





# VeraCode Technology

*From Research to Diagnostics*

June 22, 2009

# Most Complete Genomics Assay Portfolio

APPLICATION	Sequence-Based Discovery	Whole-Genome	Targeted Analysis		Validation, Screening & Standard Tests
TECHNOLOGY	Solexa Sequencing	BeadArray	BeadArray		VeraCode
PLATFORM	 Genome Analyzer	 iScan	 iScan		 BeadXpress
ASSAY	Sequencing by Synthesis	Infinium Assay Direct Hyb	Infinium Assay GoldenGate & DASL Assay		GoldenGate & DASL Assay
MARKER DENSITY	1base to >15GB	240K to 1M	6K to 60K (iSelect) 96 to 1536 (GoldenGate) Custom Standard		1 to 384-plex Custom
PRODUCT	Resequencing	Human1M-Duo	Infinium iSelect Genotyping Panels 6K-60K	Linkage-12	VeraCode GoldenGate 96- & 384-plex
	SNP & CNV Discovery	HumanHap650Y	GoldenGate Genotyping Panels 96-1536	CNV-12	DASL Expression
	De novo Sequencing	Human610-Quad	DASL Expression Panels 384-1536	MHC GG	Methylation
	Tag Expression	Exon510-Duo	GoldenGate Methylation Panels 384-1536	African American Admixture GG	Carboxyl - Protein
	Transcript Profiling	HumanCNV370-Duo		CanineSNP20	Universal Capture
	ChIP Sequencing	Human 6/Ref-8 Gex		BovineSNP50	
	MeDIP Sequencing	Mouse 6/Ref-8 Gex		Micro RNA	
	BiSulfite CpG	Rat Ref-12 Gex		Methylation Cancer Panel I	
		Genome-wide Methylation27			
2008 Release					



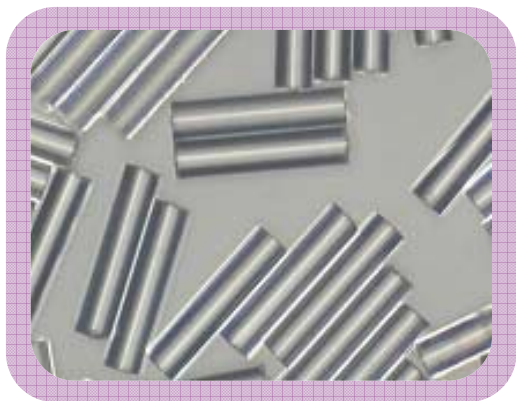
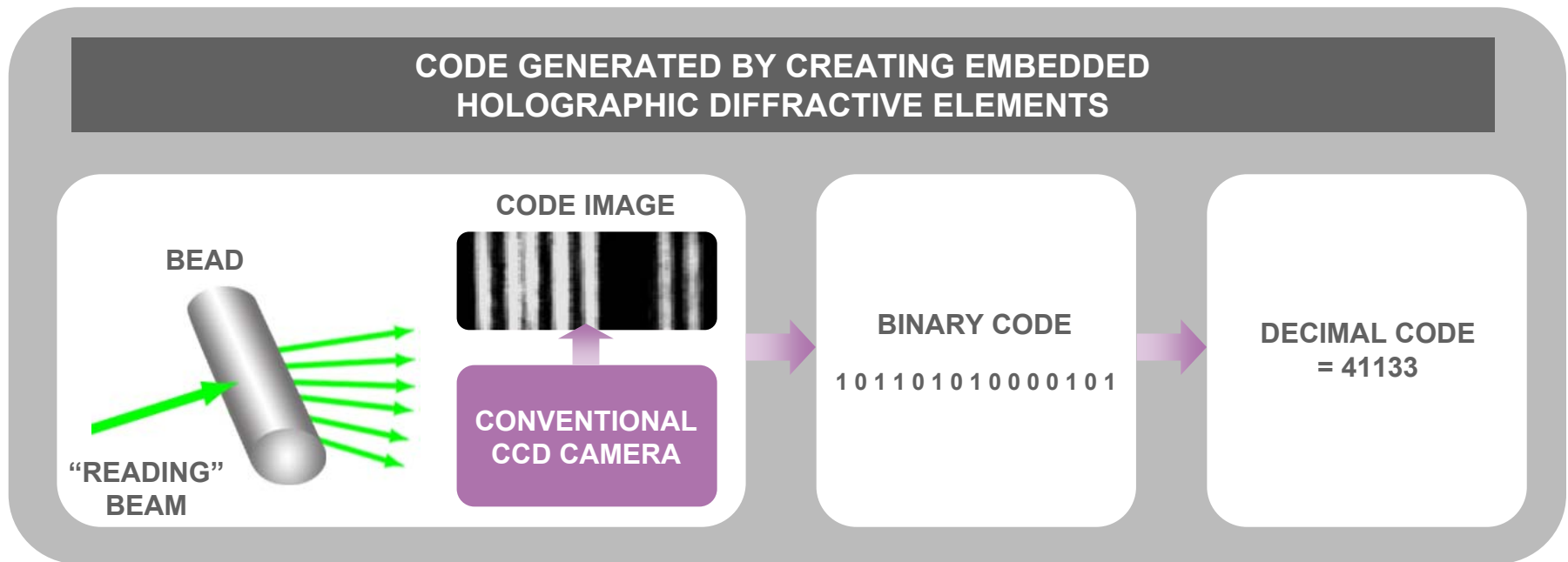
# Illumina's Solution for Diverse Applications

VeraCode® Technology



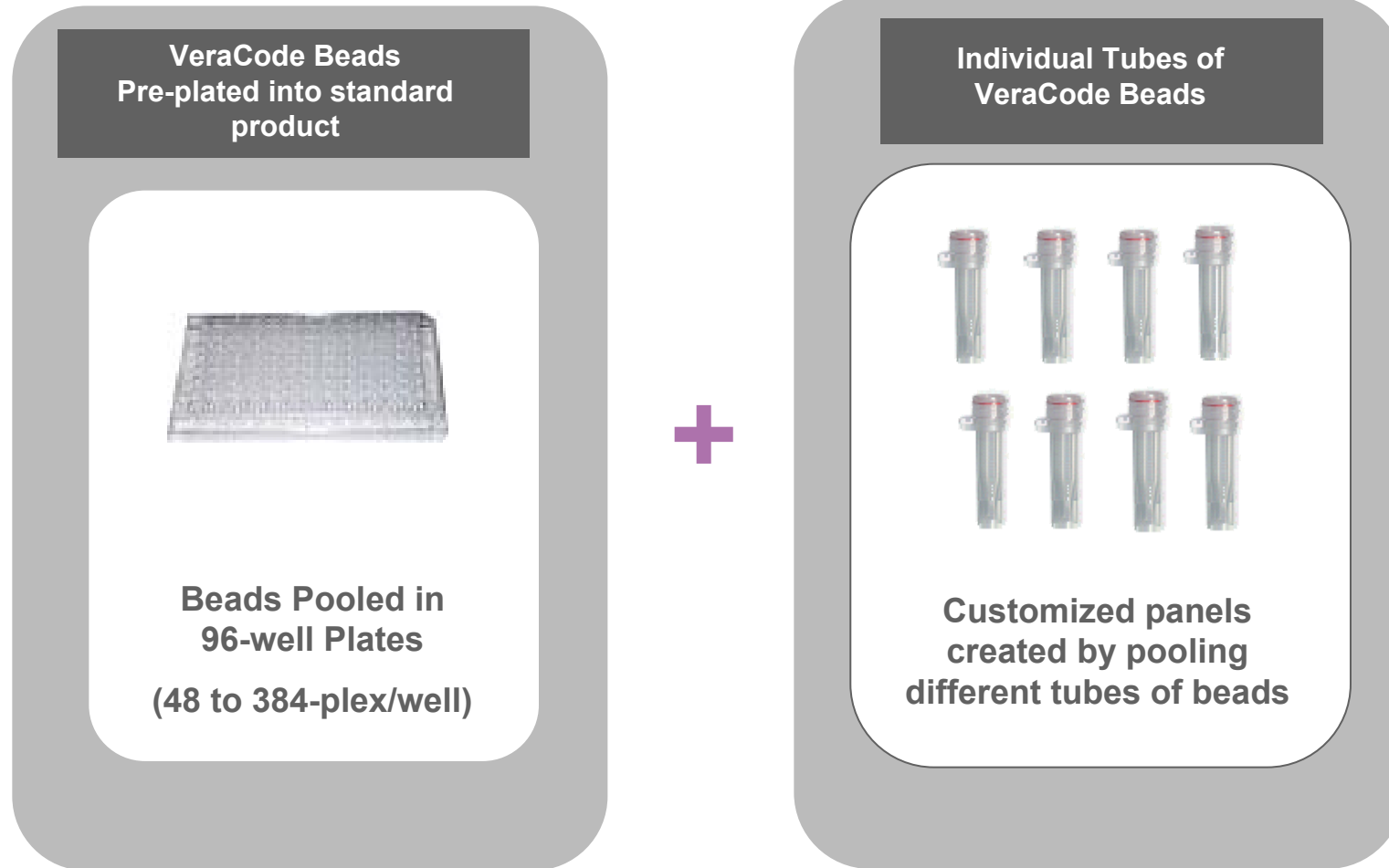
- Cylindrical glass microbeads
- 240  $\mu\text{m}$  length x 28  $\mu\text{m}$  diameter

# VeraCode Technology



- High Density Codes Easily Imprinted (24 bit)
- Virtually Unlimited Unique Bead Codes Available – 626 unique codes used in launched products
- Glass Surface of Beads Ideal for Bioassays – numerous surface chemistries available
- Specific panels created easily with addition or removal of specific bead types

# Easily adaptable to changing content requirements



# BeadXpress™ Reader

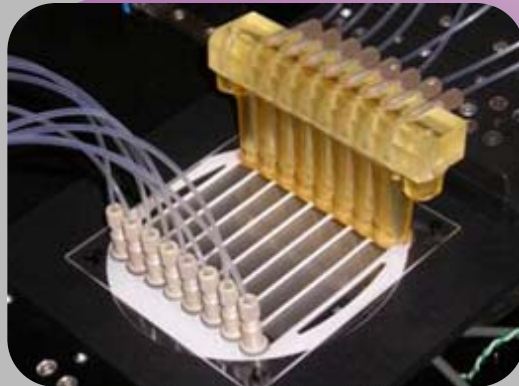


## INSTRUMENT SPECIFICATIONS

SAMPLE DELIVERY FORMAT	● Standard 96-well plate & Stripwell Plates
MULTIPLEXING PER SINGLE WELL	● 1 to 384
THROUGHPUT	● 120 samples/hr at 10-plex (two-color detection) ● 80 samples/hr at 96-plex (two-color detection)
DETECTION	● Optional 1 or 2 color detection
REGULATORY	● Regulatory Design Control

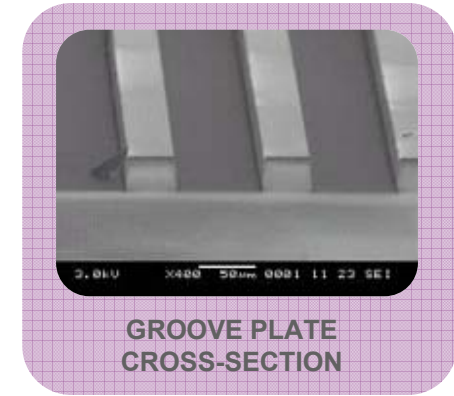
# VeraCode Technology

EIGHT WELLS ARE SAMPLED SIMULTANEOUSLY AND MICROBEADS ARE TRANSFERRED INTO A MULTI-SECTORED READER CELL.

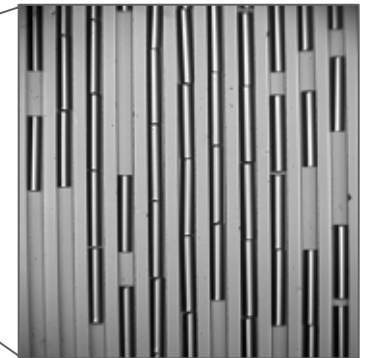
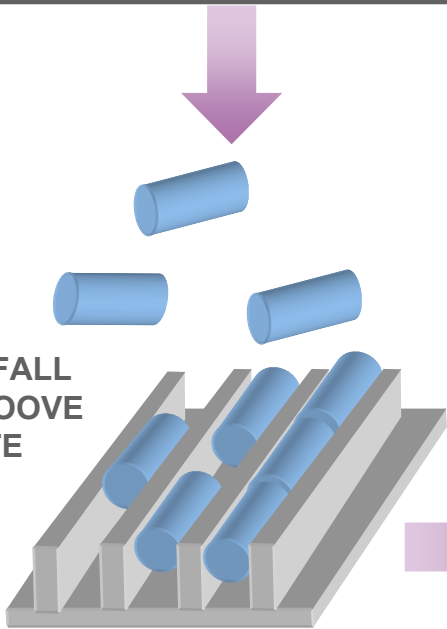


# VeraCode Technology

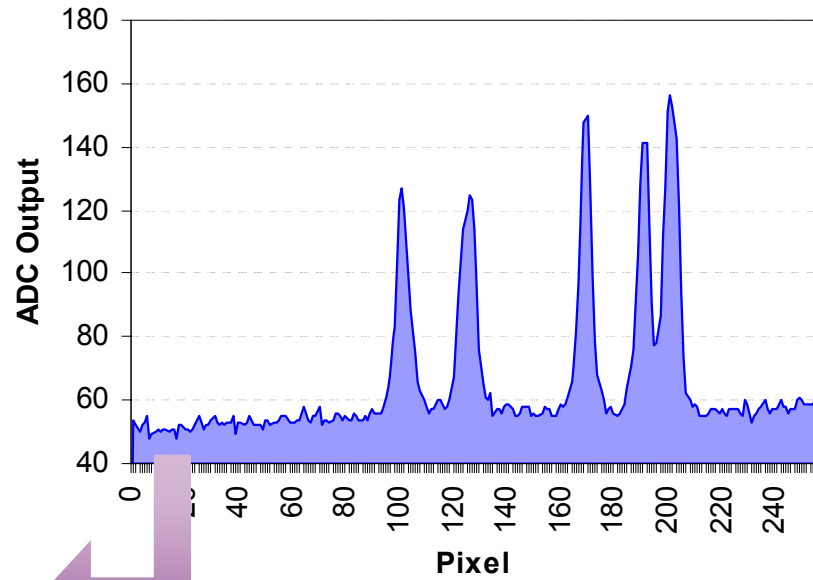
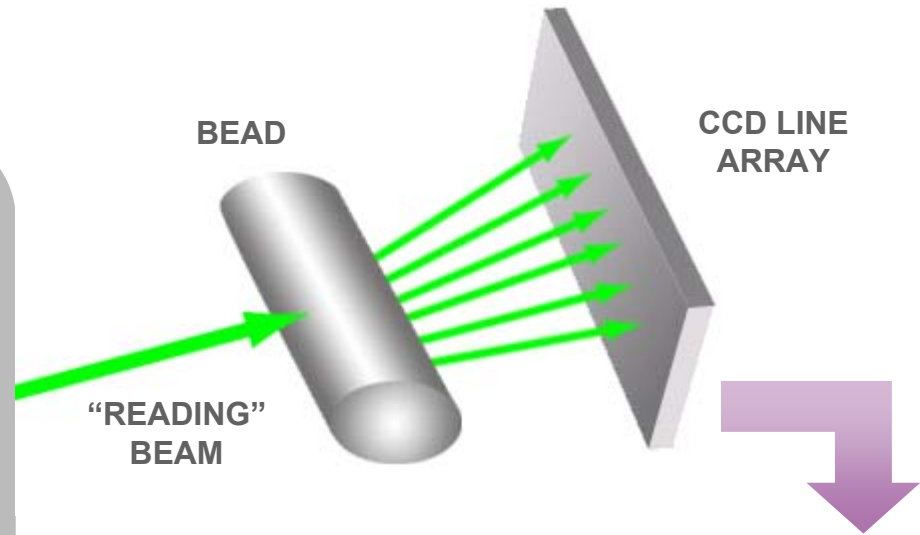
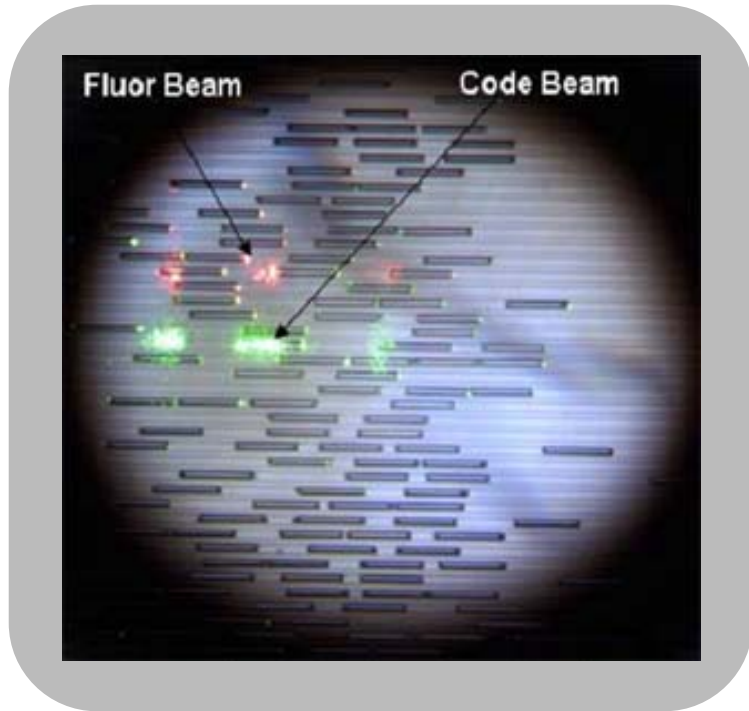
CAPILLARY FORCE ATTRACTS  
BEADS INTO GROOVES



