A low-angle photograph of two people climbing a steep rock face. One person's hand is visible, gripping a rope, while another person's leg and foot are seen on the right. The background shows a vast mountain range under a clear blue sky.

## Implementing a CLIA Laboratory for Pharmacogenomics Testing Using the VeraCode® ADME Core Panel from Illumina®

John L. Kolman, Ph.D.

Senior Director, Global Research and Development,  
BioReliance Genomics

THE WORLD RELIES ON YOU

YOU CAN RELY ON US

 BioReliance

# BioReliance Genomics

## Experience...

- 60+ years and counting
- 616 Staff; many PhDs
- 15,400 Diagnostic PCRs per year

## Regulatory...

- GLP, GMP... GCLP/CLIA
- We build and validate our own assays

## Genomics commitment and position...

- Multi-million dollar capital investment
- “R&D” staff of 12, including Statistics and BioInformatics
- Cynergene acquisition
- Automation, automation, automation...



# BioReliance Genomics Expertise

## Genomics Division -- Platforms...

**MDS** -- Development Services (QPCR)

**MPS** -- Massively Parallel Sequencing

**AT** -- Array Technology (Multiplex Genotyping)

## Equipment...

- ABI, Roche, Illumina, Agilent, Bio-Rad, MDS

## Automation...

- Tecan, QIAGEN, Eppendorf, Agilent...

## Chemistries...

- QIAGEN, ABI, Quanta, Illumina, LifeTech...

**MDS -- Development Services (QPCR)**

MPS -- Massively Parallel Sequencing

AT -- Array Technology (Multiplex Genotyping)

# MDS:

## Design, Validation and Execution of QPCR Assays

Where...

- US Rockville
- UK Glasgow

Platform...

- TaqMan® QPCR

Projects...

- Genotyping and Quantitation
  - DME, SNP, CNV, Microsatellite, Indels and multi-nucleotide polymorphisms, microRNA, custom targets

- Client custom QPCRs

- BioSafety testing
- Pre-Clinical Studies (Biodistribution)
- Clinical trials with QPCR endpoints



# BioReliance Genomics

MDS -- Development Services (QPCR)

**MPS -- Massively Parallel Sequencing**

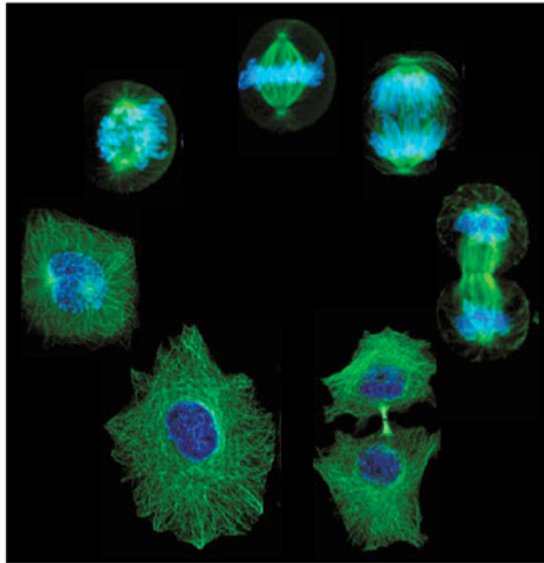
AT -- Array Technology (Multiplex Genotyping)

# MPS: Massively Parallel Sequencing

•US Rockville



# MPS Contracts and Initiatives



[http://www.cbp.pitt.edu/faculty/yong\\_wan/images/main\\_cell\\_cycle.jpg](http://www.cbp.pitt.edu/faculty/yong_wan/images/main_cell_cycle.jpg)



## Contracts

- New cell substrates – Vaccine development
- MCB, MVSS characterization
- Fermenter “Fires”
- Clinical trial targeted genotyping

