Mutation Scanning and Candidate-Gene Verification in the ORNL Regional ENU-Mutagenesis Program

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Mutation Scanning in the Mouse Genome

- **Evaluate candidate genes for mutations mapped to specific chromosomal regions**
  - derived from phenotype-driven screens
  - candidate gene(s) identified based on map position, predicted function in relation to mutant phenotype; expression profile
- **Find mutations using gene- or sequence-driven approach**
  - select genes of interest
  - screen bank of mutagenized DNAs derived from F1 progeny of mutagenized mice
    E. Michaud, Cryopreserved Mouse Mutant Bank (CMMB), ORNL
  - reconstitute mutant mice from frozen sperm after mutations are identified (eg. ICSI)

N-ethyl-*N*-nitrosourea (ENU)

- **Potent mutagen of mouse spermatogonial stem cells**
- **Induces primarily single base pair changes**
- **Gene mutations can have a range of consequences (allelic series)**
  - Amorphic (null)
  - Hypomorphic (near-null to almost full activity)
  - Hypermorphic (overactive)
  - Neomorphic (new function)
- **Slightly altered proteins for protein-complex analysis**

Temperature-gradient capillary electrophoresis

- **Qiufeng Gao and Edward S. Yeung**
  High-throughput detection of unknown mutations by using multiplexed capillary electrophoresis with poly(vinylpyrrolidone) solution. *Analytical Chemistry* 2000, 72: 2499-2506

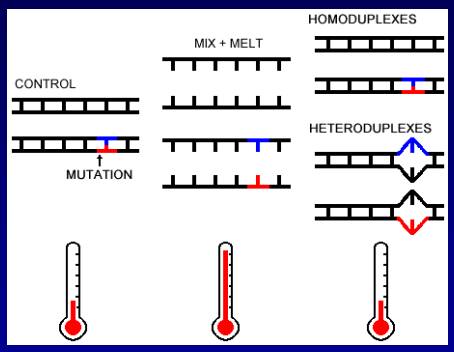
  Ames National Laboratory, USDOE
  Dept of Chemistry, Iowa State University

- **Qingbo Li, Zhaowei Liu, Heidi Monroe and C. T. Culiat**
  Integrated Platform for Detection of DNA Sequence Variants Using Capillary Array Electrophoresis. *Electrophoresis*, in press April, 2002

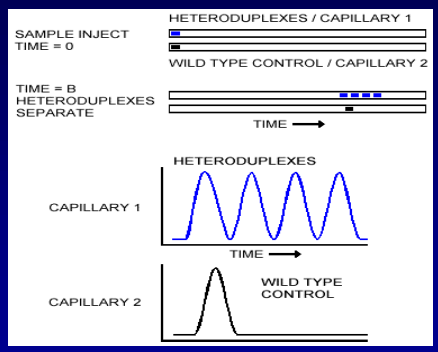
  SpectruMedix Corporation, State College, PA
  Life Sciences, Oak Ridge National Laboratory

Mutation Scanning with TGCE

Heteroduplex formation



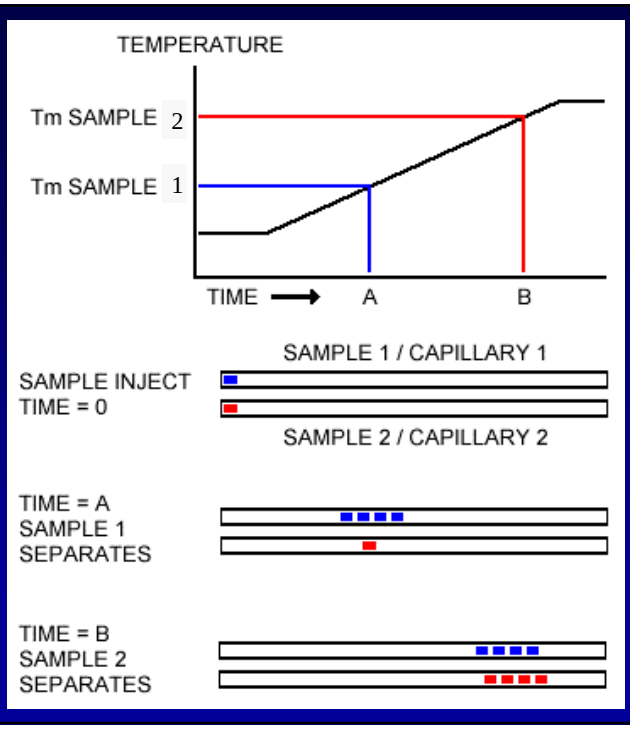
Separation by TGCE



•Optimal temperature for resolving heteroduplex from homoduplex depends on sequence of fragment

