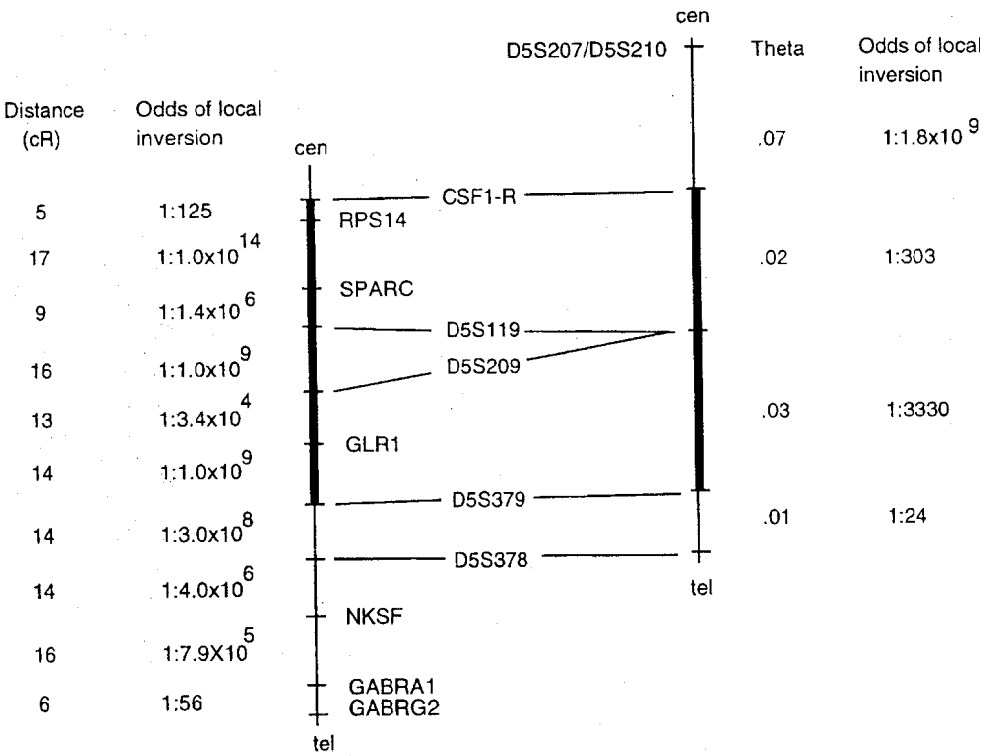
- **S. Lawrence, N. Morton, J. Teague - U. Southampton, 91-97**
- **multiple-pairwise method**
  - pairwise methods less sensitive to typing errors
  - pairwise methods have less statistical power for correct ordering
- **retention model: constant or based on distance from centromere**
- **links to the location database (LDB) for map integration**
- **machines: UNIX - requires C**
- **http://cedar.genetics.soton.ac.uk/public_html/programs.html**

Hyperekplexia

- **"Startle disease" (1958)**
  - neonatal muscular rigidity
  - startle-induced muscle contractions, may cause falling
  - responds to clonazepam,via enhanced GABA neurotransmission
- **Linkage found to chromosome 5q (1992)**
  - growth factor and GF receptor genes, hormone receptor
  - neurotransmitter receptors: GABRA1, GABRG2
- **Closest flanking markers identified through linkage**
- **An RH map of the region was constructed**
  - several genes excluded: GABRA1, GABRG2,ADRB2, DRD1
  - several genes confirmed in candidate region:
    GLUR1, SPARC, **GLRA1**, ANX6, CSF1R, RPS14
- **Spastic mutant mouse model supported GLRA1**
- **Point mutations subsequently identified in humans**