BEADS FALL  
INTO GROOVE  
PLATE

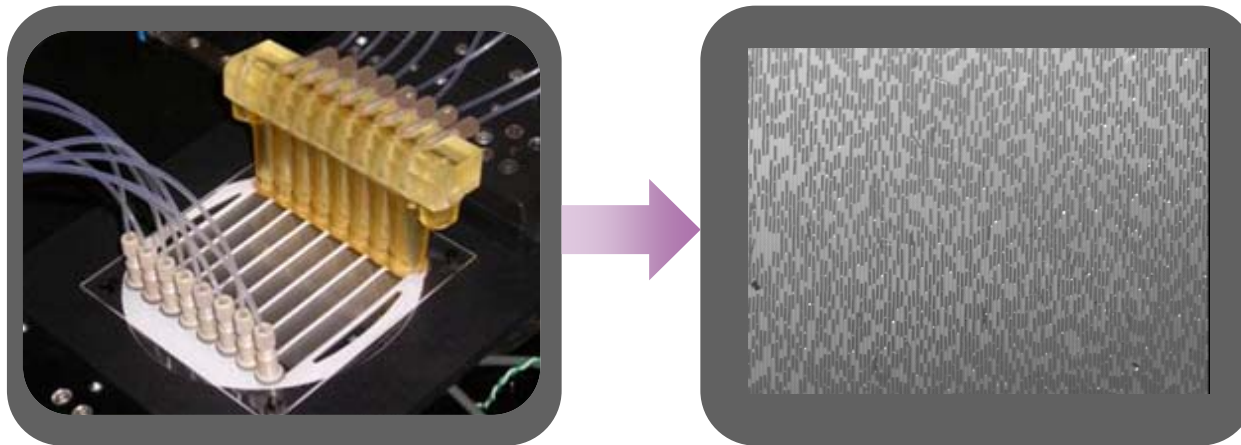


# Code Detection



# VeraCode Technology

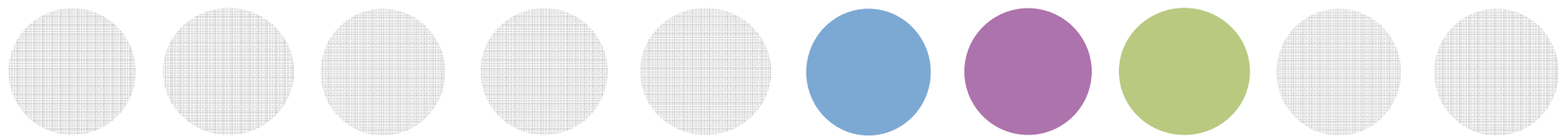
**FOLLOWING READOUT, THE BEADS ARE FLUSHED FROM THE READER CELL USING A SECOND ACTUATION PRESSURE CYCLE.**



# Data Analysis Streamlined with BeadStudio

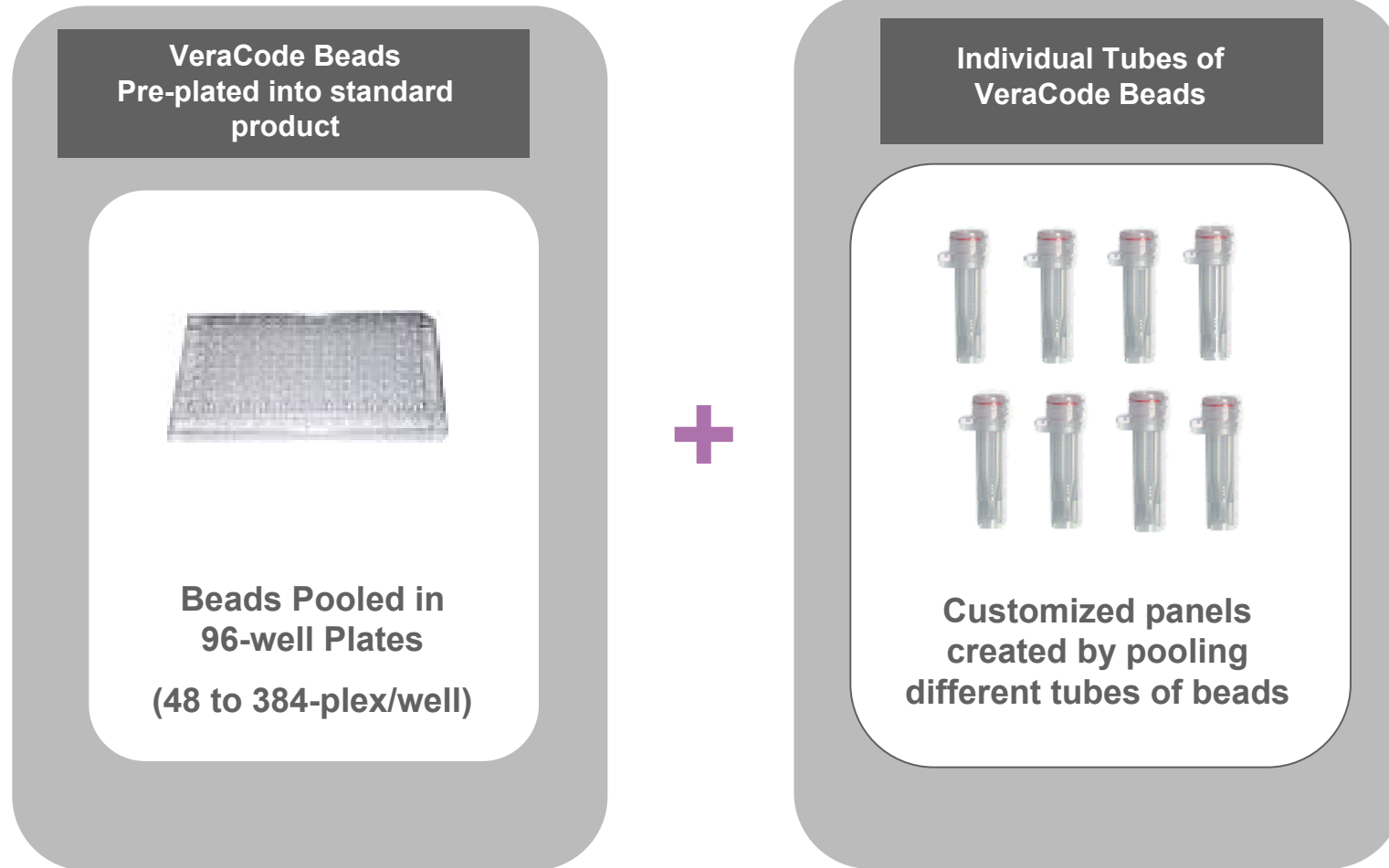
The screenshot displays the BeadStudio software interface with several key components:

- Table of Probe Statistics:** A large table with columns for TargetID, R Pearson, R2, Rr Spearman, Concordance, and four pairs of AVG\_Beta and AVG\_Signal columns for different probes (K562\_a, Raj\_a, A431\_a, Jurkat\_a).
- Bar Plot:** A plot titled 'Combined Table: 092006\_DA' showing Beta values for four probes: K562\_a, Raj\_a, A431\_a, and Jurkat\_a. The y-axis ranges from 0.0 to 1.0.
- SNP Graph:** A plot titled 'M667' showing Norm R vs Norm Theta. Three clusters are identified with counts 31, 22, and 23.
- SNP Graph Alt:** A plot titled 'M667' showing Intensity (B) vs Intensity (A).
- Full Data Table:** A table with columns for Index, Name, Address, Chr, Position, GType, Score, Theta, R, and Th. It shows data for samples like FII, FV, M1298, and M667.
- Samples Table:** A table with columns for Index, Sample ID, Sample Section, Call Rate, Genotype for M667, p05 Grn, p50 Grn, p95 Grn, and p05 Red. It lists various sample IDs and their corresponding genotypes and call rates.



## VeraCode Custom Products

# Easily adaptable to changing content requirements



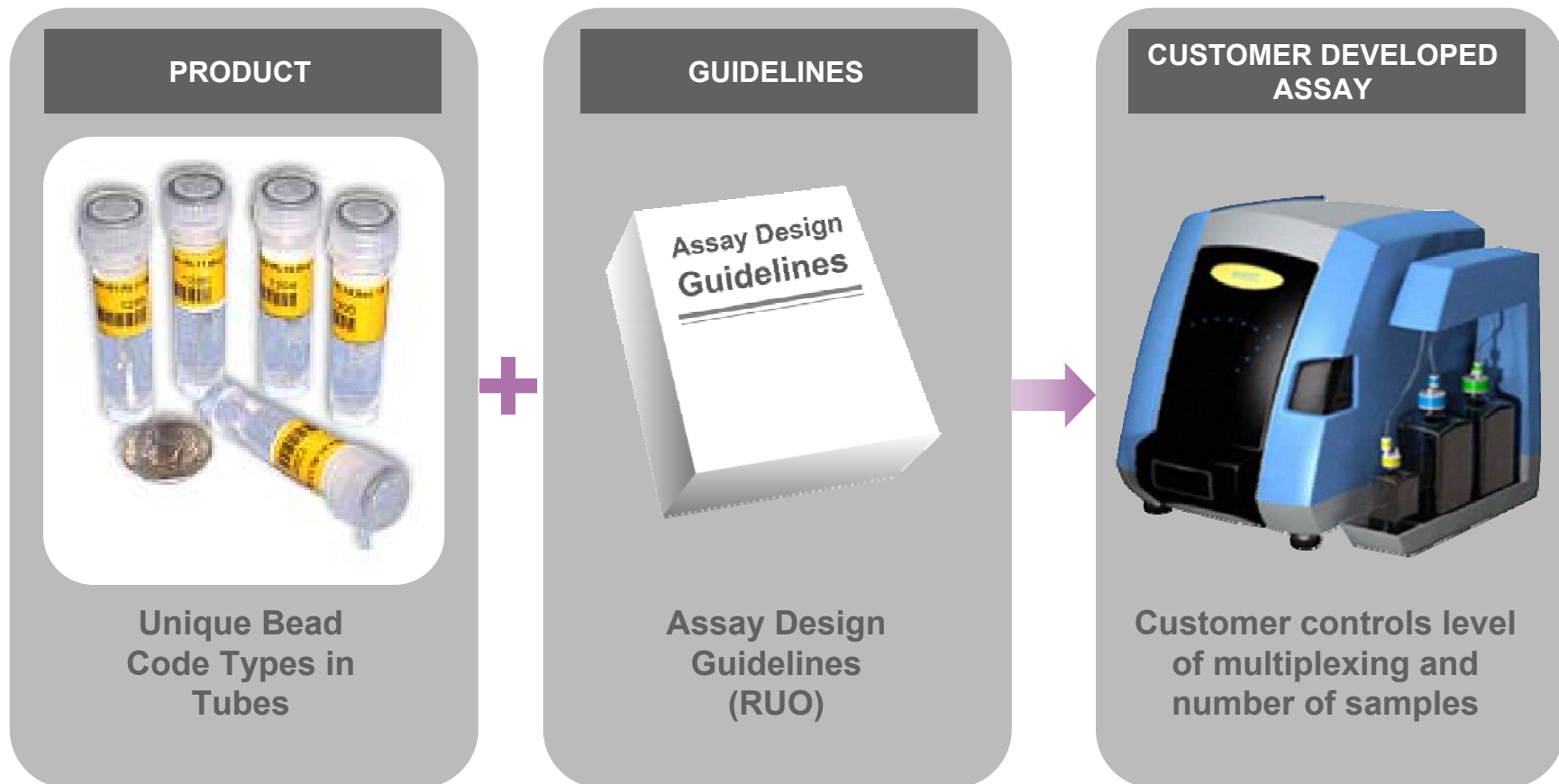
## Comprehensive VeraCode product portfolio

- VeraCode Carboxyl Bead Sets
- VeraCode Universal Capture Bead Sets
- Custom GoldenGate Genotyping, 48 to 384-plex
- DASL Custom Gene Expression, 96 & 384-plex
- Custom GoldenGate Methylation, 96 & 384-plex



# Available Products

## CARBOXYL BEAD SETS (PROTEIN BASED ASSAYS)



# Carboxyl Beads: Chemistry of Attachment



COOH<sup>-</sup>  
COOH<sup>-</sup>  
COOH<sup>-</sup>  
COOH<sup>-</sup>  
COOH<sup>-</sup>  
COOH<sup>-</sup>  
COOH<sup>-</sup>  
COOH<sup>-</sup>

