

VeraCode Technology

From Research to Diagnostics

June 22, 2009

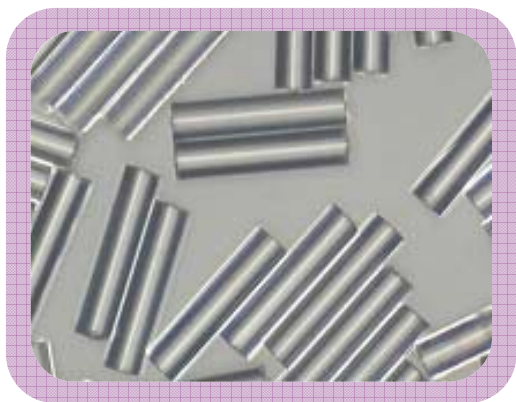
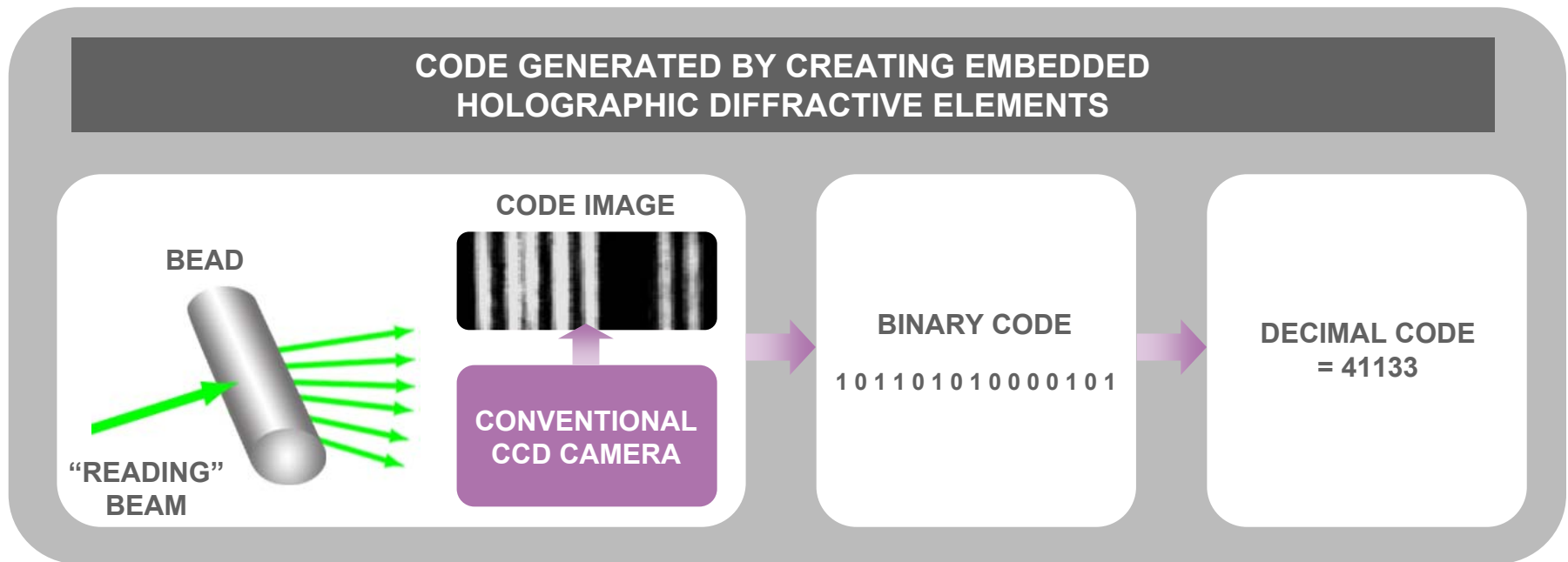
Illumina's Solution for Diverse Applications

VeraCode® Technology



- Cylindrical glass microbeads
- 240 μm length x 28 μm 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