## Innovation: Targeted primer technology

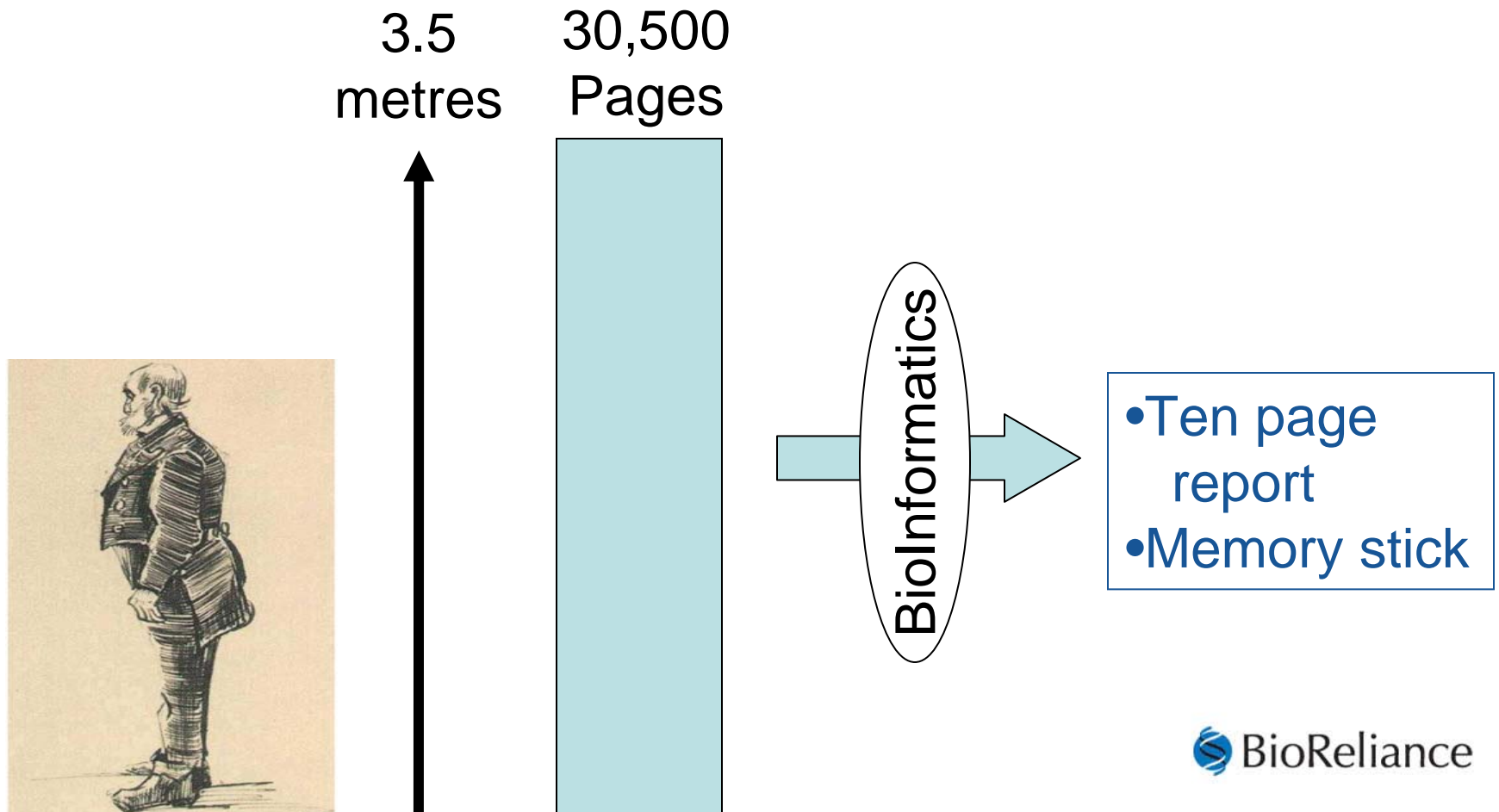
- Maximum sensitivity using algorithms

## Internal Initiatives

- |                         |              |
|-------------------------|--------------|
| • Basic MPS             | GMP ~Q3 2010 |
| • MPS Genetic Stability | GMP ~Q4 2010 |

# Why Bioinformatics? 3.5 Meters

... Height of half of one MPS run printed on A4.



# BioReliance Bioinformatics and Statistics

## Customized and interactive....

- Analysis (statistical and/or bioinformatic) workflow design and companion software development (web or desktop)
- BLAST searches/optimizations and sequence annotation
- Custom Database generation
- Primer design
- Survival analysis
- Contig assembly
- Outlier Detection and Removal
- Pattern Analysis
- Simulations
- Array Analysis
- PCR Analysis
- Nonparametric data analysis
- Image Analysis



# BioReliance Genomics

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# AT: Transcription Profiling and Genotyping

Where...

- US Rockville

Platforms...

- Expression Analysis – Agilent Array Scanner
- SNP Detection – Illumina BeadXpress

Projects... Genotyping/SNP Detection

- Custom Assays and Commercial Kits
- Low and medium density
  - 1-384 SNPs detected simultaneously ...
- Multiple niches
  - Discovery   Development   Pre-Clinical   CLIA



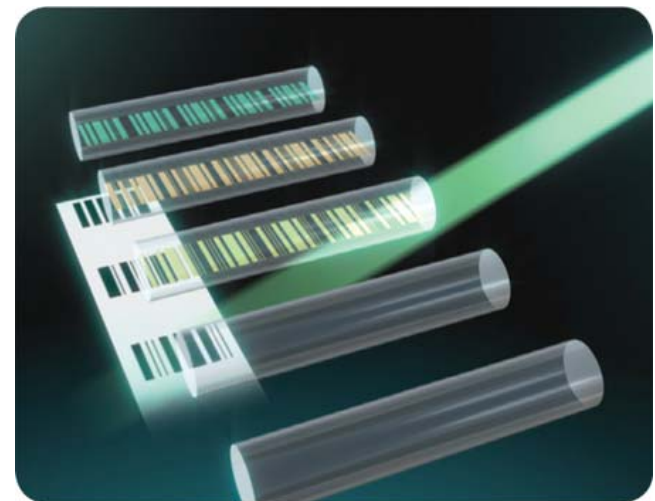
# Illumina BeadXpress®: 384-Plex SNP Detector

A two color fluorometer...

