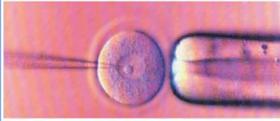


2010 2nd quarter

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Transgenic analysis

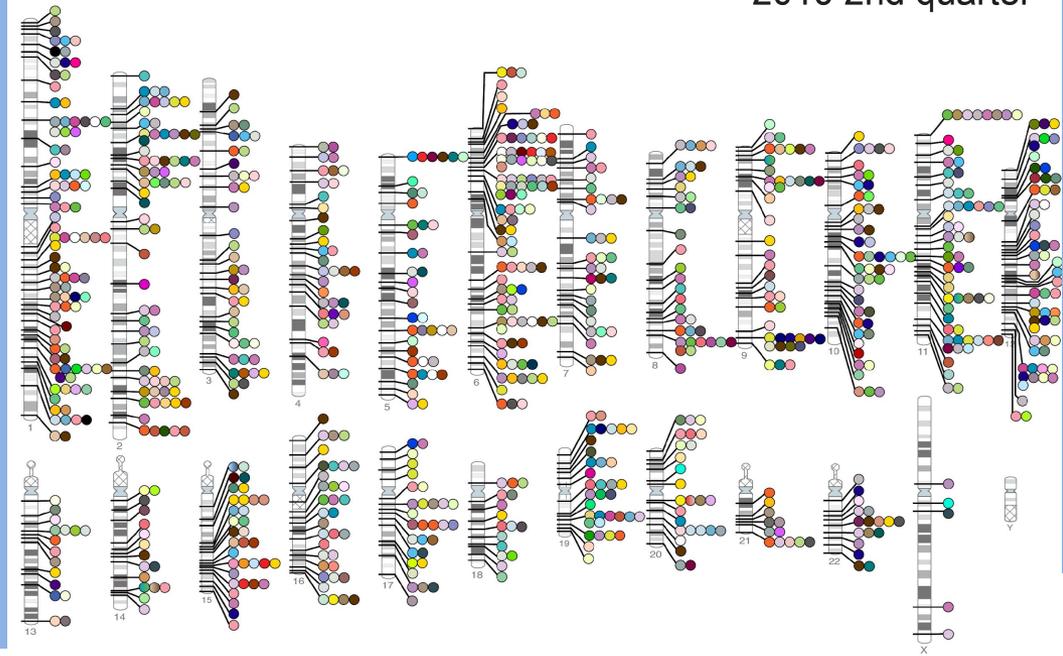


Foreign DNA Fertilized egg
♂ Pronucleus
♀ Pronucleus

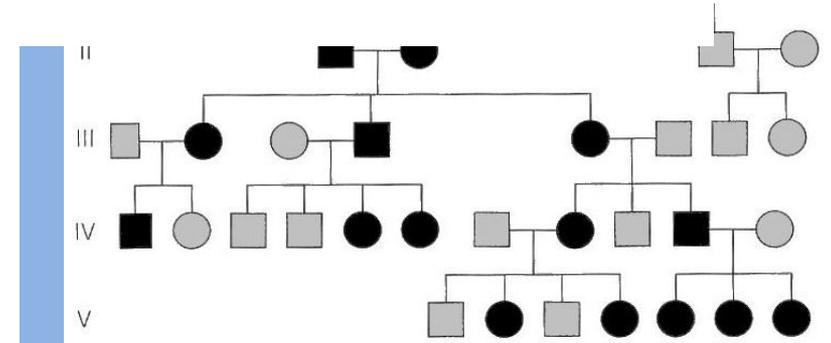
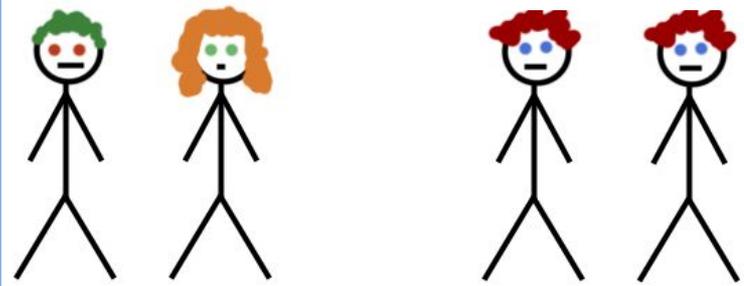
Foreign DNA injected into male pronucleus → Injected eggs surgically implanted into uterus of "foster" mother and allowed to develop. → Mice are born with foreign DNA in every cell nucleus.



(top) © Birgit Hopan, Vrije Universiteit Amsterdam
(bottom) Reproduced with permission from Dr. Robin Lovell-Badge/MRC National Institute for Medical Research

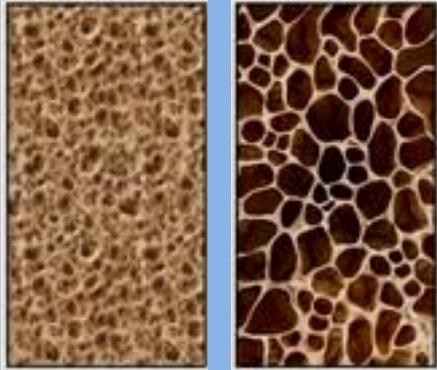


DZ Twins



Abordagens integrativas para se estudar doenças complexas

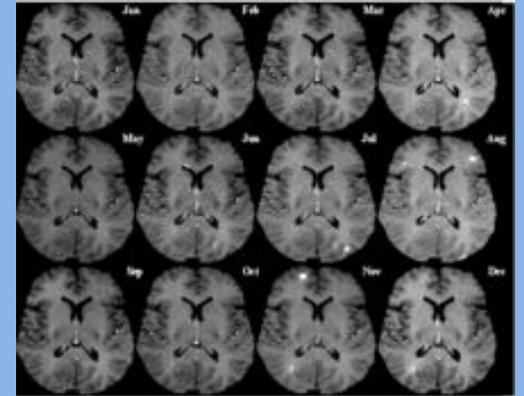
DOENÇAS COMPLEXA



osteoporos
e



lupus



esclerose
múltipla



asma



Alzheimer'
s

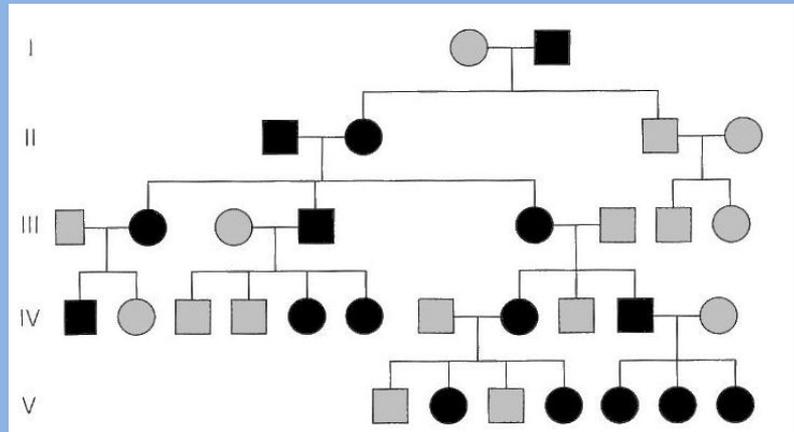


escleroderm
a

DOENÇAS COMPLEXA

Critério de diagnostico

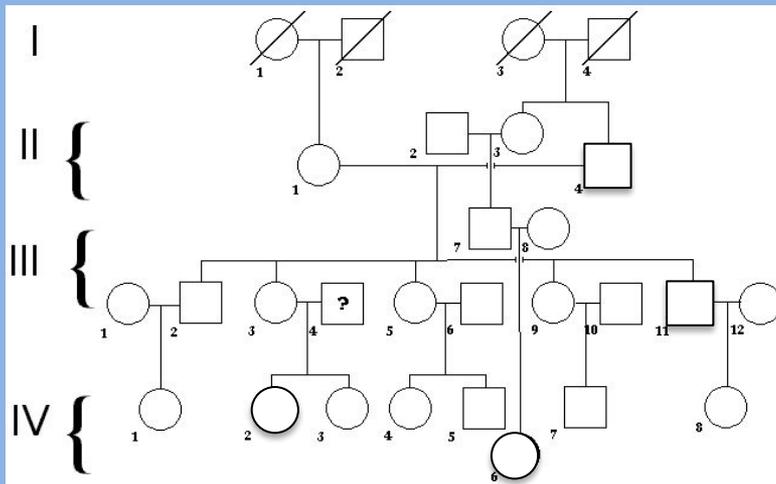
- Individuo Afetado ou Não afetado
- Doenças psiquiátricas e do comportamento



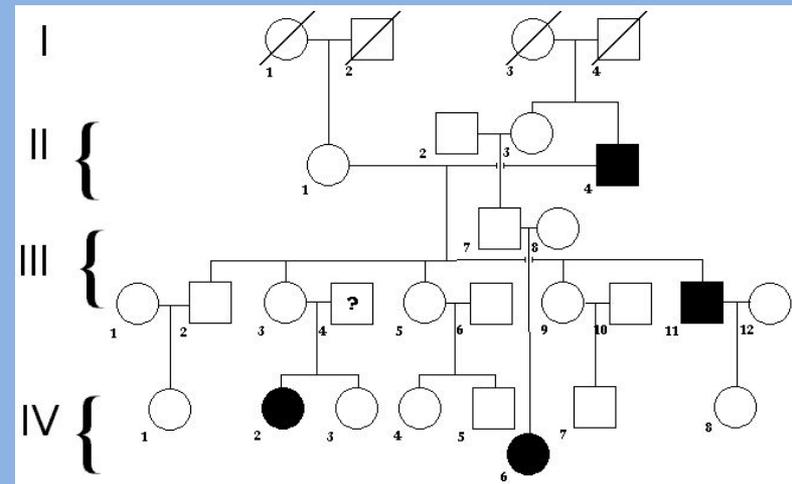
DOENÇAS COMPLEXA

Base Genética

Pessoas que compartilham DNA, tem mais chance de apresentar o mesmo fenótipo



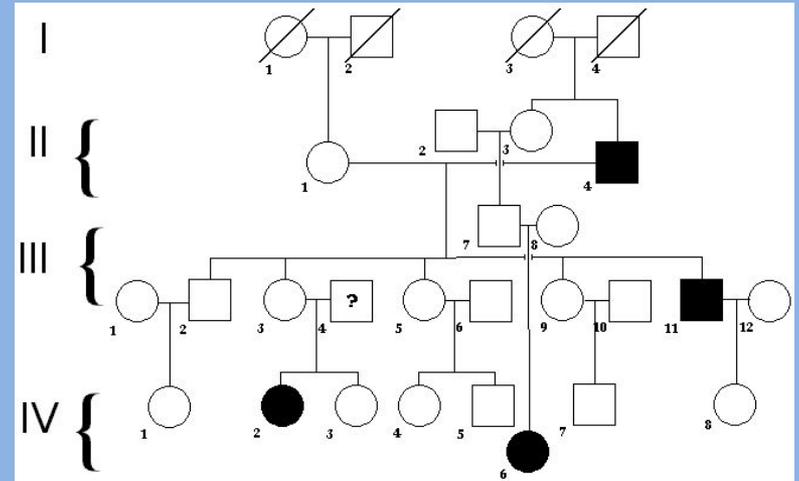
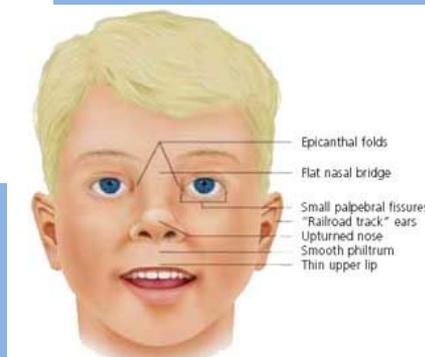
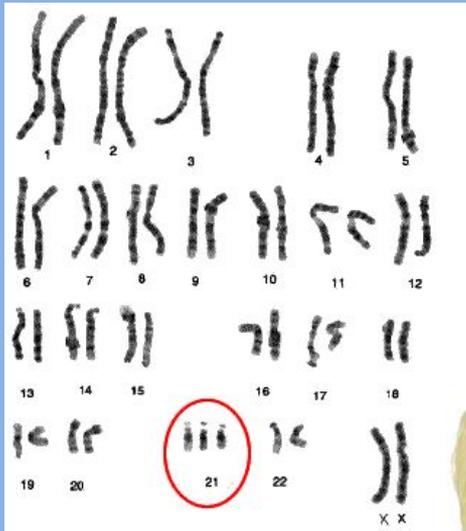
X



