

Narrowing the QTL Region Proving the QTL Gene

Bev Paigen

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Narrowing a QTL Region

- ◆ Can narrow with additional mouse crosses
- ◆ Can narrow with bioinformatics and statistical tools (later lecture by Luanne Peters)
- ◆ Finding the gene
- ◆ Proving the gene

Narrowing a QTL

- ◆ Easy to obtain 20-30 cM QTL; difficult to narrow to $< 1\text{cM}$
- ◆ Method depends on:
 - Phenotyping costs
 - Genotyping costs
 - Time
 - Mouse room space

From 25cM to 5cM: Methods*

Methods	Generations	No. of Animals	No. Phenotyped
Selective genotyping	2	1440	1440
Selective phenotyping	2	1440	285
Progeny testing	3	485	385
Overlapping congenics	6	380	80
RIST	2	200	200

*Assumes backcross and dominant QTL with allele effect of 0.25

From 5cM to 1cM: Methods*

Methods	Generations	No. of Animals	No. Phenotyped
Selective genotyping	2	7200	7200
Selective phenotyping	2	7200	345
Progeny testing	3	485	385
Overlapping congenics	6	380	80
RIST	2	200	200

*Assumes backcross and dominant QTL with allele effect of 0.25

Ways to Narrow a QTL

- ◆ **Selective genotyping: produce more mice; genotype only the extremes**
- ◆ **Selective phenotyping: produce more mice; phenotype only the crossovers**
- ◆ **Recombinant progeny testing: produce more mice; phenotype the crossovers**
- ◆ **Congenics: interval specific, overlapping**
- ◆ **Recombinant Inbred Strain Test (RIST): use RI strains with crossovers that divide the region**
- ◆ **Advanced intercross lines (AIL)**

Selective Genotyping

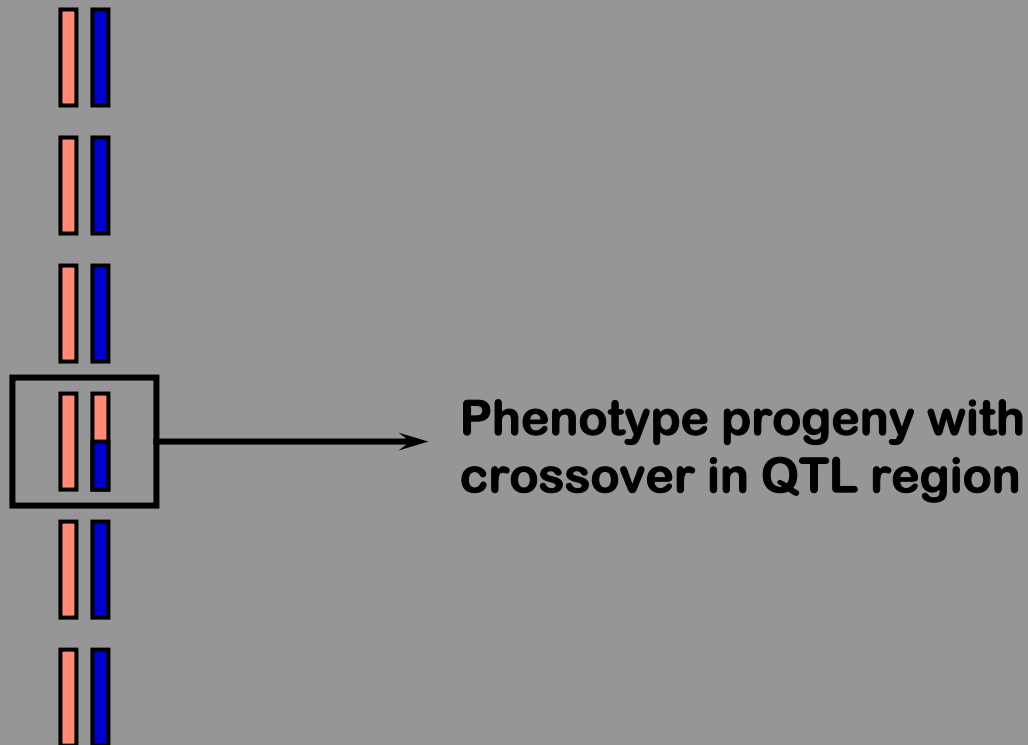
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- ◆ **Appropriate when phenotyping is cheap (look in mouse cage)**
- ◆ **Breed many mice, select those with aberrant phenotype, genotype only those.**

