Current Testing Platforms Used to Detect One/Few Gene Mutations in Routine Newborn Screening

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Special Considerations

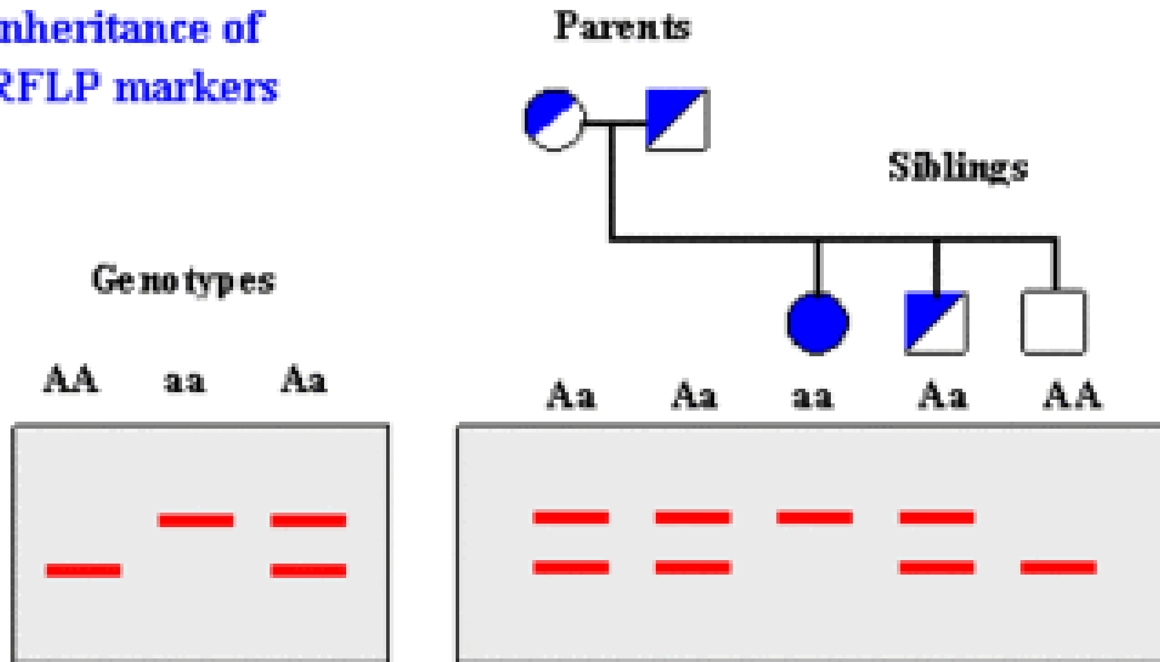
- Utilities
- Targeted mutation analysis
- Platforms
  - Available instrumentation
  - Workflow
  - Targeted gene sequences

Restriction Fragment Length Polymorphism (RFLP) Analysis

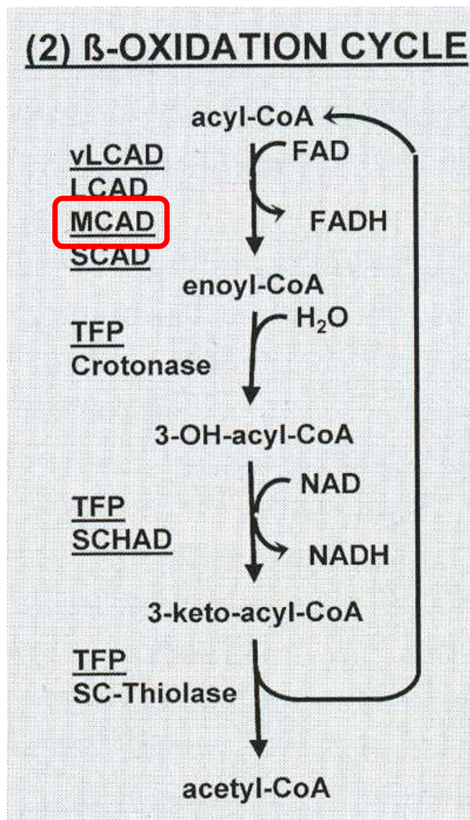
Enzyme	Recognition Site
Rsa 1	. . G T A C . . . . C A T G . .
Mbo 1	. . G A T C . . . . C T A G . .
EcoR1	. . G A A T T C . . . . C T T A A G . .

The red triangles indicate where the enzyme cuts the DNA.