DOENÇAS COMPLEXA

Base Genética

Doenças com heranças mendelianas ou associadas com aberrações cromossômicas



DOENÇAS COMPLEXA

Razão de Risco

λ = risco do parente do afetado / risco na população geral

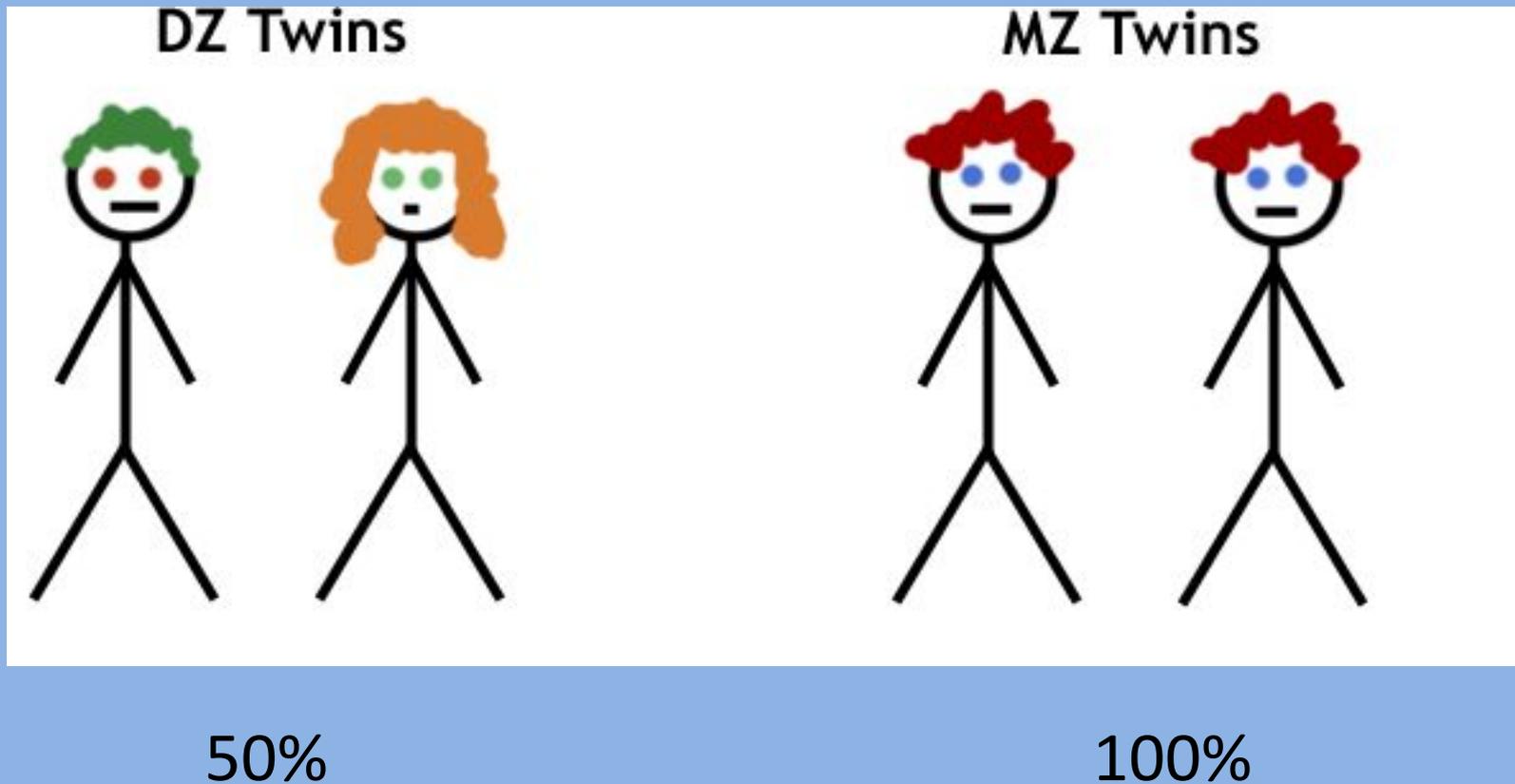
**TABLE 15.1 RISK OF SCHIZOPHRENIA AMONG RELATIVES OF SCHIZOPHRENICS:
POOLED RESULTS OF SEVERAL STUDIES**

Relative	No. at risk	Risk (%)	λ
Parents	8020	5.6	7
Sibs	9920.7	10.1	12.6
Sibs, one parent affected	623.5	16.7	20.8
Offspring	1577.3	12.8	16
Offspring, both parents affected	134	46.3	58
Half-sib	499.5	4.2	5.2
Uncles, aunts, nephews, nieces	6386.5	2.8	3.5
Grandchildren	739.5	3.7	4.6
Cousins	1600.5	2.4	3

DOENÇAS COMPLEXA

Estudo de Gêmeos

Estudo de gêmeos dizigóticos e monozigóticos



DOENÇAS COMPLEXA

Estudo de Gêmeos

Estudo de gêmeos dizigóticos e monozigóticos

Study	Country	Concordant pairs	
		MZ	DZ
Kringlen et al. (1968)	Norway	14/50 (0.28)	6/94 (0.06)
Fischer et al. (1969)	Denmark	5/21 (0.23)	4/41 (0.10)
Tienari et al. (1975)	Finland	3/20 (0.15)	3/42 (0.07)
Farmer et al. (1987)	UK	6/17 (0.35)	1/20 (0.05)
Onstad et al. (1991)	Norway	8/24 (0.33)	1/28 (0.04)

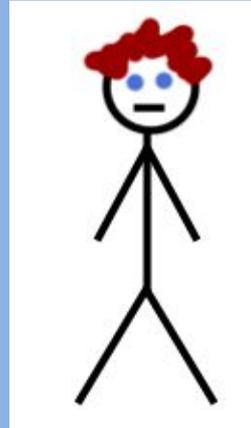
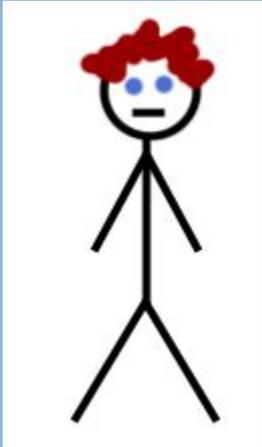
Problema

- 1) Monozigóticos tem mais chance de ter mesmo sexo
- 2) Monozigóticos são tratados mais igualmente

DOENÇAS COMPLEXA

Estudo de Gêmeos

Estudo de gêmeos monozigóticos separados ao nascimento



Problema

- 1) Tamanho amostral (N) pequeno
- 2) Separação não total
- 3) Ambiente Intra-uterino

DOENÇAS COMPLEXA

Estudo de Adoção

Estudo com pessoas adotadas



- 1) Procurar pessoas adotadas com doenças complexas e investigar se os pais biológicos ou os adotivos apresentam a mesma doença
- 2) Procurar pais adotivos com doenças complexas e investigar se os filhos adotivos apresentam a mesma doença.

DOENÇAS COMPLEXA

Estudo de Adoção

TABLE 15.3 AN ADOPTION STUDY IN SCHIZOPHRENIA

Case types	Schizophrenia cases among biological relatives	Schizophrenia cases among adoptive relatives
Index cases (47 chronic schizophrenic adoptees)	44/279 (15.8%)	2/111 (1.8%)
Control adoptees (matched for age, sex, social status of adoptive family, and number of years in institutional care before adoption)	5/234 (2.1%)	2/117 (1.7%)

Problema

- 1) Falta de histórico familiar dos pais biológicos
- 2) Semelhança de características na adoção

DOENÇAS COMPLEXA

Identificação da região do genoma

Problema

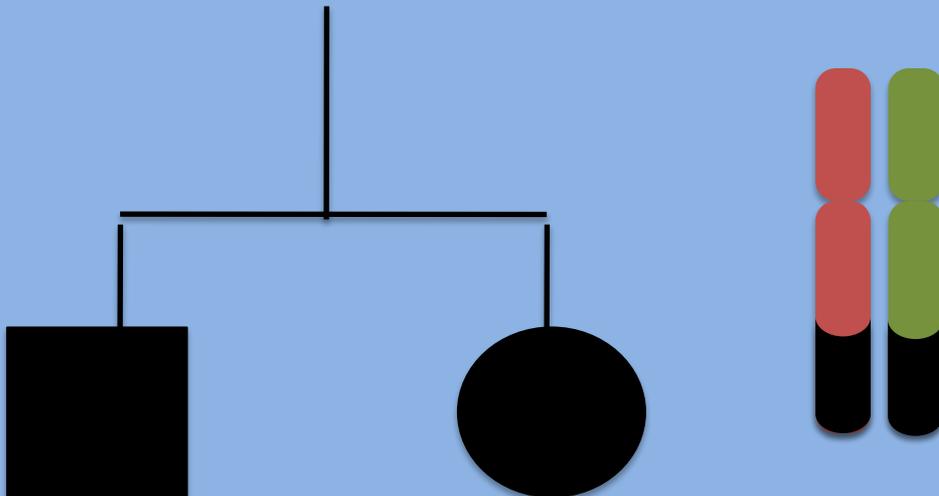
Como identificar a região do genoma responsável pelo fenótipo?