Hyperekplexia

1992



1993

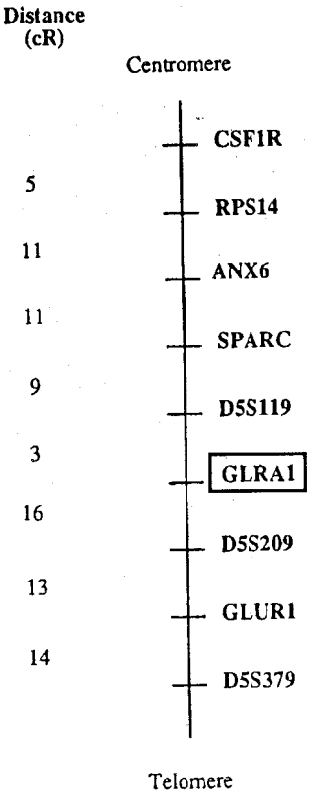


Figure 2 RH (*left*) and genetic (*right*) maps of the *STHE* region on chromosome 5q. The heavy black lines represent the *STHE* candidate region as defined by location score analysis.

Spinal Muscular Atrophy

- **2nd most common lethal autosomal recessive disease**
- **motor-neuron degenerative disease, muscle wasting**
- **linkage found to chromosome 5q11.2-13**
- **closest flanking markers indentified via linkage analysis**
- **RH map constructed of the candidate region**
  - A new polymorphic marker was detected, which subsequently reduced the candidate region by 200 kb
  - New monomorphic markers found, some of which were used to help identify additional polymorphic loci
  - Obtained a 1 mb DNA fragment from one hybrid that spans the region, used to isolate useful cosmids
  - Estimation of the size of the SMA region
  - Exclusion of nearby genes

Spinal Muscular Atrophy

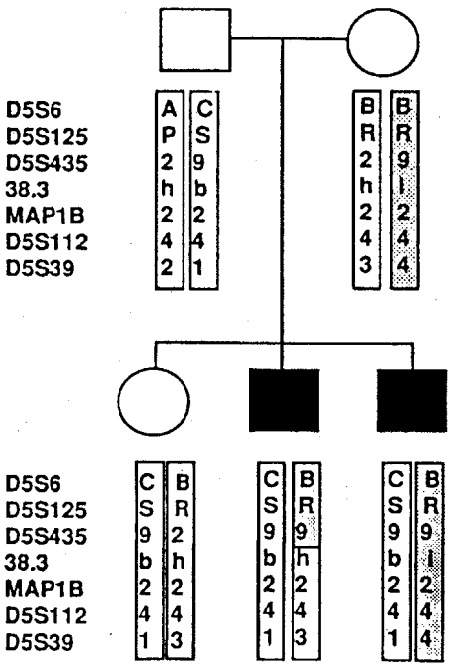
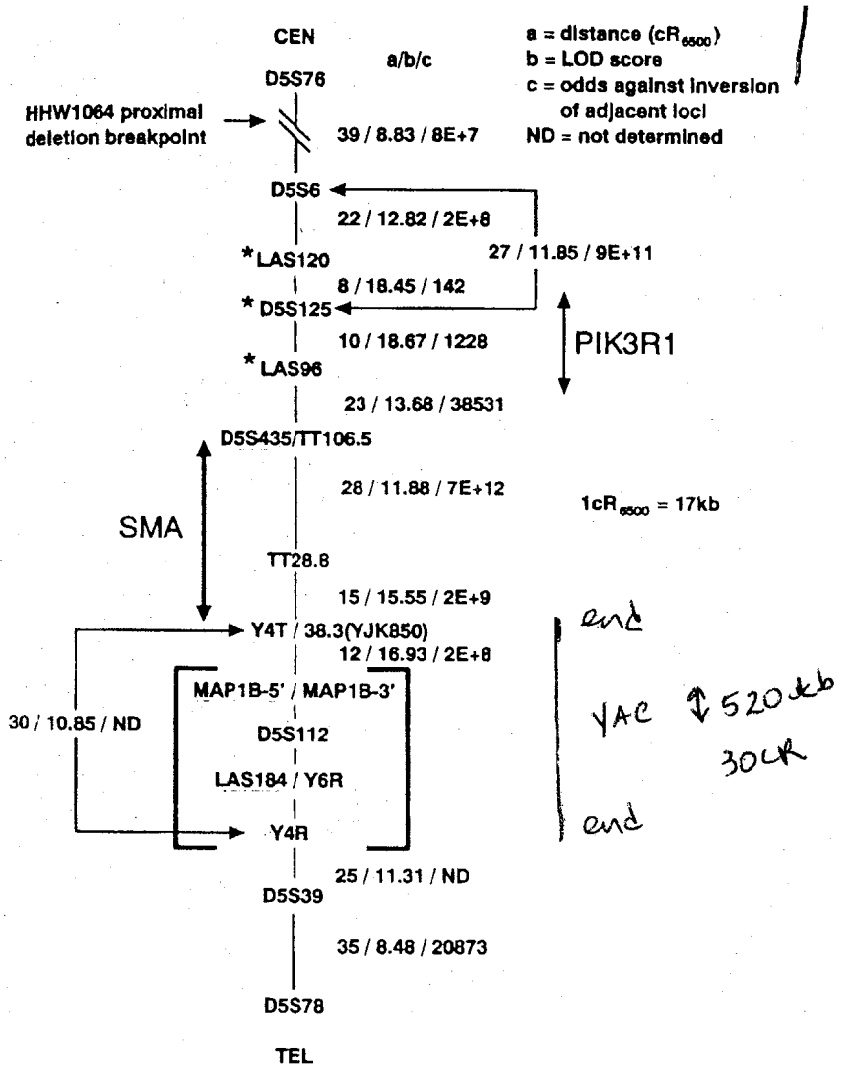