Selective Phenotyping

Breed many backcross mice; test all for markers flanking QTL; phenotype only the crossovers

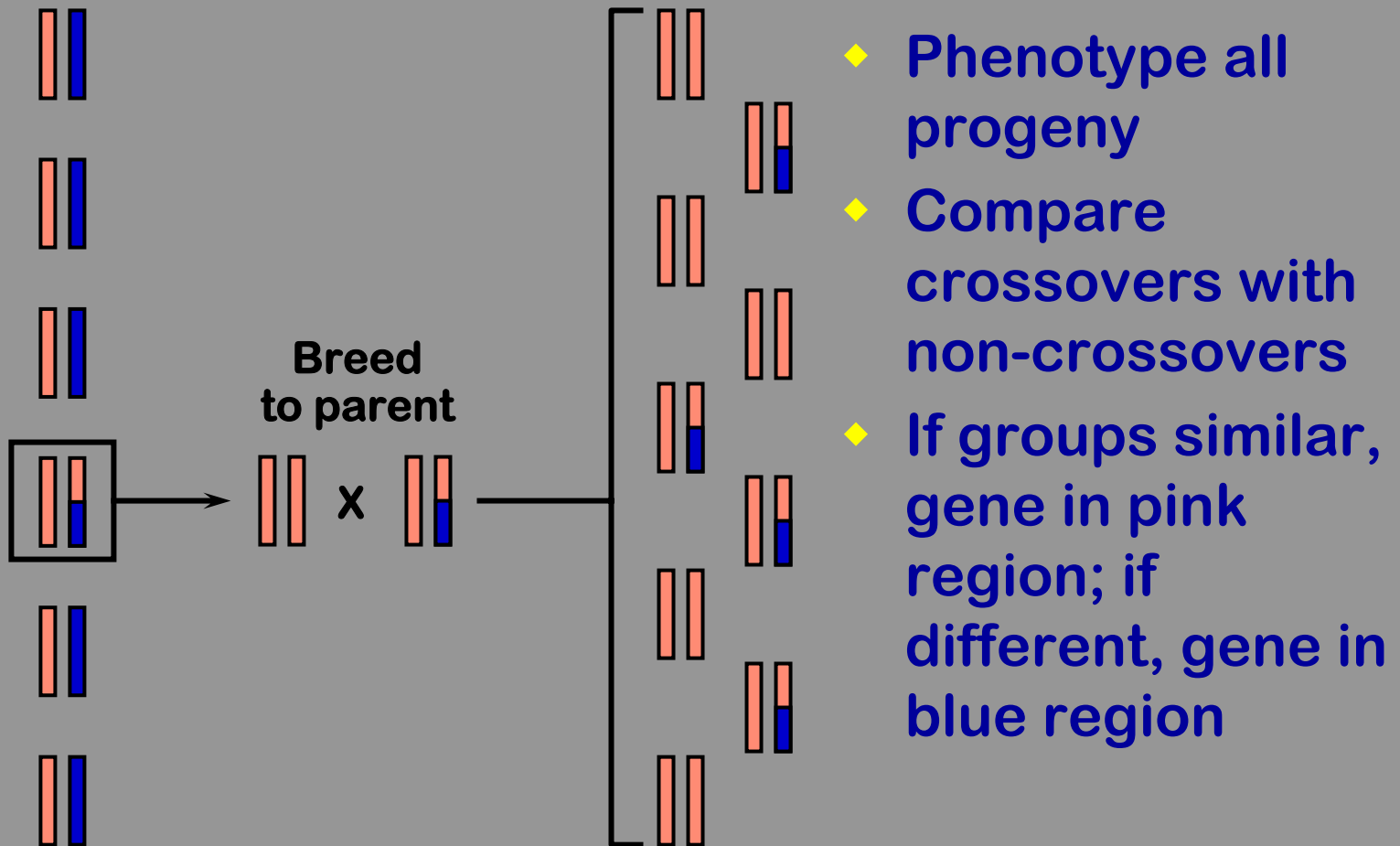
Backcross mice



When to use Selective Phenotyping

- ◆ When phenotyping is costly or time consuming
- ◆ Appropriate for QTL with large effects
- ◆ Requires only 2 generations
- ◆ Maximum resolution: 5 cM
- ◆ Requires many mice

