



# Genetic Testing Reference Material- Predicting the Future Needs?

April 24, 2008  
Geel, Belgium

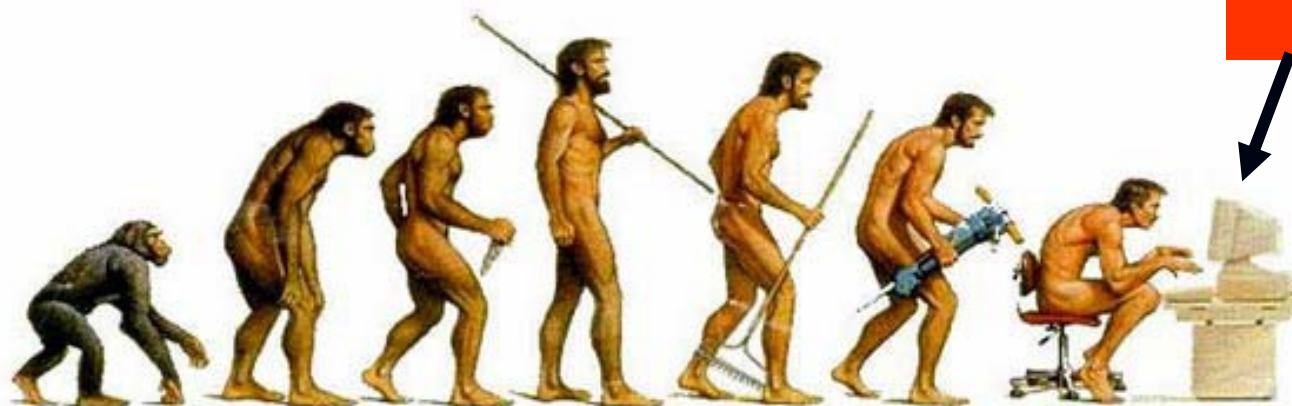
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# Evolution of Genetic Testing



**Microarray  
Data?**



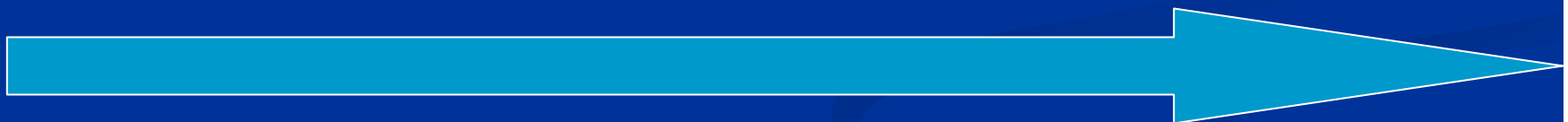
# Past, Present, Future?

**Technologies**

PCR, Allele specific amplification, sequencing, hybridization, Southern blots

Array CGH, genome wide SNP arrays, multiple gene arrays

Array CGH , SNP arrays, Whole genome sequencing?



**Types of RMs**

**One gene at a time**

Genomic DNA, Synthetic DNA, Plasmids

**A few genes and loci at a time**



**Many (all?) genes at once**





## Technologies Described at American College of Medical Genetics Meeting, March 2008

**250K SNP arrays-** Can see small deletions and duplications (few hundred bases)

**Resequencing arrays-** 11 genes on a chip for hypertrophic cardiomyopathy (also detects known variants)

**Array CGH-** looks at many genes at once. More sensitive than conventional cytogenetics can more precisely define deletions and predict gene loss.

- Predicted gene disorders based on gene loss.
- 2 step- aCGH followed by array resequencing - 11 cystic kidney disorders, Duchenne/Becker muscular dystrophy
- Mitochondrial disorders- nuclear + mito genes
- 25 Lysosomal storage disorders

**Microarray-** has target regions with 50kb resolution and coverage for the rest of genome 500 kb resolution (provides molecular karyotype)

**Cell free fetal DNA**

# RMs for New Technologies- Some Issues to Consider

- How DO you do QC for microarrays?
- How do you validate all features on an array?
- What type of material is most appropriate?
- What is a “normal genotype”???
- How do we keep up with changing technology?





