UT-BATTELLE

High-throughput Identification of Point Mutations in the Mouse Genome using Temperature Gradient Capillary Electrophoresis (TGCE)

Cymbeline T. Culiat, Ph. D.
Functional Genomics Group
Life Sciences Division

OAK RIDGE NATIONAL LABORATORY
U. S. DEPARTMENT OF ENERGY

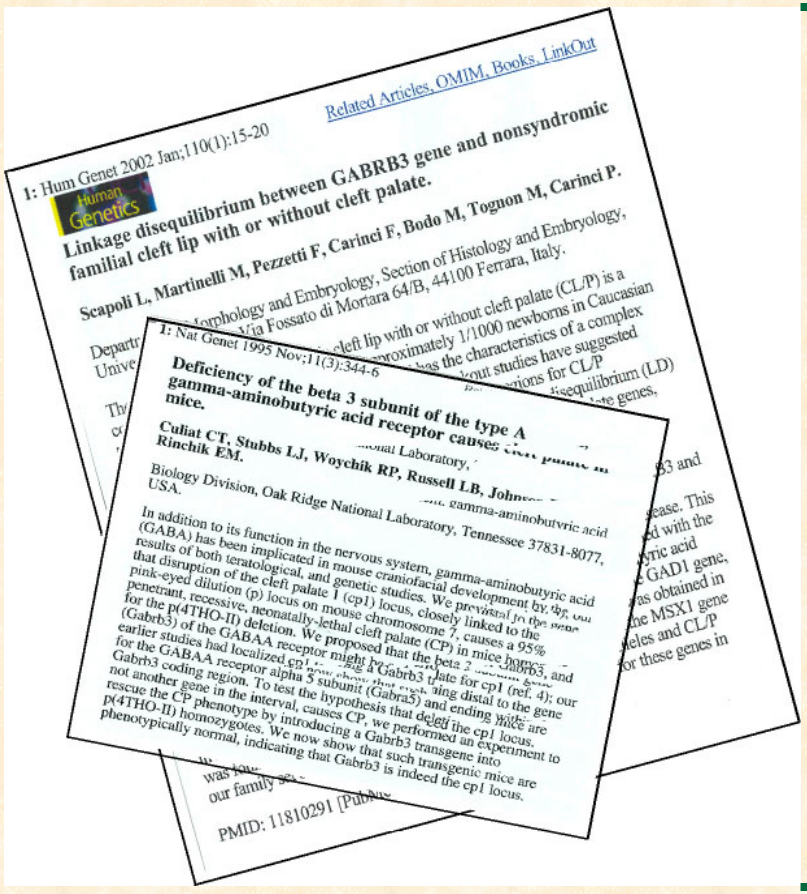
Functional Annotation of Human Genome Sequence

- Assigning function to ~30,000 - 50,000 genes
- Identifying regulatory elements (unknown number)
- Experimentally validating predictions of gene function
- Detailed analysis of mechanisms of function (complex biological pathways)

Mouse Mutagenesis: An Indispensable Approach

- Examine function by observing consequences of disrupting a gene in a mammalian system
- The mouse remains the premier mammalian animal model
- Mutant mouse serves as reagent for both basic and pharmaceutical studies
- Often yields very surprising results!

GABRB3 in Craniofacial Development



- GABRB3, a neurotransmitter receptor molecule was initially predicted to be a gene playing a major role in mental retardation
- The major role of this gene in facial clefting was first demonstrated at ORNL using a series of mouse mutations and gene-rescue experiments (Culiat *et al.,* 1995, Nature Genetics) .
- A recent publication (Scapoli et al., 2002, Human Genetics) has linked the *GABRB3* gene to non-syndromic cleft lip and palate in man.