• Electrophoreses in a temperature gradient permits separation of samples with different Tm values

eg. 55-65 ºC / 21 min
60- 70 ºC / 35 min



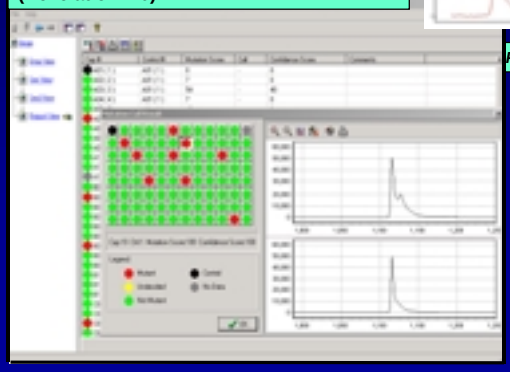
Mutation Scanning using the SCE9610

- 96 **parallel separations in a broad temperature gradient**
  - eliminates calibration runs
  - 150-800 bp PCR fragments with different Tms
- **High-throughput**
  - twelve 96-well plates/day
- **Highly sensitive**
  - low sample requirement (~200 pg/µl)
  - multiple analysis of same sample preps
  - ~95% accuracy (based on 480 amplicons of known SNPs in 6 strains; Novartis Foundation)
- **Simple and low cost sample preparation**
  - rapid mouse tail DNA preps, unpurified PCR reactions
  - detection with ethidium bromide laser-induced fluorescence
  - scanning cost: $0.10/sample
- **Multiplexing**
  - analyze different amplicons in single capillary
  - pooled samples (EtBr: 1/20 samples)

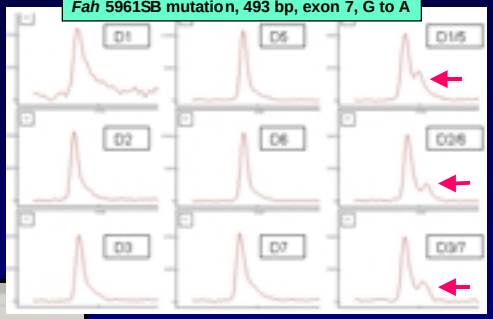
	heterozygotes	control
200bp, G>A @ 34bp	R4/6	R4
200bp, T>C @ 66bp	R4/8	R8
200bp, G>A @ 34bp & T>C @ 66bp	R6/8	R6
493bp, G>A @ 268bp	B3*/4*	B3*
341bp, G>C @ 98bp	C6*	C5*
341bp, G>C @ 98bp	C8*/9*	C8*

Protocols were optimized with known mutations in the *Fah* and *Tyr* genes, before applying TGCE to finding unknown mutations.

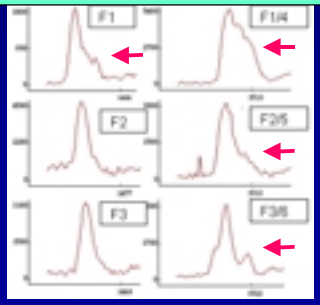
• SpectruPro 1.99 MuFinder Program (Revelation 2.0)



