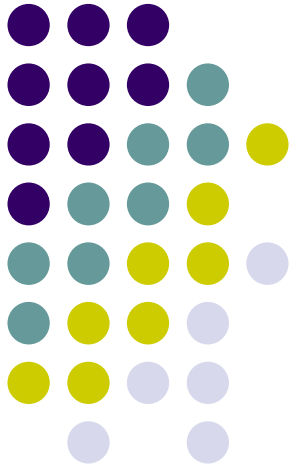
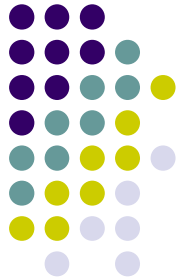
Gene Mapping Strategies

Gene422/522



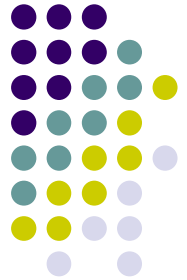
Gene Mapping



Ultimate aim is to identify gene & functional mutation

- Structural / functional studies on gene product → knowledge of biochemical pathway controlling trait of interest
- Functional mutation is a ‘perfect marker’ → GAS
- Significant resources and time are required, particularly for continuous traits

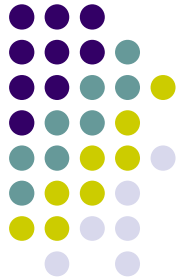
Gene Mapping



An alternative end point

- Haplotype spanning 1- 5 cM → MAS
- Information content can be similar to that of a direct marker, depending on extent of linkage disequilibrium

The steps



1cM → ~1 million bp containing ~10 genes

Unknown location to ~20cM region

- achievable via ‘broad-scale’ linkage mapping

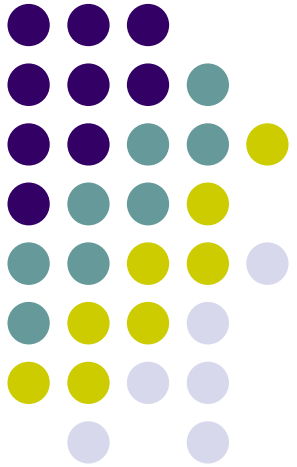
~20cM region to <2cM region

- various approaches, including LD mapping
- usually requires significant animal resources

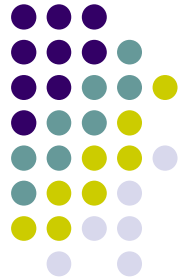
<2cM region to gene and functional mutation

- positional candidate and other approaches
- may need to sequence through large regions for a number of animals

From 20cM to <2cM



Strategies for refining region from 20cM → 2cM



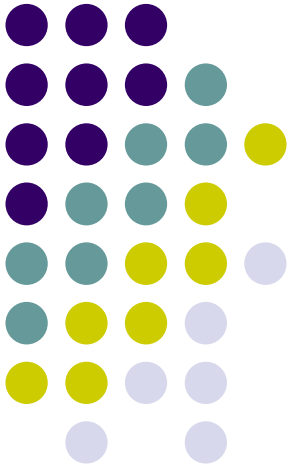
Fine-scale linkage mapping

Linkage disequilbrium mapping

Multi-generational QTL mapping e.g. targeted recombinant progeny

Overview of approaches 20cM→ 2cM

Fine-scale linkage mapping



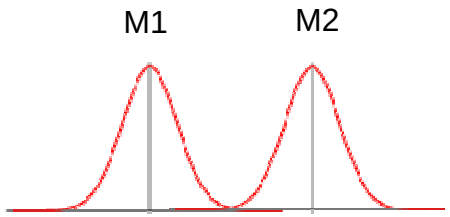
Fine-scale linkage mapping

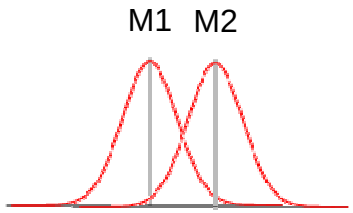
Basis of linkage mapping

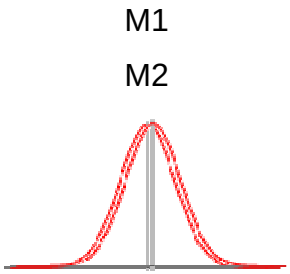
- As an example consider a half-sib mapping design and single marker analysis