Figure 2. Genotypes of members of a type II SMA family for loci around the SMA gene. The affected sibling shown in the middle has a recombination between D5S435 and 38.3 which places the SMA gene proximal to 38.3.



Additional Disease Genes

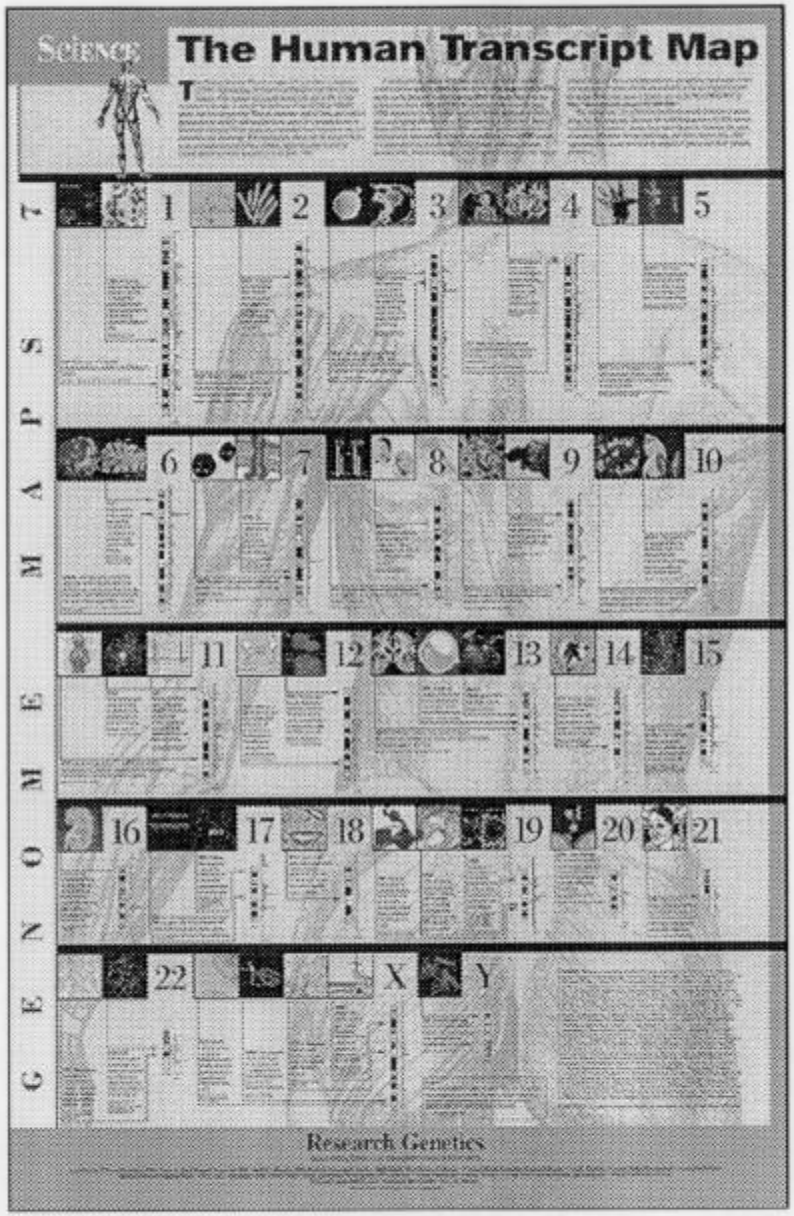
- Hirschsprung's Disease
- Ataxia Telangiectasia
- Pycnodysostosis
- Treacher-Collins Disease
- Breast Cancer - BRCA1
- Familial Cardiomyopathy
- Neurofibromatosis Type II
- Huntington Disease