Generating Mouse Mutations with ENU

William B Russell *et al.* (1979)
PNAS 76:5918-5911.
ORNL

- *N-ethyl-N-nitrosourea* (ENU), *an* alkylating agent
- Potent mutagen of mouse spermatogonial stem cells
- Induces primarily single base pair changes in DNA
- Different mutations in the same gene with a range of consequences- an allelic series
- Slightly altered proteins for protein-complex analysis

Large- scale ENU-mutagenesis Programs

- **Publicly funded**
  - USA (ORNL, Jackson Lab, Baylor Univ, NIH consortia)
  - United Kingdom (MRC, Harwell)
  - Germany
  - Japan
  - Canada
  - Australia
- **Privately funded**
  - Novartis
  - GlaxoSmithKline
  - Pharmacia
  - Ingenium Pharmaceuticals AG
  - Hypnion Inc.
  - Celltech R&D

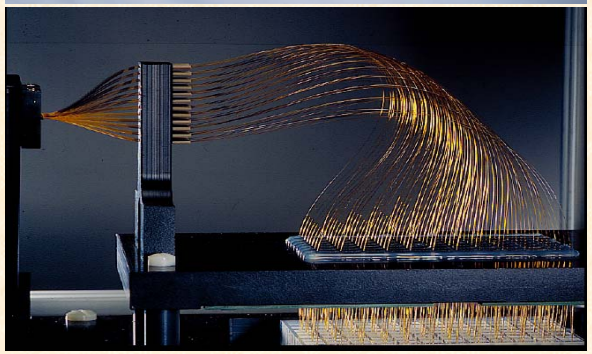
Temperature-gradient capillary electrophoresis (TGCE)

Q. Gao and E. 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

Mutation Scanning using the SCE9610 (SpectruMedix LLC)



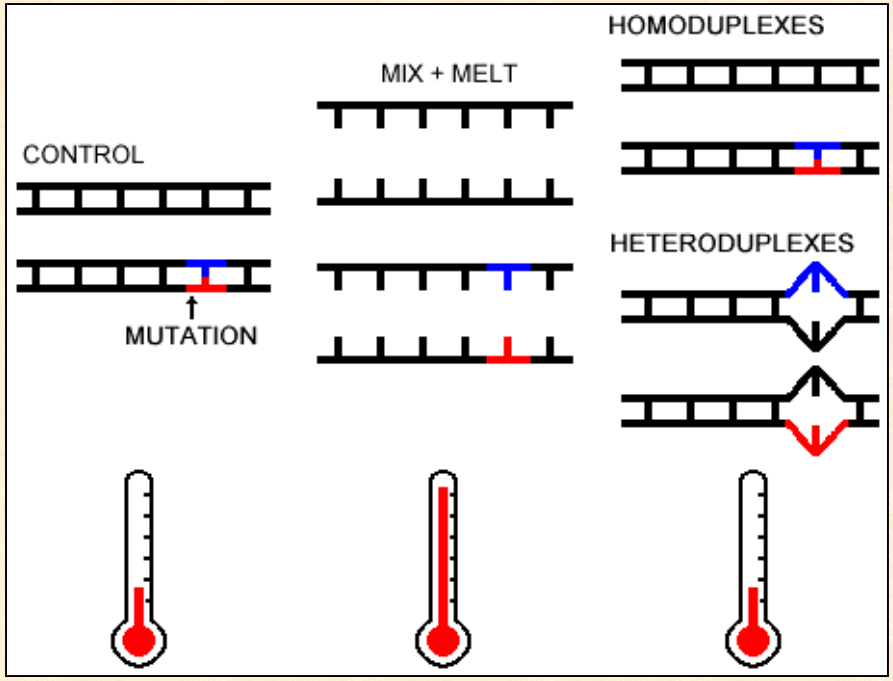
- **High-throughput**
  - twelve 96-well plates/day
  - 150-800 bp PCR fragments with <u>different Tms</u>
- **Highly sensitive**
  - low sample requirement (~200 pg/µl)
  - 97% accuracy, 3% false positive rate
- **Simple and low-cost sample preparation**
  - mouse tail DNA preps for PCR templates
  - unpurified PCR reactions for TGCE scanning
  - ethidium bromide laser-induced fluorescence
- **Multiplexing**
  - different amplicons in single capillary
  - pooling of samples

Li, Q., Liu, Z., Monroe, H. and Culiat, C. T.