## PROTEIN ATTACHMENT: TWO-STEP PROCESS

Activate Surface

EDC/Sulfo-NHS

Covalently Bind

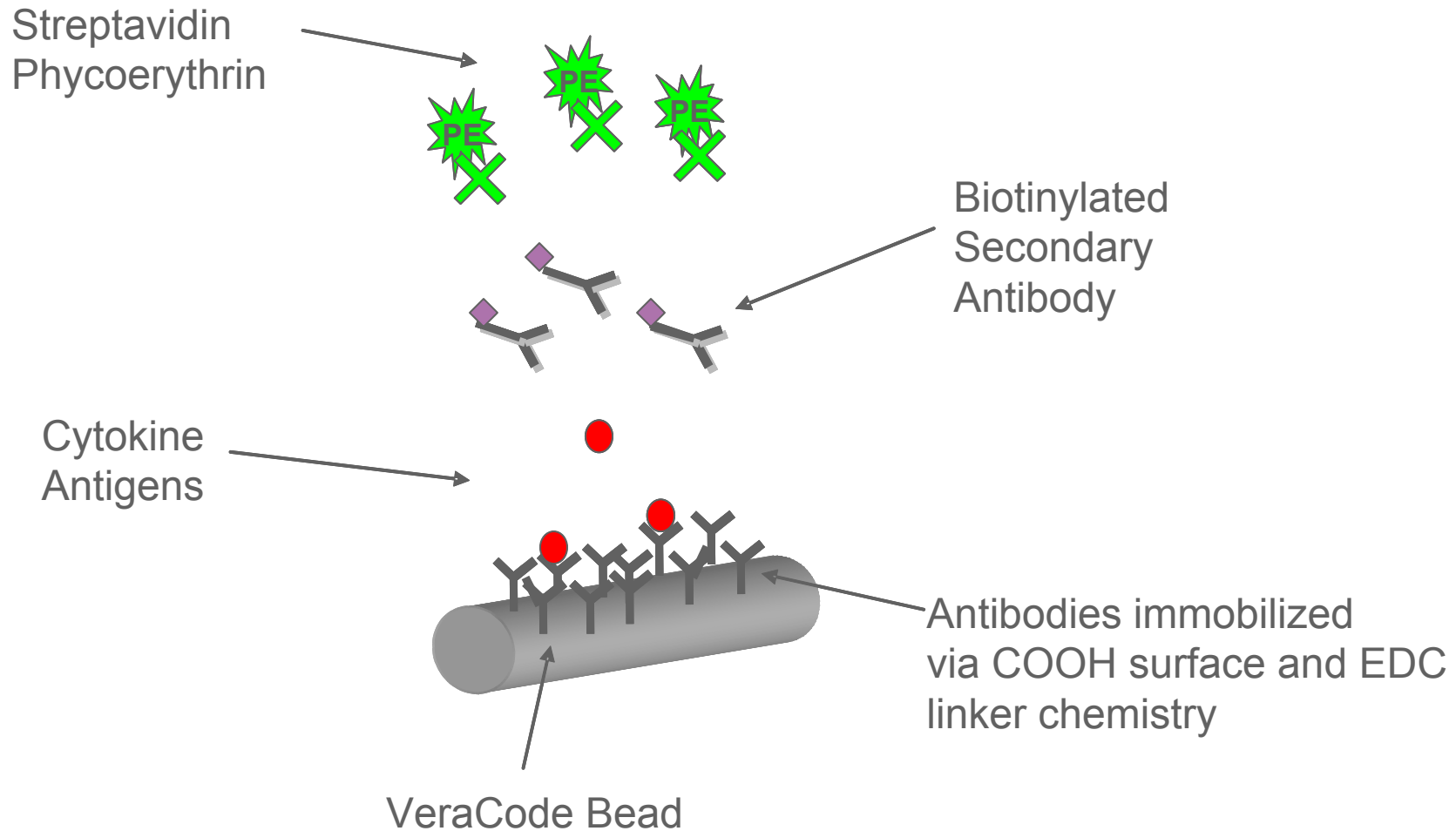
Antibodies  
Antigens  
Peptides  
Streptavidin

## NUCLEIC ACID ATTACHMENT: ONE-STEP PROCESS

EDC/Sulfo-NHS plus NH<sup>2+</sup>Oligo

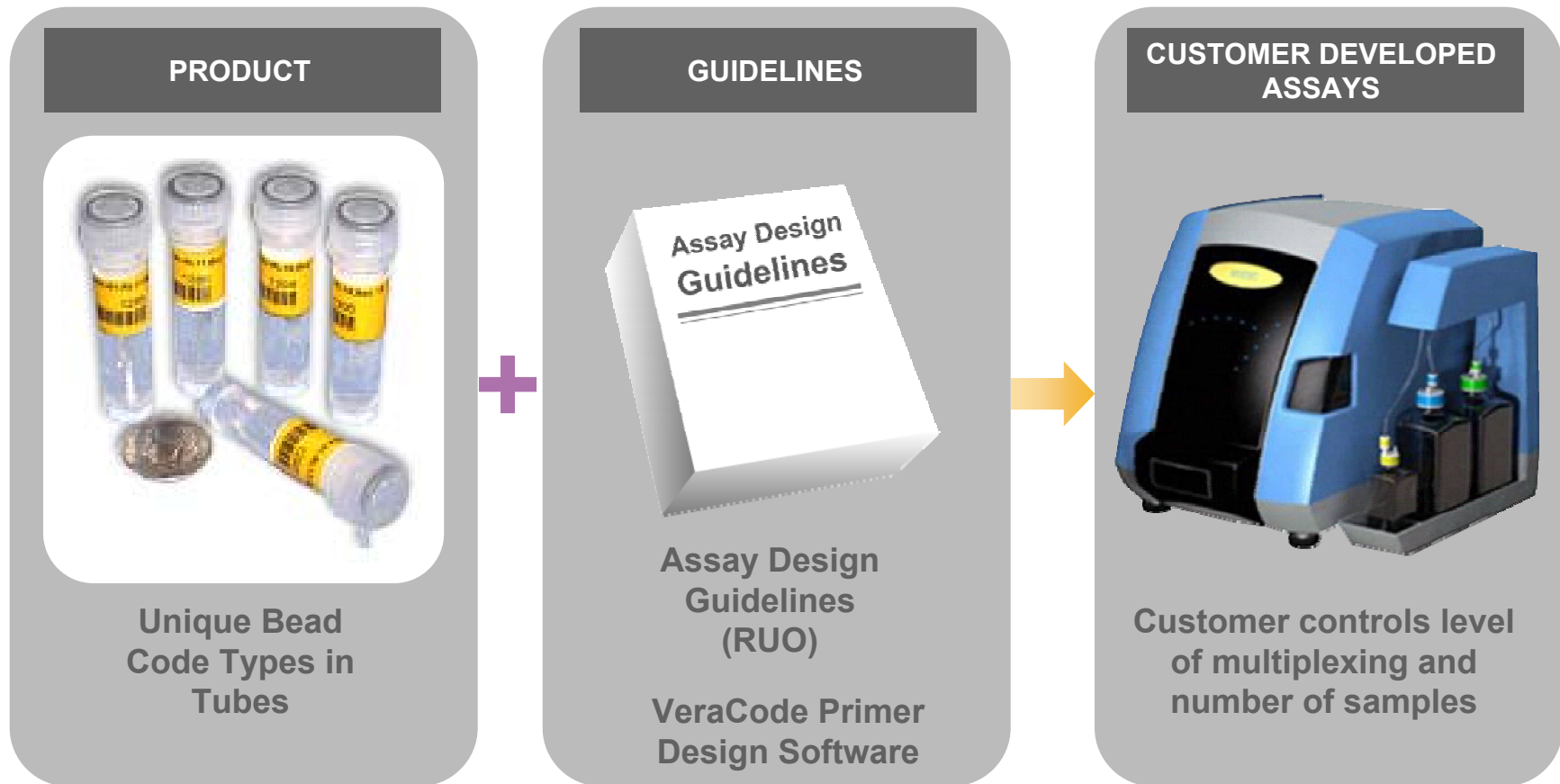
Nucleic  
Acids

# Cytokine Sandwich Assay Overview

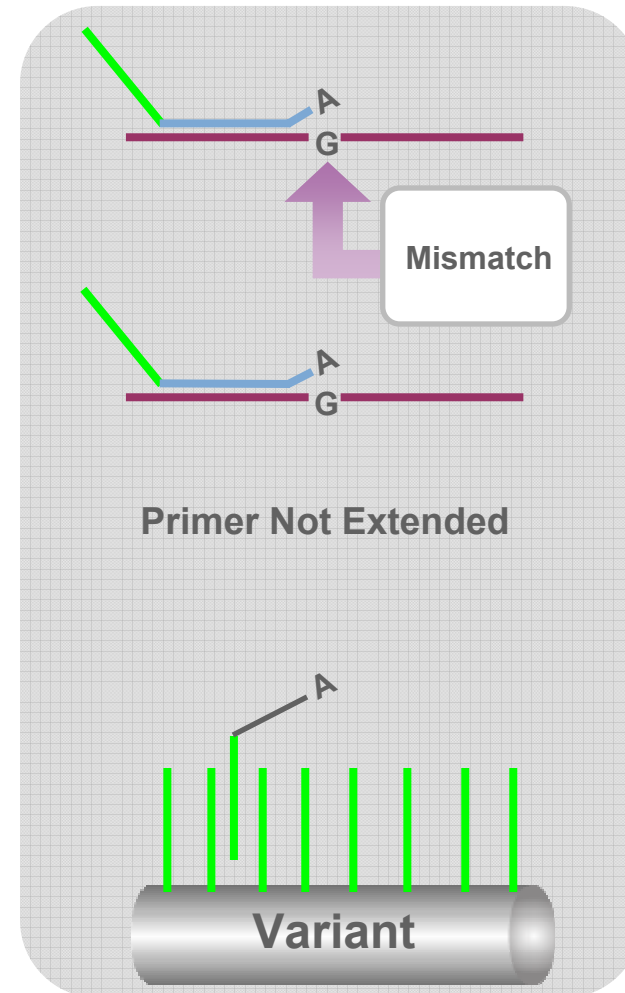
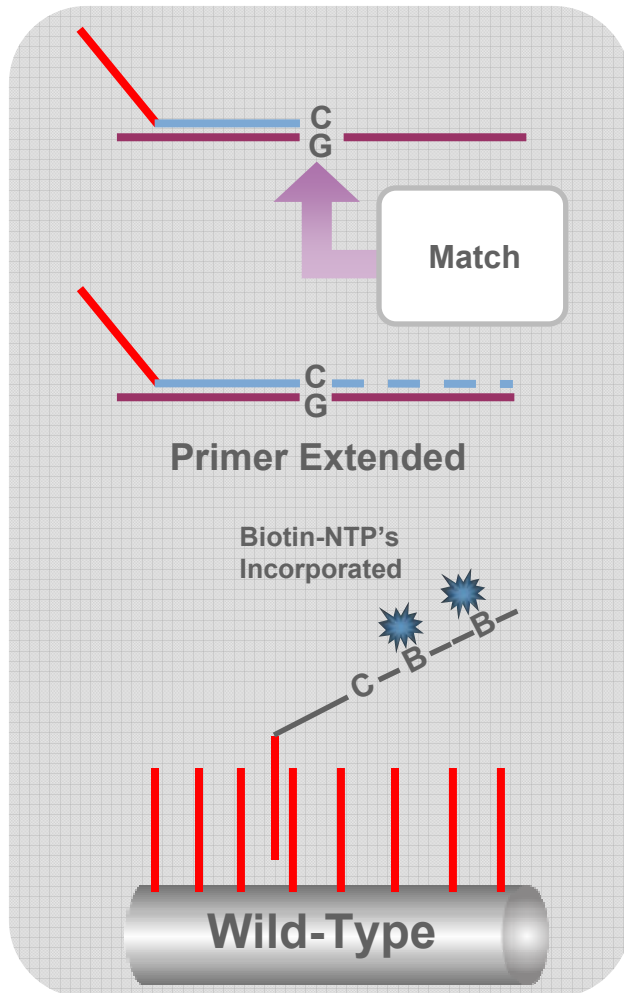


# Available Products

## UNIVERSAL CAPTURE BEAD SETS



# Allele-Specific Primer Extension (ASPE)



# The VeraCode Assay Designer Solution

- Access user-specific accounts
- Input SNP targets
- Choose workflow option
- Customize design parameters
- Select among possible assay designs
- Download detailed ordering information

illumina®

veracode<sup>™</sup>  
BY ILLUMINA<sup>®</sup>

Main Menu

Sequence Entry ?

**Help**  
Enter your sequence(s) or accession Number(s) into the sequence entry field. ▶ More...

**Design Run**  
**Method:** ASPE  
**Application:** SNP  
**Mode:** Express  
 Use Anchored Oligo(s)

**Search NCBI Databases**  
**Organism:** Human (Homo sapiens)  
 Identify and exclude potential SNPs from assay design.  
 Search Genome  
NCBI Disclaimer

**Sequence Entry**  
Enter nucleotide sequence(s) or NCBI accession number(s). ▶ More...

```
rs911903,  
rs223201,  
rs2030162,  
rs743137,  
rs1570964,  
rs1397354,  
rs649593,  
rs1981635,  
rs898249
```

<< Back   File Upload   Multiplex >>   Next >>

# Assay Design Results

- Example output of an assay design run

Summary Results

Primer Designs

**Summary Results**

**Design Run**  
 Name: rs911903, rs223201, ...  
 Method: ASPE-SNP  
 Status: Complete  
 # Sequences: 9  
 # Std Assays: 0  
 # Multiplexes: 20  
 Date: 8/16/2007 3:43:55 PM  
 Mode: Express  
 Speed: Fast  
 # With Assays: 9  
 # Submitted Sequences: 9  
 # Included Sequences: 9  
 # Failed Sequences: 0  
 # Incompatible Sequences: 0

**Results**

Multiplex 223A5B85 62.37

rs911903-Y-80  Assay 0005 62.37

Oligo	Rank	Tm	GC%	Length	5' Pos	3' Pos	5'-Sequence-3'
<input type="checkbox"/> Allele1	84.03	51.0	60	15	66	80	WM CCTCTCCAGCTCAAC
<input type="checkbox"/> Allele2	89.23	49.5	53	15	66	80	WM CCTCTCCAGCTCAAT
<input type="checkbox"/> Forward	85.67	60.4	50	18	21	38	TTCTGATGCCACGATGA
<input type="checkbox"/> Reverse	89.83	60.4	48	21	129	109	CATTTGAAGCCTCTGGTATG

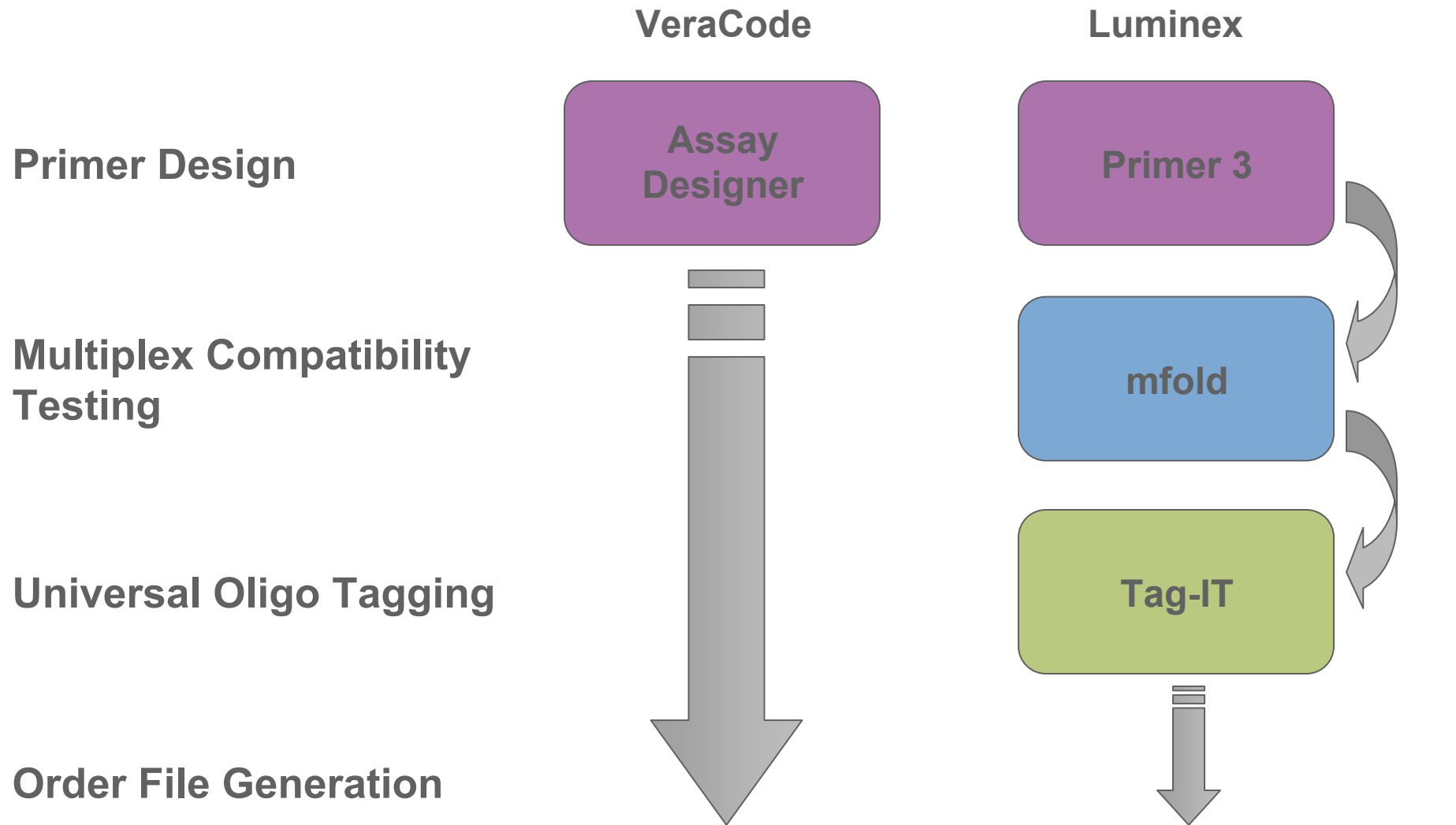
**Identified Mis-Alignments**

Self Align	Dimer	3' Align	Hairpin	Total ΔG°	Pair Align	Dimer	3' Align	Bi-ΔG°
<a href="#">Allele1</a>	4	1	2	-10.69	<a href="#">Allele1/Allele2</a>	4	1	.00
<a href="#">Allele2</a>	4	2	2	-10.63	<a href="#">Forward/Reverse</a>	3	3	.00
<a href="#">Forward</a>	2	2	2	-10.34	<a href="#">Allele1/Reverse</a>	4	4	.00
<a href="#">Reverse</a>	3	3	3	-10.34	<a href="#">Allele2/Forward</a>	3	3	.00
					<a href="#">Allele2/Reverse</a>	4	4	.00
					<a href="#">Allele1/Forward</a>	3	3	.00