Potential QTL

Means of progeny groups inheriting the M1 and M2 alleles significantly differ







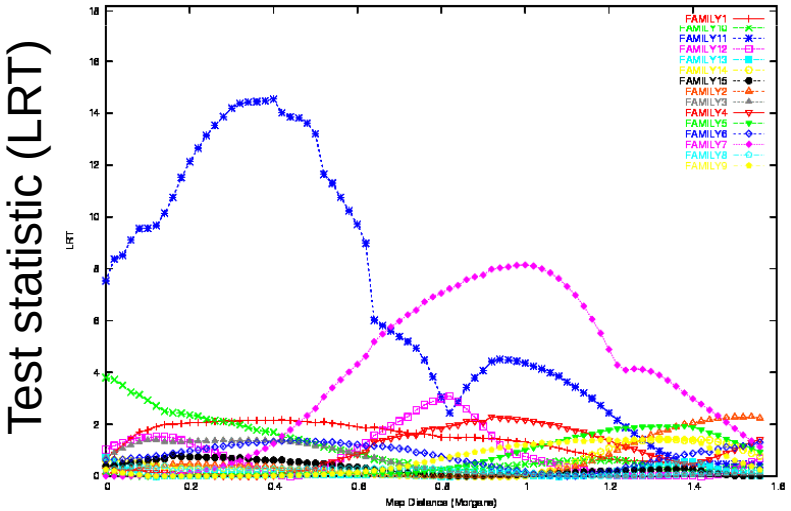
No evidence for QTL

Trait distribution of progeny inheriting either a M1 or M2 allele from sire

Fine-scale linkage mapping

Basis of linkage mapping

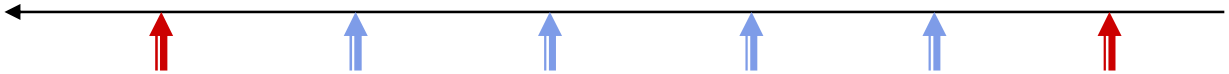
- More usual implementation is interval mapping via maximum likelihood or regression



Fine-scale linkage mapping

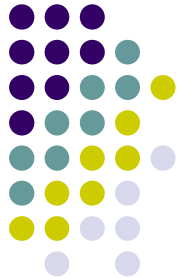
Basis of ‘fine-scale’ linkage mapping

- as for linkage mapping but with additional markers within the region of interest



- aim is to reduce the confidence interval (CI) of the position estimate
- CI calculated from LRT: decrease in one LOD score either side of best position → 96.8% CI

Fine-scale linkage mapping

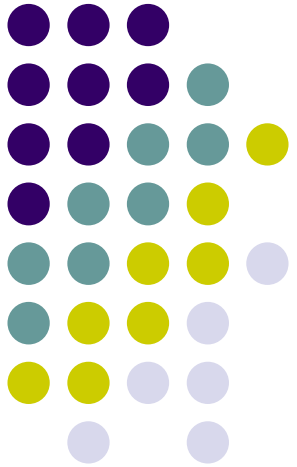


Reality

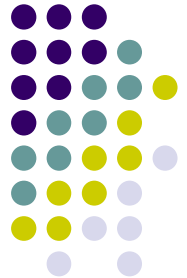
- At some point increasing marker density will not refine the QTL position
- This is because very large half-sib families are required to generate recombinants between closely spaced markers
  - e.g. markers 2cM apart → only 20 out of 1000 progeny are recombinant within this region