The Human Transcript Map

- **A Gene Map of the Human Genome, Science 274, 540-546**

- Schuler, G. D., Boguski, M. S., Stewart, E. A., Stein, L. D., Gyapay, G., Rice, K., White, R. E., Rodriguez-Tom, P., Aggarwal, A., Bajorek, E., Bentolila, S., Birren, B. B., Butler, A., Castle, A. B., Chiannilkulchai, N., Chu, A., Clee, C., Cowles, S., Day, P. J. R., Dibling, T., Drouot, N., Dunham, I., Duprat, S., East, C., Edwards, C., Fan, J.-B., Fang, N., Fizames, C., Garrett, C., Green, L., Hadley, D., Harris, M., Harrison, P., Brady, S., Hicks, A., Holloway, E., Hui, L. , Hussain, S., Louis-Dit-Sully, C., Ma, J., MacGilvery, A., Mader, C., Maratukulam, A., Matise, T. C., McKusick, K. B., Morissette, J., Mungall, A., Muselet, D. , Nusbaum, H. C., Page, D. C., Peck, A., Perkins, S., Piercy, M., Qin, F., Quackenbush, J., Ranby, S., Reif, T., Rozen, S., Sanders, C., She, X., Silva, J., Slonim, D. K., Soderlund, C., Sun, W.-L., Tabar, P., Thangarajah, T., Vega-Czarny, N., Vollrath, D., Voyticky, S., Wilmer, T., Wu, X., Adams, M. D., Auffray, C., Walter, N. A. R., Brandon, R., Dehejia, A., Goodfellow, P. N., Houlgatte, R., Hudson Jr., J. R., Ide, S. E., Iorio, K. R., Lee, W. Y., Seki, N., Nagase, T., Ishikawa, K., Nomura, N., Phillips, C., Polymeropoulos, M. H., Sandusky, M., Schmitt , K., Berry, R., Swanson, K., Torres, R., Venter, J. C., Sikela, J. M., Beckmann , J. S., Weissenbach, J., Myers, R. M., Cox, D. R., James, M. R., Bentley, D., Deloukas, P., Lander, E.S. and Hudson, T.J.

- **> 16,000 human ESTs placed on a 1000 marker framework**



http://www.ncbi.nlm.nih.gov/SCIENCE96/

RH Mapping in Other Organisms

- Mouse
- Rat
- Pig
- Baboon
- Zebrafish
- Dog*
- Chicken*

RH Maps and Gene Hunting

RHs are useful for:

- **constructing high resolution maps of candidate region**
  - genetic markers and ESTs can be placed relative to each other on the same map
- **resolving questionable or unknown marker orders**
- **identifying additional useful markers**
  - polymorphic markers that can be used in linkage analysis
  - monomorphic markers that can be used to direct production of polymorphic markers
- **confirming existing maps**
- **excluding or including neighboring candidate genes**
- **estimating the physical distance of a region of interest**
- **isolating a DNA fragment spanning region of interest**

How Can You Use RHs?