**Amplicon Detail**

Amplicon	Rank	AT Run	GC Run	GC%	Length	Variation
Allele 1	100.00	4	2	44	109	C
TTCTGATGC CACGATGATT TCCACCGCAG CCATTCTGGA GTTTTCCTCT CCAGCTCAAC AGCTATTTCA AGTGTGTTTT ATATTTTACA TACCAGGAGG CTTCAAATG						
Allele 2	100.00	4	2	43	109	T
TTCTGATGC CACGATGATT TCCACCGCAG CCATTCTGGA GTTTTCCTCT CCAGCTCAAT AGCTATTTCA AGTGTGTTTT ATATTTTACA TACCAGGAGG CTTCAAATG						

# Customer Feedback: Software Required for Multiplex Assays

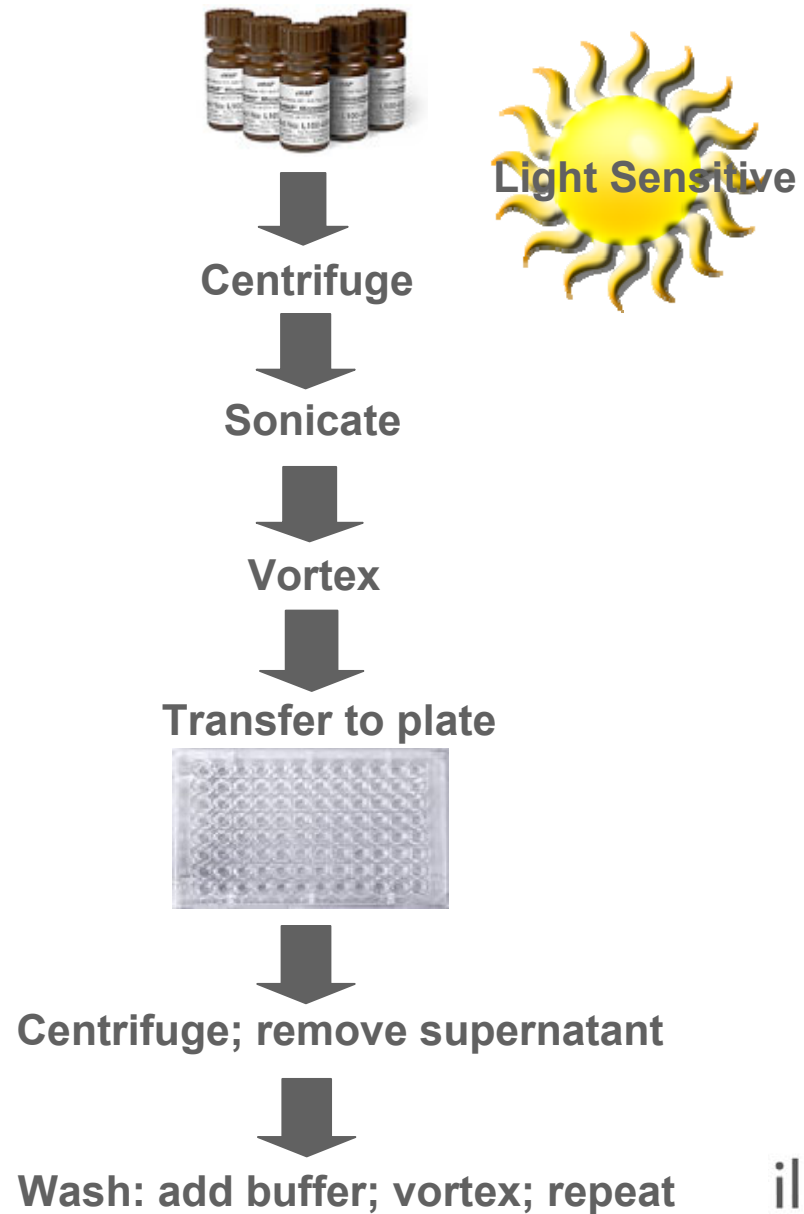


# Multiplex ASPE Genotyping Protocol

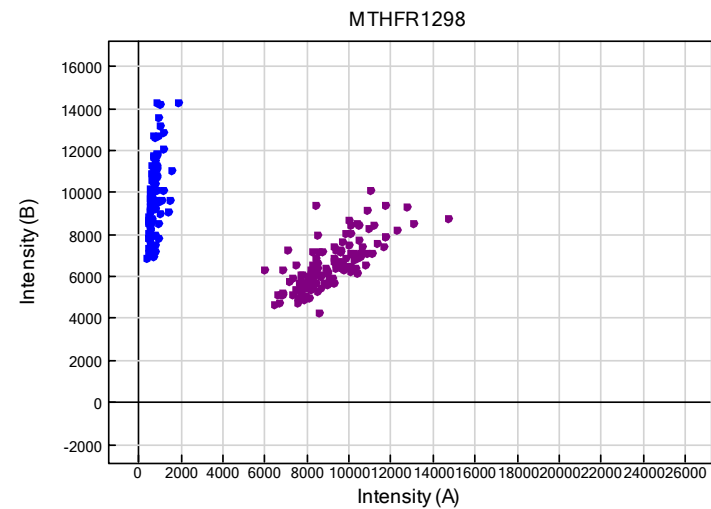
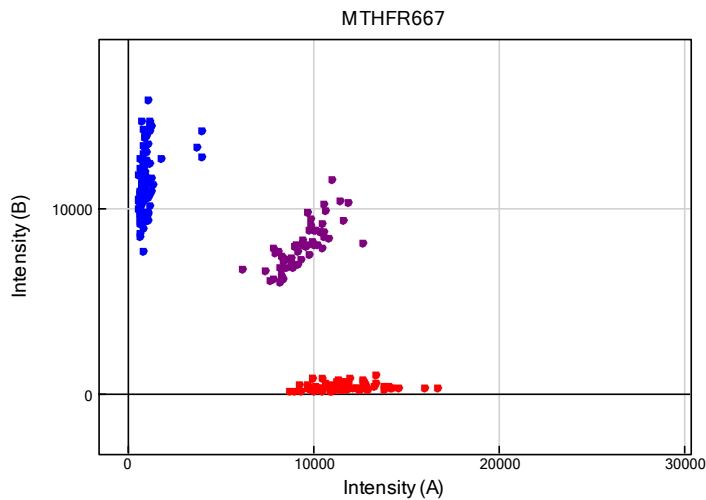
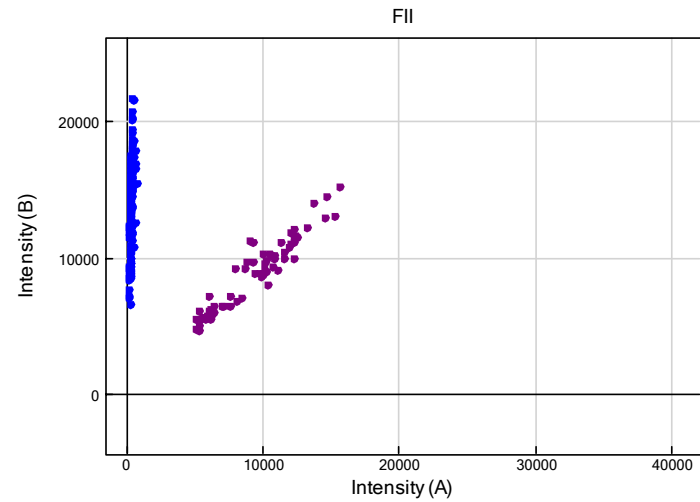
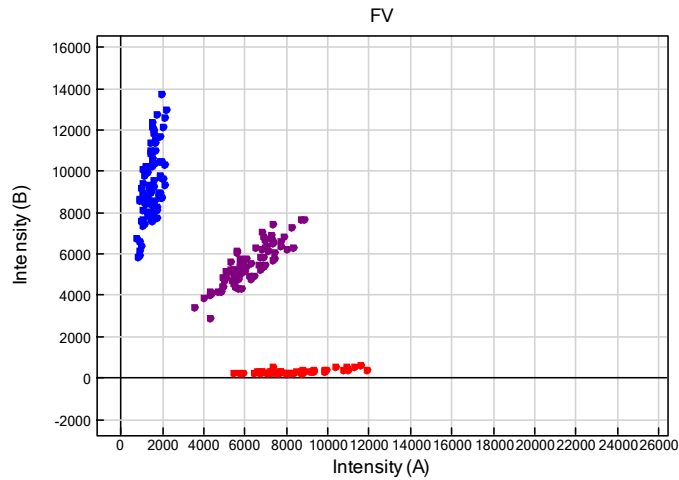


*Total Hands On Time ~ 1.5 hr/plate*  
*Total Time (Sample to Answer) ~ 6.5 hours*

# Bead Kitting Workflow – VeraCode vs. Luminex



# Low Plex ASPE Genotyping Results



- $\geq 99.99\%$  reproducibility
- 100% concordant genotypes

## Customer Success

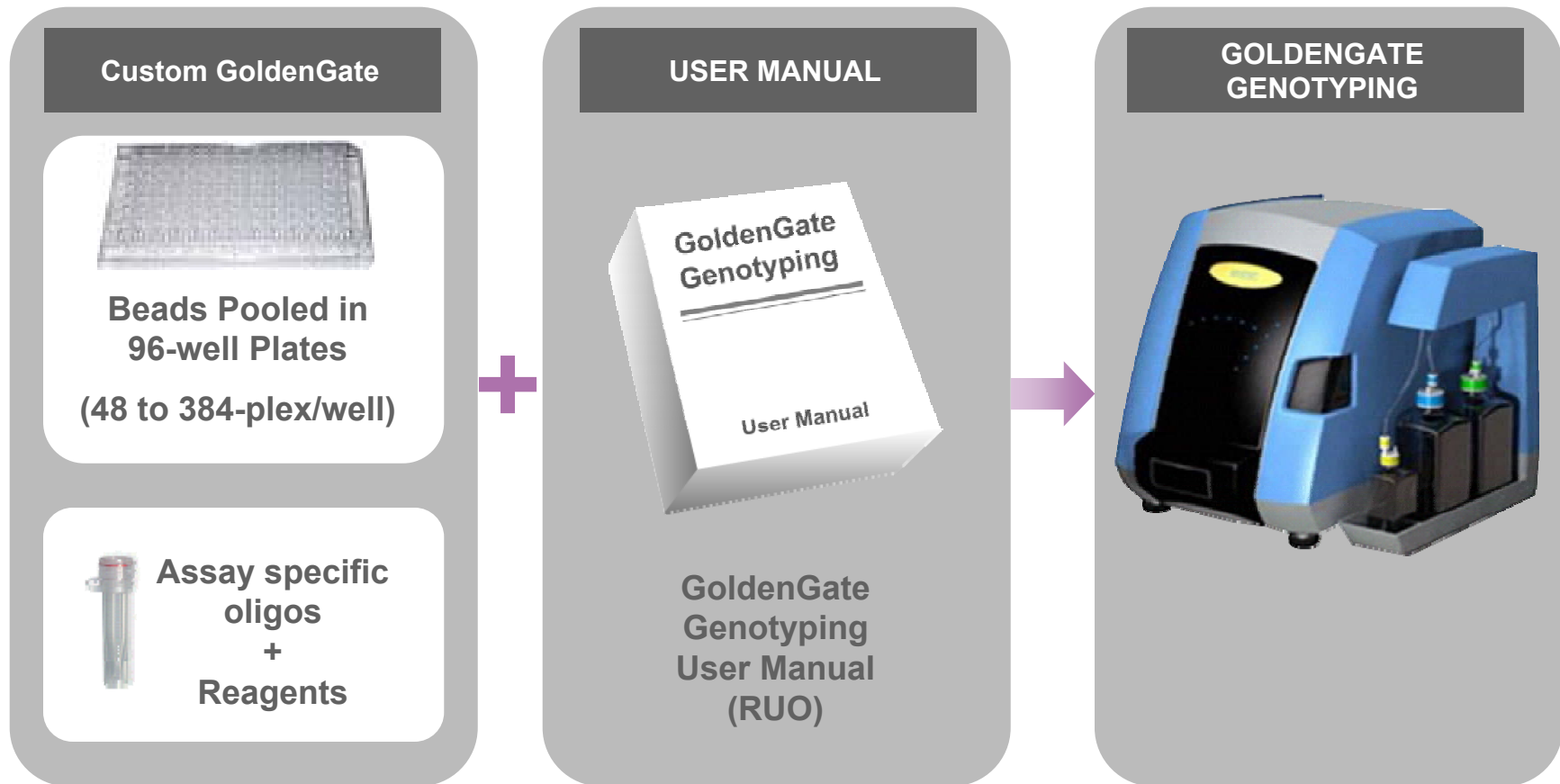
- Sciona – Health and Wellness company offering genetic testing and personalized exercise, diet and lifestyle recommendations
  - Validated assays presented in October 28, 2008 Webinar<sup>1</sup>
  - GSTM1: Deletion of GSTM1 associated with compromised detoxification
  - GSTT1: Deletion of GSTT1 associated with compromised detoxification
  - ALDH2: 487 G->A associated with alcohol intolerance
  - CYP1A1: 462 A->G associated with increased metabolism of carcinogens
- Children's Hospital of Eastern Ontario – Newborn screening laboratory testing 135,000 children per year
  - Assay development presented at 2008 Illumina Seminar Series<sup>2</sup>
  - HbC, HbE, HbSickle, HbO and HbF: Hemaglobinopathy panel
  - SMA – Spinal Muscular Atrophy assay looks at gene copy number variation associated with severity of muscular disease

1: <http://www.illumina.com/webinars/archives/archivesWebinars.ilmn?entry=50&w=20>

2: [http://www.illumina.com/seminars/archive/Crocker\\_0808\\_NewbornScreening.pdf](http://www.illumina.com/seminars/archive/Crocker_0808_NewbornScreening.pdf)