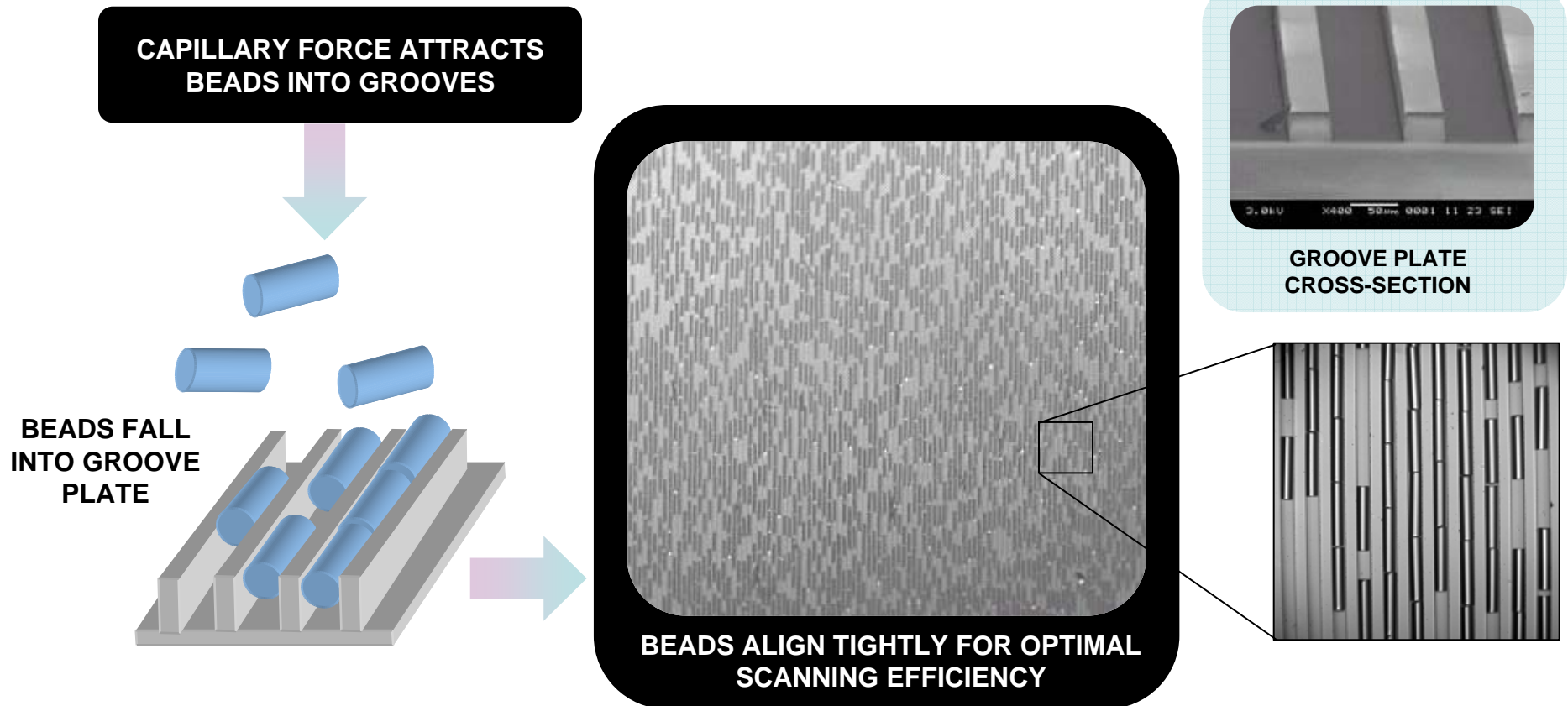


Each cylindrical “bead” has unique  
capture oligonucleotides on the  
surface...  
...And a unique holographic barcode  
within.

VeraCode® Technology



# VeraCode® Technology



Each bead is read three times: one pass reads the barcode, two others read the **green channel** and **red channel** intensities



# Illumina's VeraCode® ADME Panel on the BeadXpress®

Pharma chose:

- The markers (human ADME genes)
- BeadXpress platform...
  - Higher signal to noise
  - Fewer SNPs needed
  - Cleaner calls

Technology, built by Illumina

- Modified (1-day) GoldenGate™



- **Goal:** Pharma to standardize genotyping content for pharmacogenetic drug studies
- **Content Contributors:** Most of the influential pharma companies including Abbott, Bristol-Myers Squibb, GSK, Johnson & Johnson, Eli Lilly, Merck, Pfizer, Roche, Sanofi Aventis and Wyeth. Genome Quebec also participated.