- **Suppose you want a fine resolution map of a specific genomic region**
- **Scan publications and the Internet for useful information**
  - chromosome specific sites
  - genomic map resources
  - disease related sites
- **Identify best available maps**
- **Identify additional useful STSs/markers**
  - identify them from available maps
  - generate them from relevant YAC clones
- **Screen additional STSs against an RH panel**
- **Submit to an RH server for gross location verification**
- **Download additional RH mapping data for the region**
- **Use available programs to construct a fine map**

RH Mapping Resources
Commercial WG-RH Panels, Research Genetics

- **Stanford G3 - D. Cox**
  - number of hybrids: 83
  - average retention probability: 18%
  - average fragment size: 4 mb
  - radiation dose: 10,000 Rads
- **Genebridge 4 - Goodfellow and Weissenbach**
  - number of hybrids: 92
  - average retention probability: ~30%
  - average fragment size: ~10 mb
  - radiation dose: 3,000 Rads
- **Stanford TNG4 (The Next Generation) - D. Cox**
  - number of hybrids: 90
  - average retention probability: 16%
  - average fragment size: 800 kb
  - radiation dose: 50,000 Rads

RH Map Resources

- **Whitehead Institute**
  - Genetic, RH (GB4), STS-content, Integrated
  - RH maps avg. resolution ~2.3 Mb
  - Hudson et al., 1995, http://www-genome.wi.mit.edu/
- **Genethon/Cambridge**
  - Genetic, and RH (GB4) with ESTs only
  - RH maps avg. resolution ~7.7 Mb
  - Gyapay et al., 1996, http://www.genethon.fr/genethon_en.html
- **Stanford**
  - RH - 500 kb avg. resolution and 100 kb expected
  - G3 maps avg. resolution ~1.7Mb/500 kb
  - Stewart et al., 1997, http://shgc-www.stanford.edu/Mapping/rh/
- **Human Transcript Map**
  - >16000 ESTs mapped against Genethon genetic map.
  - Framework avg. resolution ~3.5 Mb
  - Schuler et al, 1996, http://www.ncbi.nlm.nih.gov/SCIENCE96