DOENÇAS COMPLEXA

Identificação da região do genoma

Análise de Ligação

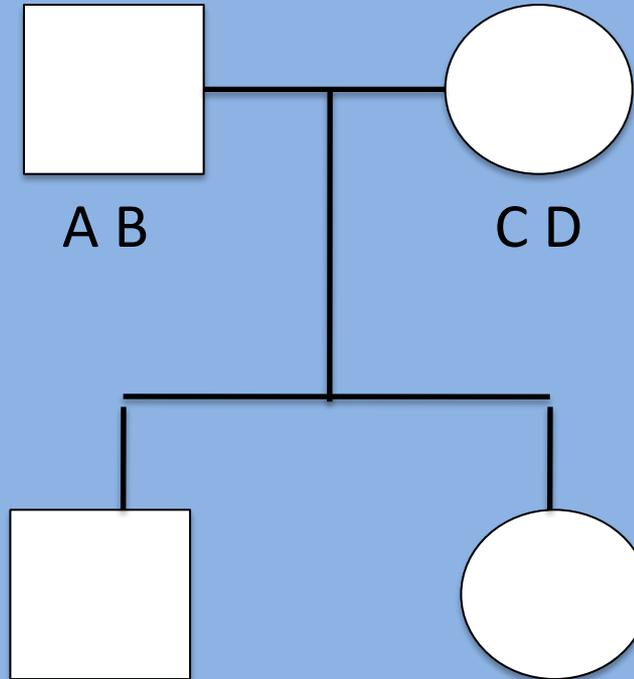
Procura encontrar segmentos dos cromossomos que são compartilhados por membros afetados nas famílias



DOENÇAS COMPLEXA

Identificação da região do genoma

Análise de Ligação



A C

A C

1/4 (Ambos)

A D

B C

B D

1/2 (Pelo menos um)

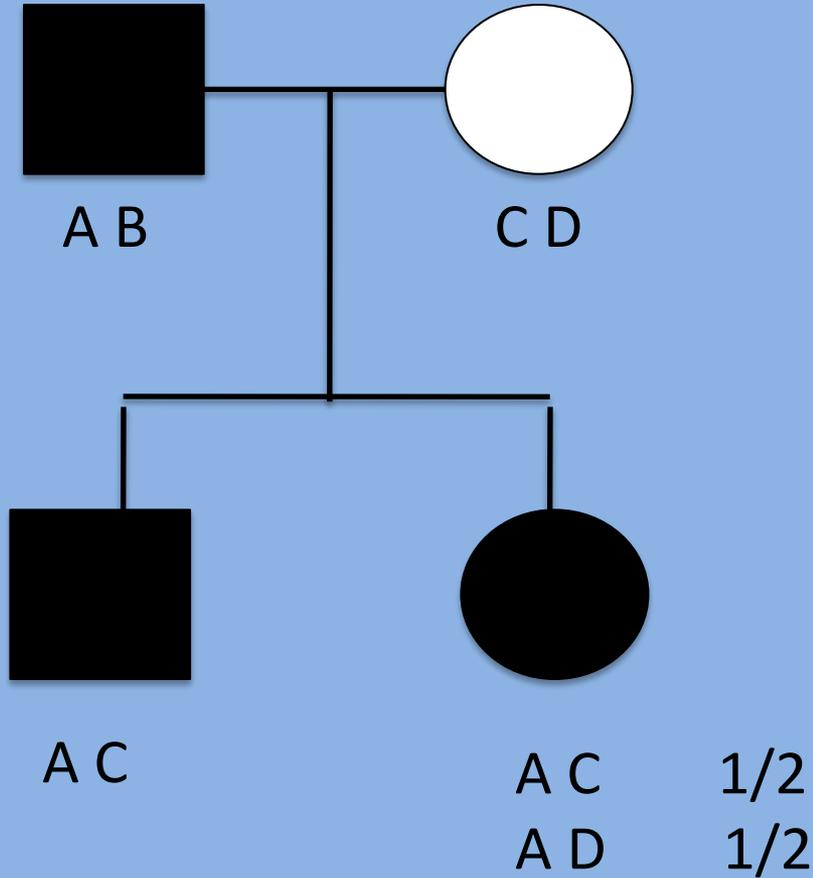
1/4 (Nenhum)

DOENÇAS COMPLEXA

Identificação da região do genoma

Análise de Ligação

Dominância

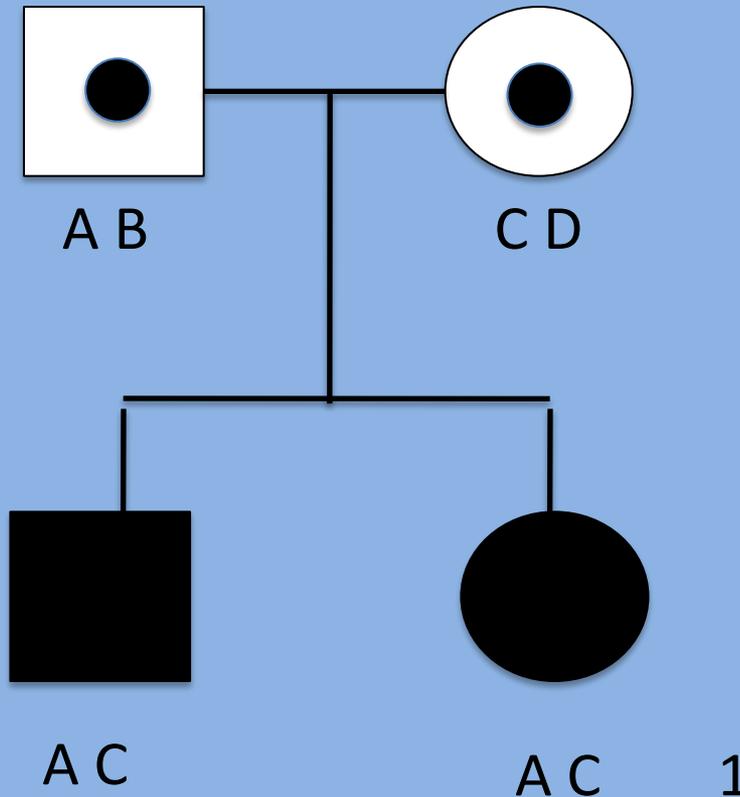


DOENÇAS COMPLEXA

Identificação da região do genoma

Análise de Ligação

Recessividade

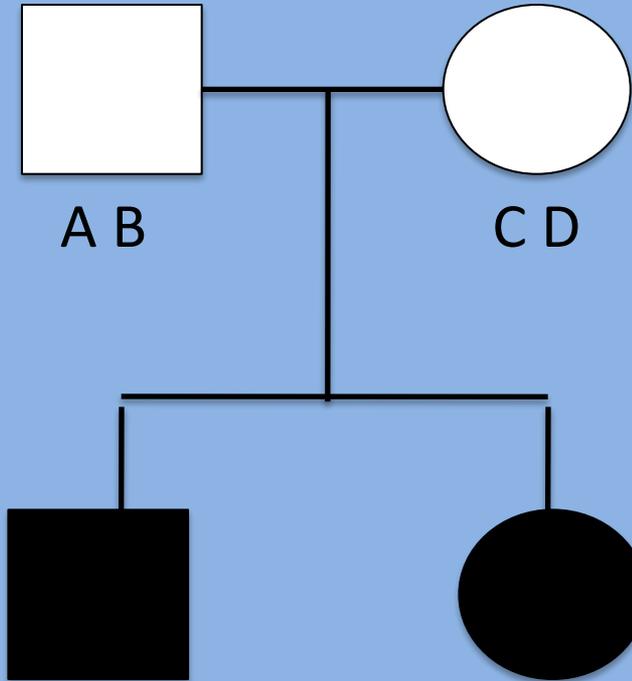


DOENÇAS COMPLEXA

Identificação da região do genoma

Análise de Ligação

Condição complexa



A C

A C >1/4

A D >1/2

B C

B D <1/4

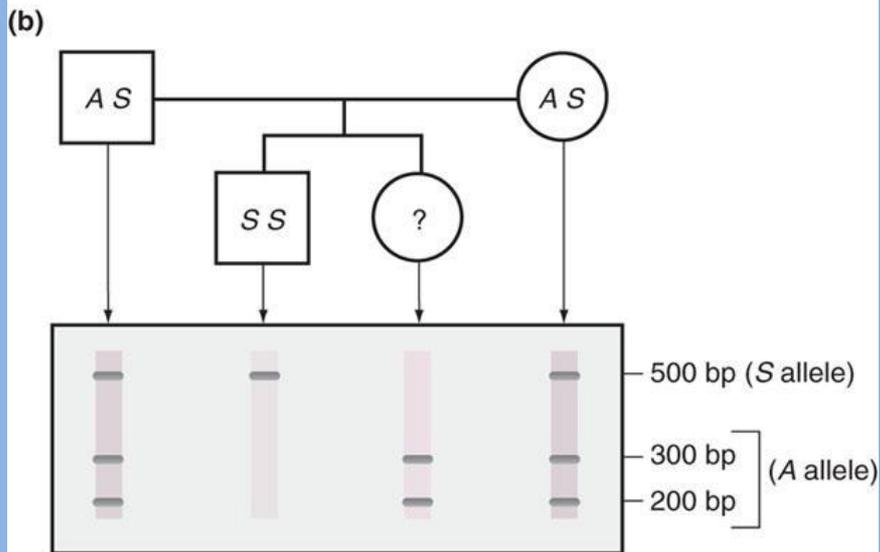
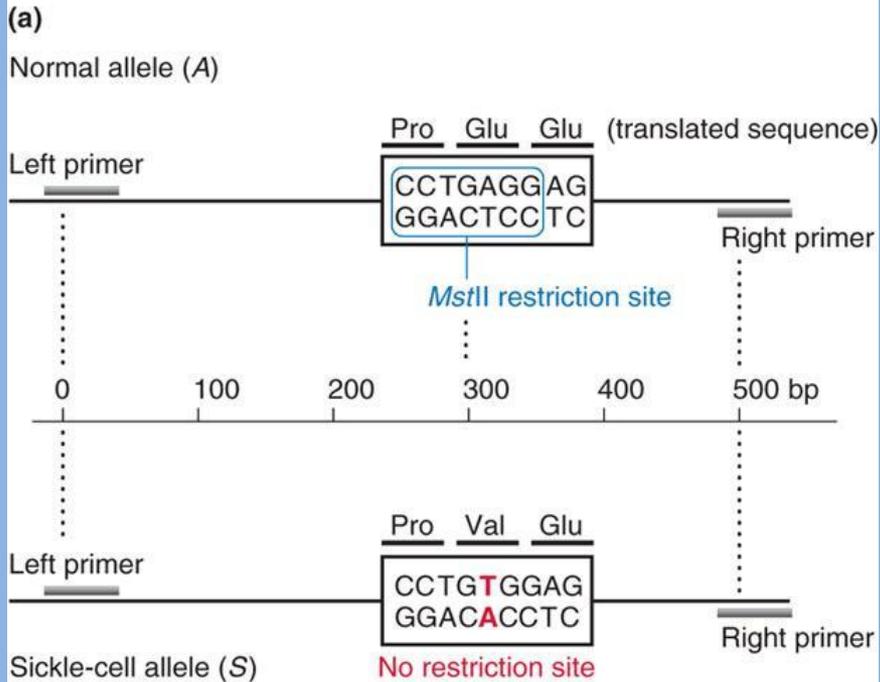
4 classes de polimorfismo de DNA

TABLE 11.1 Classes of DNA Polymorphisms

Class	Size of Locus	Number of Alleles	Number of Loci in Population	Rate of Mutation	Use	Method of Detection
<i>SNP</i>	Single base pair	2	100 million	10^{-9}	Linkage mapping	PCR followed by ASO hybridization or primer extension
<i>Microsatellite</i>	30–300 bp	2–10	200,000	10^{-3}	Linkage mapping	PCR and gel electrophoresis
<i>Multilocus Minisatellite</i>	1–20 kb	2–10	30,000	10^{-3}	DNA fingerprinting	Southern blot and hybridization
<i>Small Changes in DNA Content (deletions and duplications)</i>	1–100 bp	2	N/A	$<10^{-9}$	Linkage mapping	PCR and gel electrophoresis

Single nucleotide polymorphism (SNP)