BeadXpress™ Reader

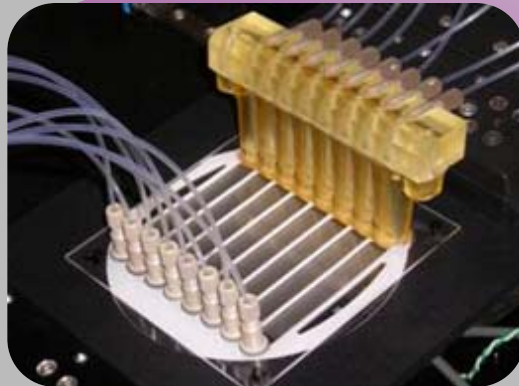


INSTRUMENT SPECIFICATIONS

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

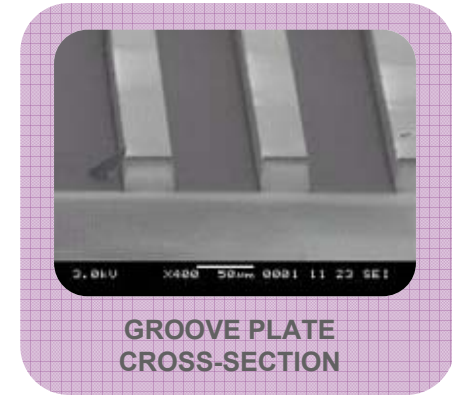
VeraCode Technology

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

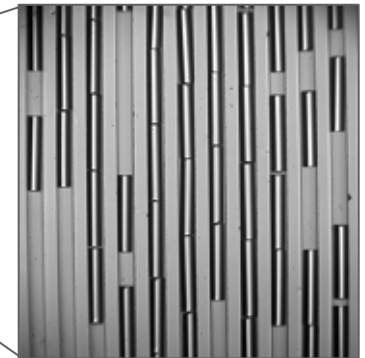
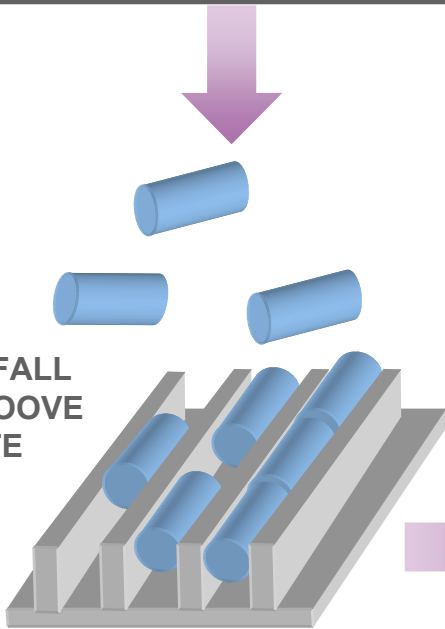