# Genes on the VeraCode ADME Core SNP Panel

## ADME Core (34 genes)

ABCB1	CYP2C9	NAT1	SULT1A1
ABCC2	CYP2D6	NAT2	TPMT
ABCG2	CYP2E1	SLC15A2	UGT1A1
CYP1A1	CYP3A4	SLC22A1	UGT2B15
CYP1A2	CYP3A5	SLC22A2	UGT2B17
CYP2A6	DPYD	SLC22A6	UGT2B7
CYP2B6	GSTM1	SLCO1B1	VKORC1
CYP2C19	GSTP1	SLCO1B3	
CYP2C8	GSTT1	SLCO2B1	

## 184 Variants by Polymorphism Type

169	Bi-allelic SNPs & Small Indels
6	Triallelic SNPs
10	Copy Number Variants
<b>184</b>	<b>Total</b>

# Validated Core Content that Matters

## ***Validated Biomarkers and the Drugs they Metabolize***

### CYP2D6

- Metabolizes codeine to active drug morphine
- Metabolizes Stratera® (atomoxetine), used for Attention Deficit Disorder
- Associated with tamoxifen metabolism

### CYP2C9

- Referenced in the labeling for blood thinner warfarin

### CYP2C19

- Referenced in label for anti-platelet Plavix® (clopidogrel)