Overview of approaches 20cM→ 2cM

Linkage disequilibrium mapping



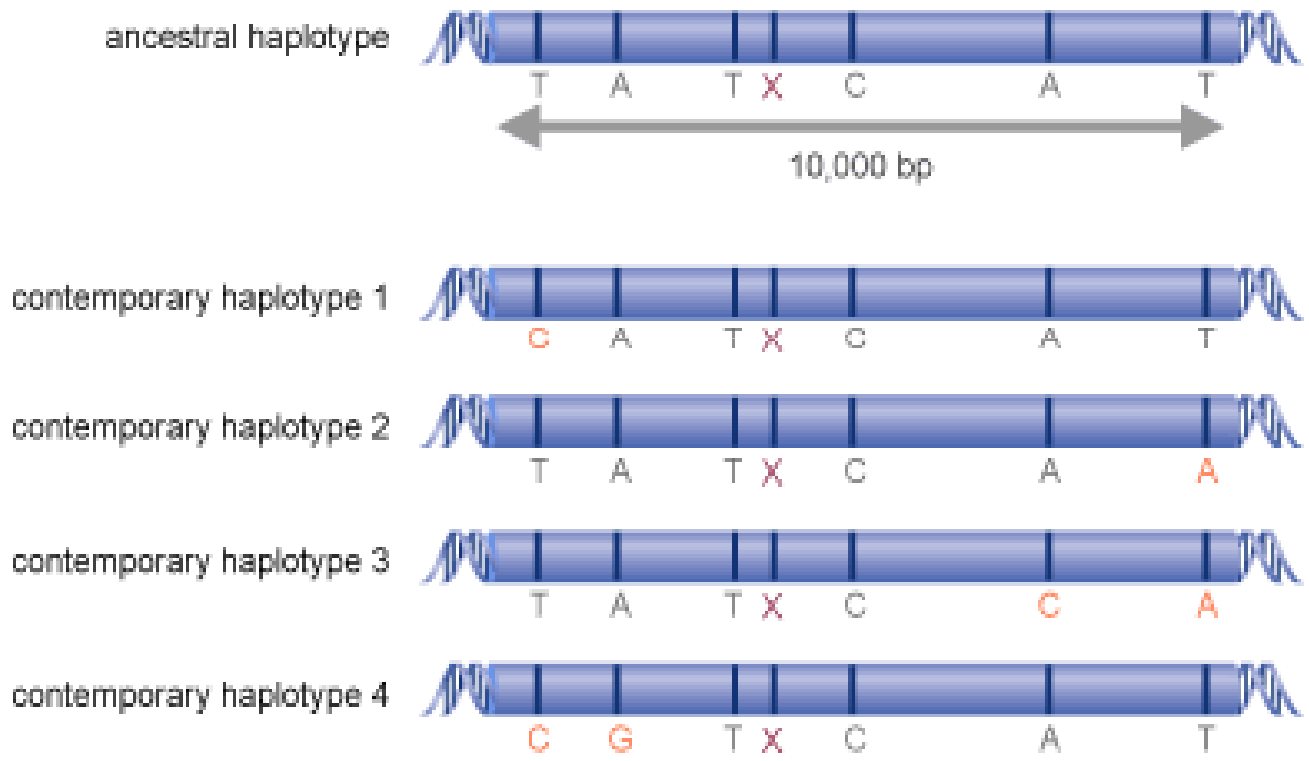
Linkage disequilibrium (LD) mapping



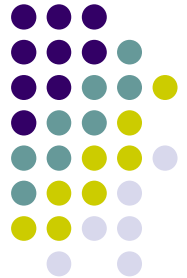
Basis

- Linkage mapping considers the linkage disequilibrium that exists *within families*
- LD mapping considers the linkage disequilibrium that occurs across the *entire population.* Pure LD mapping disregards pedigree structure
- *LD and linkage mapping can be combined → LDLA mapping*

LD mapping


ancestral haplotype
T A T X C A T
10,000 bp
contemporary haplotype 1
C A T X C A T
contemporary haplotype 2
T A T X C A A
contemporary haplotype 3
T A T X C C A
contemporary haplotype 4
C G T X C A T

Linkage disequilibrium (LD) mapping

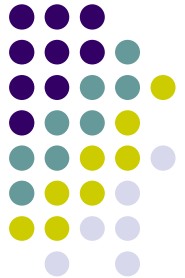


Basis

- For LD to occur across the entire population, and not be broken down over generations, the QTL and marker must be closely linked

- LD mapping is applicable to
  - region of ~20cM or less (i.e. in LD) for sheep / cattle
  - historical data, where analysis is performed over generations
  - industry data, where analysis is performed over families
  - half-sib data, if QTL is assumed to be segregating in dams