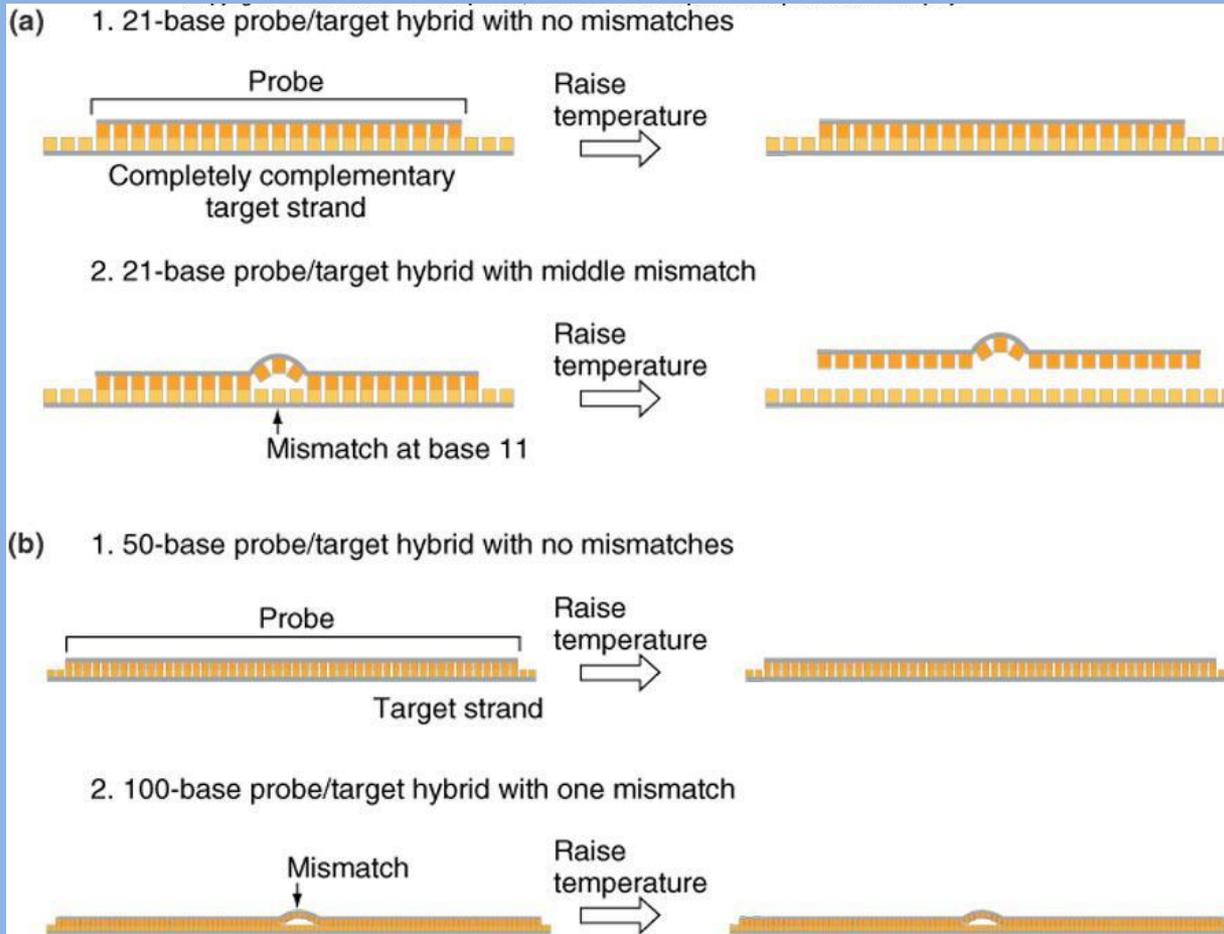
- Single base-pair substitutions
- Arise by mutagenic chemicals or mistakes in replication
- Biallelic – only two alleles
- Ratio of alleles ranges from 1:100 to 50:50.
- 2001 – over 5 million human SNPs identified
- Most occur at anonymous loci.
- Mutation rate of 1×10^{-9} per locus per generation
- Very few are thus new mutation in the species.
- Useful as DNA markers



SNP detection by PCR

- Must have sequence on either side of polymorphism
 - Amplify fragment
 - Expose to restriction enzyme
 - Gel electrophoresis
- e.g., sickle-cell genotyping with a PCR based protocol

SNP detection by ASO



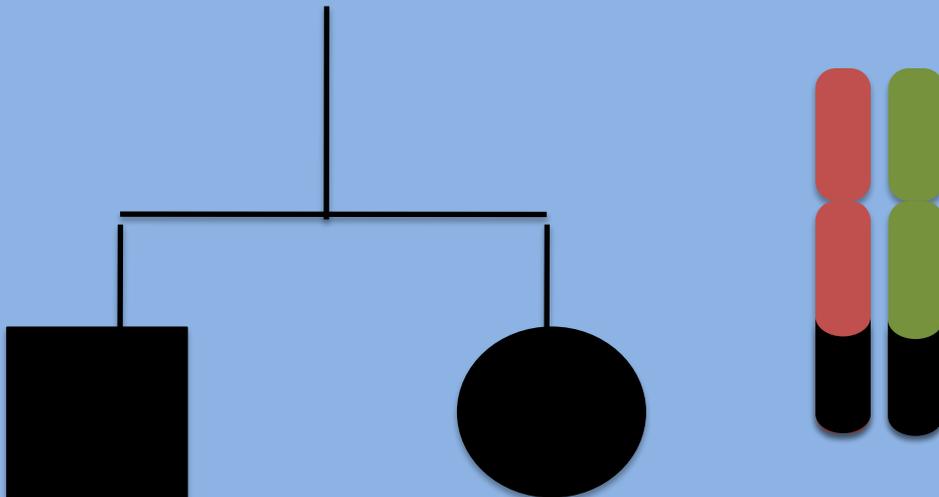
- Very short probes (<21 bp) specific which hybridize to one allele or other
- Such probes are allele-specific oligonucleotides (ASOs).

DOENÇAS COMPLEXA

Identificação da região do genoma

Análise de Ligação

Problemas: regiões muito grandes, pouco recombinantes.



Estudos de associação e desequilíbrio de ligação

Gene

AGTCCTCGTCTCAGCTCGTGAAATTTGTGCCCC
AGTCGTCGTCTCAGCTCGTGAAATTTGGGCCCC



Gene Mutado

AGTCCTCGTCTCAGCCCGTGAAATTTGTGCCCC
AGTCGTCGTCTCAGCTCGTGAAATTTGGGCCCC

Estudos de associação e desequilíbrio de ligação

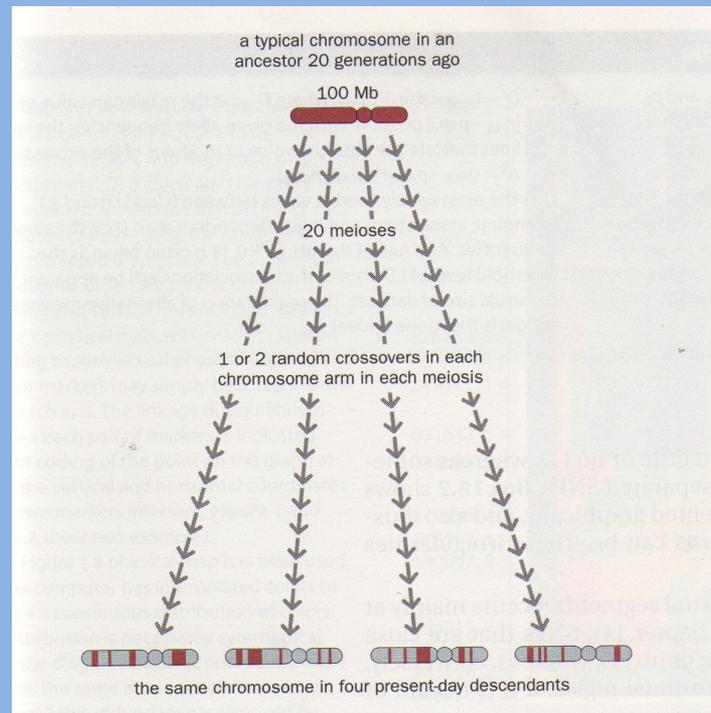
Gene Mutado

AGTCCTCGTCTCAGCCCGTGAAATTTGTGCCCC
AGTCGTCGTCTCAGCTCGTGAAATTTGGGCCCC

F1 ... F2...

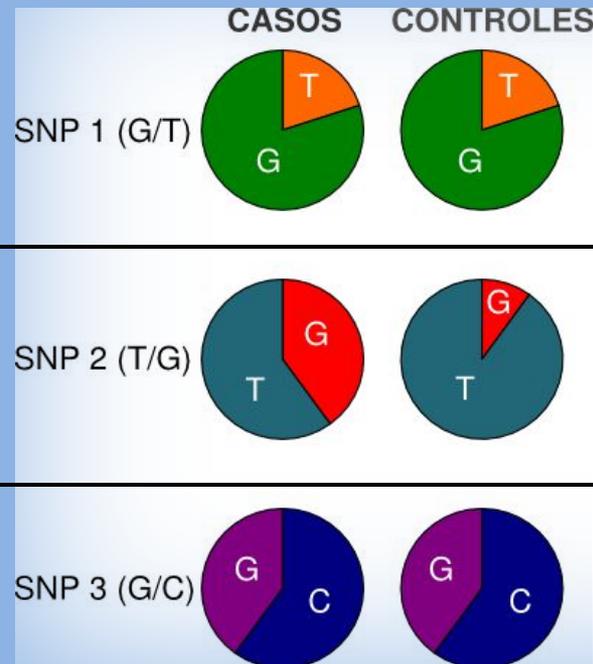
F3....Fn

Estudos de associação e desequilíbrio de ligação



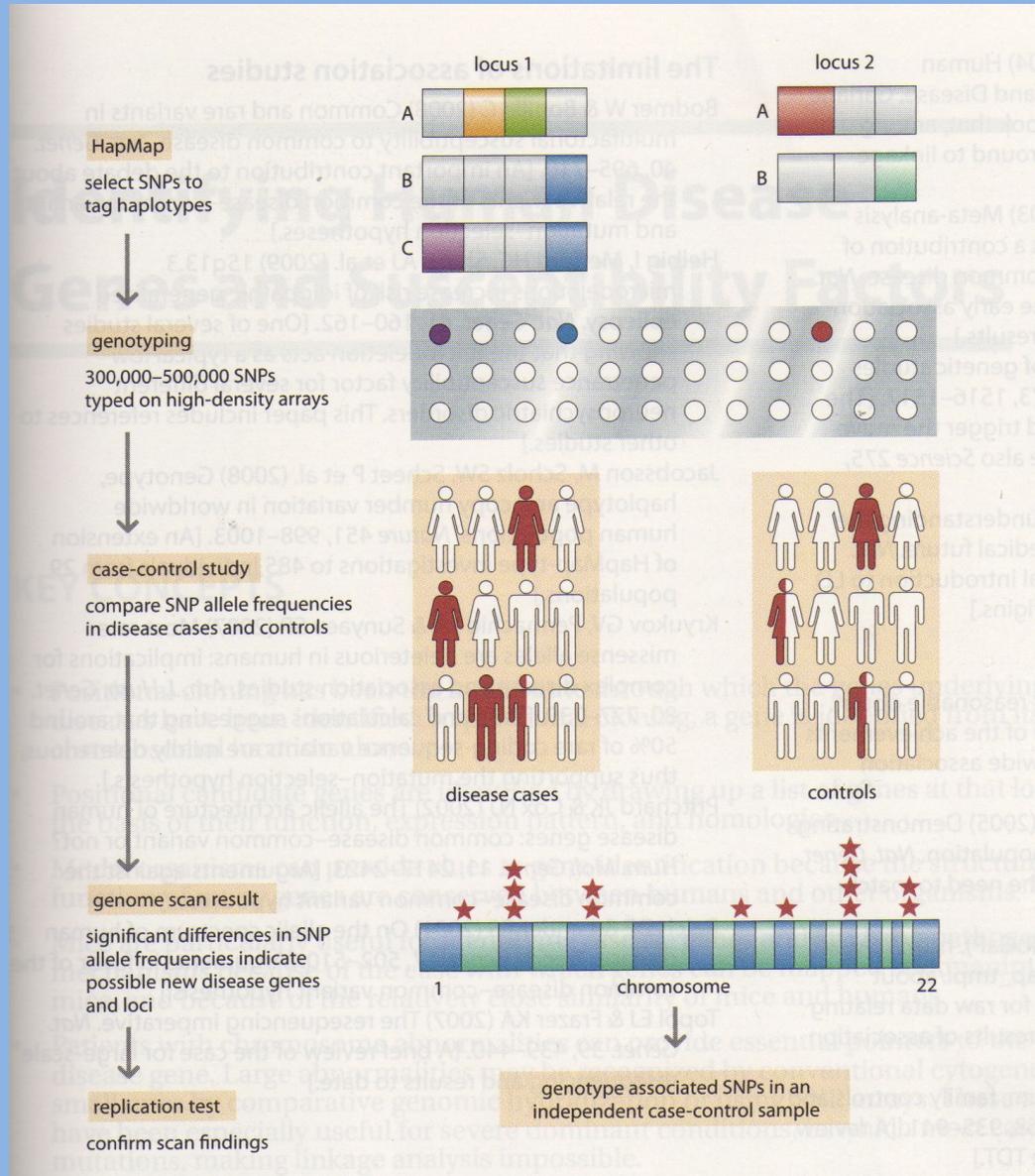
Todos humanos são relacionados em um ancestral em comum. No reino unido, por exemplo, duas pessoas não relacionadas tem um ancestral em comum não mais que 22 gerações atrás.