# Available Products

## GOLDENGATE GENOTYPING ASSAY (48, 144, 192 & 384-PLEX)



## GoldenGate Assay Used for Majority of the International HapMap Project

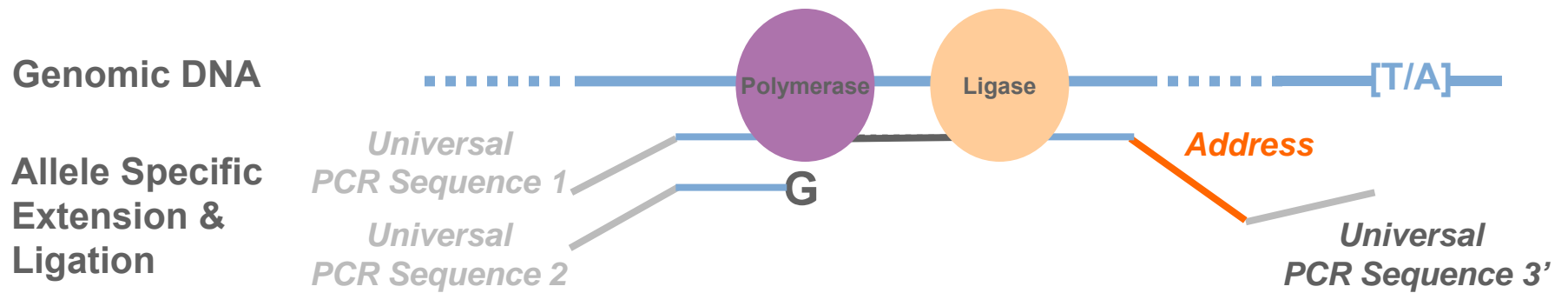
Country	Research Group and Leaders	Chromosome Assignment	% of Genome
Japan	Yusuke Nakamura, RIKEN/University of Tokyo	5, 11, 14, 15, 16, 17, 19	25.1%
United Kingdom	David Bentley, Wellcome Trust Sanger Institute	1, 6, 10, 13, 20	24.0%
Canada	Thomas Hudson, McGill University	2, 4p	10.0%
China	The China HapMap Consortium	3, 8p, 21	10.0%
United States	Illumina Broad Baylor UCSF & Washington University	8q, 9, 18q, 22, X 4q, 7q, 18p, Y 12 7p	15.5% 9.1% 4.4% 1.9%

- >1,000,000 SNP assays developed
- 285,000,000 genotype calls made
- ~70% done with Illumina GoldenGate assay using the Sentrix BeadArray Reader



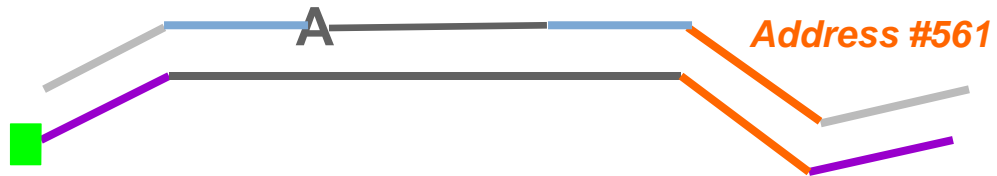
# GoldenGate Genotyping Assay

## Allele Specific Extension and Ligation

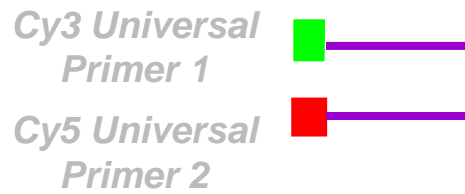


# GoldenGate Genotyping Assay Amplification

Amplification  
Template

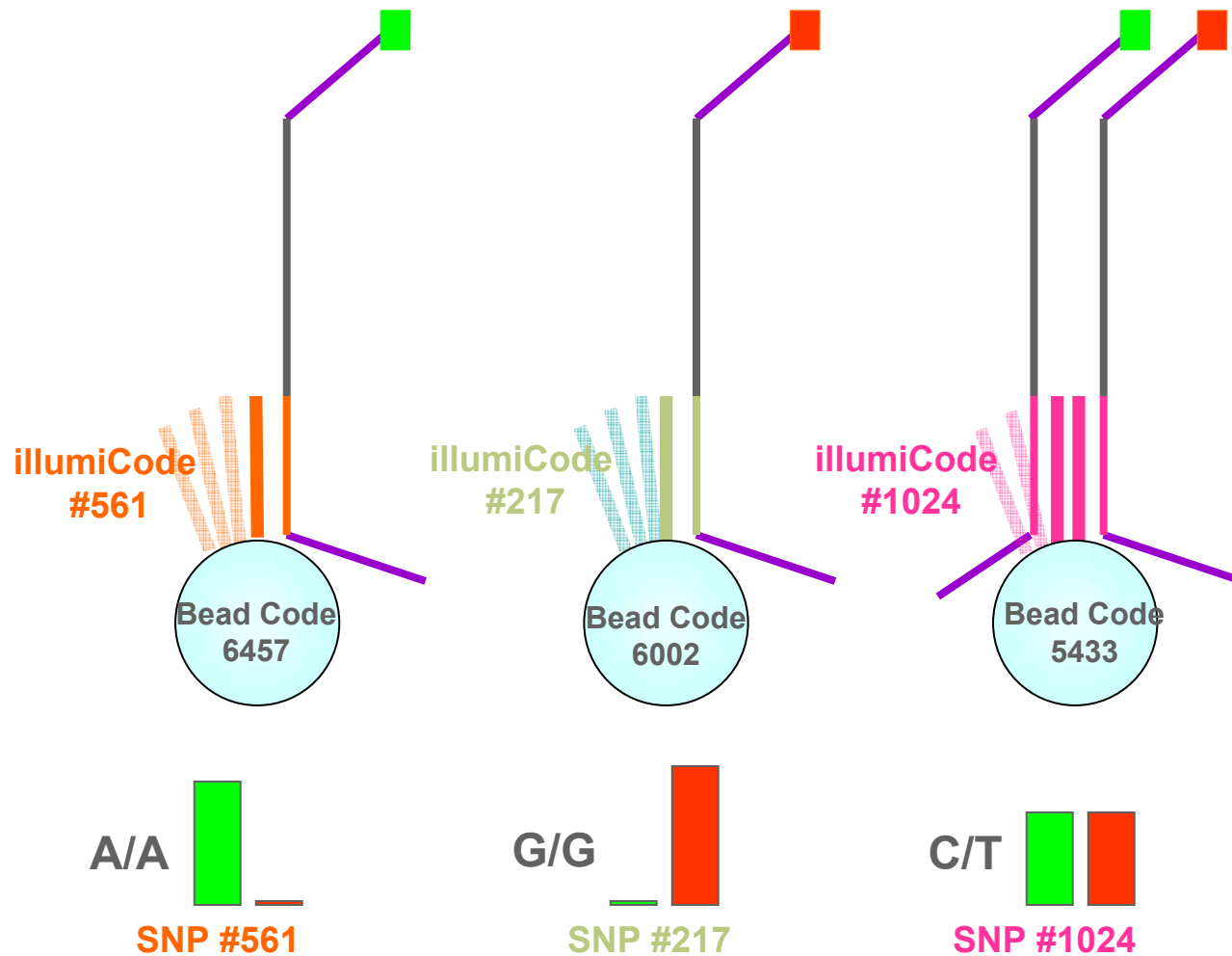


PCR with  
Common  
Primers

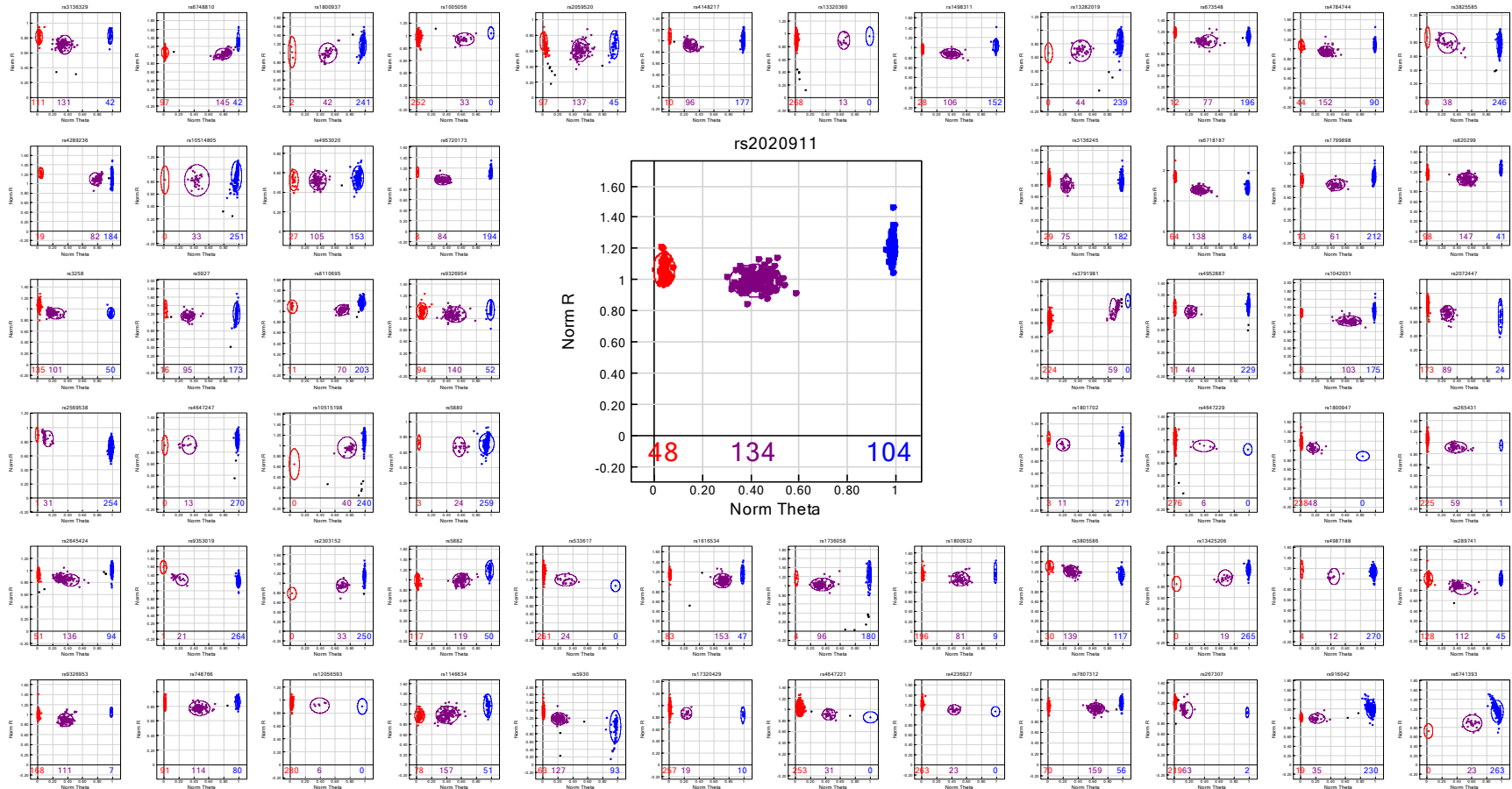


# GoldenGate Assay – two color detection

- Hybridization to VeraCode Beads – one SNP per bead type



# GoldenGate Assay Results



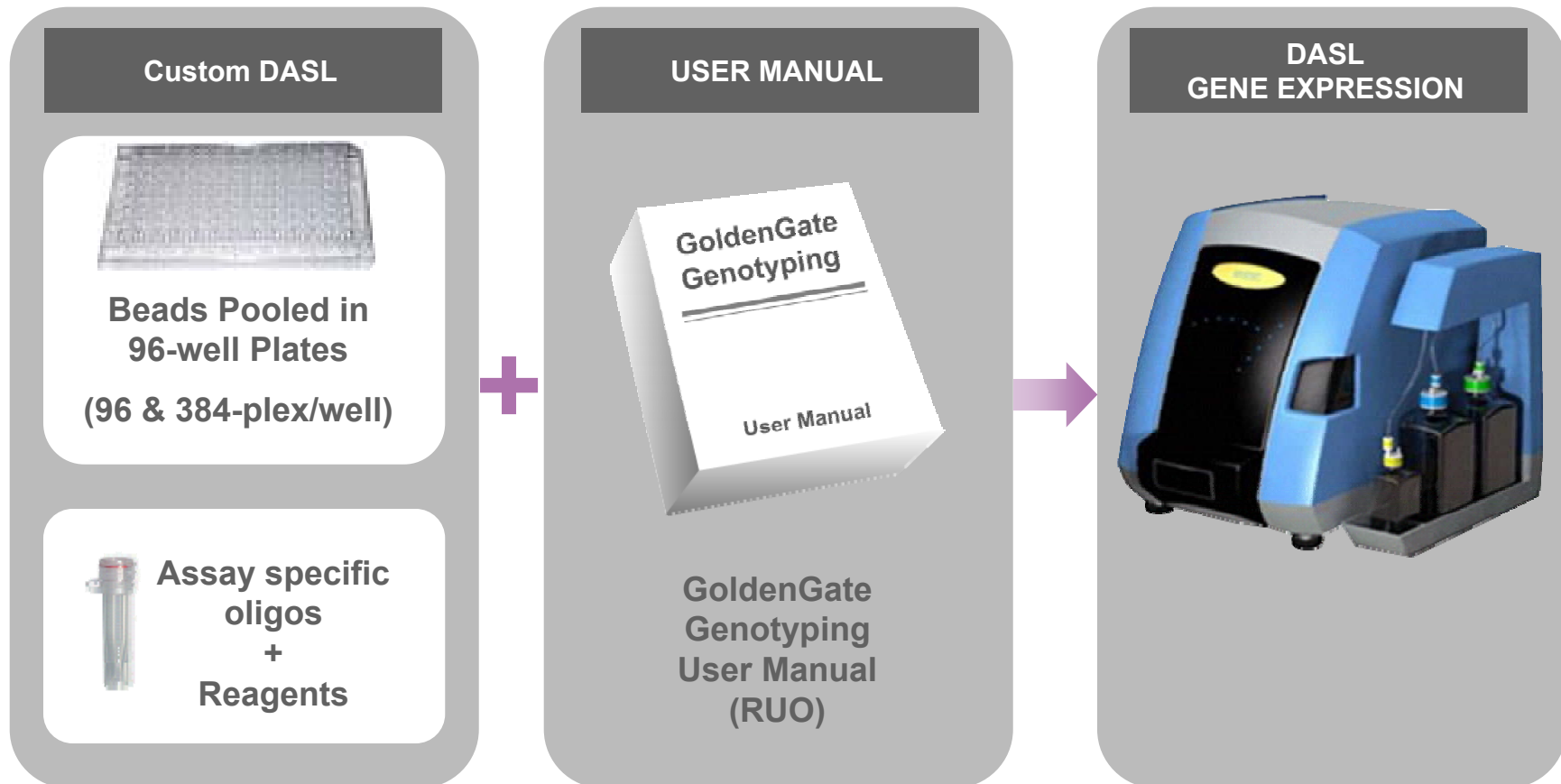
Reaction Multiplex	Samples Tested	Call Rate	Total # of Data Points	Reproducibility	Heritability
96	286	99.9%	41,184	100%	100%