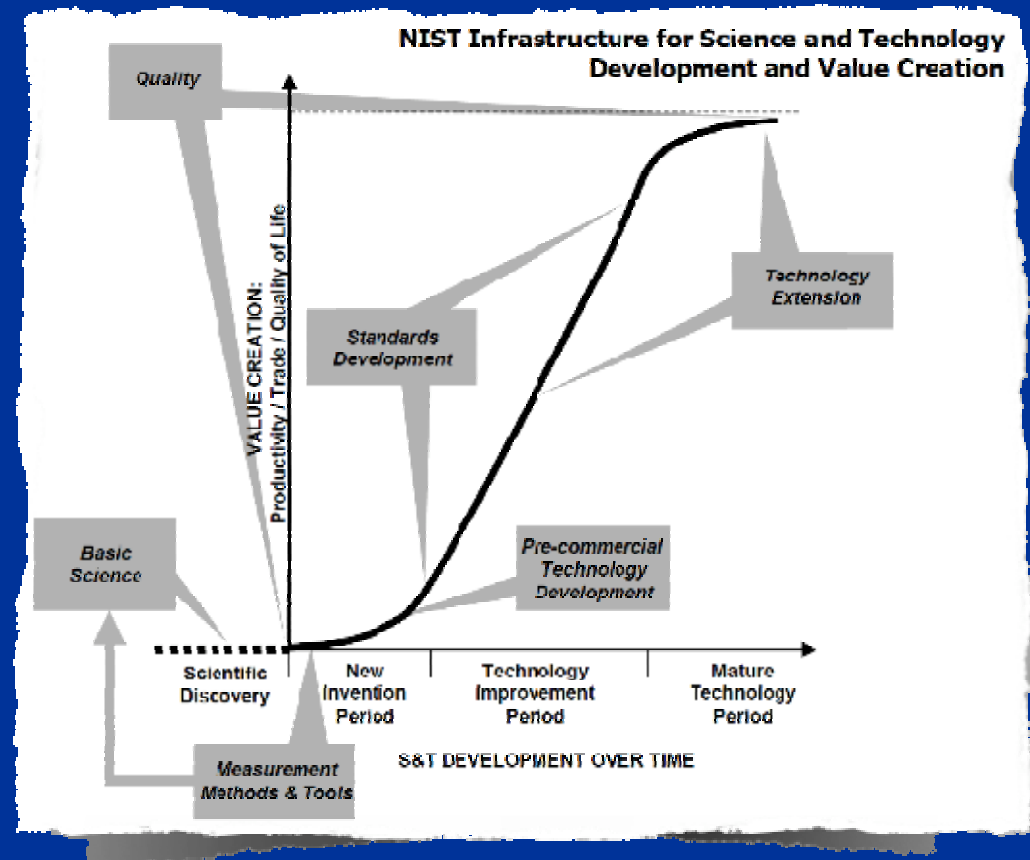
## Actual quote from question on AMP listserv that I received while preparing this talk

“How are labs addressing the issue of validation of commercial arrays that are not FDA cleared? Using Array CGH would one have to validate each BAC in the lab or each Oligo or are labs accepting the validation of each probe from the manufacturer and just validating the complete result?”

-Lab director

# NIST...

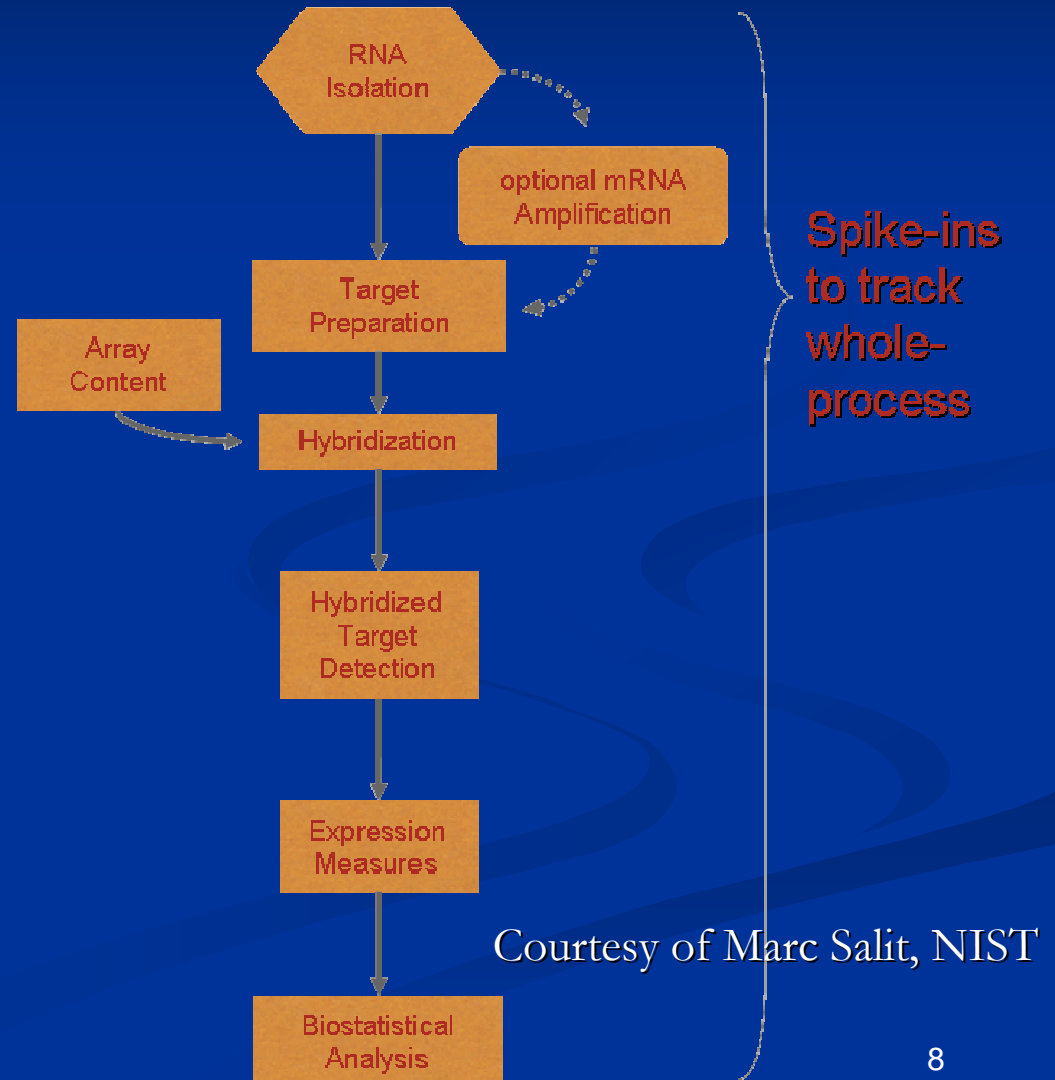
- US *National Metrology Institute*
  - established 1901
  - largely physical-science based
  - part of US Department of Commerce
- “NIST's mission is to develop and promote measurements, standards, and technology to enhance productivity, facilitate trade, and improve the quality of life.”