Restriction Fragment Length Polymorphism (RFLP) Analysis



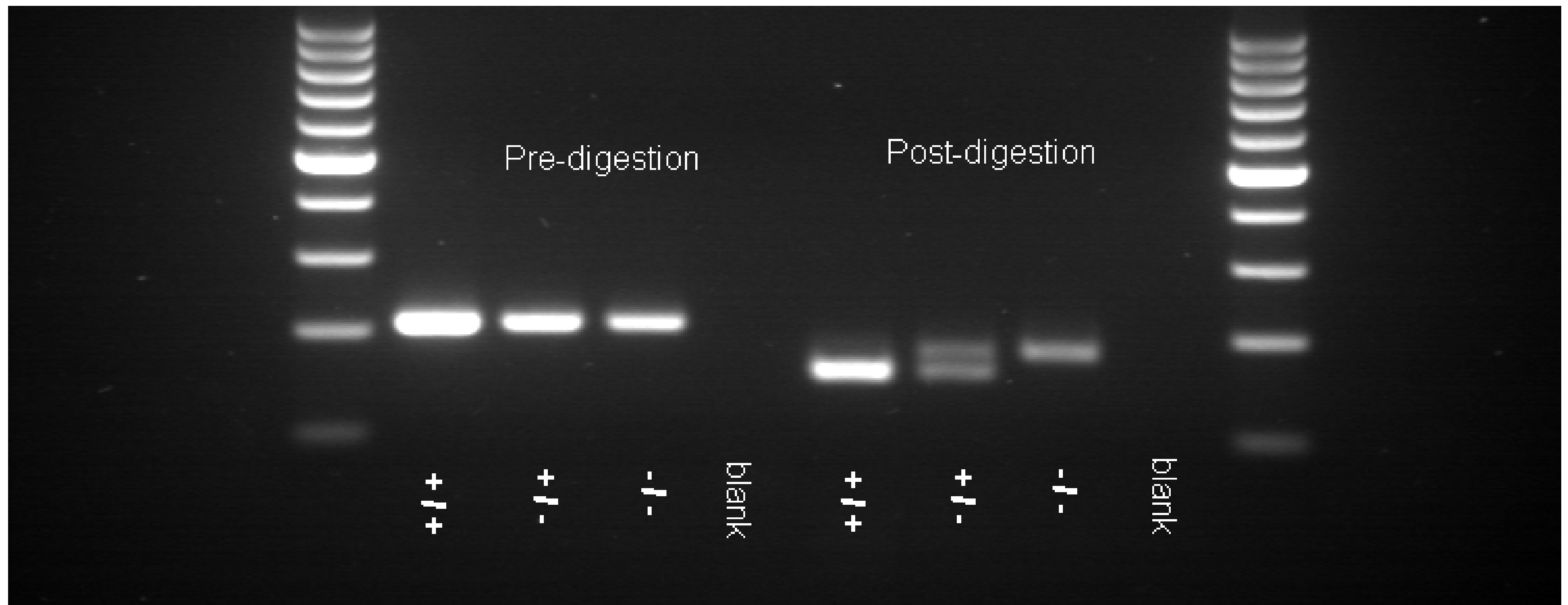
Medium-chain acyl-CoA dehydrogenase deficiency (MCADD)



A previously healthy individual presents with:

- lethargy, seizures, and coma triggered by a common illness
- Hepatomegaly and acute liver disease
- Cardiac presentation
- Sudden and unexplained death

ACADM c.985 A>G Mutation Detected by RFLP (Nco I Digestion)