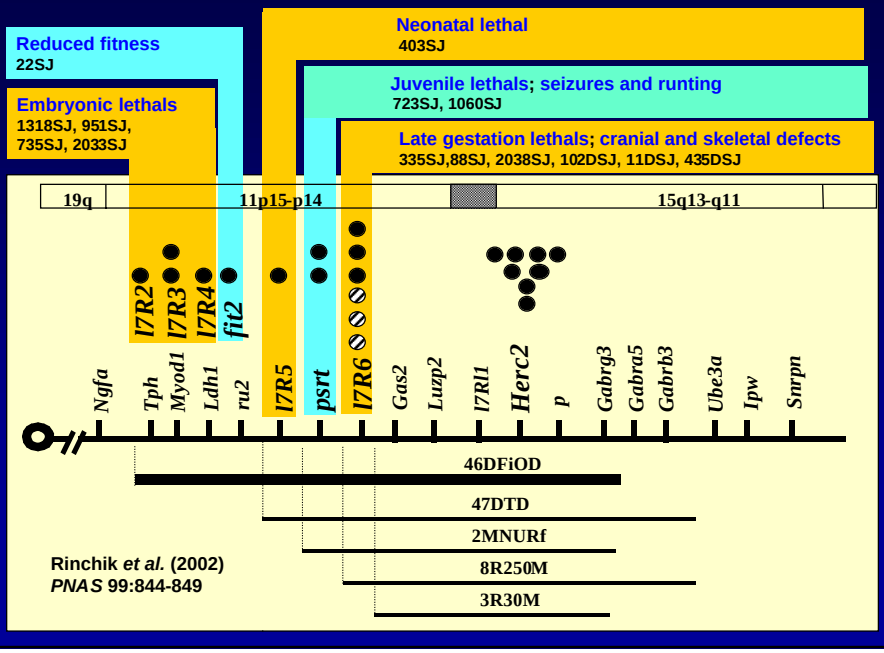
Fah 5961SB mutation, 493 bp, exon 7, G to A



Fah 6287SB mutation, 340 bp, exon 6, A to G



Mutation Map: Chr 7 proximal *p* region



Rinchik *et al.* (2002)
PNAS 99:844-849

Assigning Candidate Genes

Phenotyping

- Time of Death Studies
- Enzyme Assays
- Pathology

Transcriptomics

Expression Assays

- Normal Panel (C57BL/6)
- Mutant Panel

Bioinformatics

- Sequence availability and quality
- Sequence analysis
- Gene information*

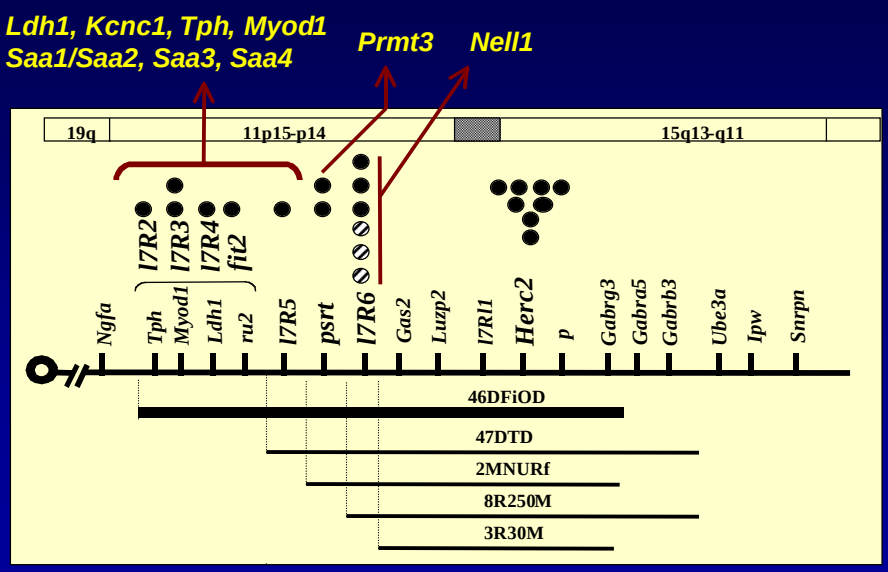
*(map position, known mutations, expression, protein domains, gene function)

CANDIDATE GENE(S)