## Inter-User Variability

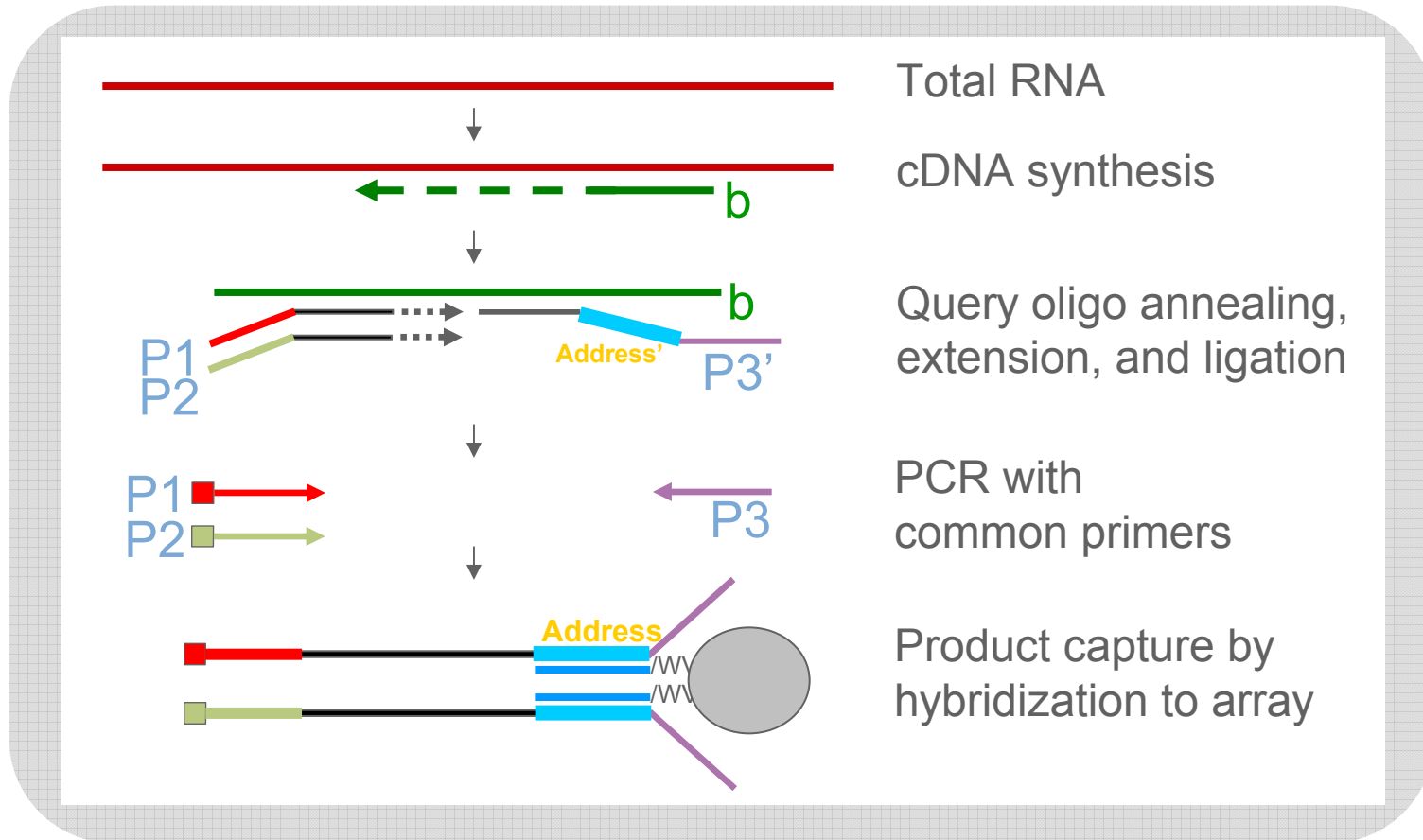
	Specification	Target	User A	User B	User C
Setup	Number of DNA Samples	95	95		
	Bead Multiplex	144	144		
	OPA multiplex	96	96		
Results	Concordance	> 99.5%	99.96%	99.92%	100%
	Reproducibility	> 99.0%	100%	100%	100%
	Heritability (Parent/Parent/Child)	> 99.5%	100%	100%	100%
	Call Rate	> 99.0%	99.97%	99.74%	99.97%
	Sample Success Rate	> 95%	100%	98.94%	100%
	Locus Success Rate	> 90%	100%	100%	100%

# Available Products

## VERACODE DASL CUSTOM GENE EXPRESSION (32 to 384-transcripts)

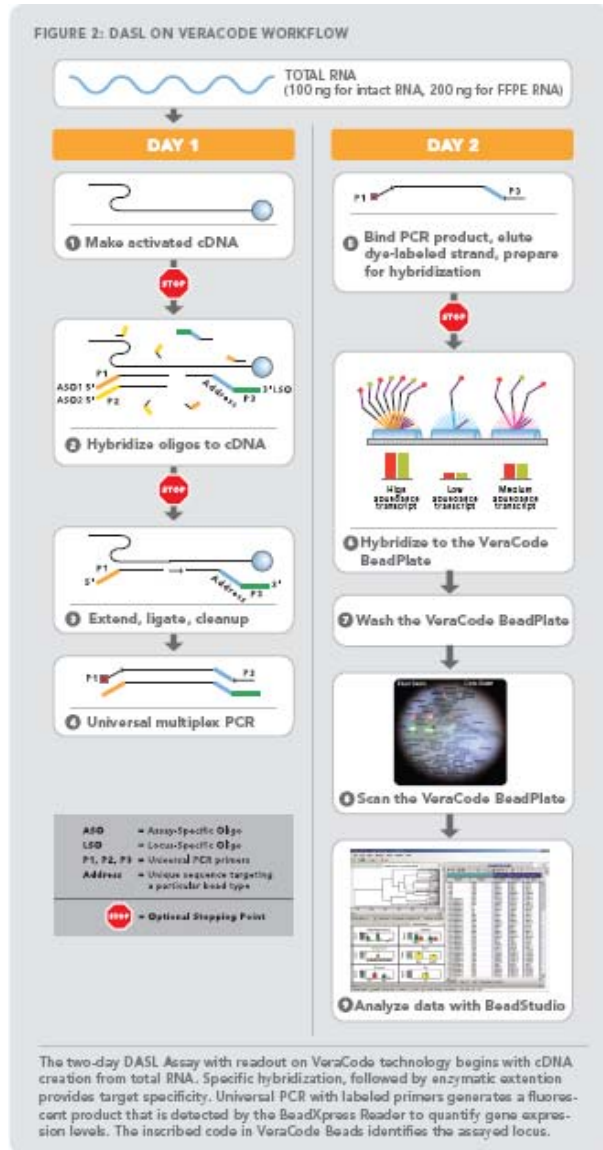


# The DASL Assay



- cDNA synthesis includes random priming for fragmented RNAs.
- Sequences of ~ 50 nt are targeted for analysis by addressed query oligos.
- PCR primers are shared among all targets, and amplicons are a uniform size.
- Reproducible across a wide range of RNA degradation.

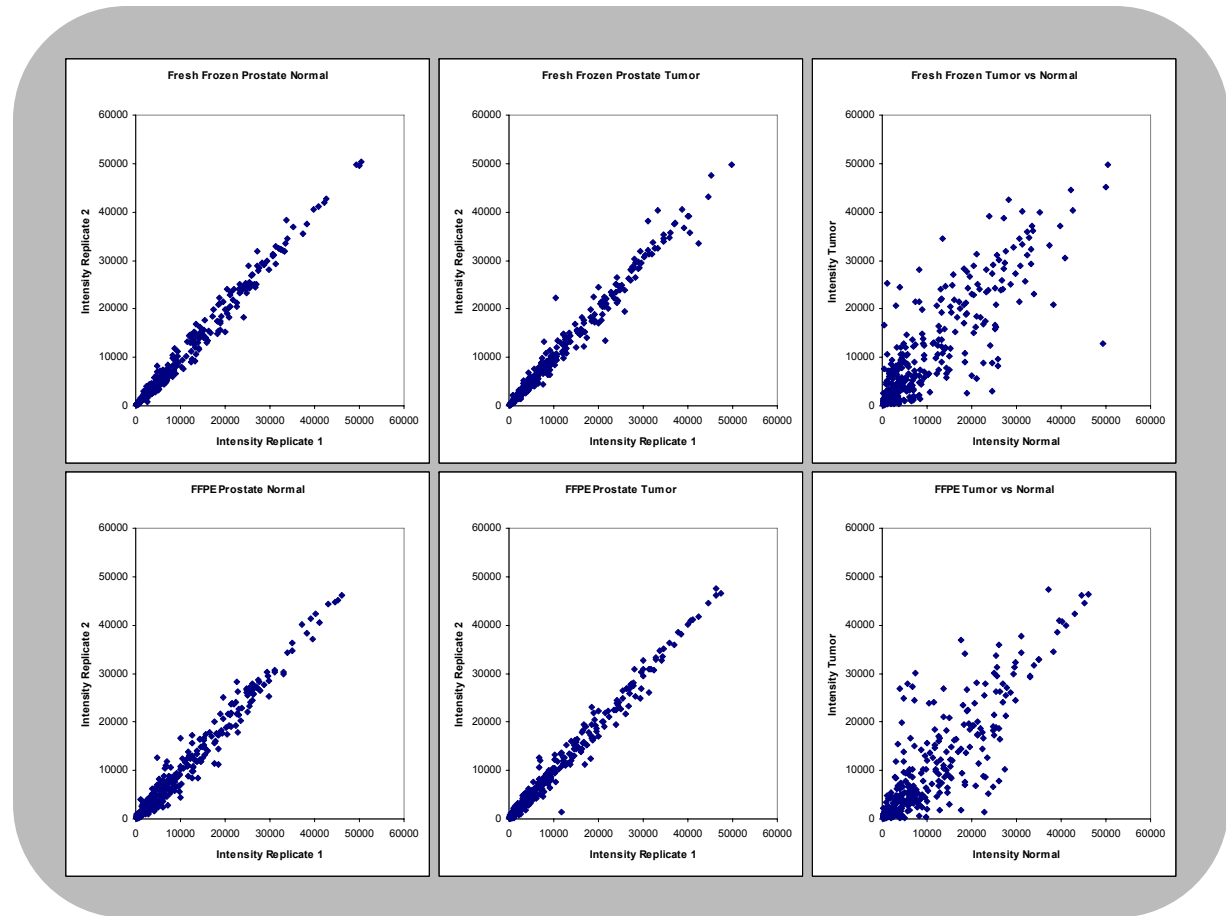
# VeraCode DASL Assay Workflow



- 2-day assay from sample to answer
- 100 ng for intact RNA; 200 ng for FFPE RNA
- Pre-kitted VeraCode bead plates in 96 or 384-plex (32 to 384 transcripts)
- Same controls as found in DASL on BeadArray
- Each bead plate contains 30-fold redundancy for each unique VeraCode bead code
- Data Analysis performed on BeadStudio GX module

# Fresh Frozen and FFPE RNA Reproducibility on VeraCode

- RNAs derived from fresh frozen or FFPE human tissues were analyzed.
- FFPE RNAs demonstrated nearly equivalent reproducibility compared to intact RNAs ( $r^2 > 0.98$ ).

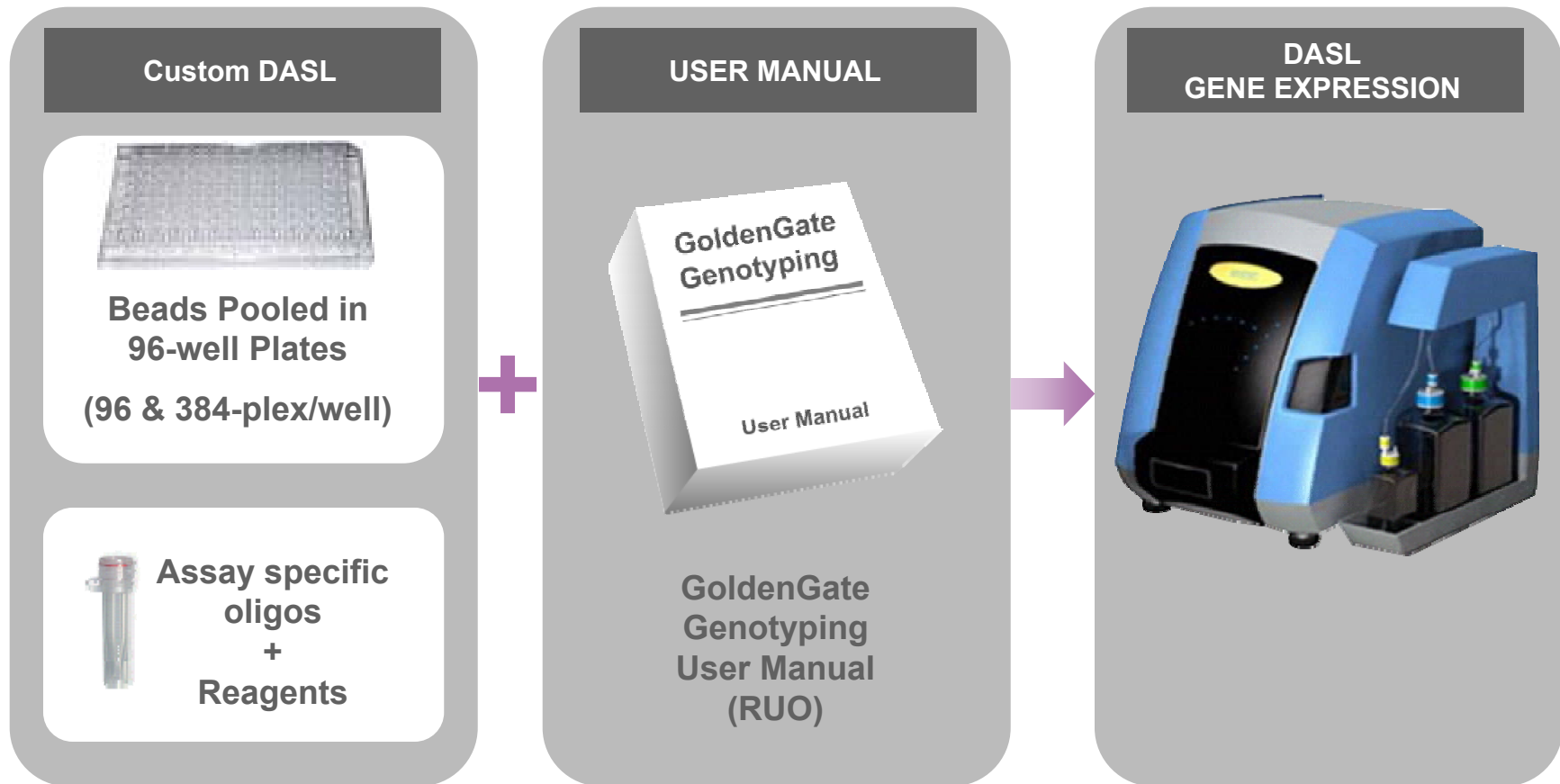


## Performance Summary

Metric	Value
Overall reproducibility of technical replicates (intact RNA)	$r^2 > 0.99$
Overall reproducibility of technical replicates (FFPE RNA)	$r^2 > 0.98$
Reproducibility between operators	$r^2 > 0.98$
Reproducibility between VeraCode bead lots	$r^2 > 0.99$
Concordance with BeadArray for Number of Probes Detected	$> 92\%$
False positive rate	$< 1\%$