Linkage disequilibrium (LD) mapping



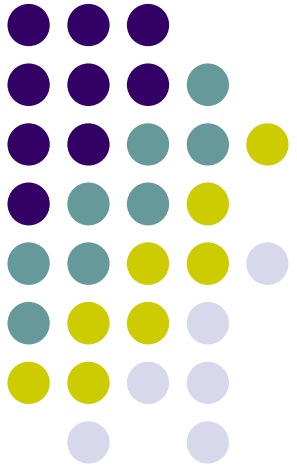
Reality

- Powerful method, although merit of *linkage* vs *LD* vs *LDLA* depends on underlying extent of LD / mutation age and data structure, and continues to be evaluated by simulation
- Only recent move to storage of DNA from breeding animals / experimental flocks, thus historical pedigree and phenotypes may be available but DNA is often not
- Successfully used to refine QTL positions e.g.
  - QTL for milk traits refined to 3cM by LD
  - QTL for twinning rate refined to <1cM by LDLA
  - numerous QTL in human literature

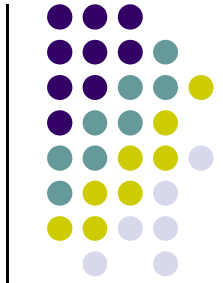
Overview of approaches 20cM→ 2cM

Multi-generational QTL mapping

“Targeted Recombinant Progeny”



Targeted Recombinant Progeny



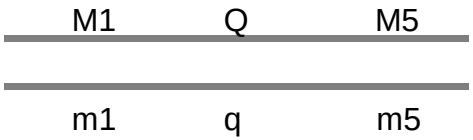
Basis

- Essentially ‘multi-generational QTL mapping’ but optimized to reduce genotypes / phenotypes

- Steps
  - Produce many progeny from a heterozygous sire
  - Identify those individuals that are recombinant within the region of interest
  - Progeny test these individuals to determine if segregating for the QTL
  - Determine QTL location via ‘breakpoint analysis’

Heifetz, Fernando and Soller 7th WCGALP

G0H



Sire known to be heterozygous for the qTL

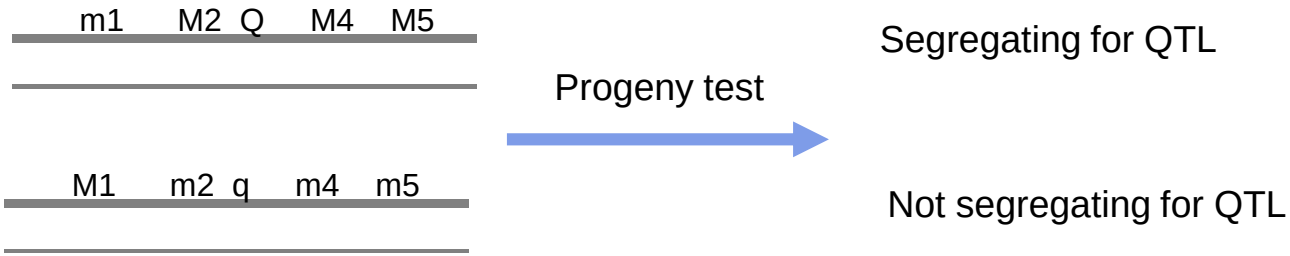
G1 (G0H x dams)

Identify sons recombinant in regions of interest (G1R)

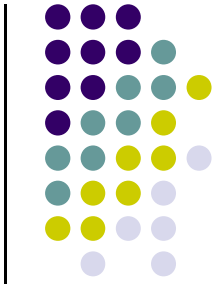
G2 (G1R x dams)

Progeny test recombinant individuals to determine if segregating for QTL

Also type these individuals with additional markers within the region of interest

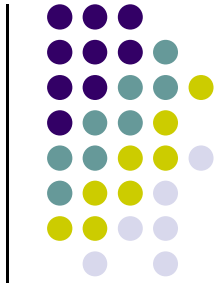


Breakpoint analysis



Sire QTL phase (G0H)	M1	M2	M3	M4	M5	M6	M7	M8	M9	
q	A	A	A	A	A	A	A	A	A	
Q	B	B	B	B	B	B	B	B	B	
										Segregating
Sons (G1R)	A	-	B	B	-	B	B	B	B	Y
	A	B	B	B	-	B	B	B	B	Y
	A	-	A	B	-	B	B	B	B	Y
	A	-	A	A	A	A	A	B	B	N
	A	-	A	A	A	A	A	A	B	N
	B	B	A	A	-	A	A	A	A	N
	B	-	B	A	-	A	A	A	A	N
	B	B	B	B	B	A	A	A	A	N
	B	B	B	B	B	B	B	A	A	Y
	B	B	B	B	-	B	B	A	A	Y
	B	B	B	B	B	B	B	B	A	Y
									Region in common	