## ***Experimental Biomarkers and the Drugs they Metabolize***

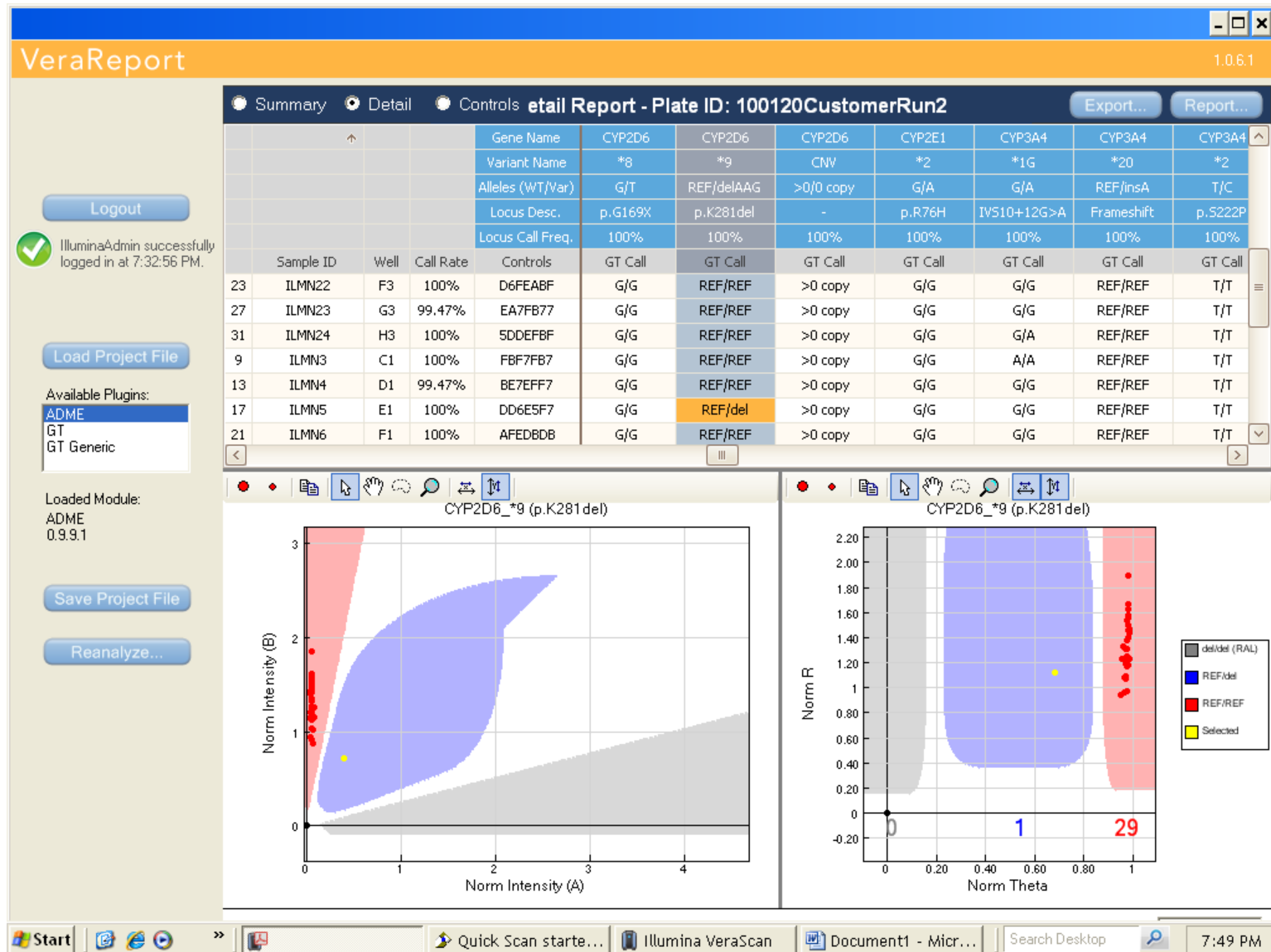
### CYP2A6

- Associated with Nicotine metabolism

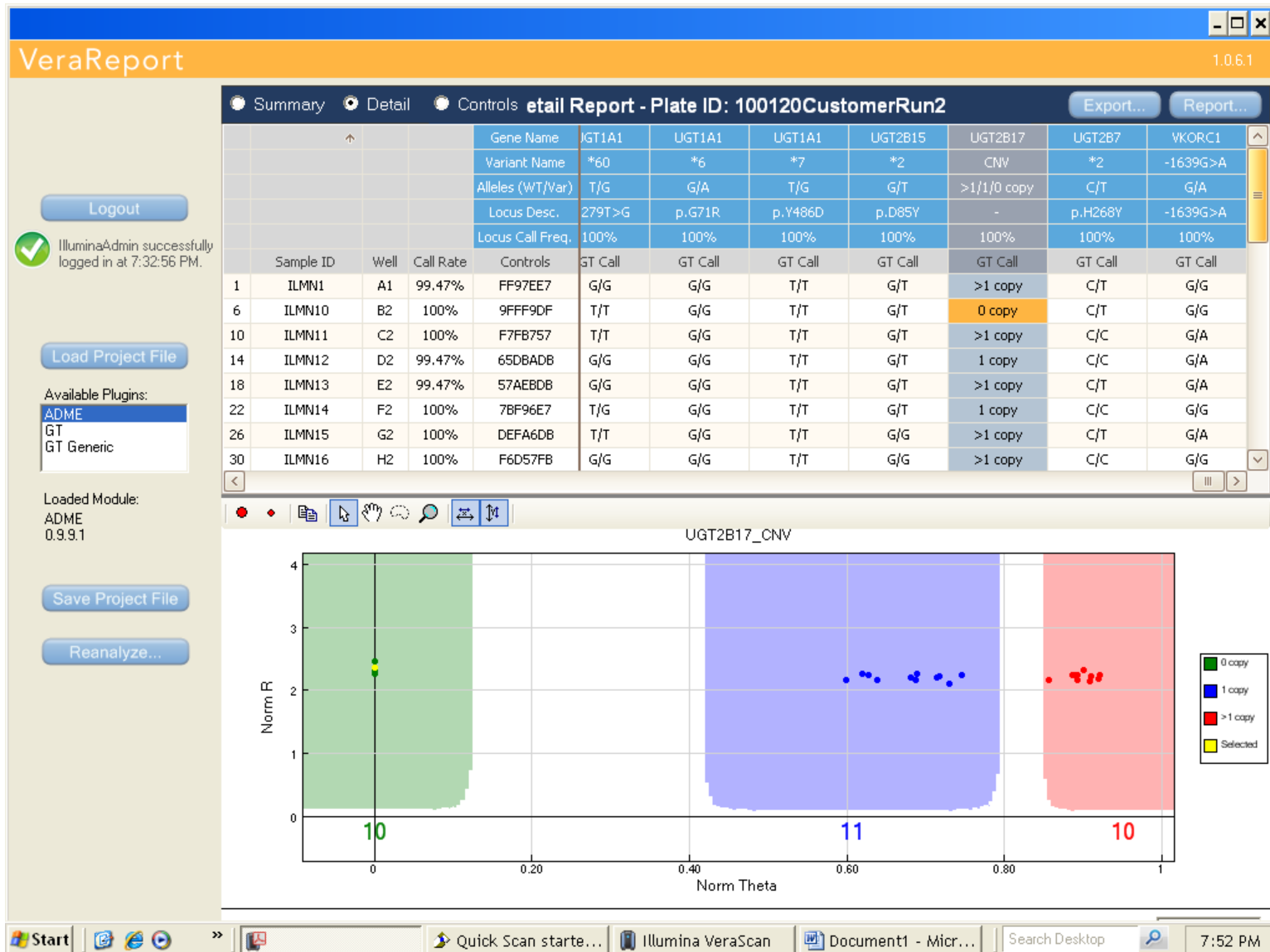
# BioReliance: β-test Results

	Training	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Average
PSC Failures	0	0	0	0	0	0	0	0
STC Failures	0	2	0	0	0	0	0	0.29
Concordance	99.96%	99.96%	99.89%	99.56%	99.78%	100%	99.46%	99.80%
Comparisons	5435	5227	4507	4495	4472	188	556	3554.29
Low Beads	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Low Signal	0.06%	0.05%	0.00%	0.00%	0.02%	0.00%	0.14%	0.04%
No Call	0.26%	0.60%	0.17%	0.36%	0.58%	0.58%	1.25%	0.54%
Calls Attempted	5452	5638	5828	5828	5828	5828	5828	5747
Average Call Rate	99.69%	99.34%	99.83%	99.64%	99.40%	99.42%	98.61%	99.42%
NTC Spikes	1.42%	18.88%	2.13%	6.38%	0.53%	1.60%	1.60%	4.65%
STC reproducibility	100%	98.48%	99.92%	100%	99.84%	100%	100%	99.75%
Re-queue rate	0.00%	6.25%	0.00%	0.00%	0.00%	0.00%	0.00%	0.89%

# BioReliance Data: An Insertion/deletion



# BioReliance Data: A Copy Number Variant



**MDS** -- Development Services (QPCR)

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GxP/CLIA Implementation

# Regulating a Laboratory

## **GLP**

### A Federal Regulation

- Facilities
- Staff Training
- Assay Validation
- SOPs
- Execution
- Quality System

## **CLIA**

### A Federal Certification

- Credentialed Personnel
- Data Interpretation
- Chain of Custody  
(Sample tracking)
- Proficiency testing

## **GCLP**

### An Evolving Regulation

- Organisation and Personnel
- Facilities
- Equipment, materials and reagents
- Standard Operating Procedures (SOPs)
- Planning, conduct and reporting
- Quality Control and Quality audits
- Retention of study records and reports.