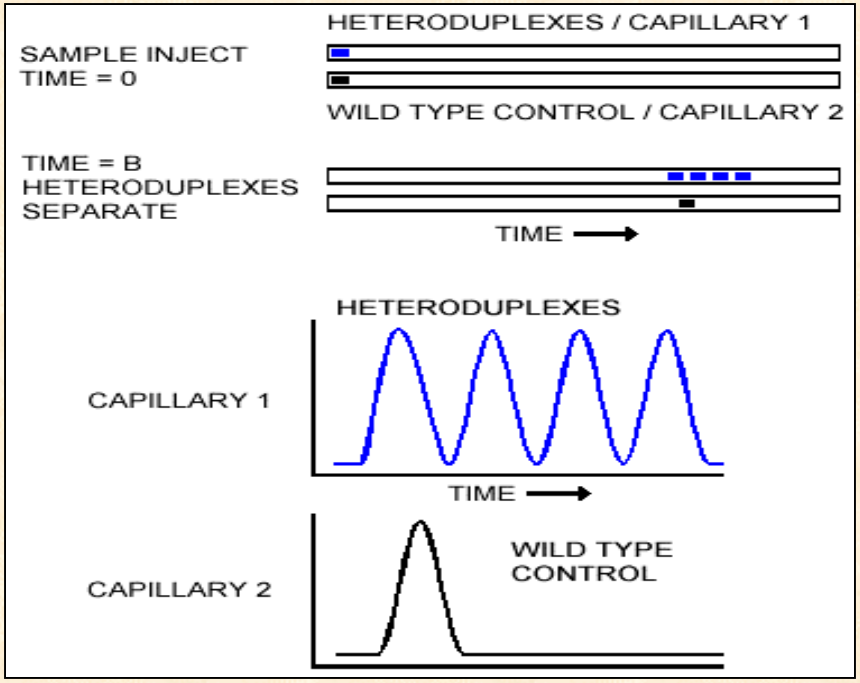
(2002) *Electrophoresis* 23:1499-1511

Mutation Scanning with TGCE

Heteroduplex formation

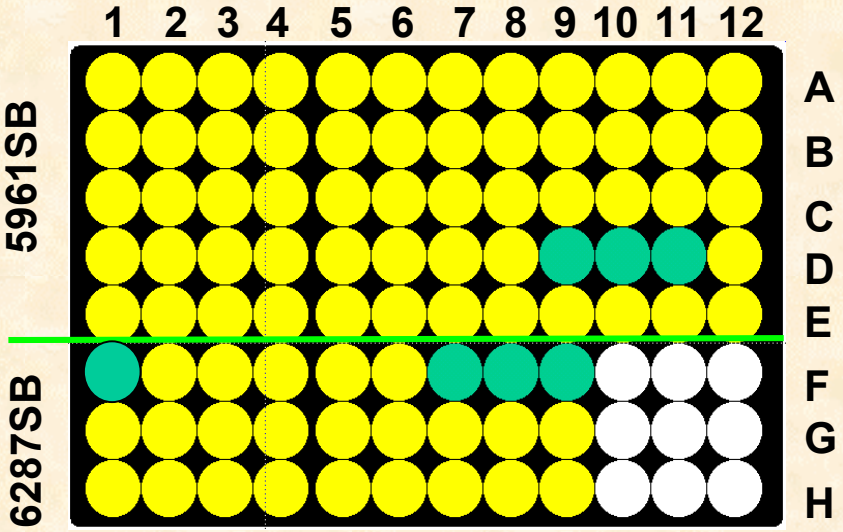


Separation by TGCE

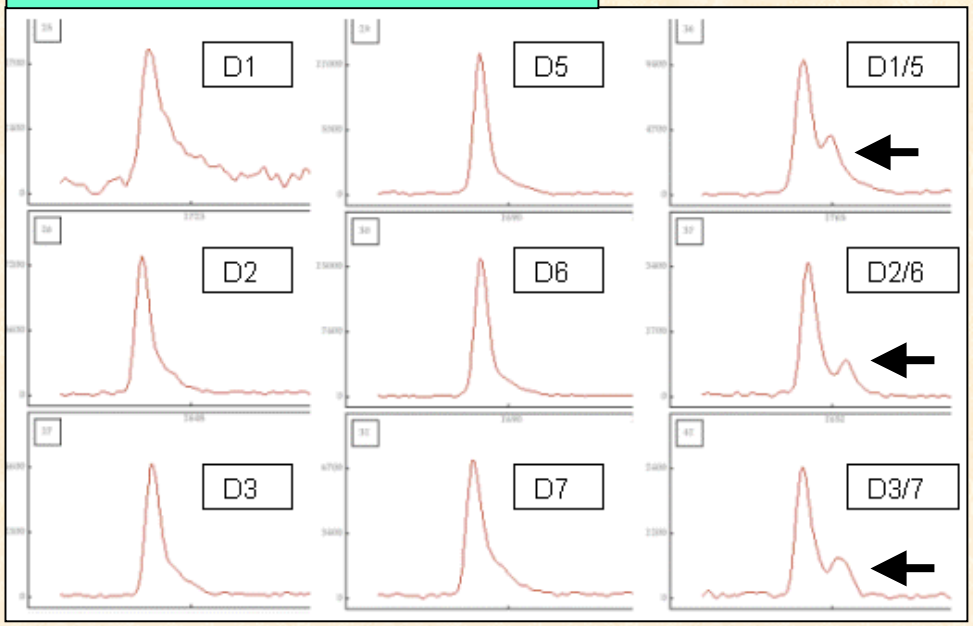


- Retarded mobility of heteroduplexes compared to homoduplexes
- Optimal temperature for resolution is <u>sequence-dependent</u>