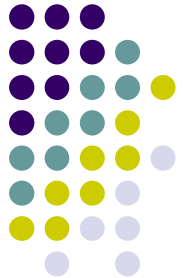
Targeted recombinant progeny



Reality

- Powerful method, but need sufficient G1R
  - 200 G1 progeny gives ~20 recombinants in 10cM region, half of which are male
- No need to progeny test if G1R individuals can be classified as segregating or not on their own phenotype
- Long time-line (several years)
- Success
  - Carwell narrowed to <1cM region using 8 G1R sires (AgResearch)
  - Callipyge (used own phenotype on G1R)

From 20cM → 2cM Approach taken depends on



Animal resources

- for fine-scale mapping, the number of required animals is large
- industry / historical data can be used if DNA is available

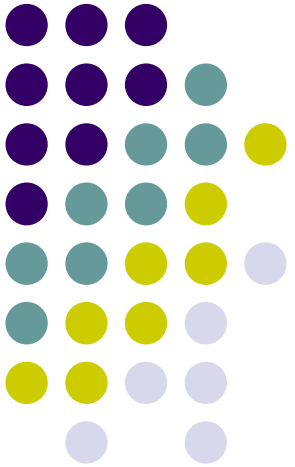
Map and markers

- new markers within the region of interest will likely be required

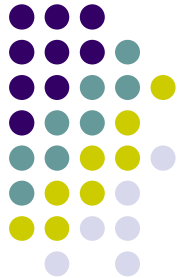
Time-scale

- different approaches have different time-scales

From <2cM to gene and functional mutation



General gene identification strategies



Positional cloning

- Uses knowledge of the mapped location of the gene

Functional cloning

- Uses knowledge of the protein encoded by the gene

Candidate gene

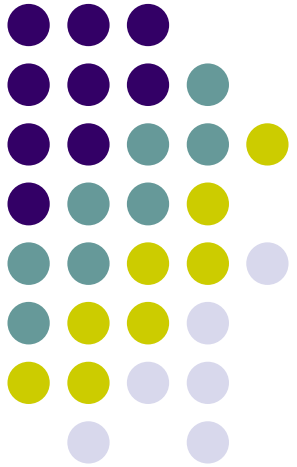
- Gene identified as good candidate

Approaches can be taken in combination

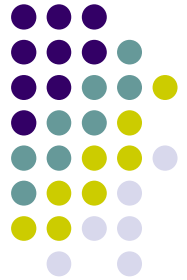
- “Positional candidate” approach

Overview of approaches 2cM to gene

Positional candidate



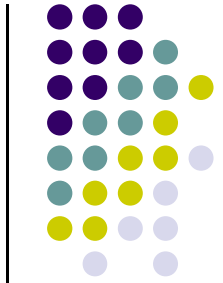
Positional candidate approach



Basis: ‘Sequence’ comparisons

- Search public databases for genes within region of interest via comparisons to:
  - Human genome – fully sequenced
  - Cattle genome – fully sequenced
- Expect to find a number of genes, some of which may be candidates
  - ~10 genes / cM (human)
  - Candidates identified after literature search for evidence of gene involvement in trait biology