# Building a GLP/GCLP Laboratory

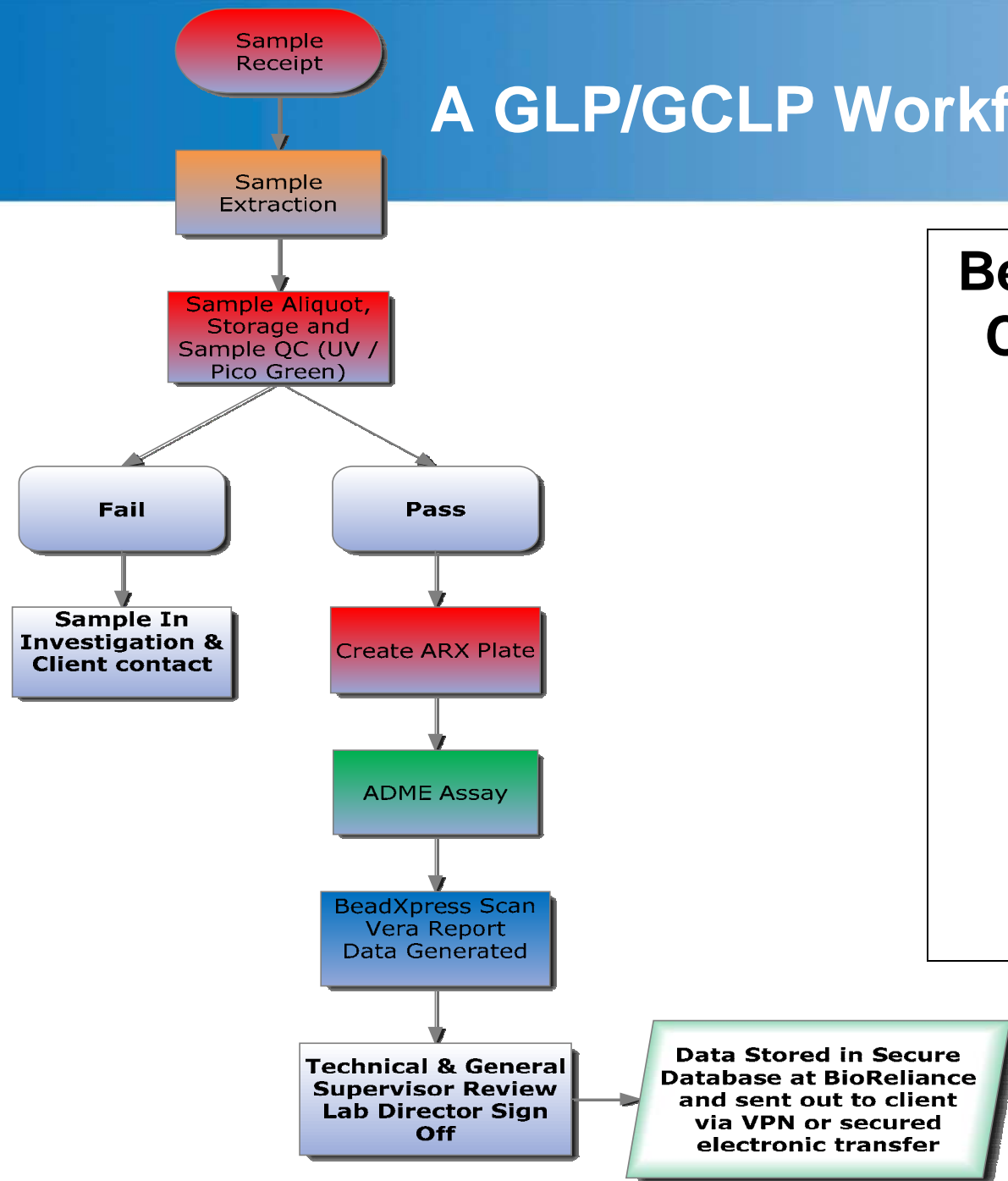
## Facility

- Security (Cypher-Locks, Security System)
- Disaster recovery program
- Sufficient work and storage areas
- Proper secured storage for lab and computer systems
- Accepted standards of electrical safety

## Safety

- All chemicals stored in appropriate places

# A GLP/GCLP Workflow



## BeadXpress CLIA Suite

Sample Prep Room

Extraction Room

Pre PCR Room

Post PCR Room

# Quality System (GLP/CLIA)

## Operator training

- Personal training binders
- Periodic refresher courses

## Study Protocols: For custom SNP Panels or the ADME Assay

- Reviewed signed off by Study Director or Lab Director
- Procedures and test criteria
- Controls
- Sponsor details

## SOPs

- Specific procedures for ADME assay or custom assay, equipment, QA & QC system, sample handling, privacy policies, computer systems, LIMS, archiving and etc.
- Reviewed sign off Lab Management

## Quality Assurance

- Effectiveness of policies and procedures periodically evaluated
- Evaluation identify problems and suggest solutions
- Solutions monitored to ensure their effectiveness
- *BioReliance BRIQS System to capture events and CAPAs*

# Equipment and Assay Validation (GLP/CLIA)

## Equipment

- BeadXpress, PCR and satellite equipment validation (IQ/OQ/PV) as well as periodic and preventive maintenance (procedure and log)
- LIMS and ancillary computer and data storage software systems
  - 21 CFR 11

## Two Levels of Assay Validation

- Obtain (native) samples for all SNPs in triplicate
- Perform assay validation per ICH Topic Q 2 (R1) Validation of Analytical Procedures (ICH 381)

# Custom BeadXpress Assays (GLP/CLIA)

## Control Samples

- Client or commercial sources
- Verified by sequencing if needed

## New V2 BeadXpress software

- Specify call zones and signal intensities based on control samples
- Lock the design in a custom Kit Manifest

## The result:

- A Controlled and secure data workflow
- Validated custom assay

# Good News for Illumina's BeadXpress

May 4, 2010

- Food and Drug Administration (FDA) Clears Illumina's BeadXpress System for Clinical Use

May 18, 2010

- FDA grants 510(k) market clearance for the VeraCode® Genotyping Test for Factor V (Leiden) and Factor II (Prothrombin)

# BioReliance CLIA Implementation

Working closely with Maryland's CMS representative (HHS)