Estudos de associação genômica em larga escala



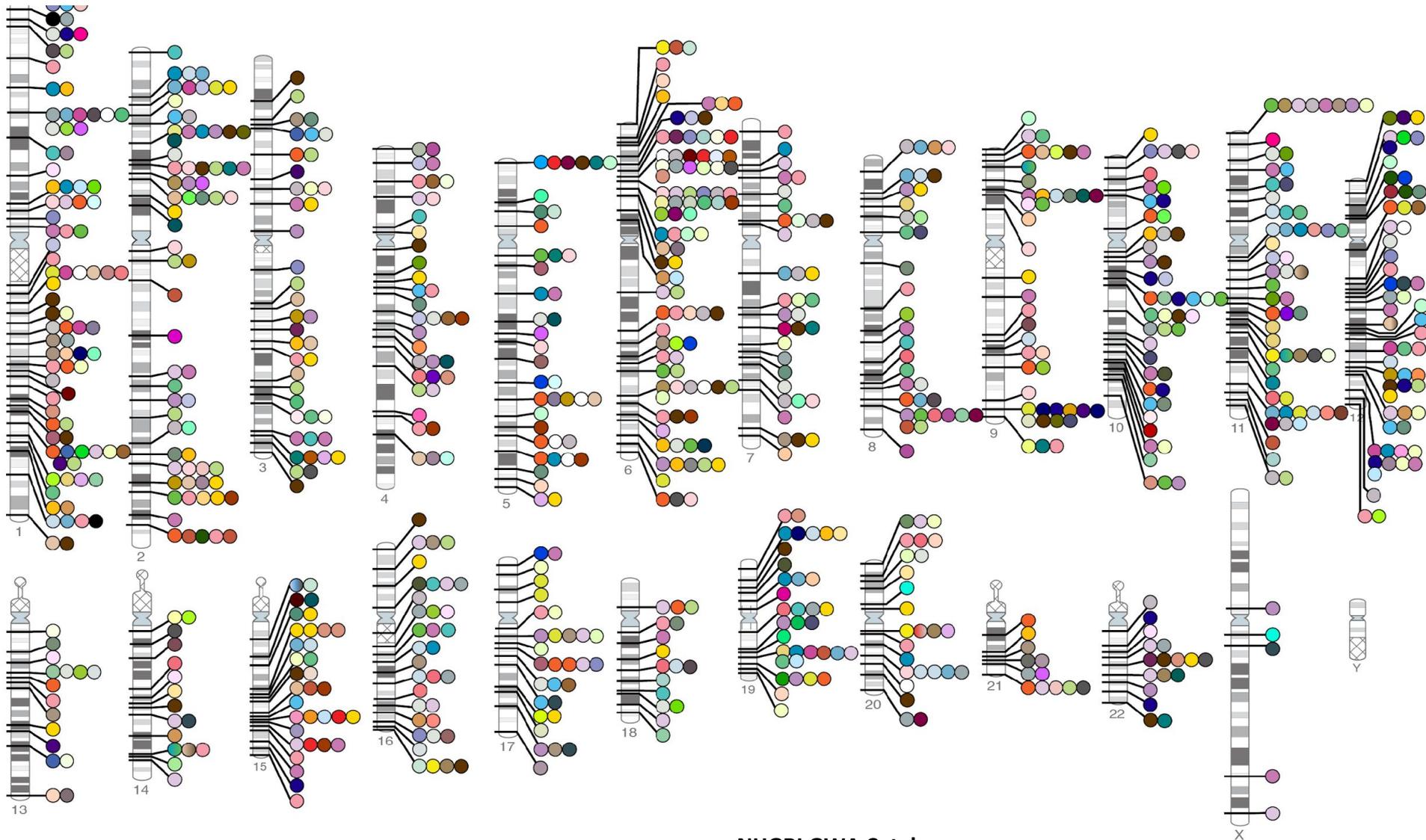
Associação

Estudos de associação genômica em larga escala (GWA)



Associações genômicas em larga escala publicadas

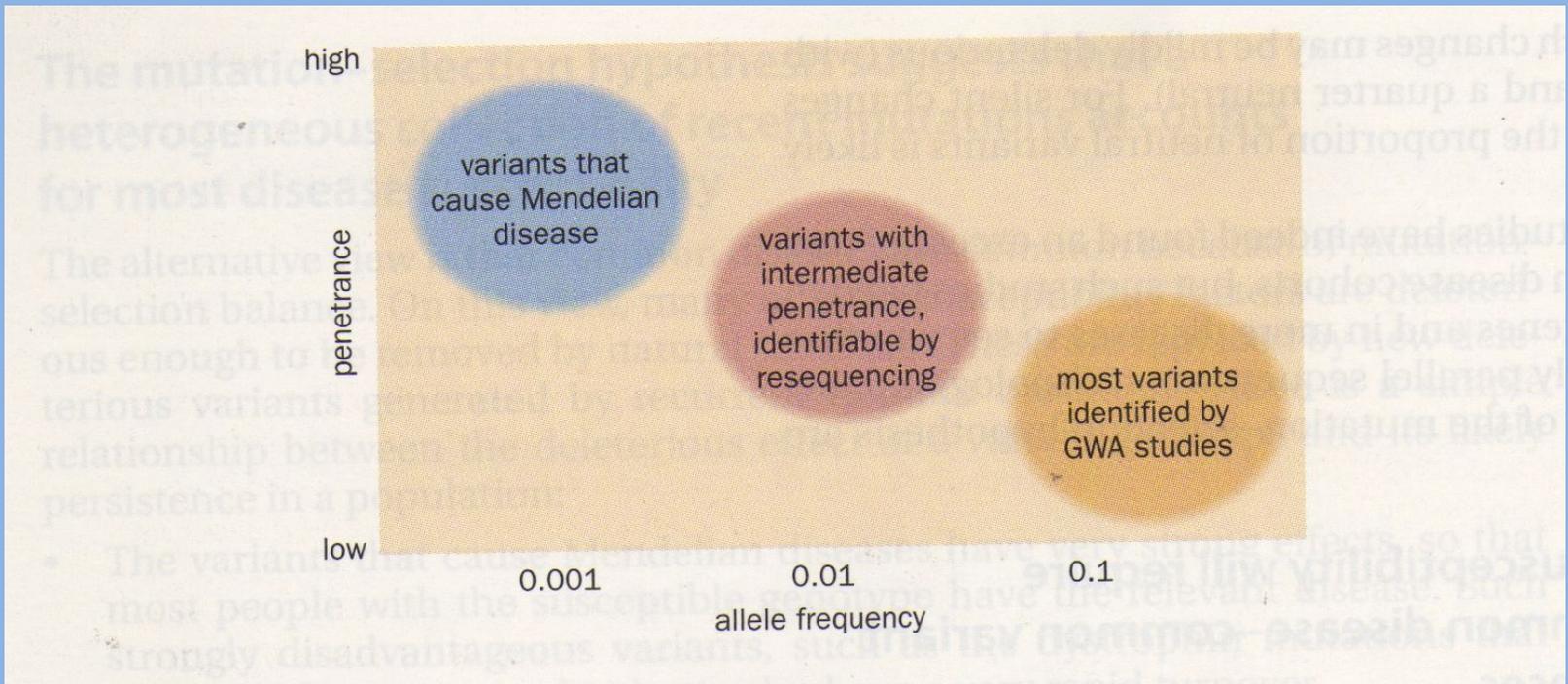
165 traços



NHGRI GWA Catalog

www.genome.gov/GWASudies

Estudos de associação genômica em larga escala



Abordagens integrativas

- Após uma região cromossômica ser identificada, os genes dentro da região devem ser investigados
- Genes candidatos
 - Usualmente existem 10 genes por fragmentos de 1kb
 - Identificar regiões codificantes
 - Análises computacionais para identificar regiões conservadas entre espécies
 - Análises computacionais para identificar seqüência que se parecem com exons, (ORFs, sítios de splice, uso de determinado de codons)
 - Aparecimento de um ou mais clones de ESTs