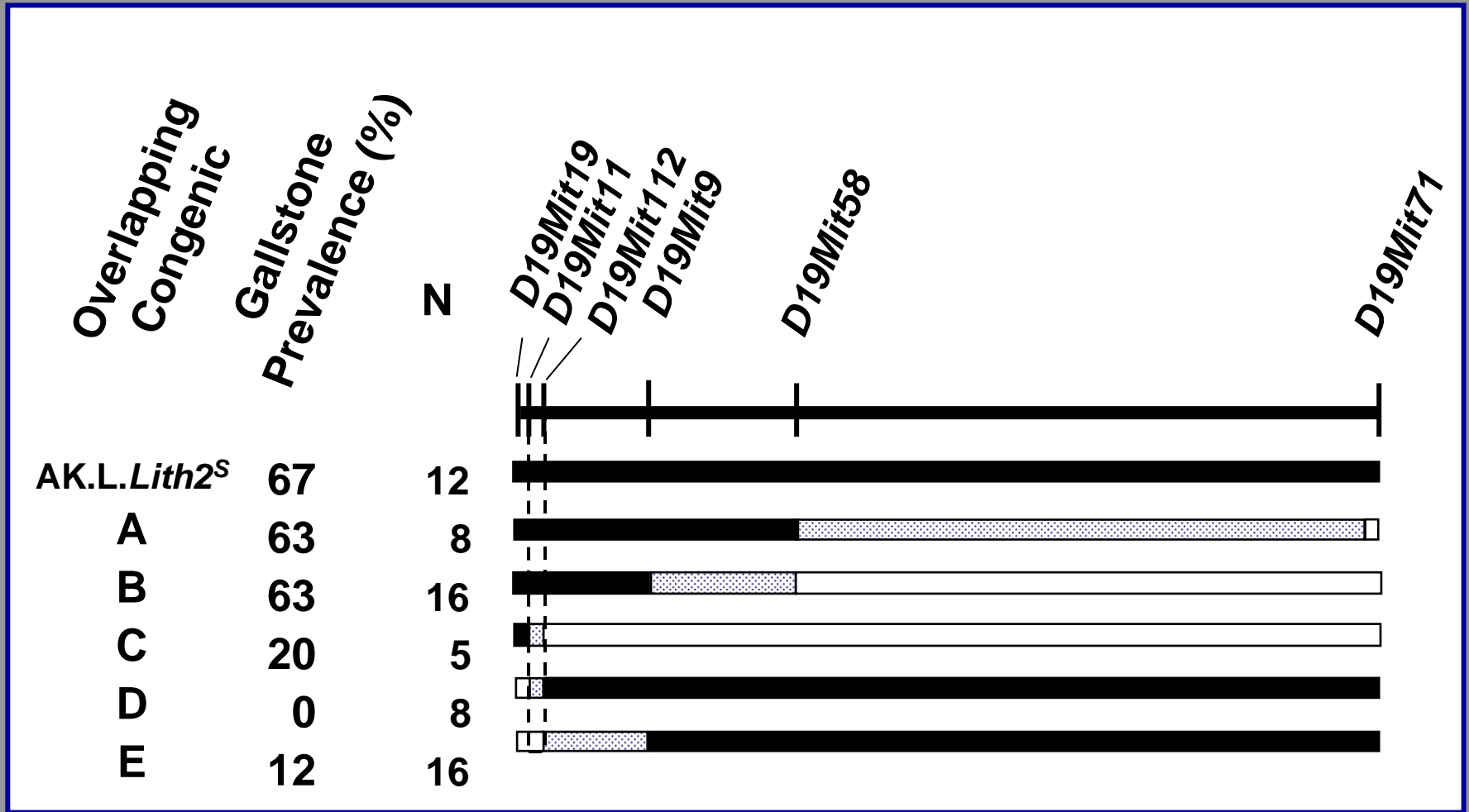
Recombinant Progeny Testing



When to Use Recombinant Progeny Testing

- ◆ When selective phenotyping yields ambiguous results
- ◆ When resolution below 5 cM is needed
- ◆ Appropriate for QTL with dominant effects
- ◆ Requires 3 generations
- ◆ Requires many mice