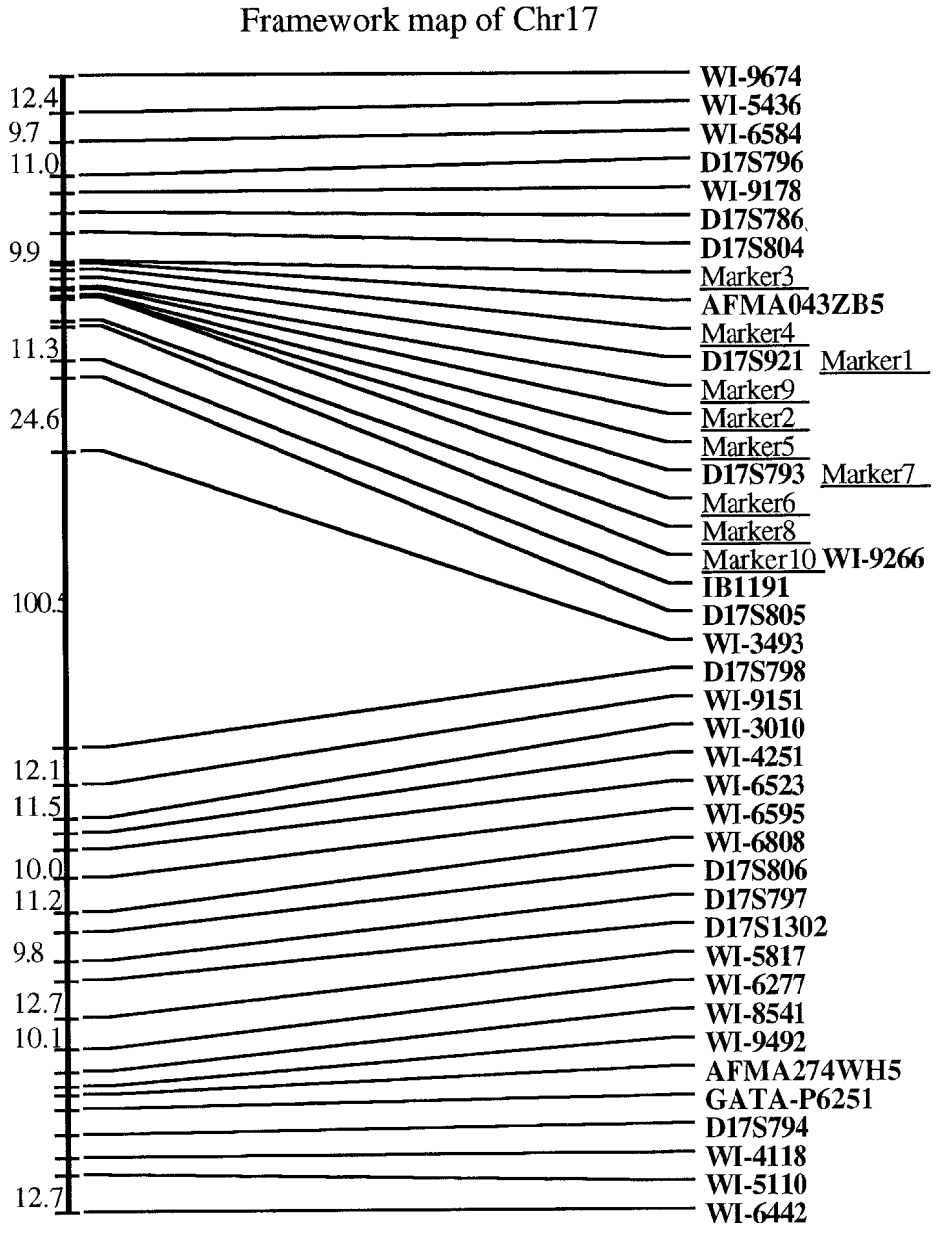
RH Map Resources

Mapping Servers and Databases

- **Stanford**
  - http://shgc-www.stanford.edu/RH/index.html
  - Submit your STS screening on their version of the StanfordG3 RH panel, server maps against their framework
- **Whitehead**
  - http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl
  - Submit your STS screening on their version of the Genebridge4 RH panel, server maps against their framework
- **RHdb**
  - http://www.ebi.ac.uk/RHdb/index.html
  - Radiation Hybrid Database - Release 10.0 - 26 September 1997

    This release contains: 7 panel entries, 75 experimental conditions, 46 maps and 70662 RH entries.