VeraCode Technology

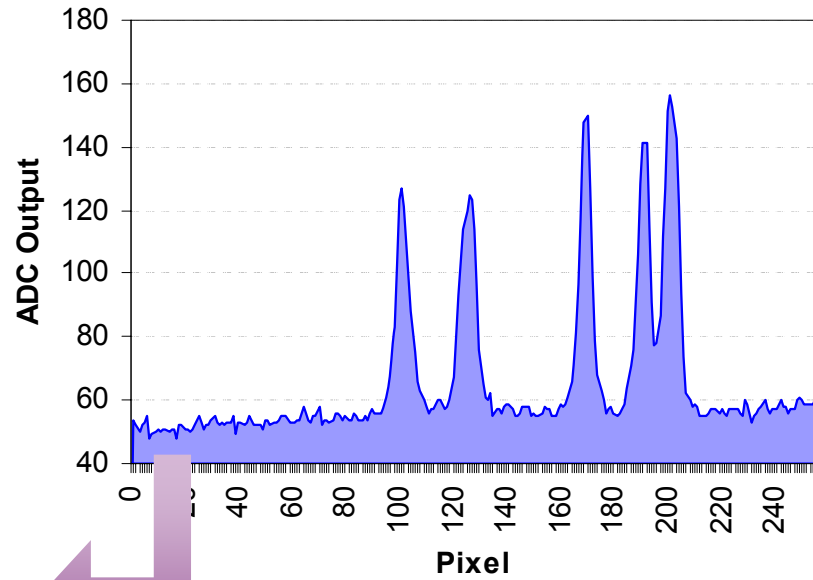
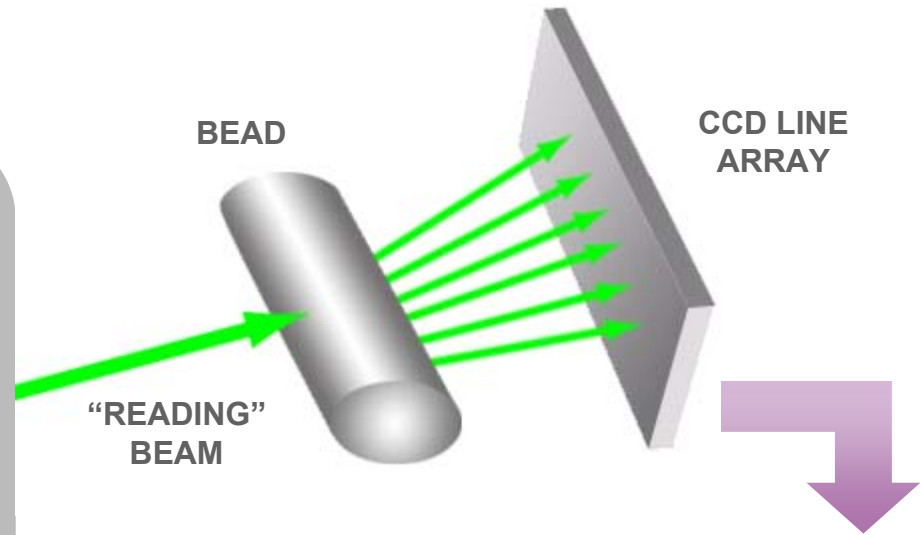
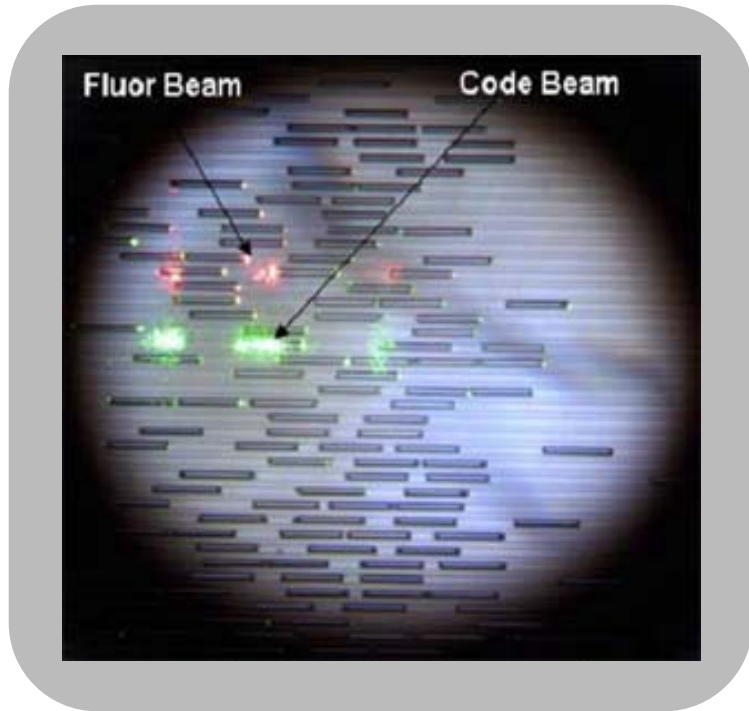
CAPILLARY FORCE ATTRACTS
BEADS INTO GROOVES