Courtesy of Marc Salit, NIST

# “Spike-In” Controls

- Spike-in controls for Validation
  - External, Exogenous
    - sequences that do not naturally appear in sample
  - Synthetic
  - Known [RNA]
    - traceable to SI
  - Known Sequence
    - traceable to reference library RM
- Track “whole-system” technical performance



# Status of NIST “Spike-in” Control

- RM will be plasmid DNA with control sequence (non human) inserts. The RM user produces RNA in-house
- Developing RM with 96 sequences (250-2000 nucleotides)
- Cloning of 96 clones completed
- Sequencing in progress
- Development of certification plan underway
- Working with reagent manufacturers to make RNA available commercially- individual and/or pooled RNA?
- “Initially working to validate, or “qualify” the measurement process, intending to be generically useful. The question remains of how to qualify a whole genome array measurement efficiently and effectively -- this is a first pass”. (Marc Salit)



# RMs- Inherited Genetic Disorders

## Present Status

- Have a few CRMs and SRMs – (eg. Prothrombin, fragile X)
- Have a few highly characterized materials, FDA approved – (eg. CF, pharmacogenetics)
- Have some characterized genomic DNA/cell lines – (eg. Huntington, Fragile X, CF, Ashkenzi Jewish)

**We are still lacking RMs for most single gene tests....**



# Reference Material Development in US: Who is doing what?



- **National Institute for Standards and Technology (NIST)**  
Working on SRMs for HER2, CMV, Gene Expression, P53
- **Acrometrix**  
Expressed interest in developing traceable, commutable CRMs for quantitative molecular infectious disease testing CMV
- **American Type Tissue Collection (ATCC)-**  
Wants to develop CRMs for infectious and inherited disease testing
- **Commercial Reference Material producers (Paragondx, Maine Molecular, etc)**  
FDA approved QC materials- pgx, inherited disorders, infectious disease
- **GeT-RM (CDC)**  
Characterizing publicly available genomic DNA samples



# Current GeT-RM Projects



## Newborn screening

- Currently characterizing 12 gDNA samples in 8 labs (MCAD, Gal, CAH, MSUD)- using panels
- DNA sequence analysis of 22 gDNA samples representing many of the disorders on the recommended ACMG newborn screening panel

## Odds and Ends - 19 labs using screening panels

- MTHFR 1 sample
- AAT 4 samples
- MEN2 2 samples
- BRCA1/2 3 samples

## Ideas for future projects:

- Duchenne/Becker, Myotonic Dystrophy
- Biochemical Genetic Testing
- Molecular oncology
- Newborn Screening

# Examples of Current Needs



# Newborn Screening in the United States



- 4,000,000 babies/yr are born in the United States each year ~95-98% are screened for inherited metabolic disorders.
- Every state has a program to screen newborns. Babies with abnormal newborn screening results are referred to metabolic centers for follow up



# Newborn Screening in the United States



In 2006, the American College of Medical Genetics published a document called “Newborn Screening: Toward a Uniform Panel and System”

This document recommends that states screen for 29 core metabolic disorders and suggests an additional 25 conditions that will be detected during the screening process.