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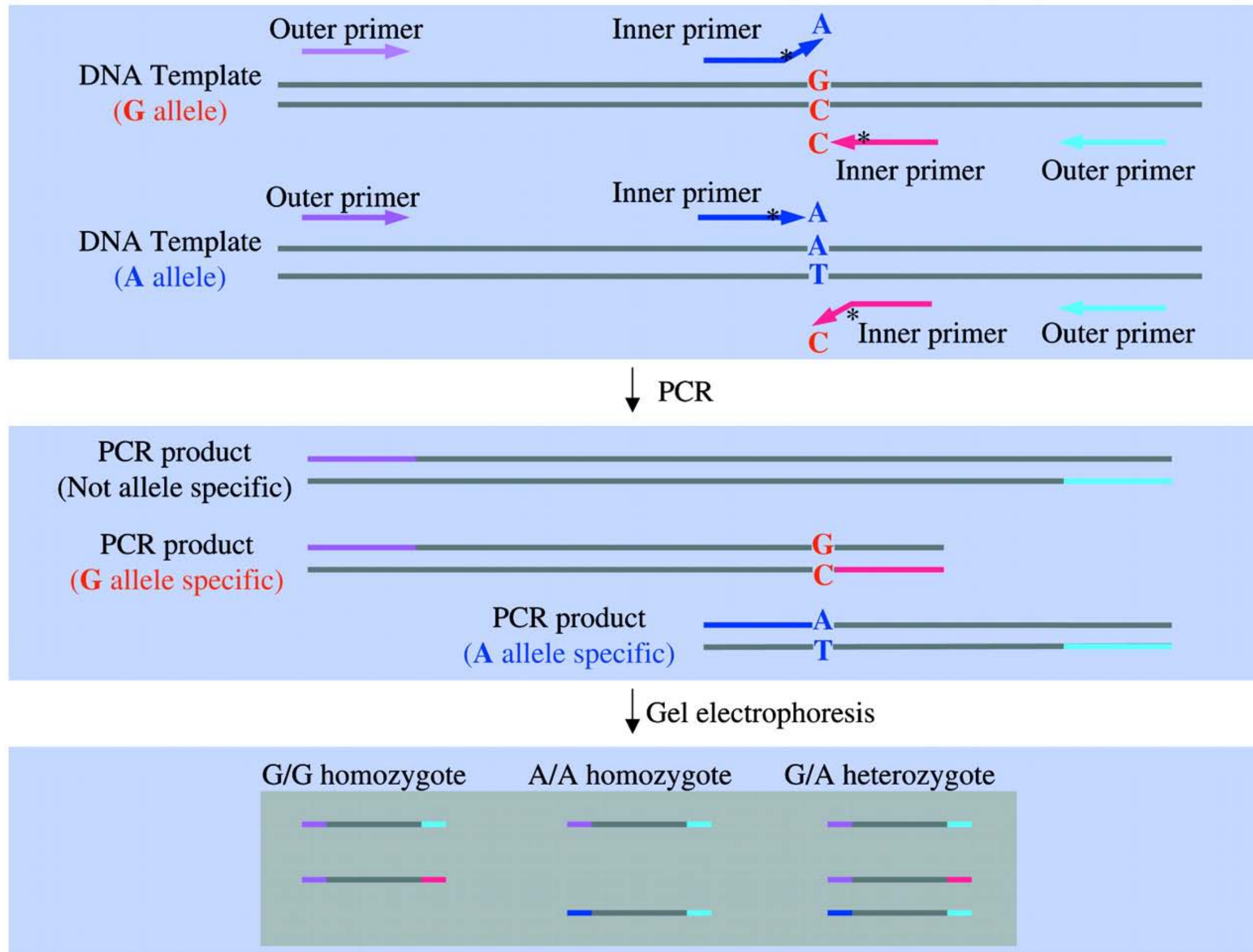
Tetra-primer ARMS-PCR



Ye, S. et al. Nucl. Acids Res. 2001 29:e88; doi:10.1093/nar/29.17.e88

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Nucleic Acids Research

Primer Design

http://cedar.genetics.soton.ac.uk/public_html/primer1.htm

Source sequence (up to 1,000 bases)

Position of SNP from start of sequence

Allele 1

Allele 2

Optimum (inner) product size

Maximum (inner) product size

Minimum (inner) product size

Maximum relative size difference of two inner products

Minimum relative size difference of two inner products

Shu Ye, Nucleic Acid Research, 2001, Vol. 29, No. 17, E88-8

Tetra-primer ARMS-PCR Reaction

- **Reaction Mix: (25 µl)**

1X PCR buffer	
Forward inner primer	1.0 µM
Reverse inner primer	1.0 µM
Forward outer primer	0.1 µM
Reverse outer primer	0.1 µM
DNTPs	200 µM
$MgCl_2$	2.5 mM
Taq DNA polymerase	2.5 U
Genomic DNA	4 µl

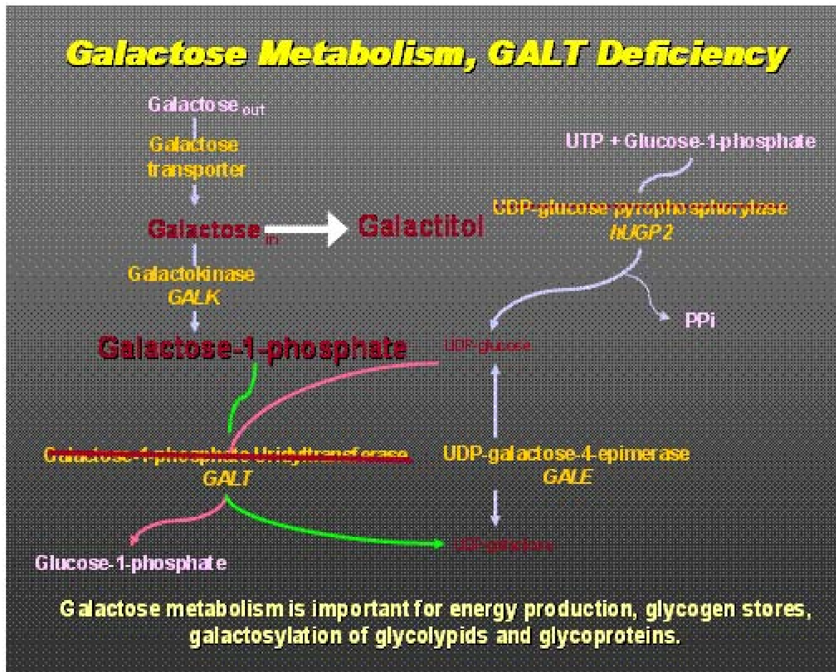
- **Thermal Cycler Condition**

1. 95ºC for 5 minutes
2. 95ºC for 30 second
3. 64ºC for 30 second
4. 72ºC for 40 second
5. repeat 2-4 for 32 cycles
6. 72ºC for 2 minutes