Overlapping Congenics



Reduce region to 1.5 Mb

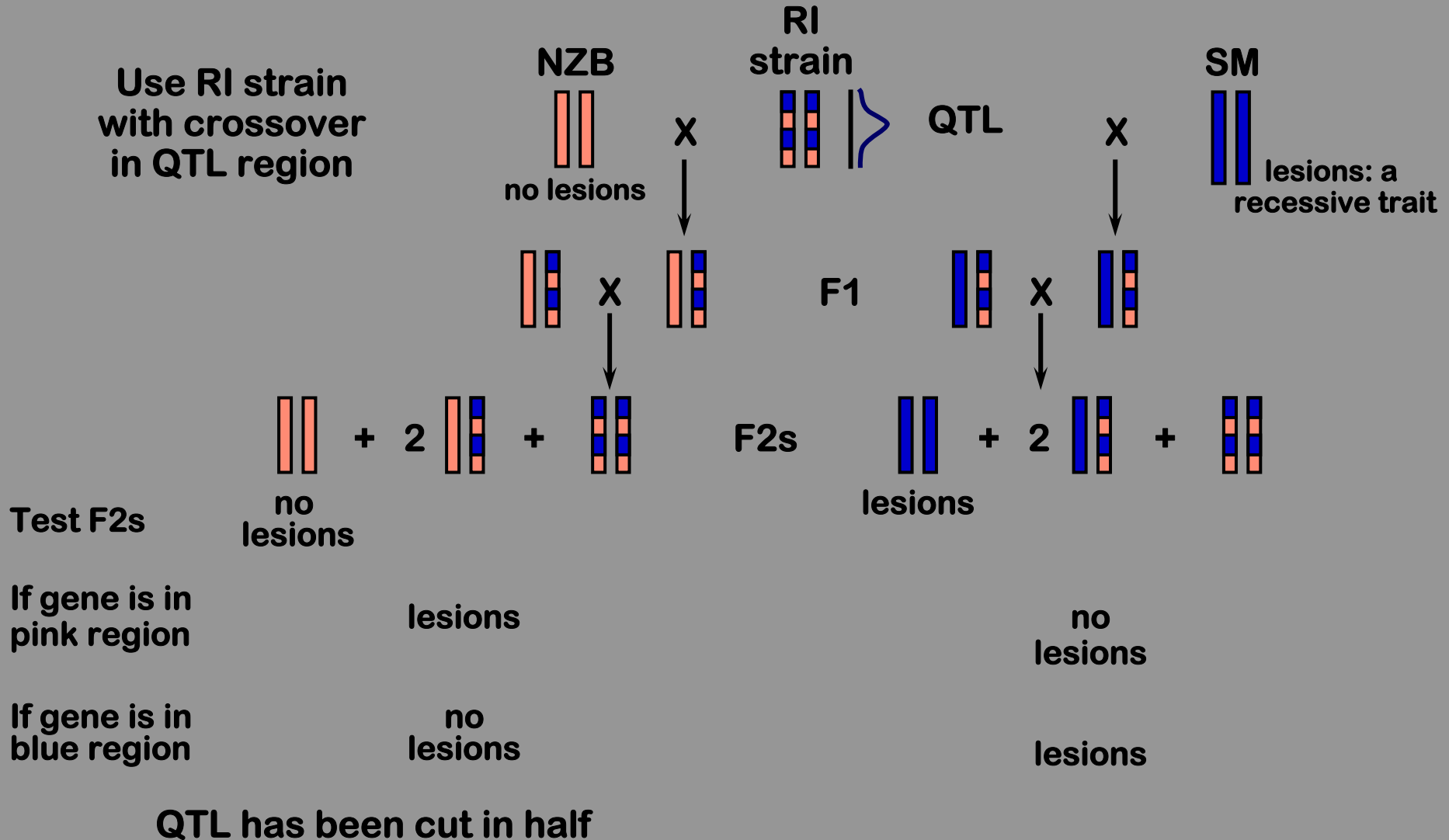
When to Use Overlapping Congenics

- ◆ **Appropriate for QTL with small to moderate effects**
- ◆ **Requires few mice**
- ◆ **Slow**
- ◆ **Cannot take advantage of dominant effects**
- ◆ **Able to separate QTL located on same chromosome**

Usefulness of Congenics

- ◆ Can confirm the QTL
- ◆ Can be used for physiological studies
- ◆ Can dissect the phenotype
 - Lith1* had cholesterol hypersecretion
 - Lith2* had increased bile flow