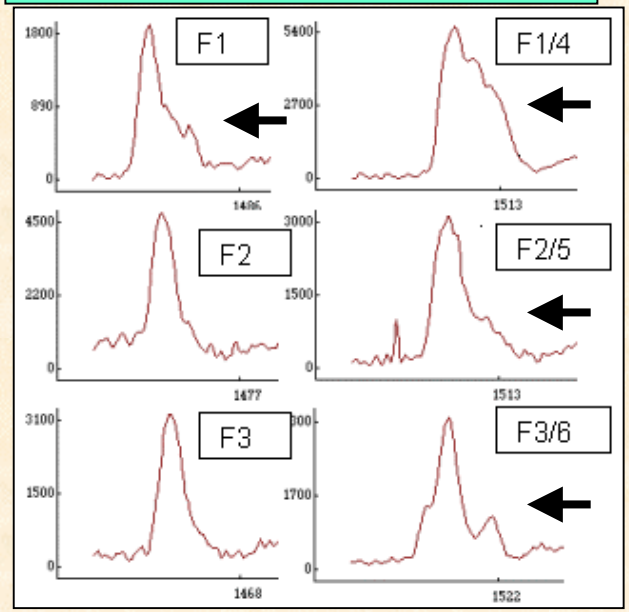
Optimizing TGCE



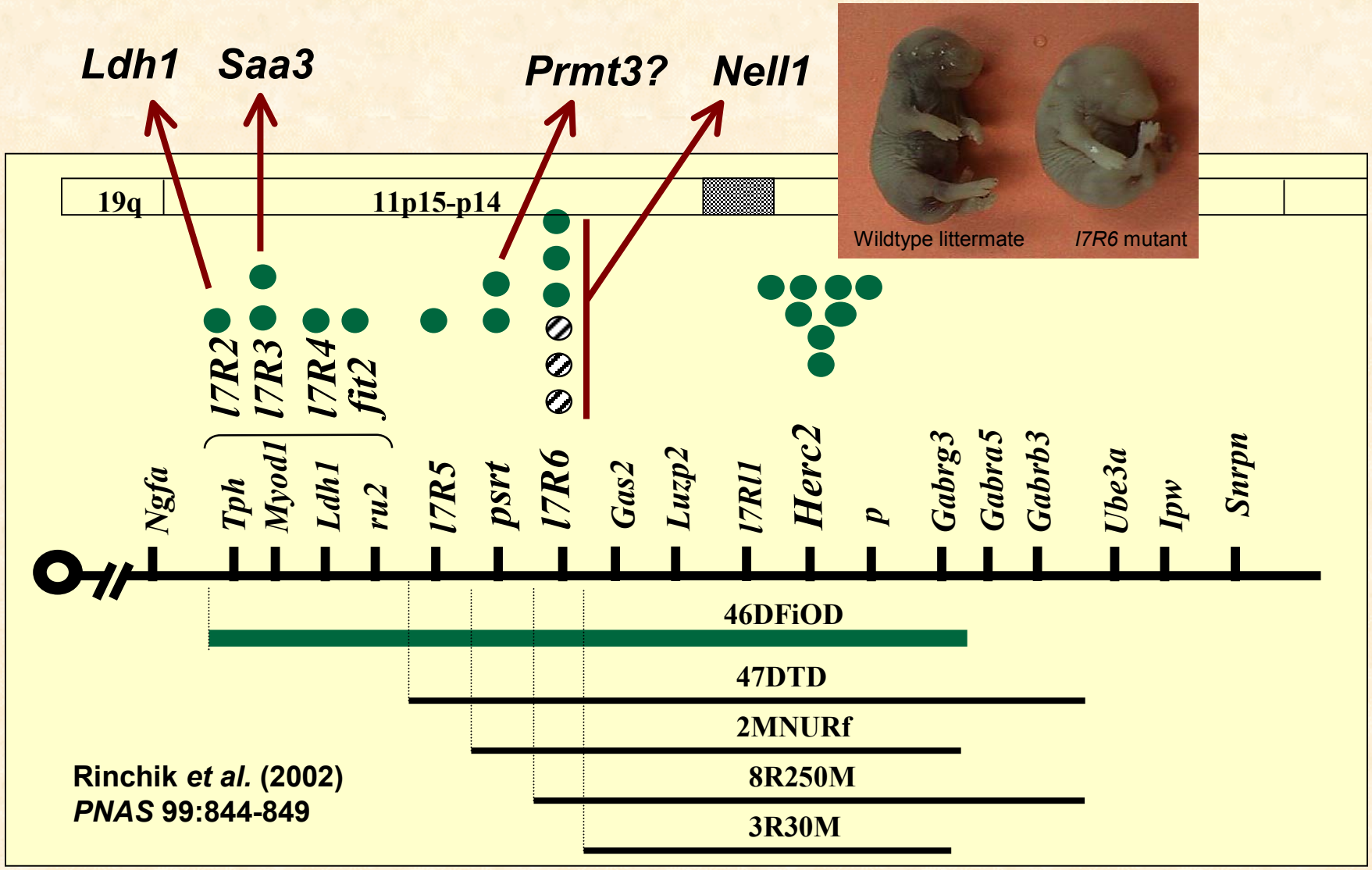