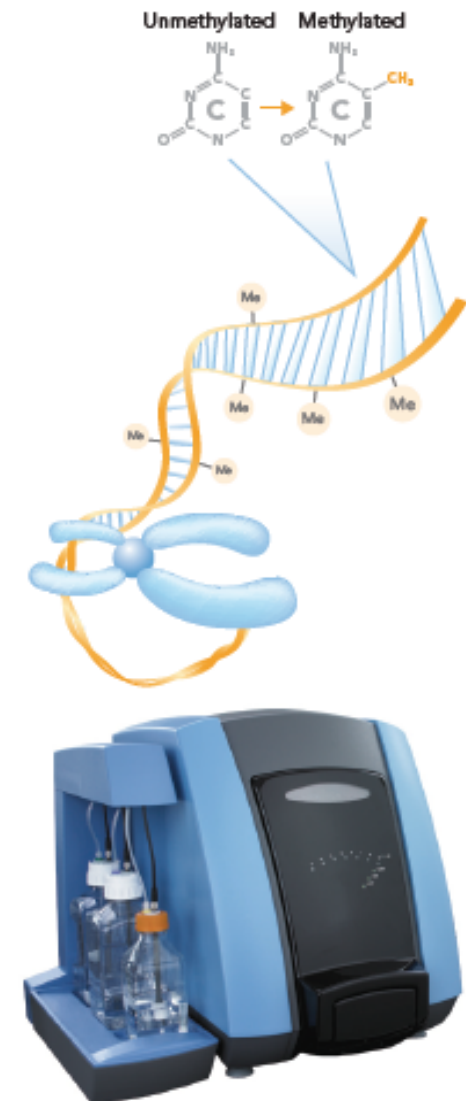
# Available Products

## VERACODE GOLDENGATE METHYLATION (48 to 384-promoter regions)



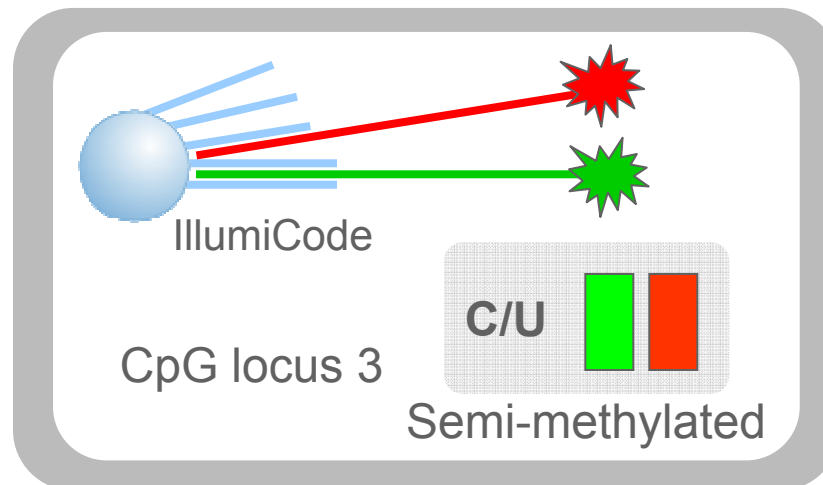
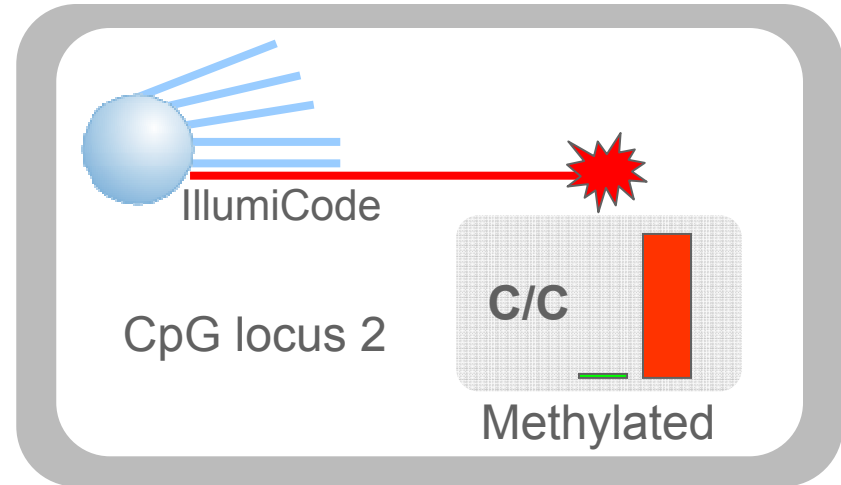
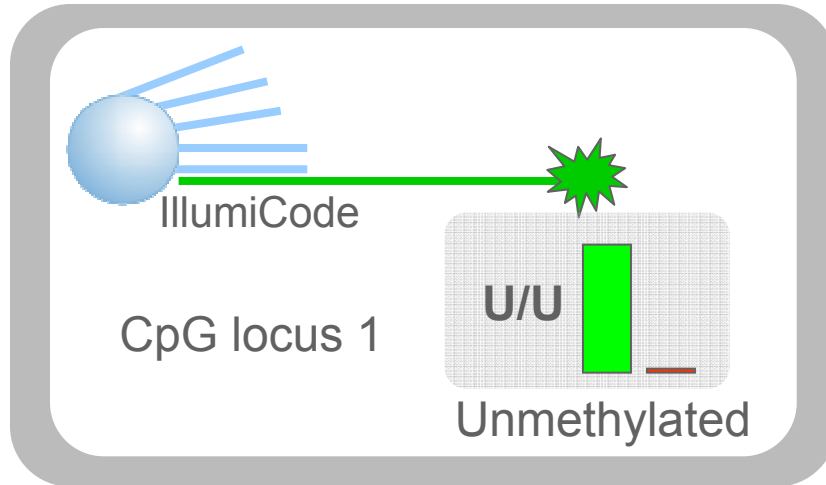
# GoldenGate Methylation Assay

1. Bisulfite treatment of DNA
  - Convert unmethylated cytosine to uracil
2. GoldenGate Assay
  - DNA Activation
  - Allele-Specific Extension (ASE) & Ligation
  - PCR Amplification
  - Hybridize to Bead Substrate
  - Scan



# GoldenGate Methylation Assay

## Hybridization & Detection



# VeraCode Methylation Data Analysis

FIGURE 3: ASSAY CONSISTANCY ON BEADARRAY OR VERACODE PLATFORMS

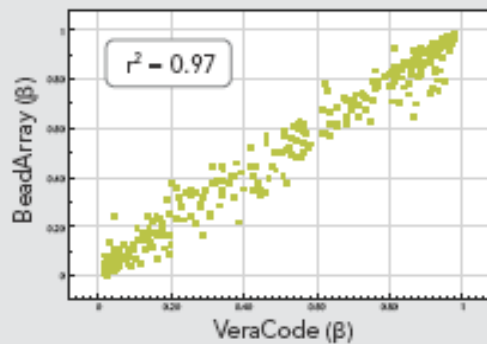


FIGURE 4: HIGH ASSAY REPRODUCIBILITY

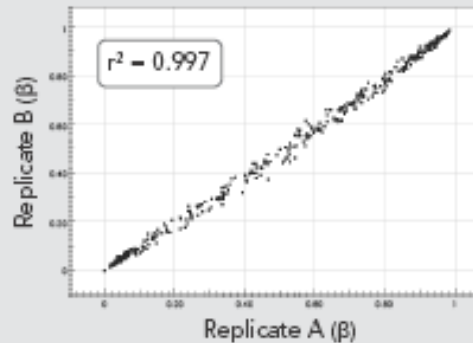
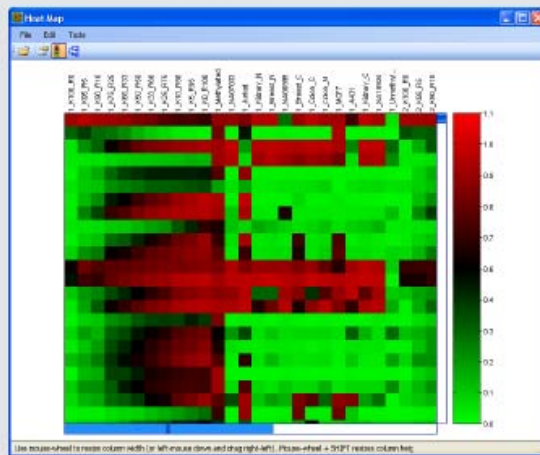


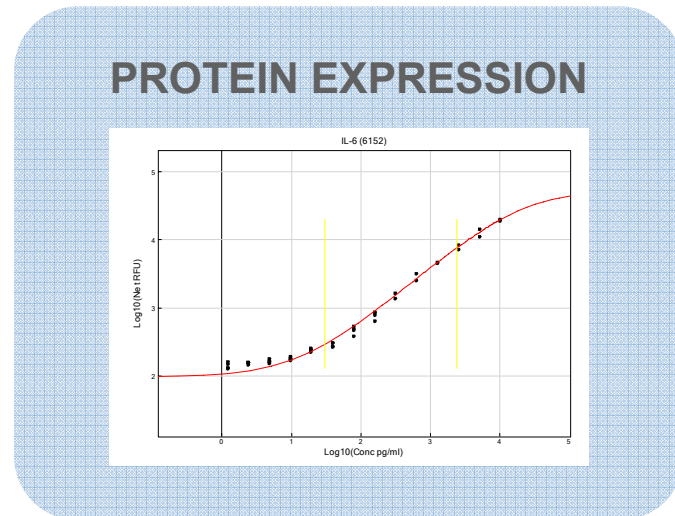
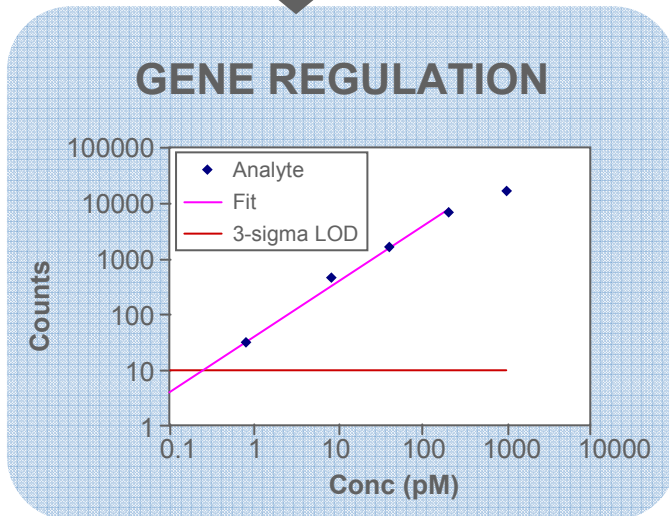
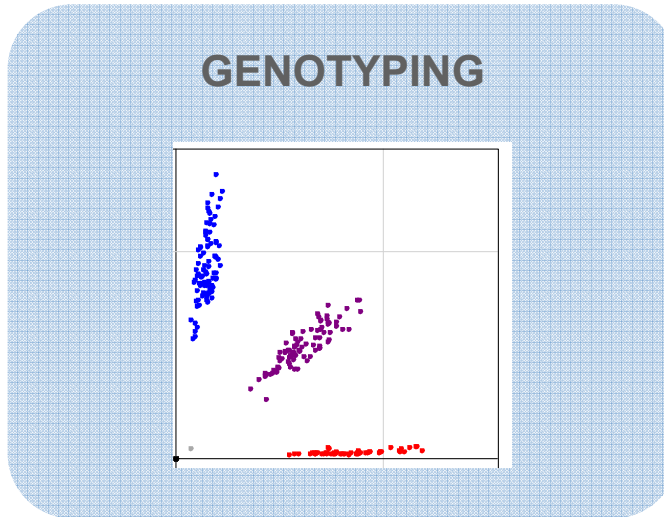
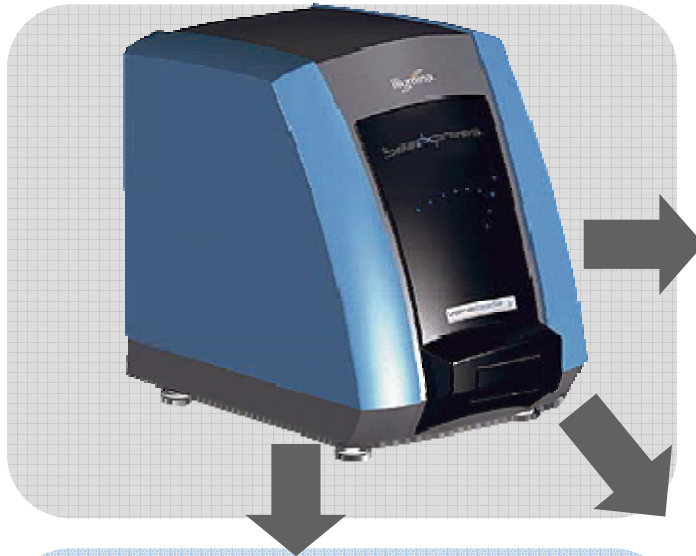
FIGURE 5: DATA ANALYSIS WITH ILLUMINA BEADSTUDIO SOFTWARE



Methylation status ( $\beta$ ) is shown for many loci (y-axis) across many samples (x-axis) in graphical heat map format to easily see trends and outliers.

- Data analysis performed on BeadStudio Methylation module
- Internal replication experiments show an  $r^2$  correlation of 0.997
- Experiments comparing methylation results for the same loci on BeadArray and VeraCode show high concordance ( $r^2 = 0.97$ )

# Ability to Analyze Multiple Applications



# Bridging from the lab to the clinic

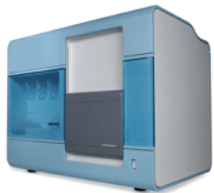
## Laboratory



## Translate



## Clinic



Genome Analyzer



iScan

- Collaborative approach to research
- Constantly improving throughput
- Excellent cost position
- Highly multiplexed
- Multiple markers on one system



Services



BeadXpress



# illumina Diagnostics Strategy

*To be the leader in cancer translational diagnostics*



## Oncology Discovery – Identify novel biomarkers

- Identify novel biomarkers for the early detection of ovarian cancer
- Identify novel biomarkers to indicate response to platinum therapy in late stage ovarian cancer



## Sequencing Services – Utilize service lab for rapid translation

- Establish a CLIA laboratory
- Begin to offer services in the oncology area based on the ovarian biomarkers



## Platform Partnering – Build a large installed base

- Partner with content providers
- Enable broad adoption with removal of royalty burden
- Internally develop products to create breadth of menu

# illumina Diagnostics Vision

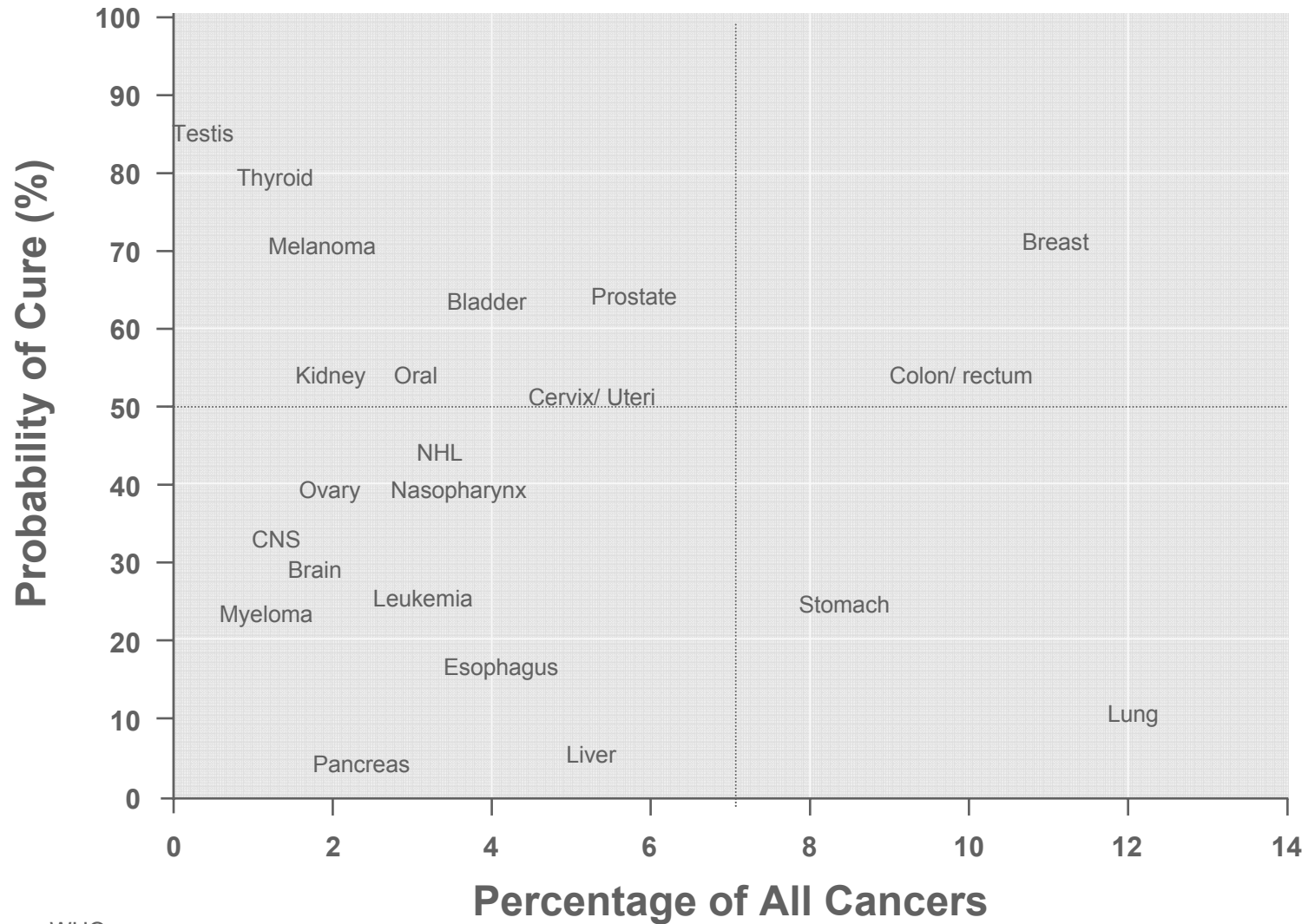
To Be the Leader in  
Cancer Translational Diagnostics