Recombinant Inbred Segregation Test (RIST)



Recombinant Inbred Segregation Test (RIST)

- ◆ Requires RI strains with recombinations in QTL region
- ◆ Requires only 2 generations
- ◆ Requires moderate number of mice

- ◆ Now that we have dense SNP maps, can use the same strategy with common inbred strains. Called a Ying-Yang test. Shifman & Darvasi *Genetics* 169:849, 04

Advanced Intercross Lines

- ◆ Mate two strains that differ, obtain F_1 s and F_2 s
- ◆ Use F_2 s to produce at least 50 breeding pairs of F_3 s
- ◆ For F_3 and beyond, set up at least 50 breeding pairs, making sure mates do not share a common grandparent
- ◆ By F_8 , many recombinations have accumulated
- ◆ Phenotype and genotype progeny in region of **previously discovered QTL**

When to use Advanced Intercross Lines

- ◆ Use only for previously discovered QTL
- ◆ Requires relatively few mice
- ◆ Requires many generations
- ◆ Generates many recombinations
- ◆ Resolution very high

- ◆ Need patience and lots of mouse room- has only been used a few times

Strategies for Different Steps

- ◆ **Narrowing to 5cM**
 - Selective phenotyping
 - Recombinant progeny testing
 - Overlapping congenics
- ◆ **From 5cM to 1cM**
 - Recombinant progeny testing
 - Overlapping congenics
 - RIST

Narrowing QTLs

Narrowing with mouse crosses takes 3-12 months and is slow

Bioinformatic methods are much faster.

Practical approach is to use bioinformatics as much as possible and then use mouse genetics guided by the bioinformatics.

Major New Resources

Thousands of SNPs for most common inbred strains

16 sequenced strains

In silico QTL mapping (equivalent to GWAS in humans)

Expression QTLs

New Challenge

Used to be hard to find QTL genes

Using combination of bioinformatics, microarrays, and mouse genetics, we are finding QTL genes.

New challenge is to prove the gene.

Knockouts are not the “gold” standard.

What Proves a QTL Gene

Nature Genetics 2003 4:911

Consensus paper by the Complex Trait Consortium lists 8 different lines of evidence

Suggests that multiples lines of evidence are needed

Since then, two additional kinds of evidence are available.

What Proves a QTL Gene

1. Polymorphism in coding or regulatory region
2. Expression in tissue or link to function
3. In vitro functional test
4. Transgenesis
5. Knockin

What Proves a QTL Gene

6. Deficiency complementation
7. Mapping in second species
8. Mutational analysis
9. Small inhibitory RNA
10. Distribution of alleles

Polymorphisms in coding or regulatory regions

Coding region: does it change amino acid, is it a significant change, is it in a conserved region, a known domain?

Regulatory region: harder to analyze the DNA change, but can determine if expression is different with microarrays or RT-PCR

Could also be a splice site, UTR, protein stability change

Link to Function

Expression in relevant tissues or at relevant time.

Known link to function.

In vitro evidence

Some evidence showing that the activity of the two alleles is different.

Transfection experiments

Podocin story- signalling exp.

Transgenesis

If overexpression of gene causes phenotype, then a transgene can provide proof.

This test requires a dominant trait and an alternative allele.

Will be easier as more BAC libraries are available.

Knockin

Replacement of one allele by another and change in phenotype.

This test requires the right strains- basically a knockout and a knockin of a gene.

Deficiency Complementation

If a knockout of the candidate is available, mate to both strains.

There should be a differential impact on the trait if it is the correct gene.

Mapping in second species

Test candidate in human or rat.

Mutational analysis

If there is a second mutation in the gene causing a phenotype, that is additional evidence.

ENU mutagenesis often finds the same genes as QTLs

Sometimes strains have more than 1 mutation.

si RNA

Determine if si RNA against the gene can reproduce the phenotype in cell culture or in the whole mouse.

Distribution of alleles

Distribution of alleles of candidate gene accounts for finding the QTL or failing to find the QTL in multiple crosses.

- For *Apoa2*, explained 13 crosses
- For *Abca1*, explained 9 crosses
- For podocin, explained 4 crosses

Most Useful Evidence

Polymorphism

Function

Second species

Transgene

Deficiency complementation