Positional candidate approach



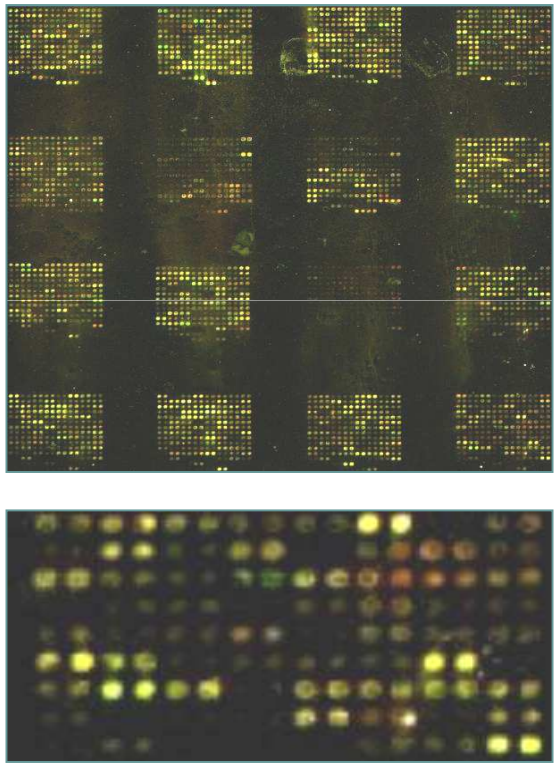
Reality: Sequence comparisons

- Better success with smaller confidence interval about QTL
- Better success if homologous region in human sequence is well defined
- Success:
  - Inverdale QTL spanning ~10cM region to gene (BMP15) on positional candidate basis
  - Milk composition QTL spanning ~3cM to gene (DGAT-1) on positional candidate basis

Positional candidate approach

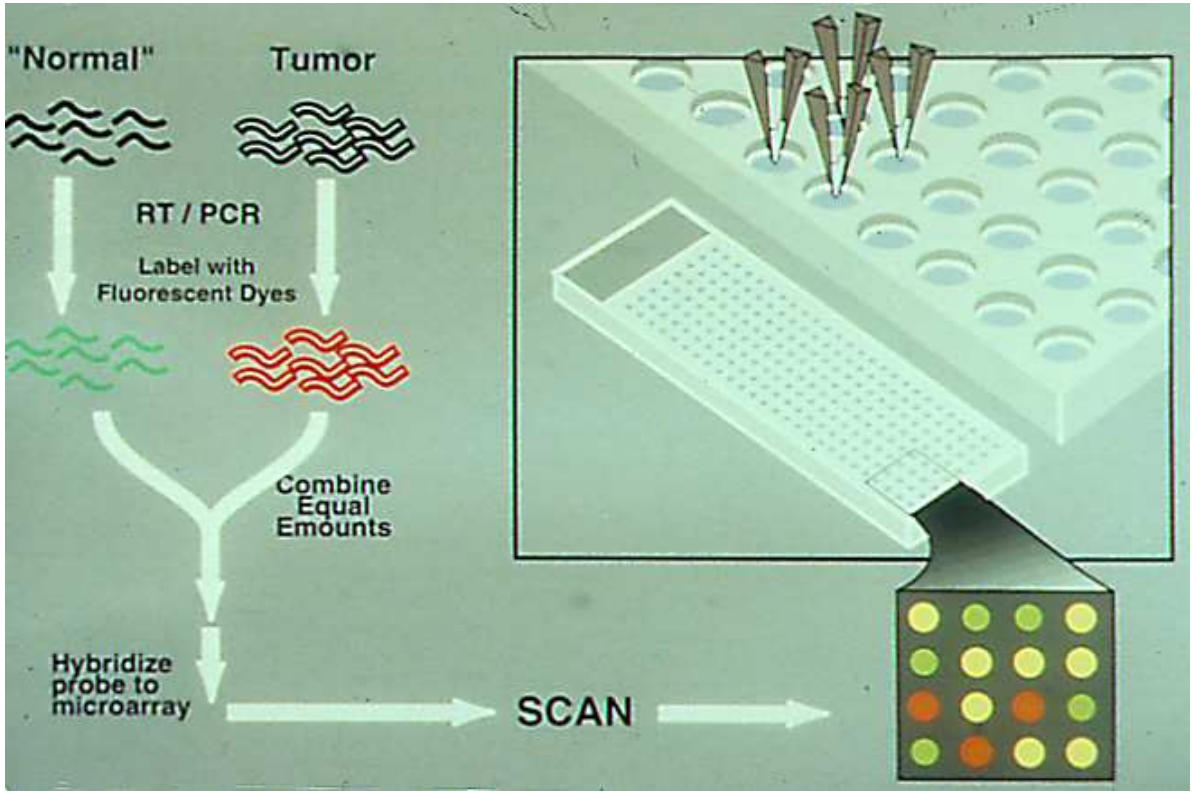
Basis: Expression data

- Detection of differential expression of genes in ‘Q’ versus ‘q’ animals
- If differentially expressed gene is then mapped to region of interest → positional candidate
- Popular current technology is microarrays