Analises computacionais de seqüência genômicas para identificar genes candidatos

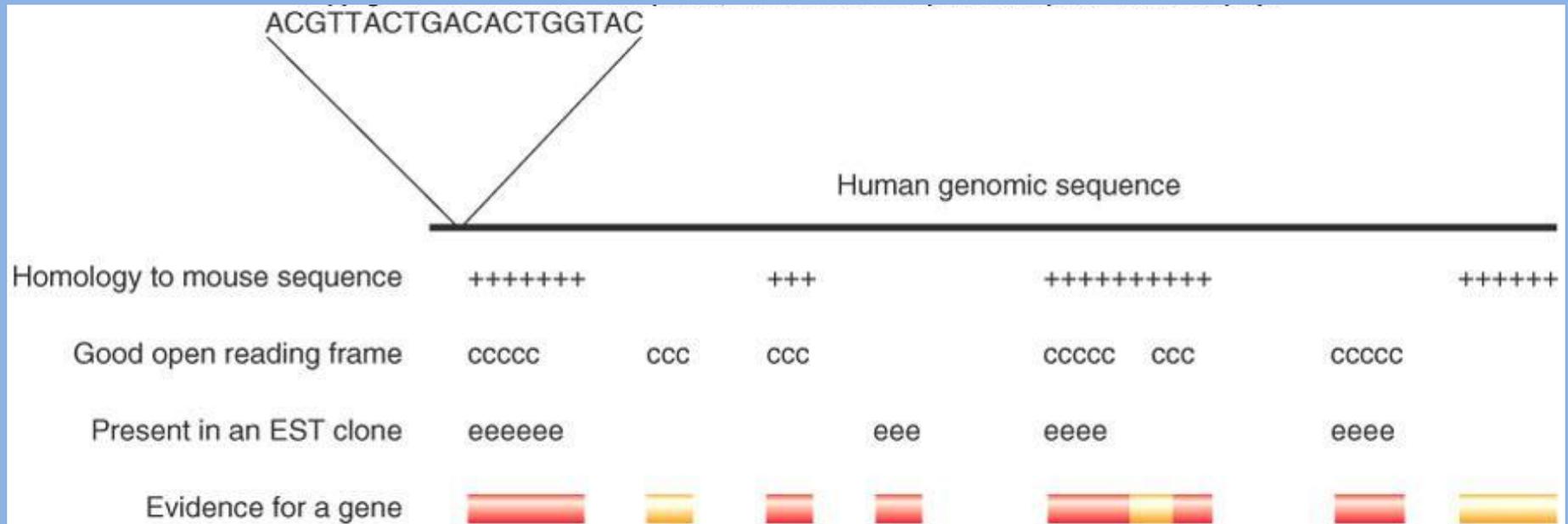


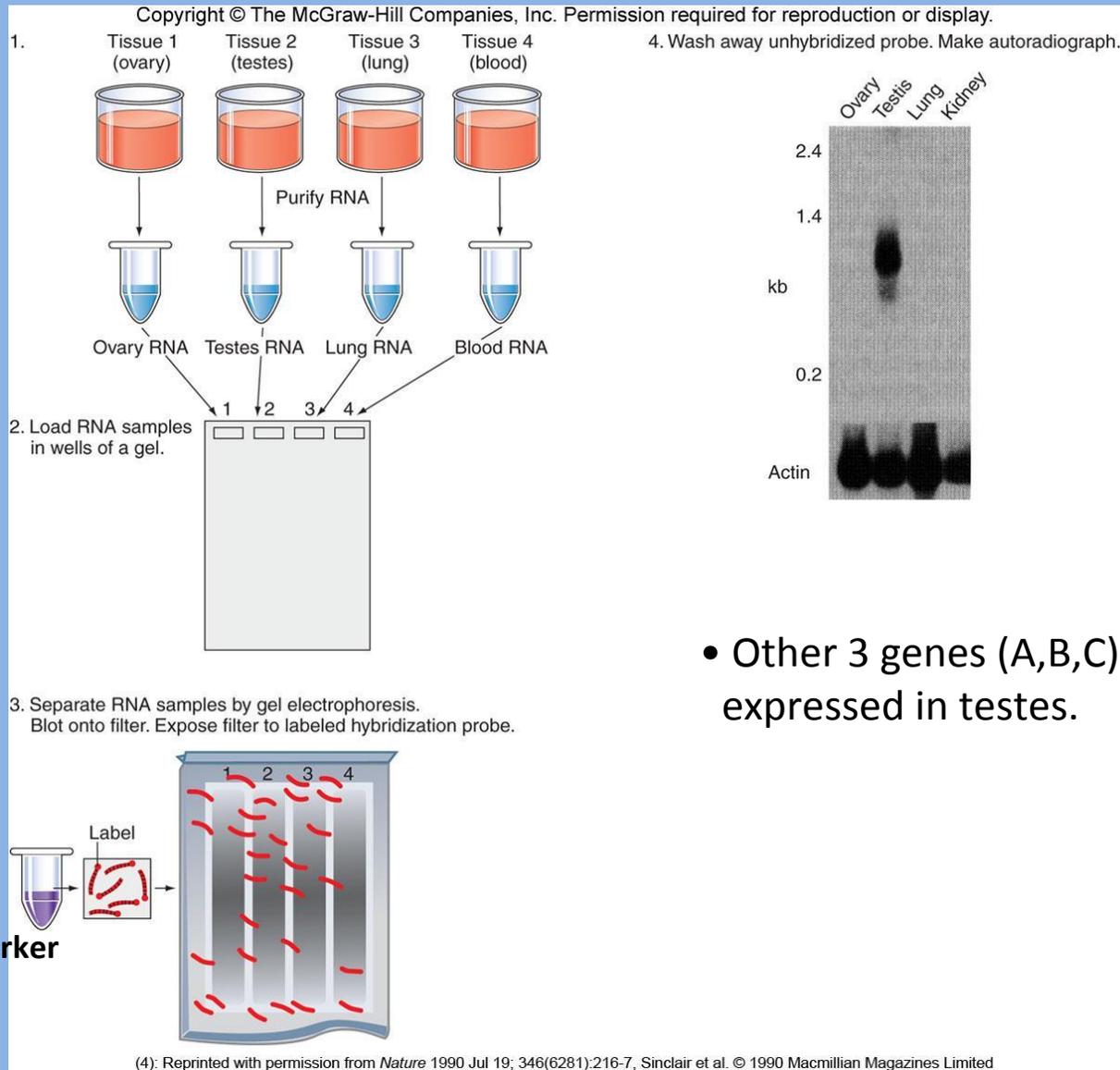
Fig. 11.19

Padrões de expressão gênica podem apontar para genes candidatos

- Sequências públicas de banco de dados de EST específicos de certos tecidos
- Northern blot
 - Transcritos de RNA de células de um tecido em particular (e.g. com doença) separados por eletroforese e a sonda do gene candidato

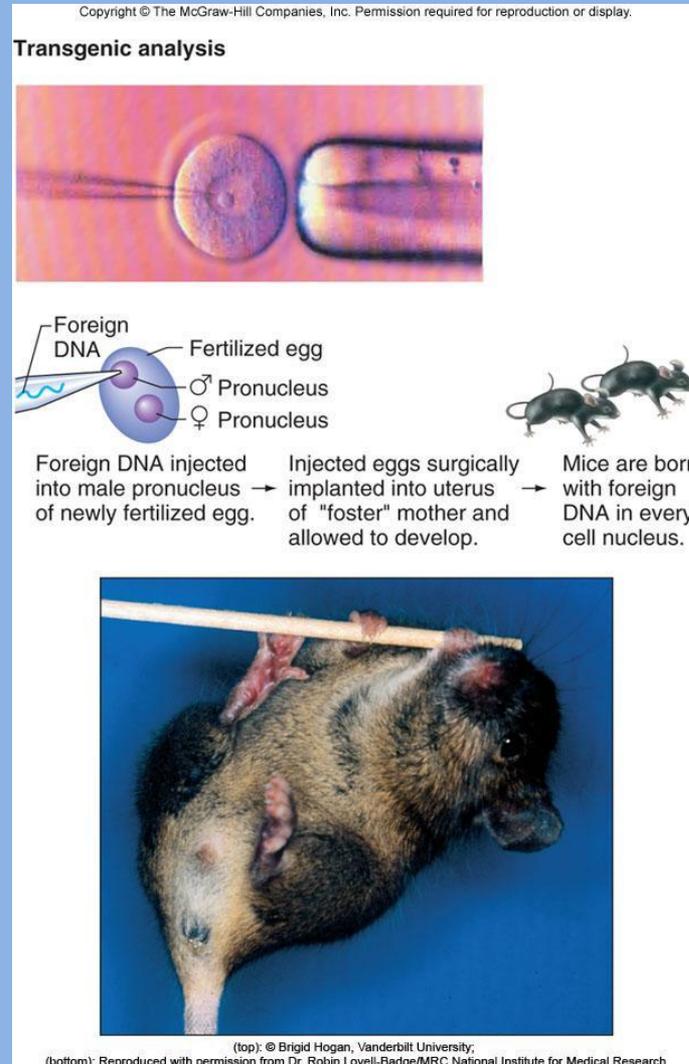


Northern blot example showing *SRY* candidate for testes determining factor is expressed in testes, but not lung, ovary, or kidney.



- Procurar os genes responsáveis pelo fenótipo.
 - Padrões de expressão
 - Ensaio de expressão de RNA por Northern blot ou amplificação de PCR de cDNA com primers específicos do gene candidato
 - Procurar por “misexpression” (no expression, underexpression, overexpression).
 - Diferenças nas seqüências
 - Identificar mutações sem sentido ao seqüenciar gene candidato em indivíduos afetados e não afetados
 - Modificações transgênicas do fenótipo
 - Inserir gene mutante em organismos modelos.

Transgenic analysis can prove candidate gene is disease locus.



Variantes em 8q24 são associadas com câncer de próstata

Multiple regions within 8q24 independently affect risk

Identification of a new prostate cancer susceptibility locus on chromosome 8q24

Meredith Yeager^{1,2,*}, Nilanjan Chatterjee², Julia Ciampa², Kevin B Jacobs³, Jesus Gonzalez-Bosquet², Richard B Hayes², Peter Kraft⁴, Sholom Wacholder², Nick Orr², Sonia Berndt², Kai Yu²

Matthew L Freedman^{2,3}, Simon R Myers², Malcolm C Pike^{1,2,4}, Arti Tandon^{2,4}, Christine Schirmer^{2,4}, Gavin J McDonald^{2,4}, Loic Le Marchand⁶, Laurence N Kolonel⁶, Melissa Frasco¹, Ingrid Ardlie^{2,7}, Ingrid Oakley-Girvan^{8,9}, Alice S Whittemore⁹, Sue A Ingles¹, David Altshuler^{2,4,12,13}

- Cinco independentes 8q24 loci associados ao câncer de próstata

Um novo gene conhecido nessa região

Laurence Kolonel⁶, Loic Le Marchand⁶, Arslan Sidiq¹⁰, Nicole Rabol¹⁴, Timothy J Key¹⁵, Bradford L Cooke¹⁵, William Isaacs⁶, Sarah Isaacs¹⁶, Kathleen E Wiley¹⁶, Henrik Gronberg¹⁷, Fredrik Wiklund¹⁷, Pär Stattin¹⁸, Jianfeng Xu¹⁹, S Lilly Zheng¹⁹, Jielin Sun¹⁹, Lars J Vatten²⁰, Kristian Hveem²⁰, Merethe Kumle²¹, Margaret Tucker², Daniela S Gerhard²², Robert N Hoover², Joseph F Fraumeni Jr², David J Hunter^{4,23}, Gilles Thomas² & Stephen J Chanock²

We report a genome-wide association study in 10,286 cases and 9,135 controls of European ancestry in the Cancer Genetic Markers of Susceptibility (CGEMS) initiative. We identify a new association with prostate cancer risk on chromosome 8q24 (rs620861, $P = 1.3 \times 10^{-10}$, heterozygote OR = 1.17, 95% CI 1.10–1.24; homozygote OR = 1.33, 95% CI 1.21–1.45). This defines a new locus associated with prostate cancer susceptibility on 8q24.

association, and controlling for non-genotypes susceptibility to cancer risk variants in the presence of an

significant signal is not centromeric to the previously reported association with prostate cancer risk variants in African Americans (Table 1). We genotyped the same variants in 465 European American cases and 446 European American controls. Analysis of these data identified a cluster of genetic variants that we denote 'region 2' in a span of linkage disequilibrium from 128.14–128.28 Mb. These variants are hundreds of kilobases away from the region 1 described in ref. 2, and the strongest single-SNP association is significant at $P = 6.5 \times 10^{-7}$ (Fig. 1b and Supplementary Table 2). We conducted a genome-wide association study (GWAS) of 550,000 SNPs in 1,172 affected individuals (484 with nonaggressive prostate cancer, Gleason <7 and stage A/B; 688 aggressive prostate cancer, Gleason >7 and/or stage C/D) and 1,157 controls using an incidence