1. The assays for different mutations are run simultaneously using the same thermal cycler conditions.

2. For N314D, inner primers concentration is 0.068 µM, and outer primers concentration is 0.25 µM (Rachel Lee)

Galactosemia

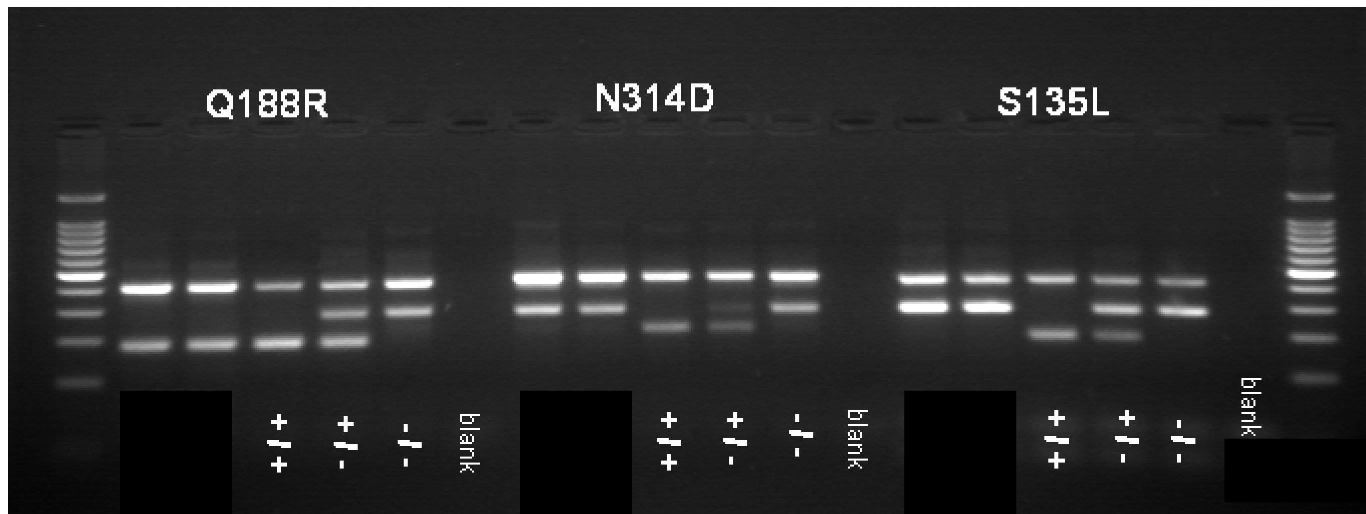


Common *GALT* galactosemia (G) mutations:
p.Gln188Arg, p.Ser135Leu, p.Lys285Asn, p.Leu195Pro, p.Tyr209Cys, p.Phe171Ser, 5kbdel, c.253-2A>G

Neonates with Classic Galactosemia

Finding	Percent
Hepatocellular damage	89%
Food intolerance	76%
Failure to thrive	29%
Lethargy	16%
Seizures	1%
Sepsis	10%

GALT mutations Detection Using Tetra-primer ARMS-PCR



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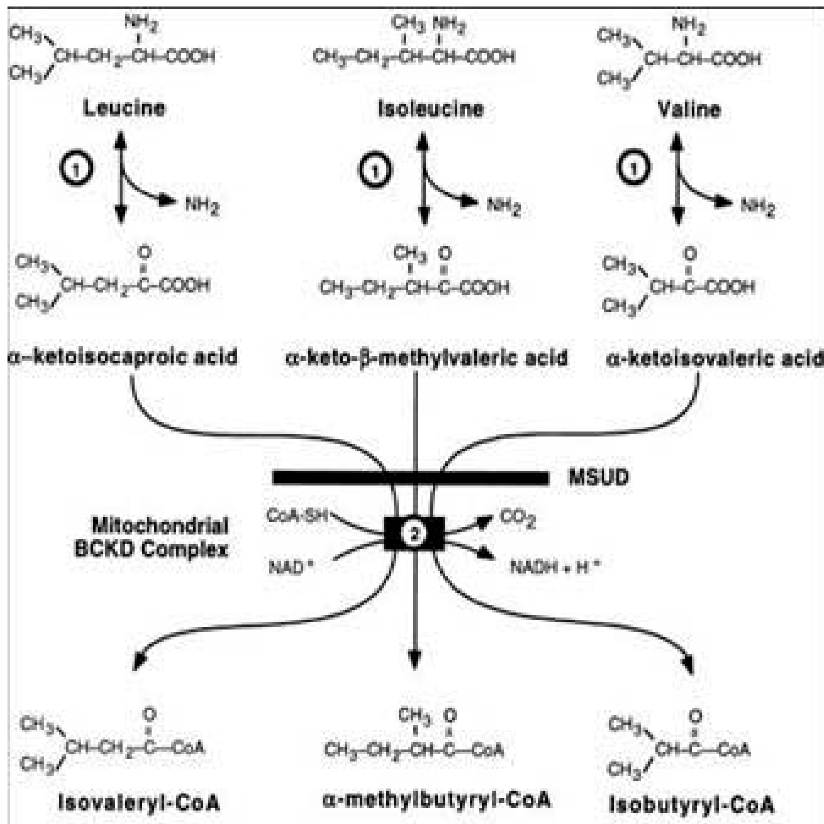
Notes:

1. Q188R—one of common galactosemia disease causing mutations.
2. N314D--Duarte (D_2) variant, and reducing enzyme activity by 25%
3. S135L is associated with a mild phenotype.
4. Newborns who are G/D heterozygotes may have a positive newborn screen

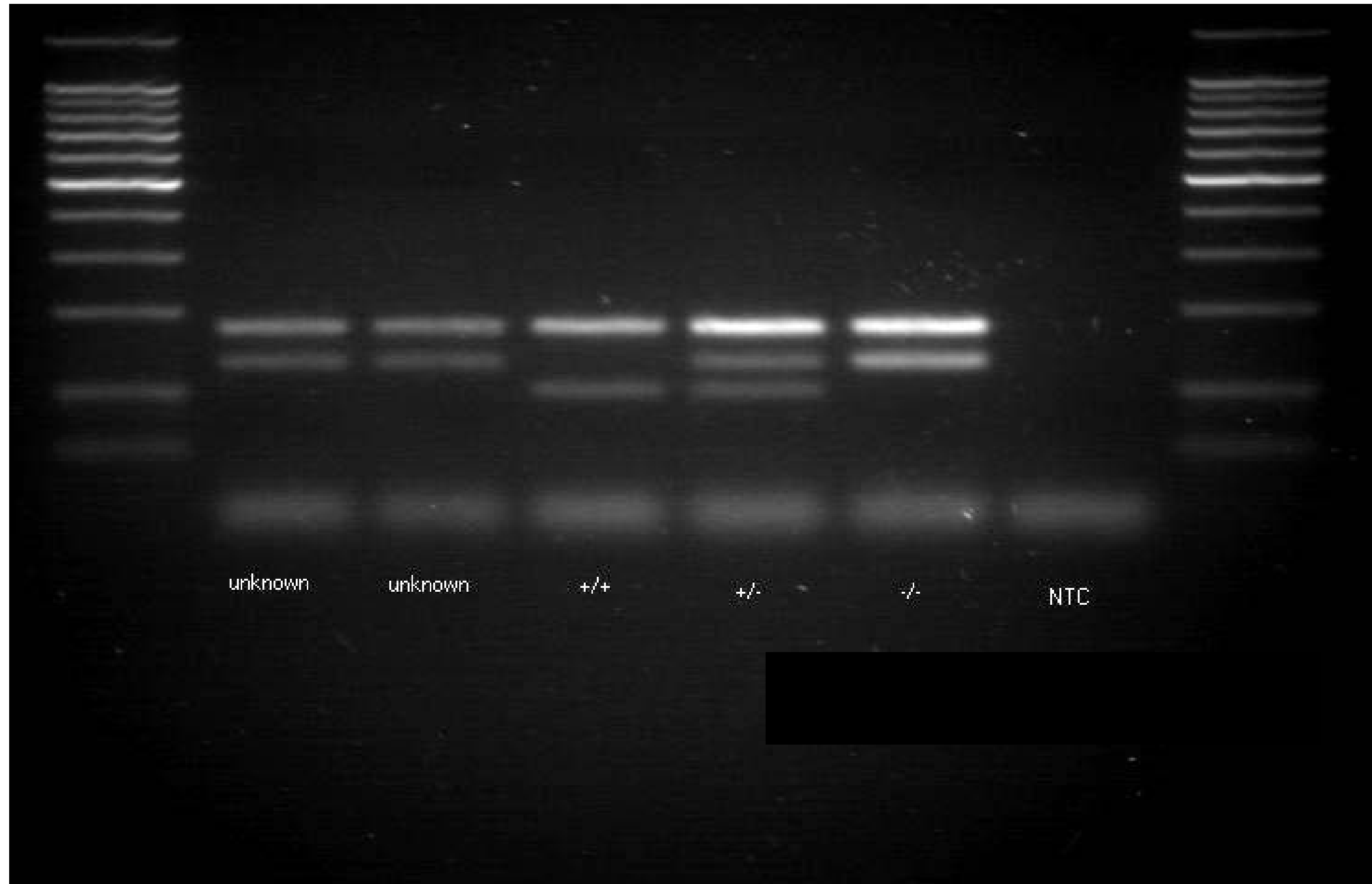
Maple Syrup Urine Disease (MSUD)