Mutation Scanning

DHPLC, TGCE, Sequencing

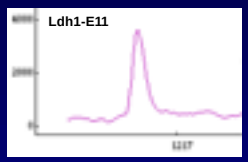
Mutation Map: Chr 7 proximal *p* region

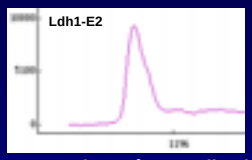


TGCE Analysis of Chr 7 Unknown Mutations

- **1318SJ (*Ldh1*)**
  - early embryonic lethal
  - exon 5 mutation

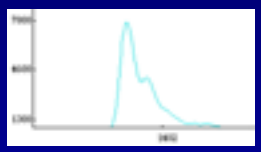
Controls **Heterozygotes**





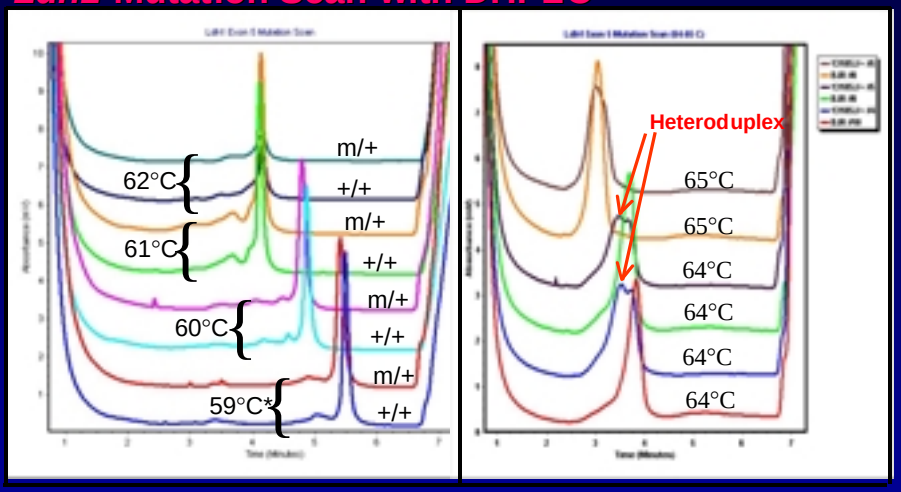
(60-68° C gradient)

BJR (+/+)	TGG	GTC	CTG	GGA	GAA	CAT	GGC
	W	V	L	G	E	H	G
1318SJ/+	TGG	GTC	CAG	GGA	GAA	CAT	GGC
	W	V	Q	G	E	H	G



(64-68° C gradient)

Ldh1 Mutation Scan with DHPLC

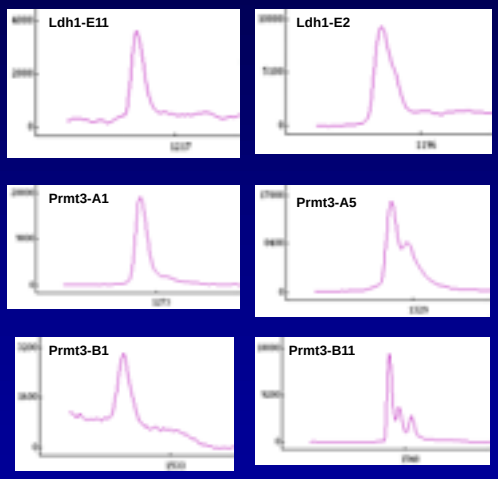


Exon 5 mutation was detected five degrees above the predicted temperature. In contrast, a single TGCE run with a broad temperature gradient (60-68 °C) successfully pinpointed this mutation with MuFInder analysis.

Chr 7 unknown mutations scanned by TGCE

- **1318SJ (*Ldh1*)**
  - embryonic lethal
  - exon 5 mutation
  - CTG to CAG
  Leu to Gln substitution
- **1060SJ(*Prmt3*)**
  - seizures and runting; juvenile lethal
  - 251 bp cDNA segment
- **723SJ(*Prmt3*)**
  - seizures and runting; juvenile lethal
  - 424 bp cDNA segment

Control | **Heterozygote**



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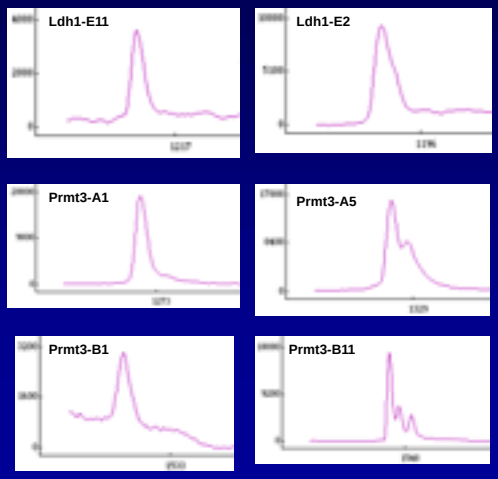
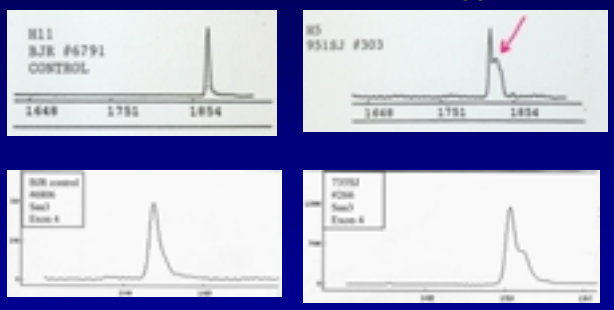