***Fah* 5961SB ; 493 bp, exon 7, G to A**



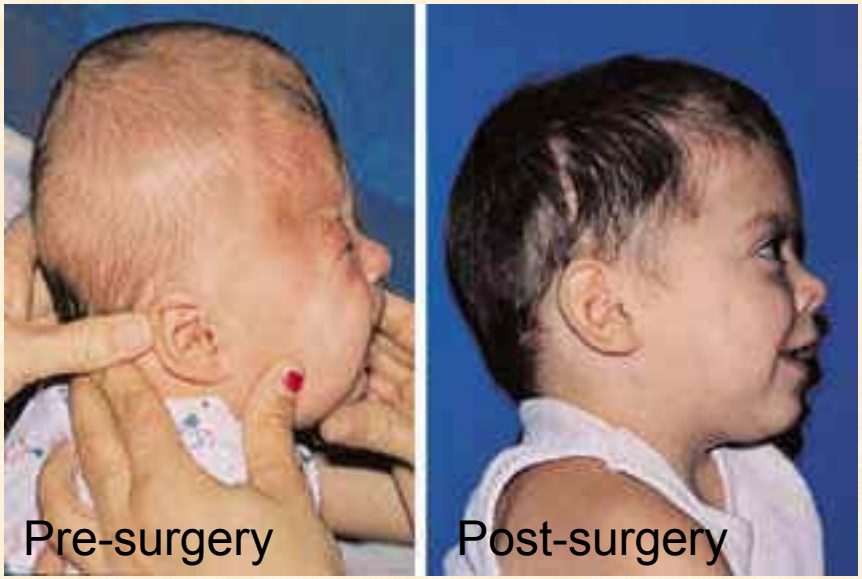
***Fah* 6287SB ; 340 bp, exon 6, A to G**