The majority of these tests use tandem mass spectrometry (MS/MS).

All state programs are implementing these recommendations

**Table 2**  
Newborn screening panel: core panel and secondary targets

MS/MS				
Acylcarnitines		Amino acids		
9 OA	5 FAO	6 AA	3 Hb Pathies	6 Others
CORE PANEL				
IVA	MCAD	PKU	Hb SS <sup>a</sup>	CH
GAI	VLCAD	MSUD	Hb S/βTh <sup>a</sup>	BLOT
HMG	LCHAD	HCT <sup>a</sup>	Hb SC <sup>a</sup>	CAH <sup>a</sup>
MCD	TFP	CIT		GALT
MUT <sup>a</sup>	CUD	ASA		HEAR
SMCC <sup>a</sup>		TYR I <sup>a</sup>		CF
CS I, A, B <sup>a</sup>				
PROP				
BKT				
SECONDARY TARGETS				
6 OA	8 FAO	8 AA	1 Hb Pathies	2 Others
CS C, D <sup>a</sup>	SCAD	HYPER-PHE	Var Hb <sup>a</sup>	GALK <sup>a</sup>
MAL	GA2	TYR II		GALE
IBG	M/SCHAD	BLOFT (BS)		
ZM3HBA	MCKAT	ARG		
ZMBG	CPT II	TYR III		
ZMGA	CACT	BLOFT (REG)		
	CPT IA	MET		
	DE RED	CIT II		

NOTE: Codes are as follows: OA, disorders of organic acid metabolism; FAO, disorders of fatty acid metabolism; AA, disorders of amino acid metabolism; Hb Pathies, hemoglobinopathies.

<sup>a</sup> Identifies conditions for which specific discussions of uniquenesses are found in the main report.

## ACMG Newborn Screening Panel

23 of the Core 29 and 22 of the 25 Secondary targets are identified by MS/MS



# Newborn Screening in the United States



- The CDC's Newborn Screening Quality Assurance Program periodically provides QC and PT materials for the screening labs all primary + most secondary + all non ACMG disorders (HIV, Toxoplasmosis)
- The chemical analytes are commercially available for most of the MS/MS tests (not pure or quantitated), but no certified reference materials are available
- There are no reference materials for analytes such as enzymes
- Very few characterized genomic DNA samples are available (CDC's GeT-RM Program is working on this)

**The follow-up labs do not have the needed reference materials!**



# Reference Materials for Biochemical Genetic Testing



- "Quality, Access, and Sustainability of Biochemical Genetic Testing" working meeting was held on Oct. 6-7, 2006, in Atlanta, GA
- Organized by the Centers for Disease Control and Prevention (CDC), National Institutes of Health- Office of Rare Diseases (NIH-ORD), Society of Inherited Metabolic Disorders (SIMD), American Society of Human Genetics (ASHG), American College of Medical Genetics (ACMG), Human Resources and Services Administration (HRSA), the Genetic Alliance, Emory University, and several other key groups.
- Discussed the current inadequacy of reference materials (to be used for quality control, proficiency testing and test development/validation) for biochemical genetic testing.



# Reference Materials for Biochemical Genetic Testing



## Outcomes from Quality, Access, and Sustainability of Biochemical Genetic Testing" working meeting

- Participants agreed that there is a need to improve the availability of reference materials .
- SIMD, together with GeT-RM, have developed a survey for clinical biochemical genetic laboratory directors to identify currently available reference materials and assess urgent reference and proficiency testing material needs.
- Hope to work with the biochemical genetic testing community to collect useful patient samples and create a publicly available repository of biochemical genetic testing reference materials



# GeT-RM /SIMD Project

- Currently drafting a Needs Assessment survey with the Society for Inherited Metabolic Disorders (SIMD)
- Will collect information on tests/methods offered, RMs used and RM needed
- Hope to establish a repository of reference materials at Coriell for QC, PT etc
  - Samples will be non-DNA/cell line
  - urine, serum, CSF, enzymes, muscle
- Hope to obtain funding with the help of the NIH Office of Rare Diseases



## RM Needs- Other Areas of Molecular Testing

We did surveys to identify reference material needs for molecular oncology and molecular infectious disease testing.

Did not come up with definitive needs, however the results indicate that quantitative standards (including bcr/abl, PML/RARa and CMV) are really needed



# RM's - Some Issues to Consider

- Do we have the necessary reference materials for current tests? (No- not for most)
- Which technologies will be important in the future?
- How do you validate, standardize and perform QC/PT for complex multigene or whole genome arrays?
- Do we need to consider new ways to assess assay performance?
- What RMs will we need for upcoming technologies?
- How do we decide what the needs are?





THANK YOU