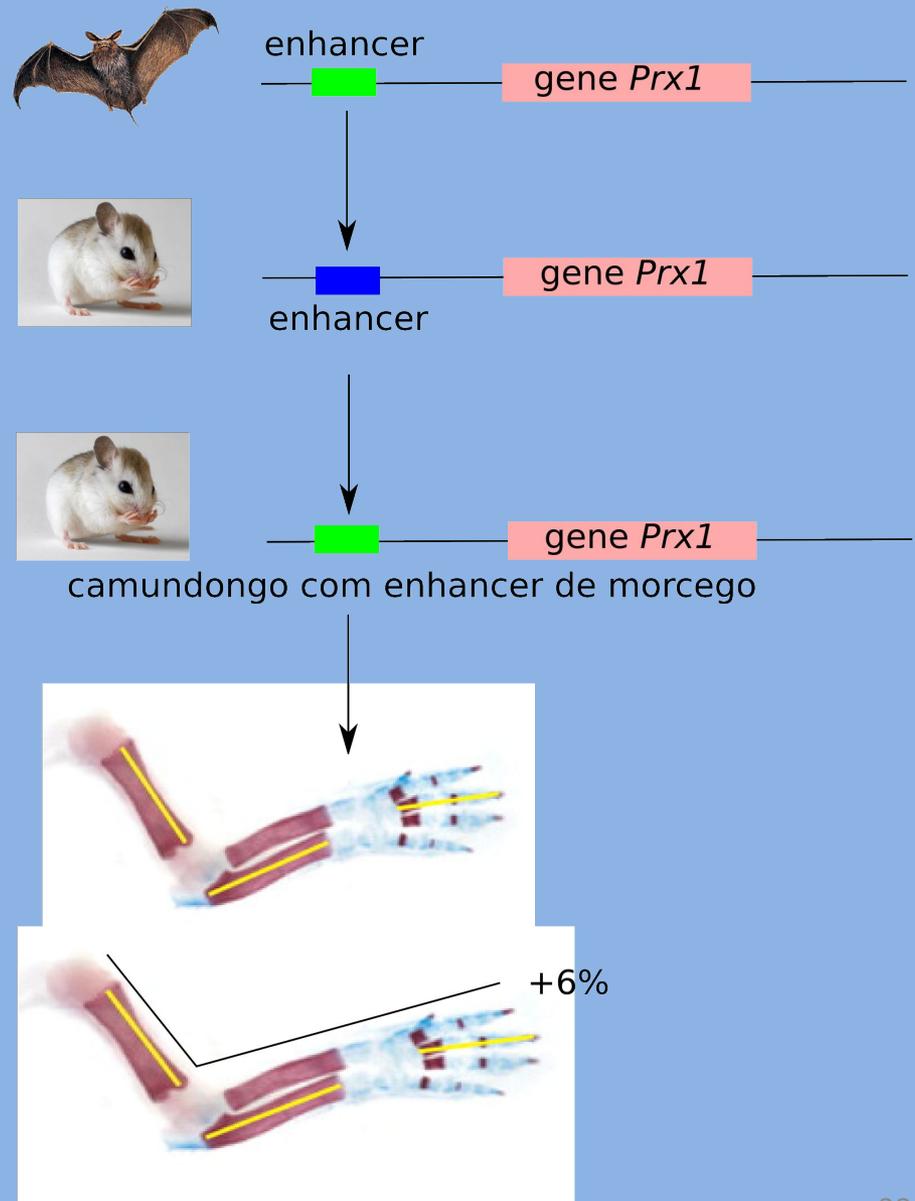
American affect of individuals with age at diagnosis <72 years and 837 African American controls (Table 1). We genotyped the same variants in 465 European American cases and 446 European American controls.

Analysis of these data identified a cluster of genetic variants that we denote 'region 2' in a span of linkage disequilibrium from 128.14–128.28 Mb. These variants are hundreds of kilobases away from the region 1 described in ref. 2, and the strongest single-SNP association is significant at $P = 6.5 \times 10^{-7}$ (Fig. 1b and Supplementary Table 2). We conducted a genome-wide association study (GWAS) of 550,000 SNPs in 1,172 affected individuals (484 with nonaggressive prostate cancer, Gleason <7 and stage A/B; 688 aggressive prostate cancer, Gleason >7 and/or stage C/D) and 1,157 controls using an incidence

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Fenótipos nem sempre são produto da alteração de genes codificadores de proteínas.

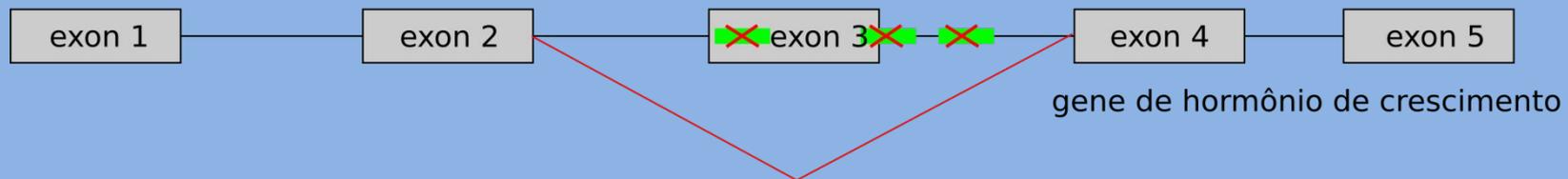
Elementos reguladores



Splicing Alternativo

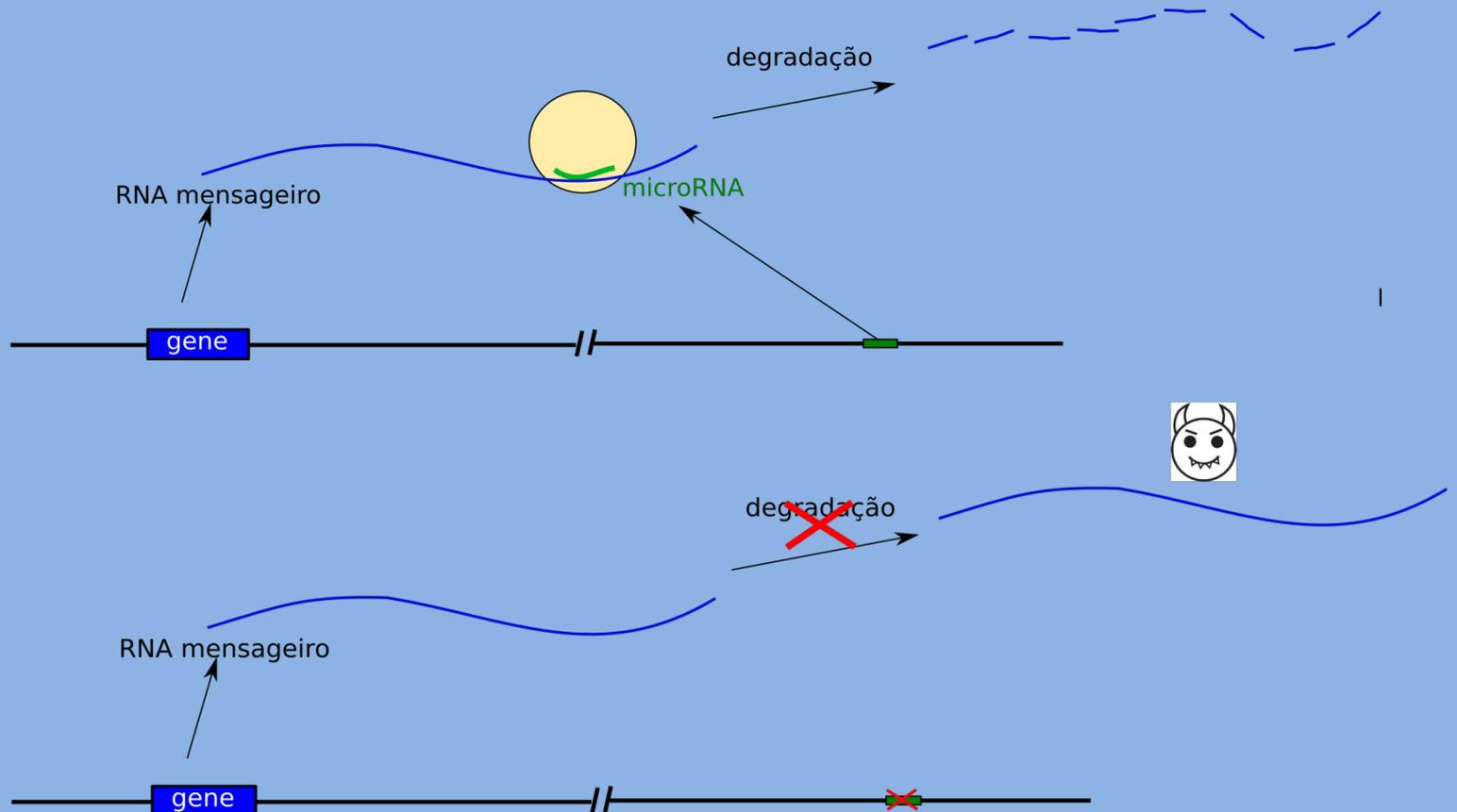
Polimorfismos em sinais de splicing resultam em deficiência de hormônio de crescimento familiar tipo II