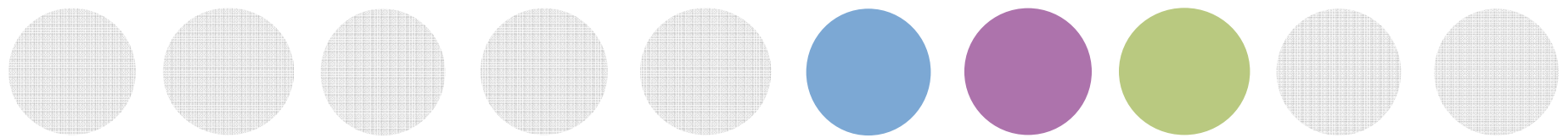


BEADS FALL
INTO GROOVE
PLATE



Code Detection





VeraCode Custom Products

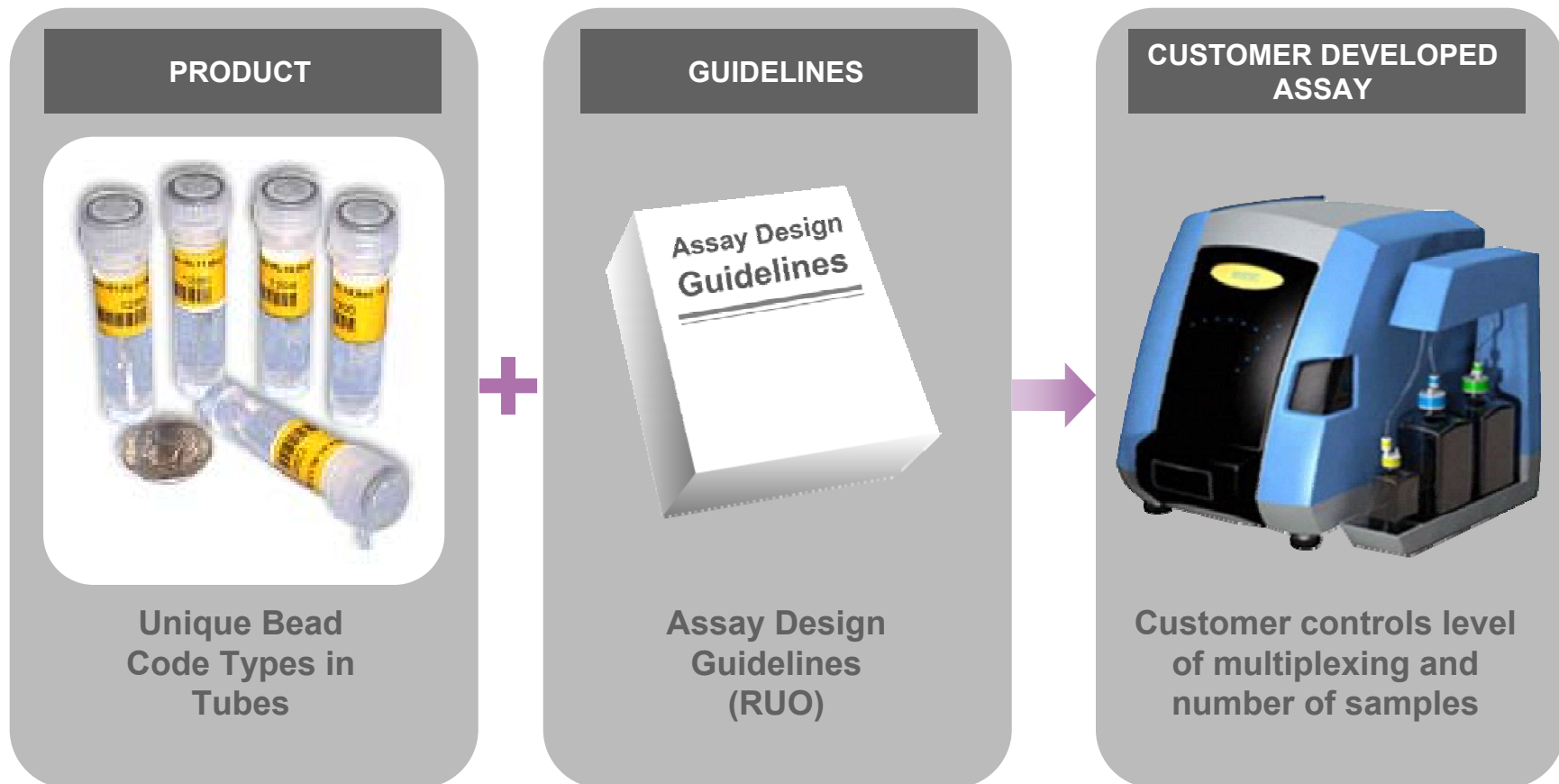
Comprehensive VeraCode product portfolio

- 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⁻
COOH⁻
COOH⁻
COOH⁻
COOH⁻
COOH⁻
COOH⁻
COOH⁻