BAA Metabolism Pathway



- **Neonates with Classic MSUD**
  - maple syrup odor (12-24 HR)
  - irritability, and poor feeding (2-3 days)
  - lethargy, intermittent apnea, opisthotonus, "fencing" and "bicycling" (4-5 days)
  - central respiratory failure (7-10 days)
- **MSUD in Old Order Mennonites**
  - Carrier frequency is as high as one in ten
  - Disease incidence of approximately one in 380 live births
  - Founder mutation (c.1312T>A) in *BCKDHA*

BCKDHA c. 1312T>A Mutation detection using Tetra-primer ARMS-PCR



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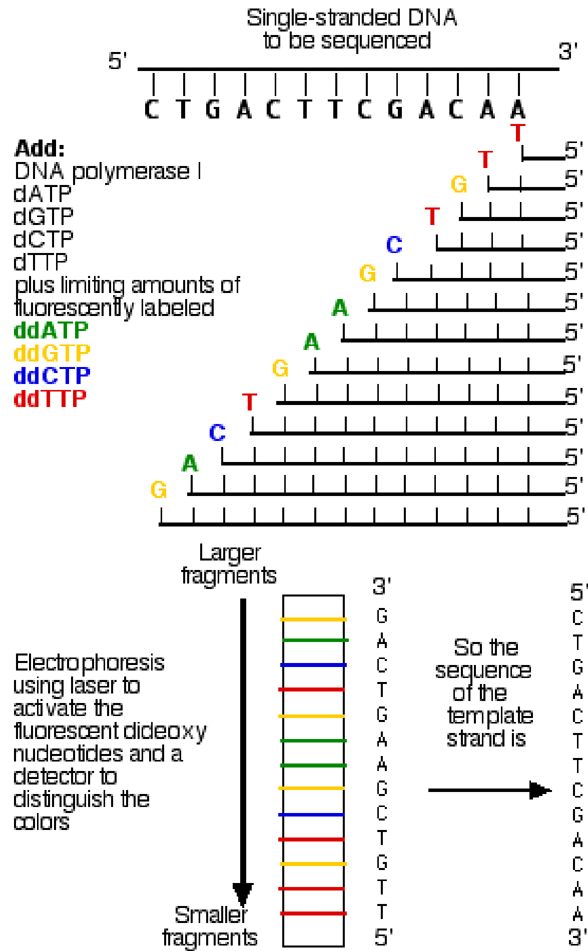
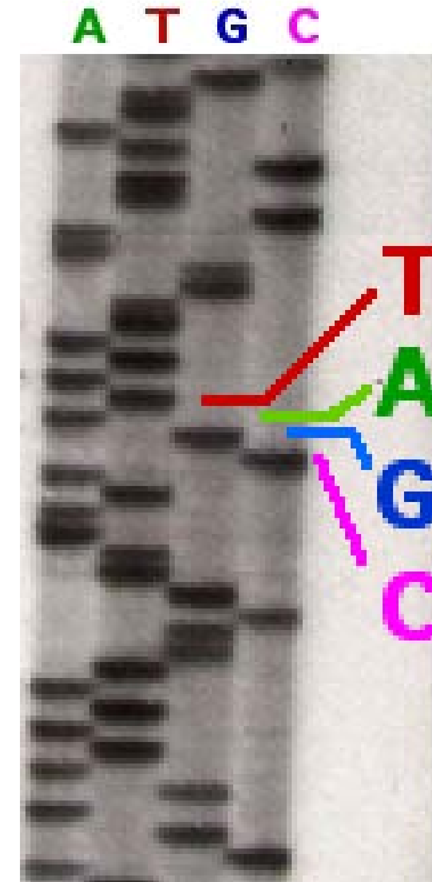
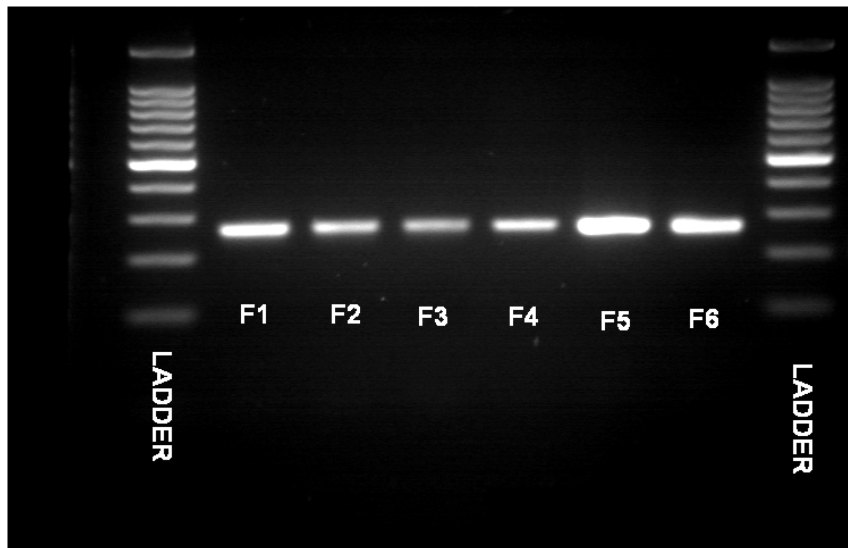
Sanger's Method of DNA Sequencing