-  sinal de splicing
-  polimorfismo em sinal de splicing

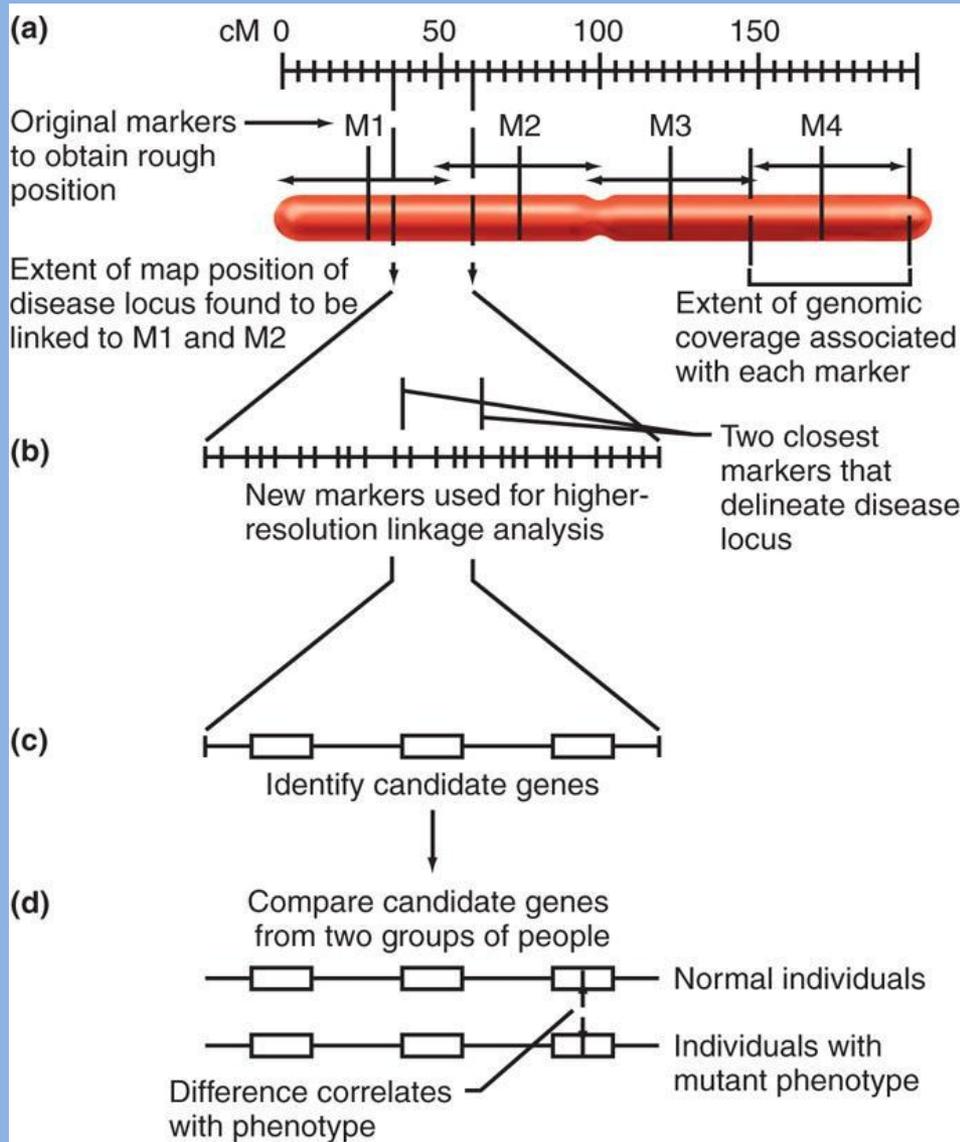


RNA não codificante

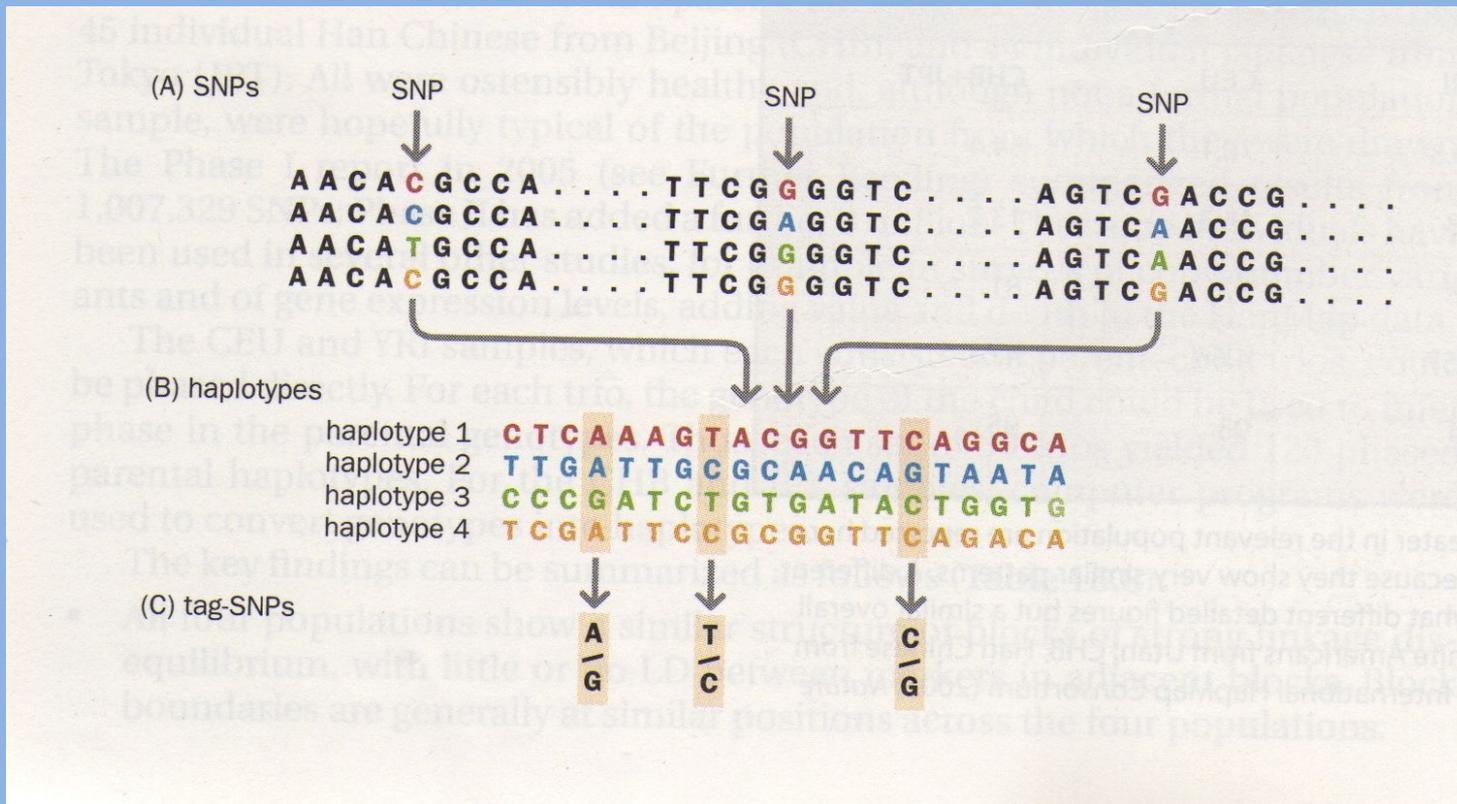
microRNAs, siRNAs (small interfering), lncRNAs (long non-coding), soRNAs (small nucleolar).



Positional Cloning

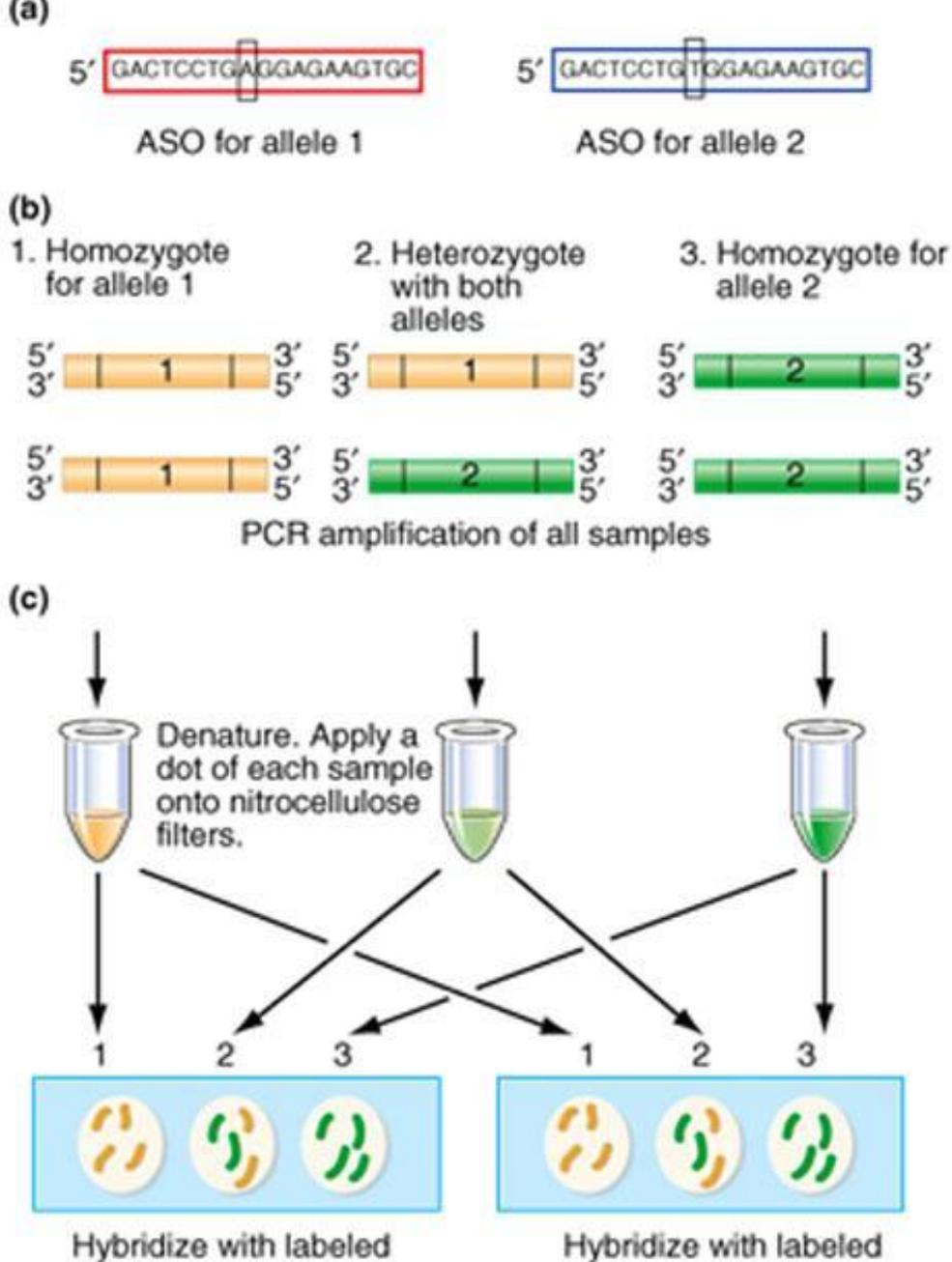


Estudos de associação genômica em larga escala



Positional Cloning – Step 1

- Find extended families in which disease is segregating.
- Use panel of polymorphic markers spaced at 10 cM intervals across all chromosomes.
 - 300 markers total
- Determine genotype for all individuals in families for each DNA marker.
- Look for linkage between a marker and disease phenotype.



ASOs can determine genotype at any SNP locus.

Hybridized and labeled with ASO for allele 1

Hybridized and labeled with ASO for allele 2

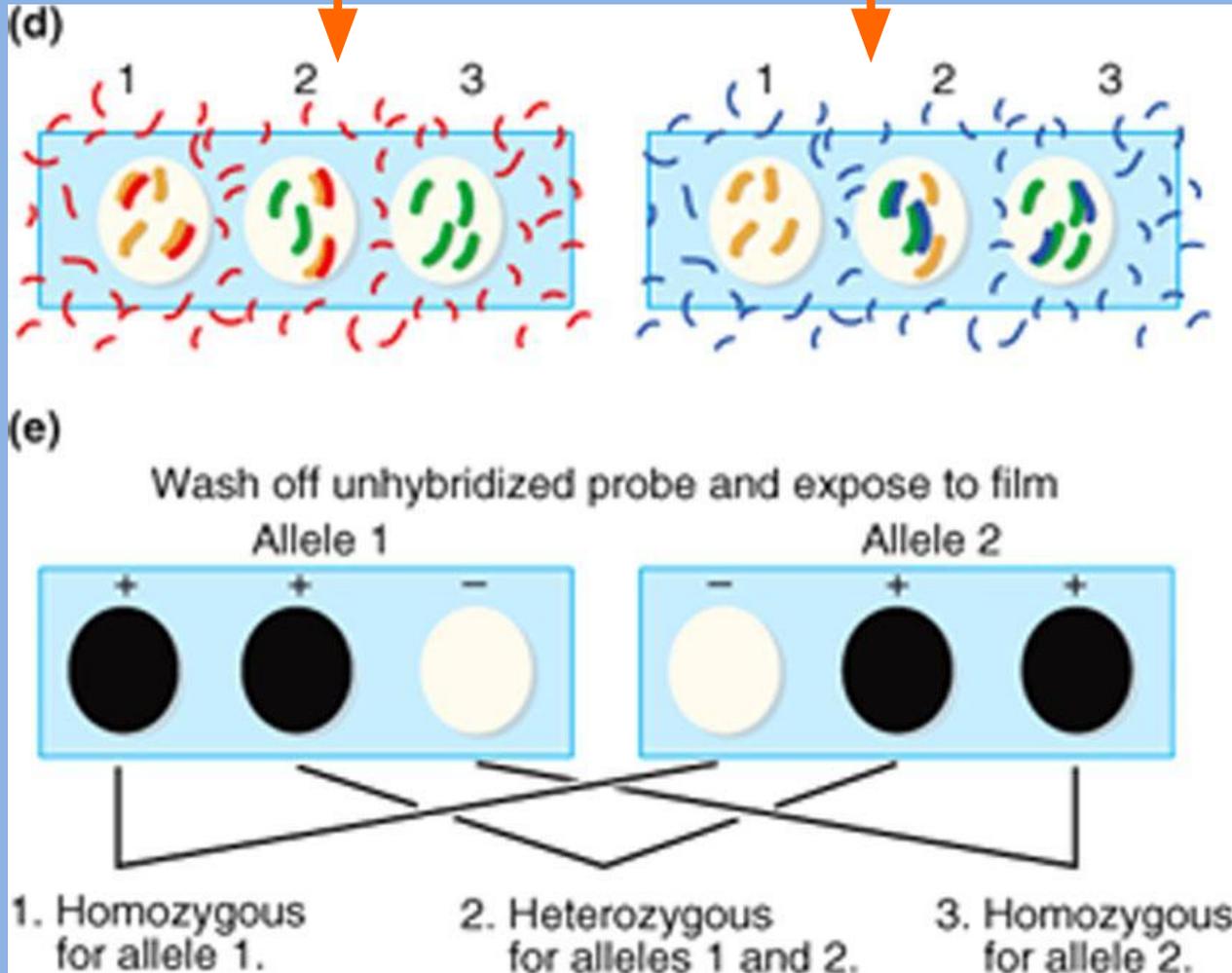


Fig. 11.9 d, e