Regional Mutagenesis: Chromosome 7 *p* region

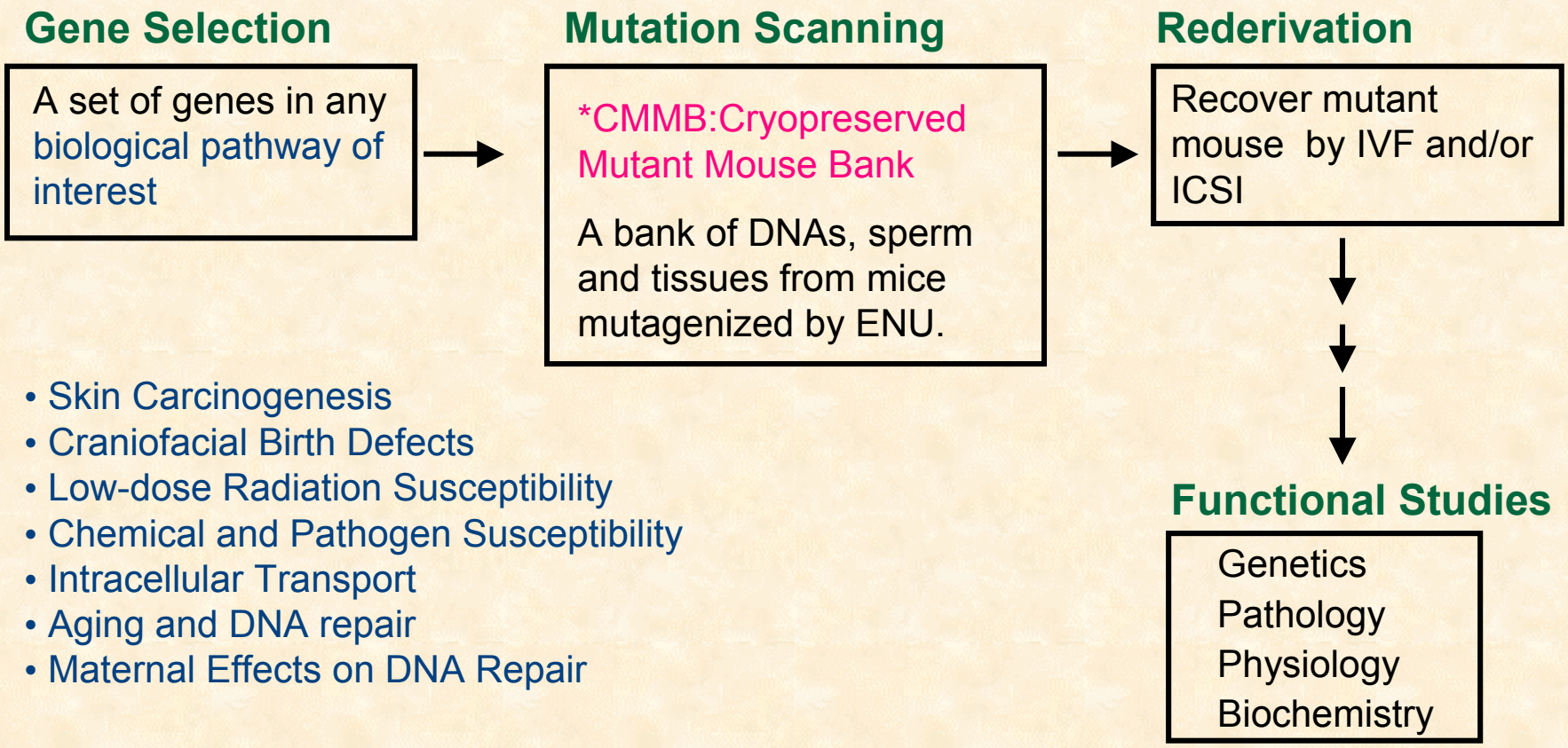


Craniosynostosis: Premature Fusion of Skull Bones



- Intracranial pressure
- Impaired cerebral flow
- Impaired vision and hearing
- Learning disabilities
- Psychological defects