## Focus Core Technologies on Cancer

- Next Generation Sequencing
- Whole Transcriptome Analysis
- Methylation Profiling



# Initial Targets



Source: WHO

# Ovarian Cancer Will be Our Entry Point

## Deadly

- ▶ Leading cause of death from gynecological cancer
- ▶ Fifth leading cause of cancer mortality in women
- ▶ Mortality: 15,520 (US Only)

## Incurable

- ▶ Laparotomy/Hysterectomy/Remove ovaries
- ▶ Followed with six cycles of chemotherapy
- ▶ Less than 40% are cured

## Difficult Dx

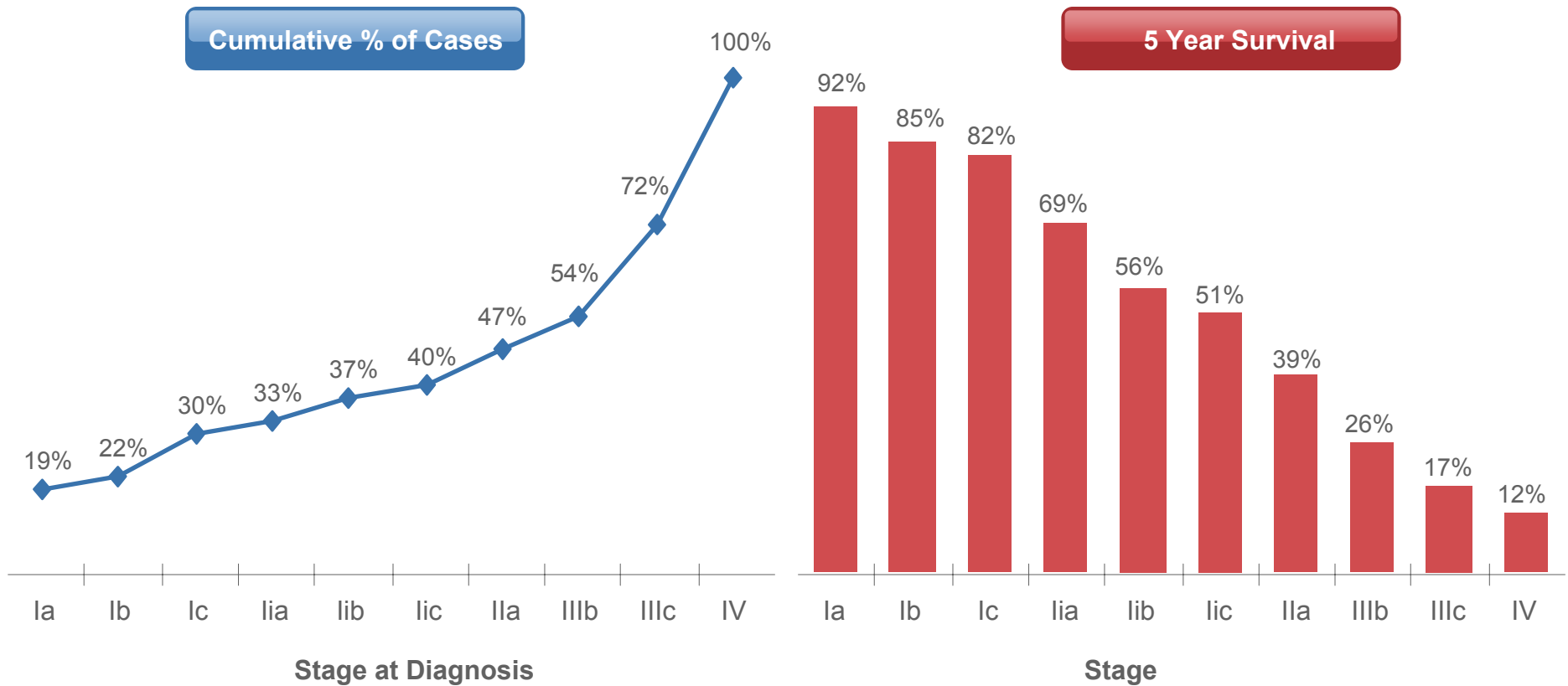
- ▶ Abdominal/pelvic exam, Ultrasound, CT, CA-125
- ▶ Patients present with a suspicious/palpable mass
- ▶ BRCA 1/2 positive in a limited number of cases



# Early Detection for Ovarian Cancer is Critical

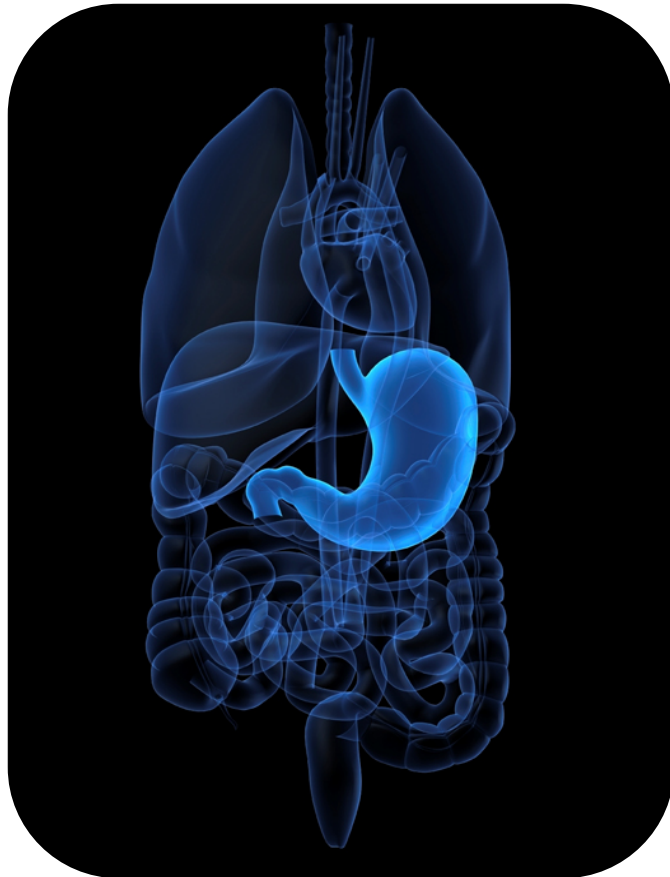
Late stage diagnosis ...

... results in poor prognosis

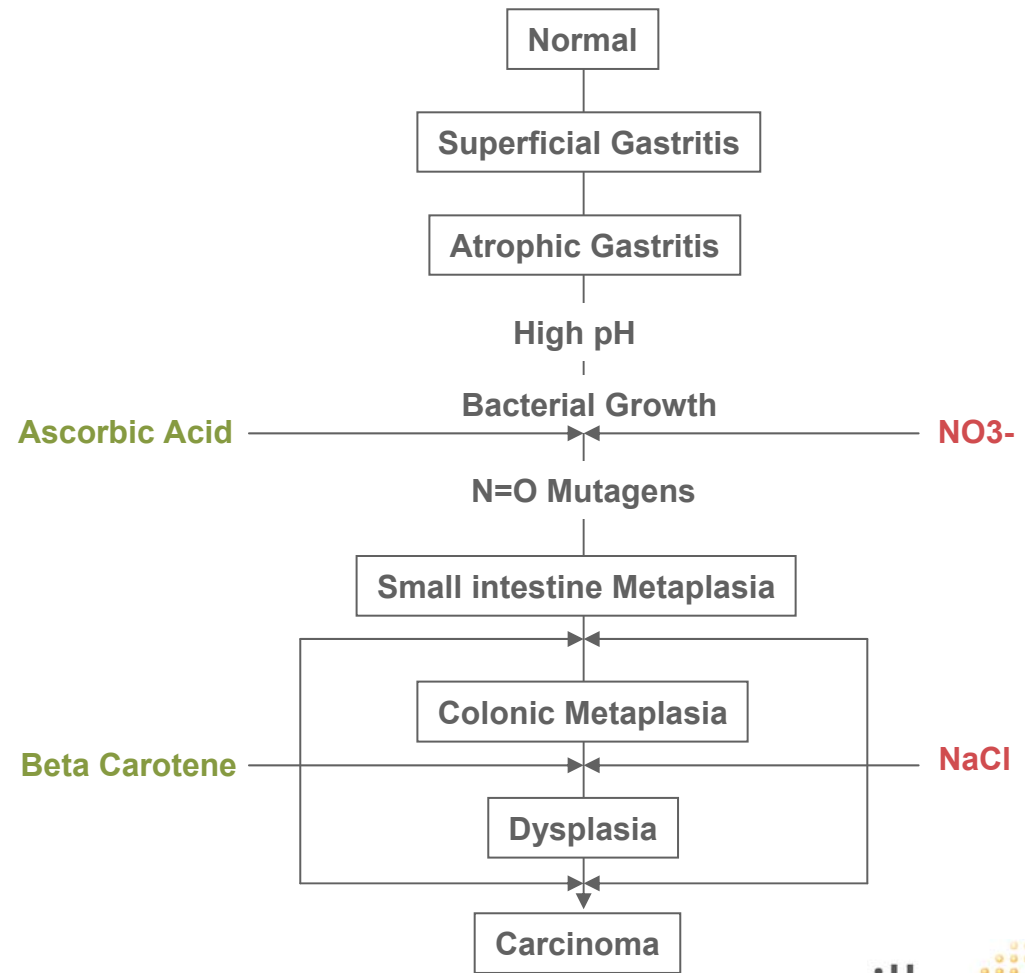


# Gastric Cancer will be Our Second Target

Cardia 15% of Gastric Cancers  
Increasing due to obesity

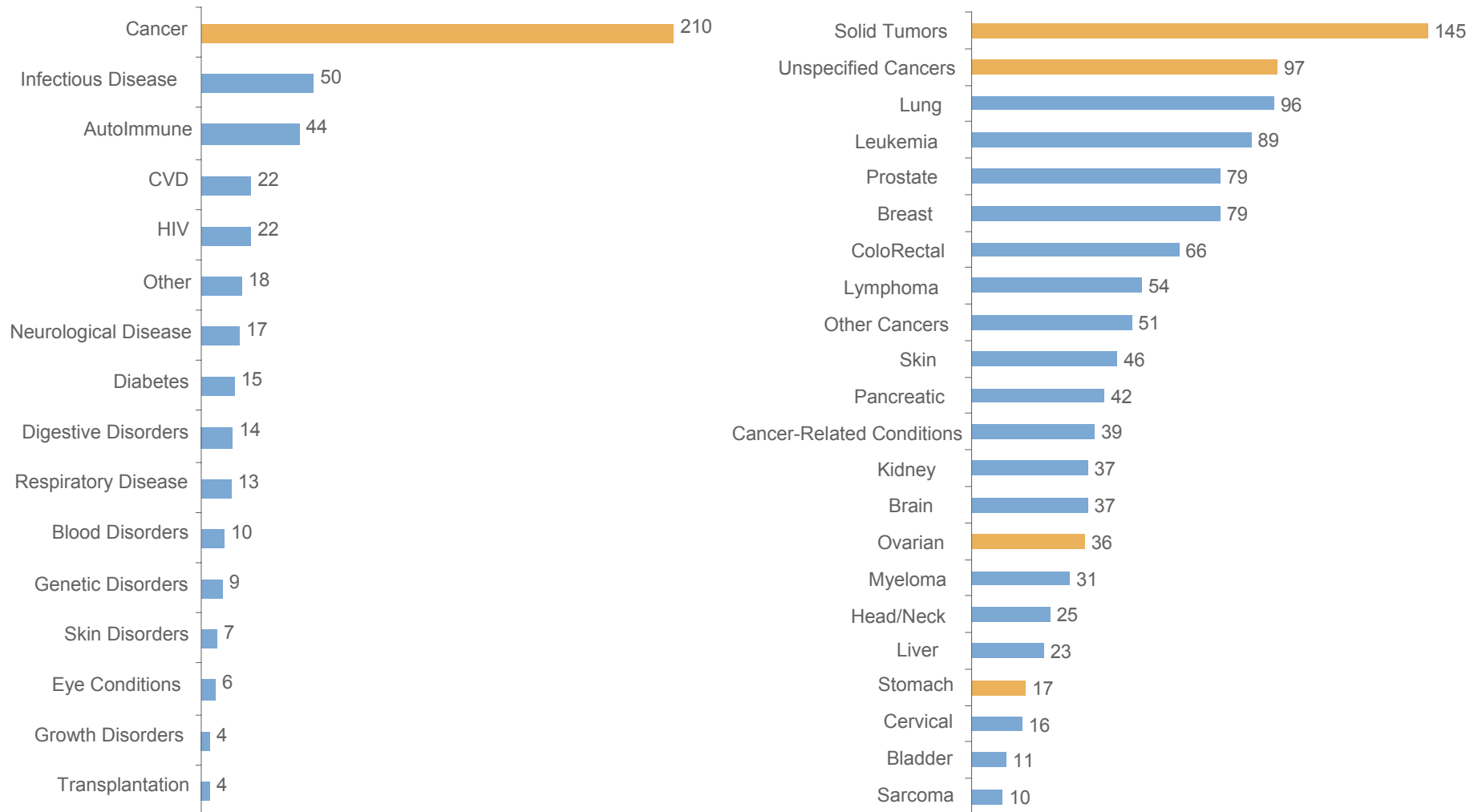


## Gastric Carcinogenesis



# More Therapies Means More Choices

## Drug Pipeline



Source: PhRMA: 2006 Medicines in Development Biotechnology

# Discovery, Strategy & Advantages

*Focused Approach Addresses Unmet Medical Needs*

## Strategy

- Begin with 25 Tumor/Normal pairs
- Comprehensive genetic analysis
- Sequence to 30x coverage whole genome
- Analyze full methylome and transcriptome
- Validate findings on larger sample size
- Implement on flexible platform



## Illumina Advantage

- Low cost internal sequencing
- Significant throughput improvements
  - Currently 1 genome/week internally
- Unique bioinformatics capability
  - Multiple genome analysis
- Capable of analyzing across methods
- Ability to deploy content on arrays
- BeadXpress optimal for multiple methods

# illumina's Diagnostic Division Branding

illumina*Dx*<sup>TM</sup>