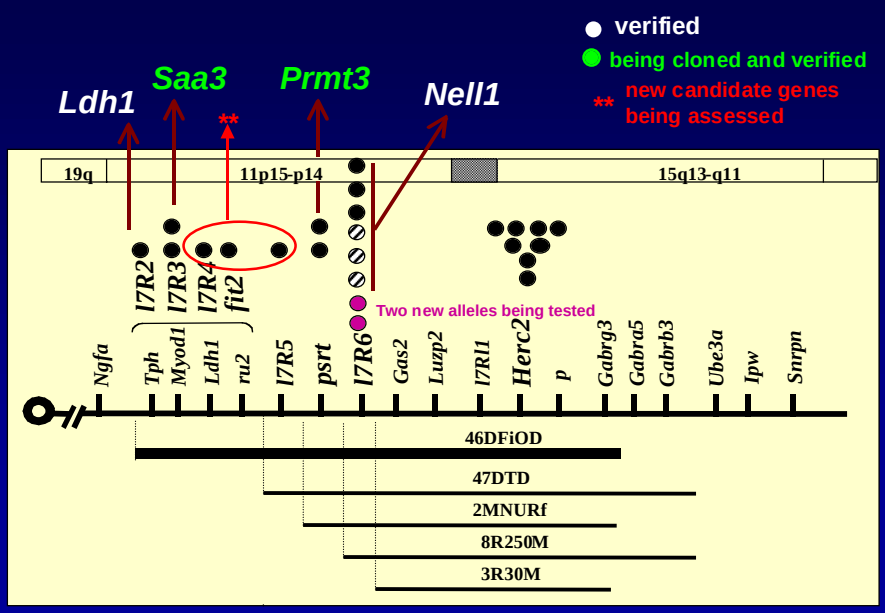
Chr 7 unknown mutations scanned by TGCE

- **951SJ (*Saa3*)**
  - embryonic lethal
  - exon 3 mutation
- **735SJ (*Saa3*)**
  - embryonic lethal
  - exon 4 mutation

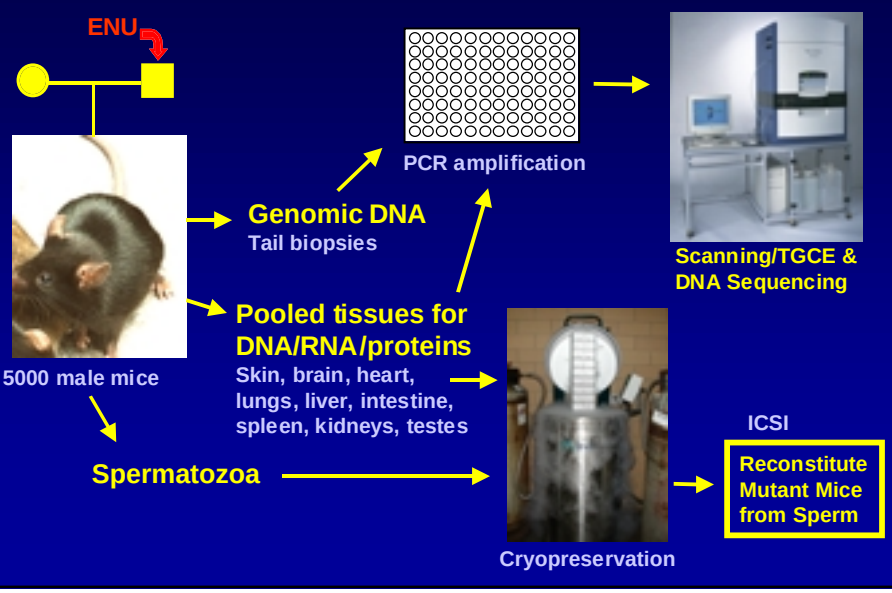
Control | **Heterozygote**



Mutation Map Update



Creating the CMMB



Summary

- TGCE is an effective, high-throughput technology for assessing candidate genes for mapped ENU-induced mutations.
- Candidate gene assignment and mutation scanning strategies that are successfully used in the chr 7 proximal *p* region will be applied to other targets of the ORNL ENU-mutagenesis screens (Chrs 7, 10, 15 and X)
- Further characterization of known mutations will contribute to functional annotation of corresponding genes in the human genome.
- Application of TGCE protocols to scan ENU-mutagenized DNA banks in a gene-driven approach for recovering mouse mutations.