A Gene-Driven Approach of Mouse Mutagenesis



- Skin Carcinogenesis
- Craniofacial Birth Defects
- Low-dose Radiation Susceptibility
- Chemical and Pathogen Susceptibility
- Intracellular Transport
- Aging and DNA repair
- Maternal Effects on DNA Repair

*Initial Funding: LDRD

PIs: E. Michaud, C. Culiat and E. Rinchik

Generating the CMMB Resource

5000 male mice

Genomic DNA
Tail biopsies

PCR amplification

cDNA

Pooled tissues for DNA/RNA/proteins
Skin, brain, heart, lungs, liver, intestine, spleen, kidneys, testes

Spermatozoa

Cryopreservation

Scanning/TGCE & DNA Sequencing

Reconstitute Mutant Mice from Sperm

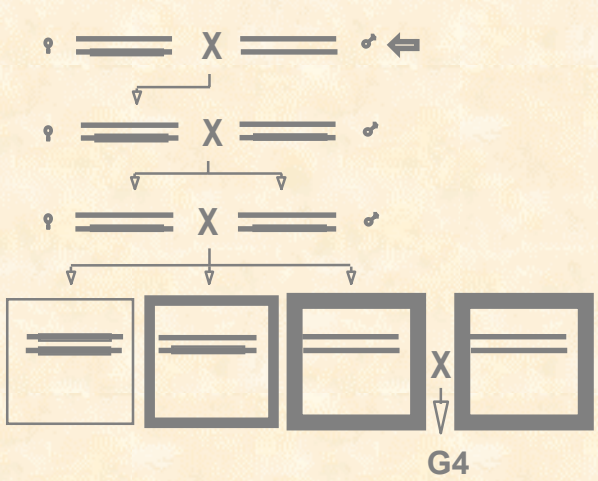
CMMB Progress

- First 2780 (55%) mice have been banked
- Established conditions for high-throughput PCR and TGCE mutation scanning
- **A 25-Mb screen under way; largest gene-driven mutation scanning experiment attempted to date.**
- Re-derivation from frozen sperm set-up
- FileMaker Pro Database

Mouse Mutagenesis at ORNL

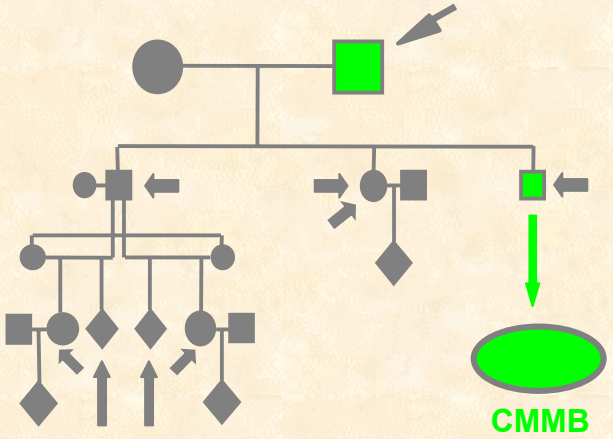
Regional
Phenotype-driven
Chrs 7, 10, 15, and X

Aberrant Phenotype → Gene



Whole-genome
Gene- and sequence-driven

Gene → Aberrant Phenotype



TGCE is a breakthrough in finding ENU-induced mutations in the mouse genome!

Acknowledgments

ORNL Staff

Ed Michaud
Bem Culiat
Dabney Johnson
Gene Rinchik
Mitch Klebig
Jay Snoddy
Frank Larimer

ORNL Technical Staff

K. T. Cain
Debra Carpenter
Lori Easter
Alysyn Gardner
Kay Houser
Bob Olszewski
Irina Pinn
Beverly Stanford
Ginger Shaw
Sarah Shinpock
Ann Wymore
Marilyn Kerley
Lori Hughes
Shen Lu
Melissa York
Carmen Foster