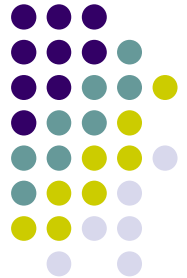
Microarray showing differential display

Microarrays



From, Bioinformatics course notes, J. McEwan, AABSS 2004

Positional candidate approach

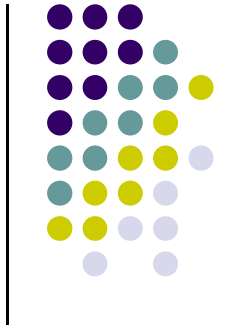


Reality: expression data

- Micrarrays generally produces numerous candidates, a number of which may also be positional, but
  - Arrays are expensive, therefore mRNA often represents tissue from single space / time
  - Not all genes in region may be represented in array
  - Genes identified in microarray may be ‘downstream’

    e.g. mutation in gene A (the QTL) causes change in expression level of gene B (detected in the microarray analysis), can only trace back to gene A if pathway knowledge exists

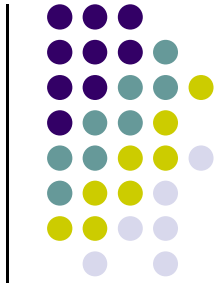
Positional approach



If genes within region of interest are unknown (and thus no candidates)

- Obtain clones of genomic DNA within region of interest
- Identify which of these contain protein coding (gene) sequences by various molecular techniques
  - zoo blots
  - exon trapping
  - cDNA selection (also achieves point below)
- Attempt to determine which of these represents the gene of interest
  - for example, test clones for hybridisation to cDNA derived from cells known to express the mRNA of interest

Identifying functional mutation

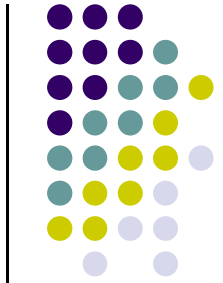


Identifying the functional mutation is usually required as ‘proof’ that the candidate gene is actually the gene of interest

Achieved by

- sequence ‘Q’ and ‘q’ individuals and look for mutations
- predict whether mutation will make a functional difference
- confirm by e.g. sequencing different populations, transgenic studies

Functional mutation



Note:

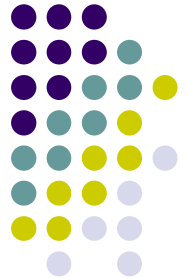
Not all mutations result in an altered phenotype

- Mutations may be silent, cause a conserved amino acid substitution, or alter a non-critical part of the protein

Not all functional mutations are in protein coding sequences

- Mutation may be in regulatory region

Gene Cloning: is it successful?



Numerous examples in literature for discrete traits

- Identification of the Inverdale gene was described as

"the culmination of many years of research involving breeding and segregation studies, genetic linkage mapping, physiology, molecular biology and comparative links to studies in humans and mice" (Galloway et al., 2001)

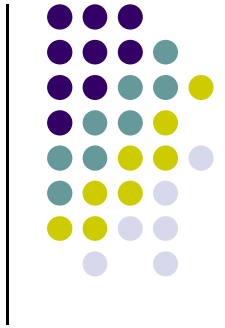
Limited (one) example for quantitative traits

- QTL for milk yield (DGAT1 gene) identified by positional cloning approach (Grisart et al., 2002)

Timeframe and resources is substantial

- e.g. DGAT1 seven + years

The DGAT-1 story



History

- Genome wide linkage analysis identified a region on chromosome 14, with a confidence interval of 20-40 cM

  Georges et al., 1995

- Region narrowed to 3cM by LD and LDLA mapping

  Riquet et al., 1999 and Farnir et al. 2002

- DGAT-1 identified as candidate within this region, subsequent sequencing detected a functional mutation within this gene

  Grisart et al., 2002