Acknowledgments

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  - Alysyn Gardner
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  - Lori Hughes
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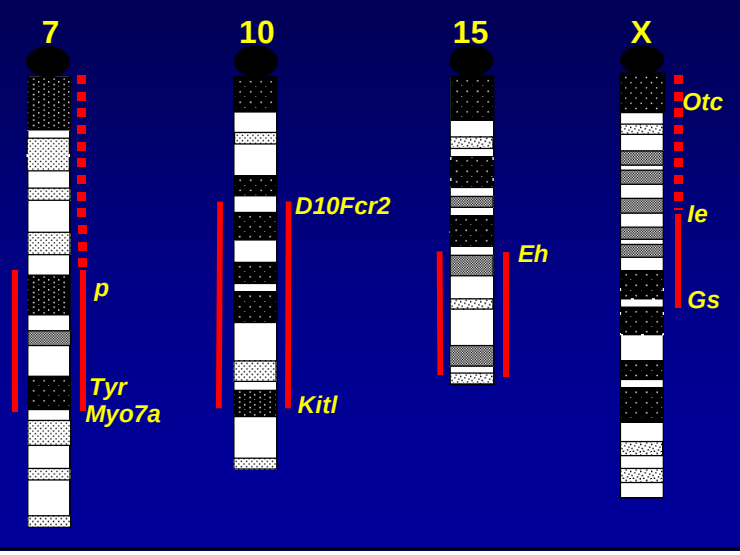
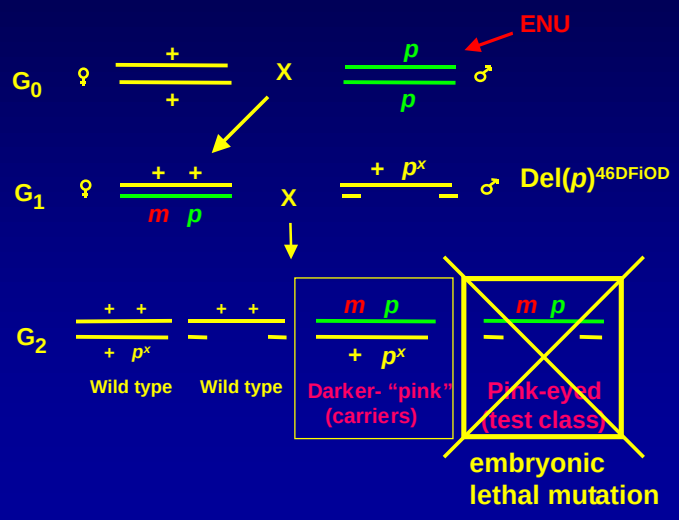
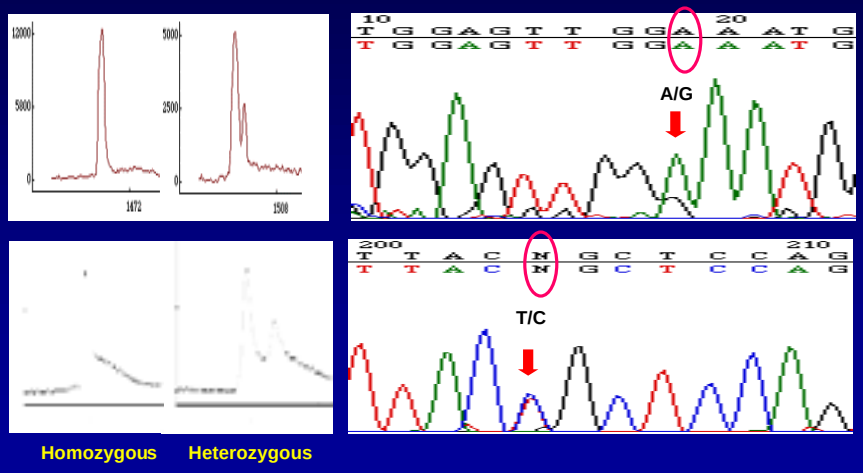
ORNL Regional Screens: Current Targets



Mutation Recovery in Regional Screens: Hemizygosity



TGCE-Powered Mutation Scanning for CMMB