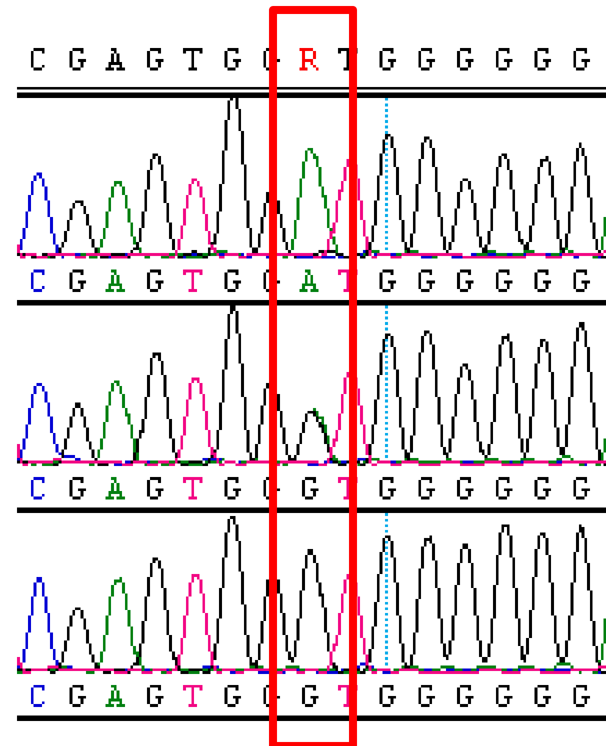


ACADSB c.1165A>G Mutation Detected by Sanger Sequencing

PCR products flank *ACADSB* c.1165A>G site

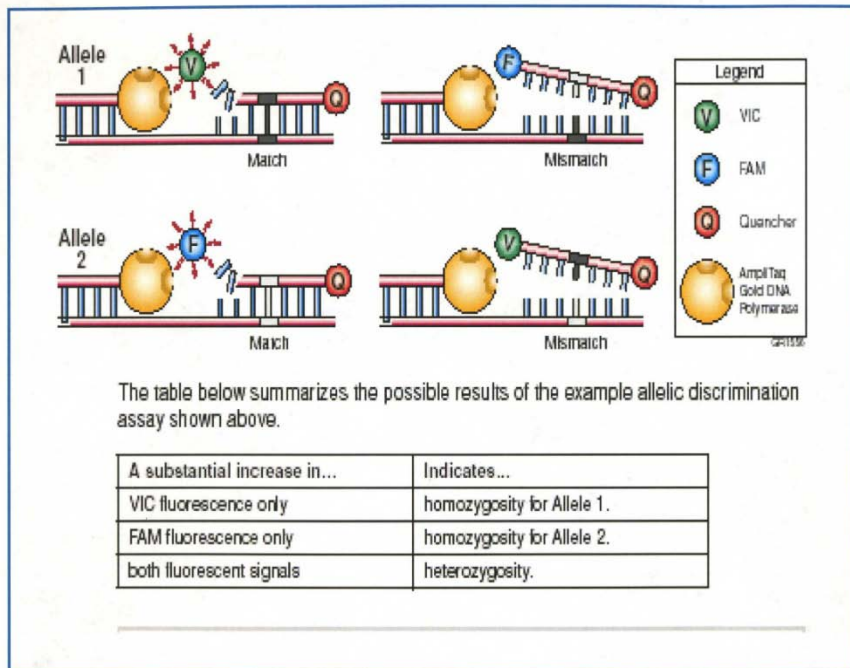


Sequencing Results



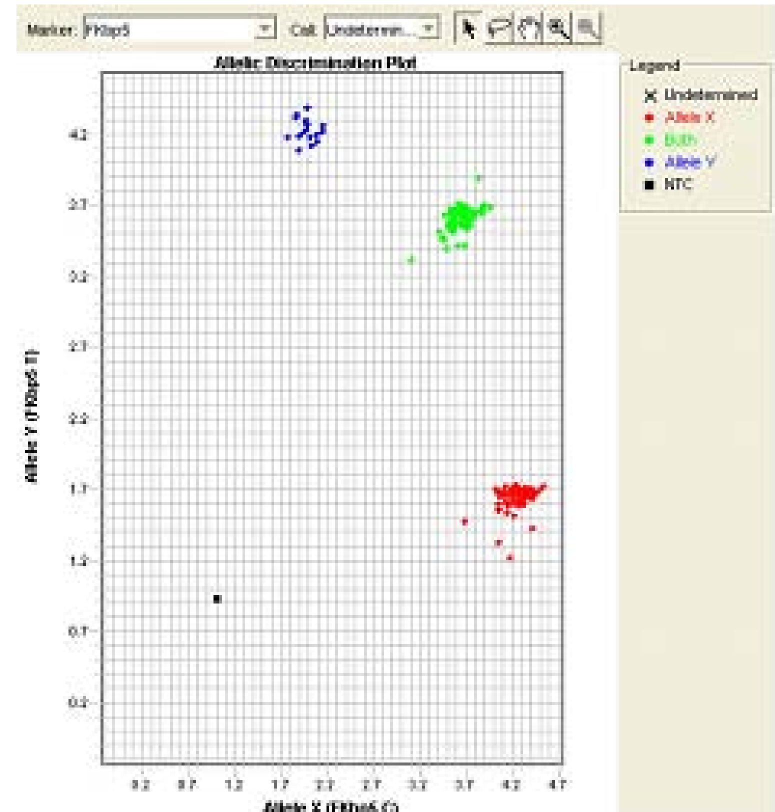
Greg Kopish & Timothy Davis

Allelic Discrimination Using TaqMan Probes

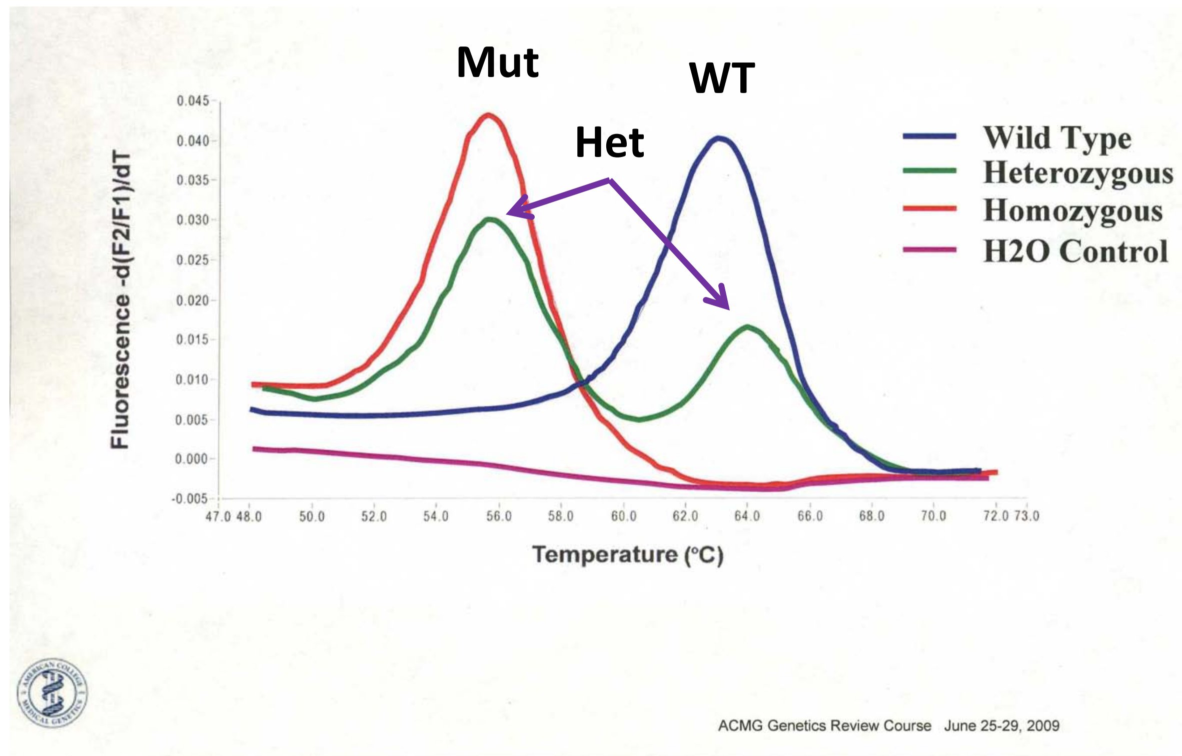


The table below summarizes the possible results of the example allelic discrimination assay shown above.

A substantial increase in...	Indicates...
VIC fluorescence only	homozygosity for Allele 1.
FAM fluorescence only	homozygosity for Allele 2.
both fluorescent signals	heterozygosity.



Fluorescence Resonance Energy Transfer (FRET) Real-time PCR Assay



Summary

Assay	Procedures	Instrument
RFLP	Conventional PCR Restriction enzyme digestion Agarose gel electrophoresis (**Size-based discrimination**)	Thermal cycler Gel electrophoresis unit
ARMS-PCR	Conventional PCR Agarose gel electrophoresis (**Size-based discrimination**)	Thermal cycler Gel electrophoresis unit
Targeted gene sequencing	Conventional PCR PCR products treatment Sequencing Reaction Capillary electrophoresis	Thermal cycler DNA analyzer
TaqMan real-time PCR	Real-time PCR	Real-time PCR system
FRET real-time PCR	Endpoint PCR	Real-time PCR system

Success is the sum of a lot of small things correctly done.

Chef Fermand Point