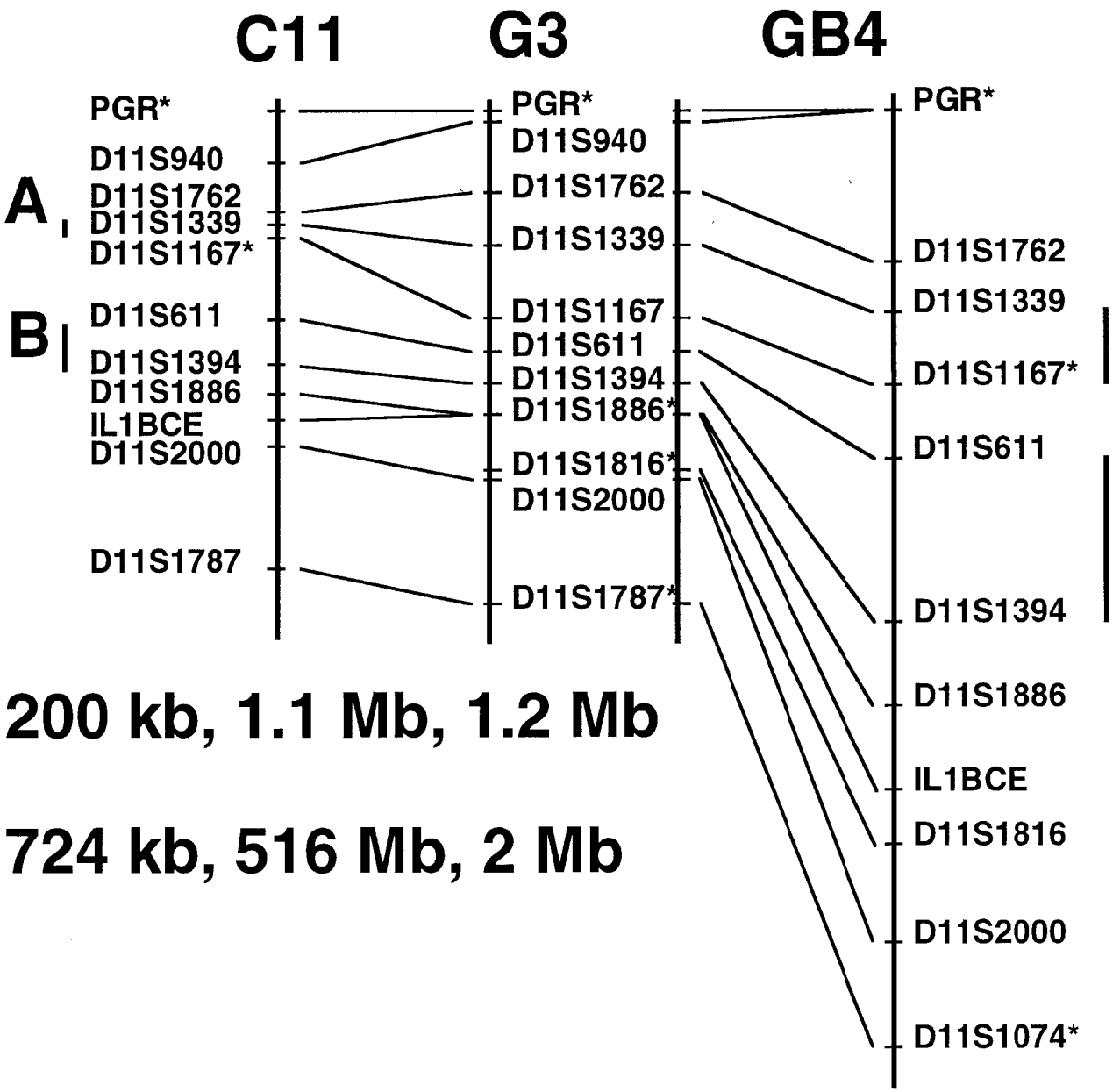
MIT-Whitehead Server Results



Current RH Research

- **Improved mapping efficiency**
  - how to rapidly and accurately map thousands of markers
- **Comparison of distance estimates**
  - relative distances can vary from one panel to another
- **Analysis of relationship between RH and physical map distances**
  - are there breakage hot/cold spots?
- **Combining multiple RH panels**
  - can this improve efficiency, resolution
- **Map integration**
  - linkage, radiation hybrid, physical

Distance Comparisons



A. 200 kb, 1.1 Mb, 1.2 Mb

B. 724 kb, 516 Mb, 2 Mb

Additional Resources

- **Likelihood analysis, lod score**
  - Analysis of Human Genetic Linkage, J. Ott
  - Handbook of Human Linkage Analysis, J. Terwilliger and J. Ott
  - Likelihood, A.W.F. Edwards
- **Map construction protocols and examples**
  - Computer program documentations
- **RH construction and screening protocols**
  - Current Protocols in Human Genetics
  - Stanford RH WWW site:

    http://shgc-www.stanford.edu/Mapping/rh/procedure/index.html

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T.C.M. - 10/2/97

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