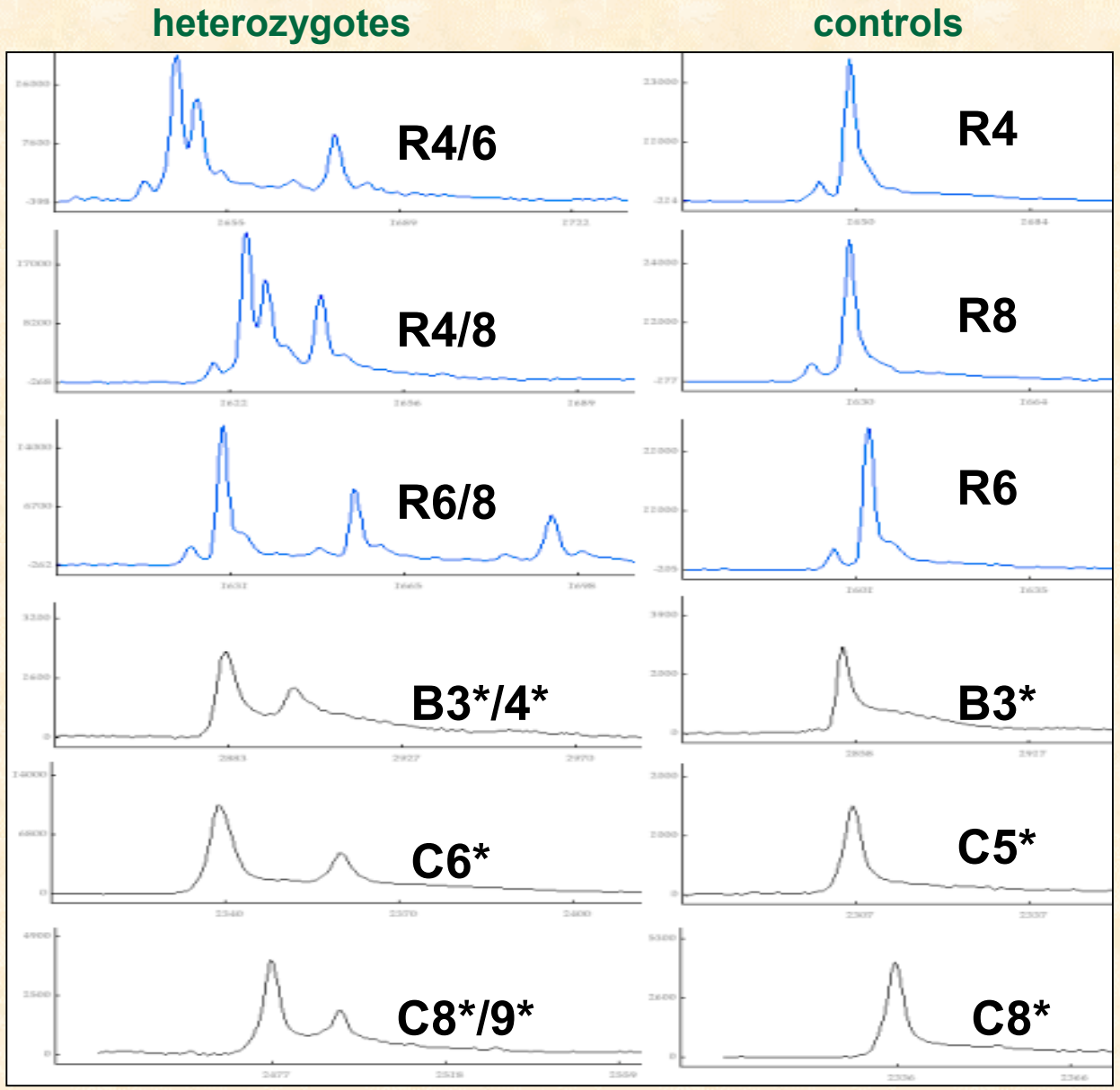
SpectruMedix LLC

Qingbo Li
Zhaowei Liu
Heidi Monroe
ZY Guo
Kevin Gutshall

DOE-BER

Marvin Frazier

LDRD Program


heterozygotes
controls
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*

A Crude Estimate of Mutation Frequency in the CMMB

- **1 mutation per 0.5 -2.5 Mb**
  - whole animal mutagenesis (Russell, Rinchik, ORNL)
  - mutagenized ES cell bank (T. Magnuson, UNC)
  - whole animal mutagenesis (S. Brown, Harwell)

- **An "average" gene (2.5 kb DNA) will have ~5 to 25 independent ENU-induced mutations**

- **Single SCE9610 can screen an amplicon/ week in entire bank**