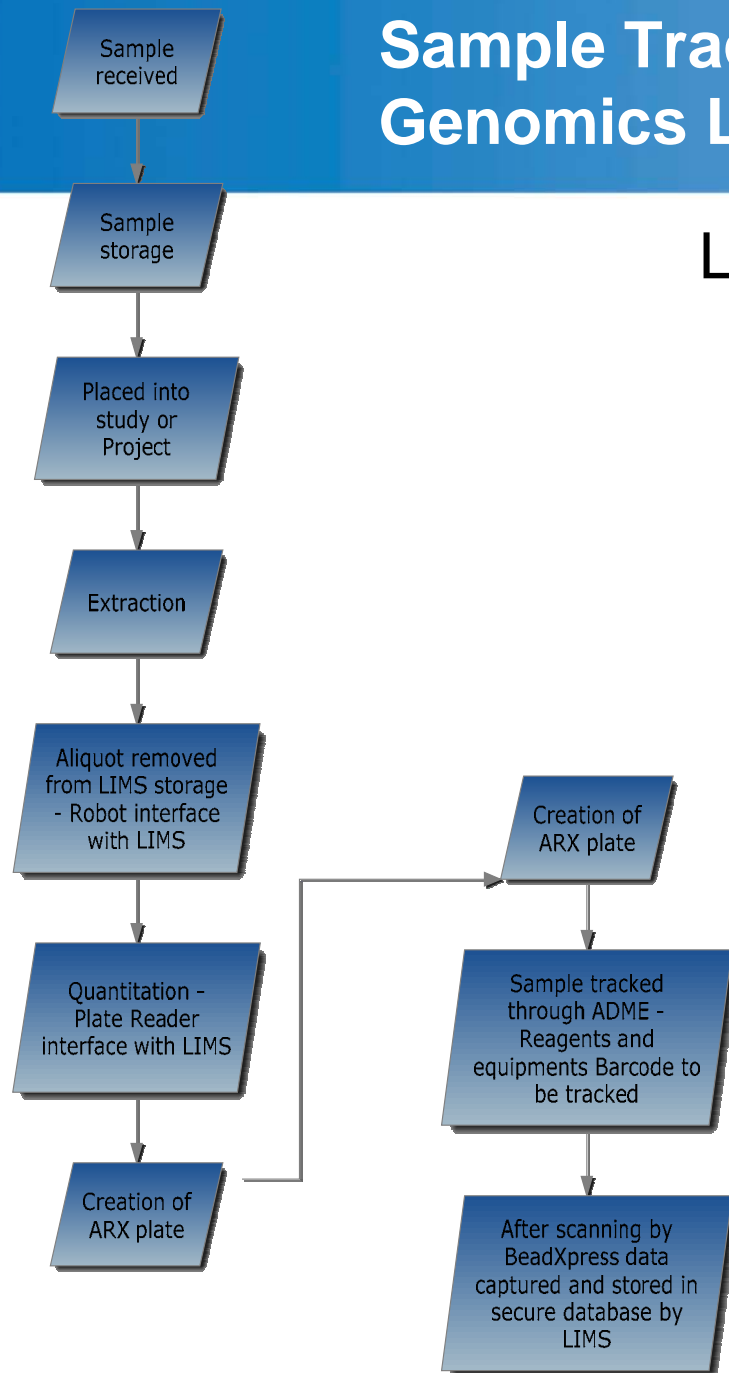
- Clinical Studies
- Direct to Consumer prohibition
- Non-waived; High complexity; Molecular Biology
- Qualified Personnel
  - Lab Director
  - General Supervisor
  - Technical Supervisor
  - Technical Personal
- Proficiency Testing
  - Timely manor (ParagonDx or Coriell samples)
  - Reviewed by Lab Director
  - Normal or expected ranges for the test

# BioReliance CLIA Implementation

## Patient Sample Management

- Uniquely identify individual samples
- Identify the sponsor
- List the test to be performed
- Automated ID and bar code generation
- Define storage location
- Monitor storage temperature
- Have backup procedure in case of freezer malfunction
- Verify the sample during analysis by scanning barcodes
- Track sample to disposal
- Require authorization for data manipulation
- Archive and sFTP data transfer

# Sample Tracking: Genomics LIMS



## LabWare v6...

- Sample barcoding (internal)
- Sample storage location
- Plate assembly (barcoded)
- Sample tracking through workflow
- E-signatures
- Automated data backup

# BioReliance ADME Core Genotyping

Equipment Validation

***June 2010***

GLP Service

***July 2010***

- ADME and Custom

Assay Validation

***Aug 2010***

CLIA Certificate of  
Registration

***Aug 2010***

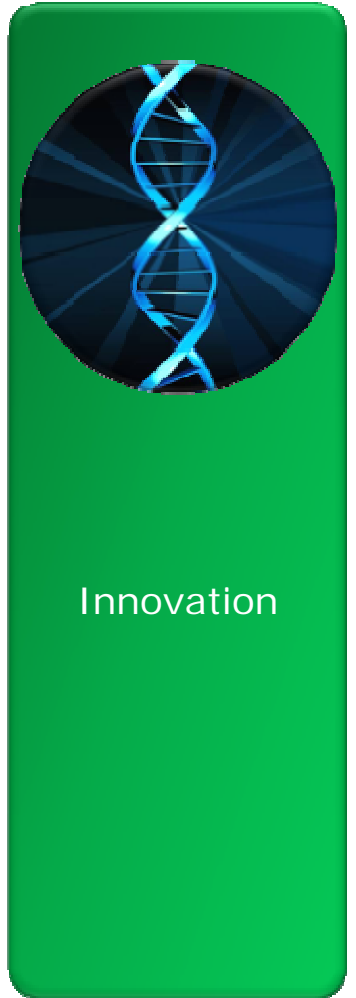
# Summary

BioReliance Genomics:

- Custom Quantitative PCR
- Massively Parallel Sequencing
- VeraCode ADME Panel
- Custom BeadExpress/VeraCode

Nucleic acid testing tools for every multiplex  
and every stage...

Thank you.



# Acknowledgements

## BioReliance

- Colette Cote
- Sailaja Koduri
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- Alice Corrigan
- Rob Goodier
- Bradley Hasson
- Bradley Toms
- Audrey Chang
- Chuck Pals
- Ming Li Cheng
- Diane McLeavey
- David Onions