PROTEIN ATTACHMENT: TWO-STEP PROCESS

Activate Surface

EDC/Sulfo-NHS

Covalently Bind

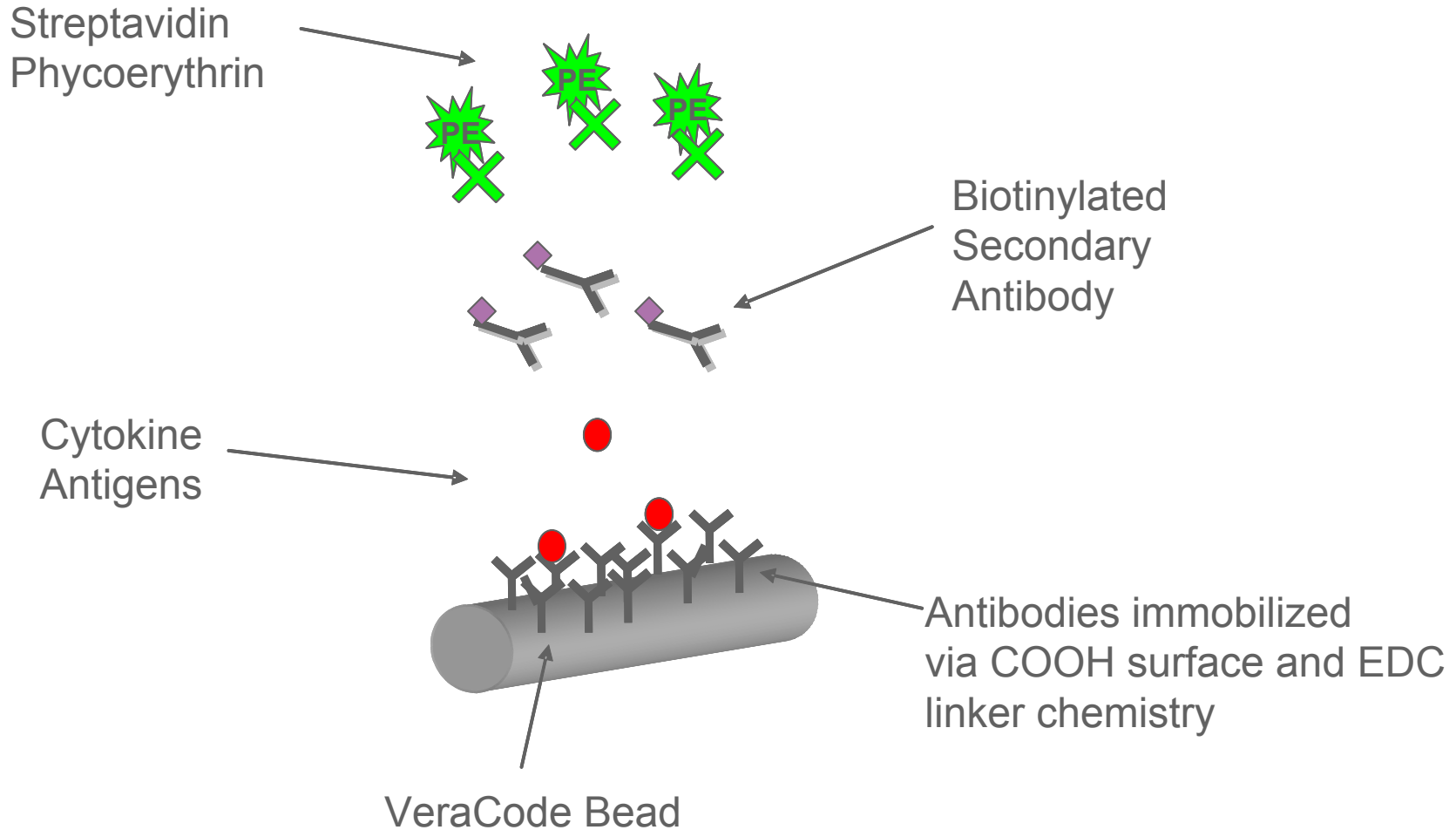
Antibodies
Antigens
Peptides
Streptavidin

NUCLEIC ACID ATTACHMENT: ONE-STEP PROCESS

EDC/Sulfo-NHS plus NH²⁺Oligo

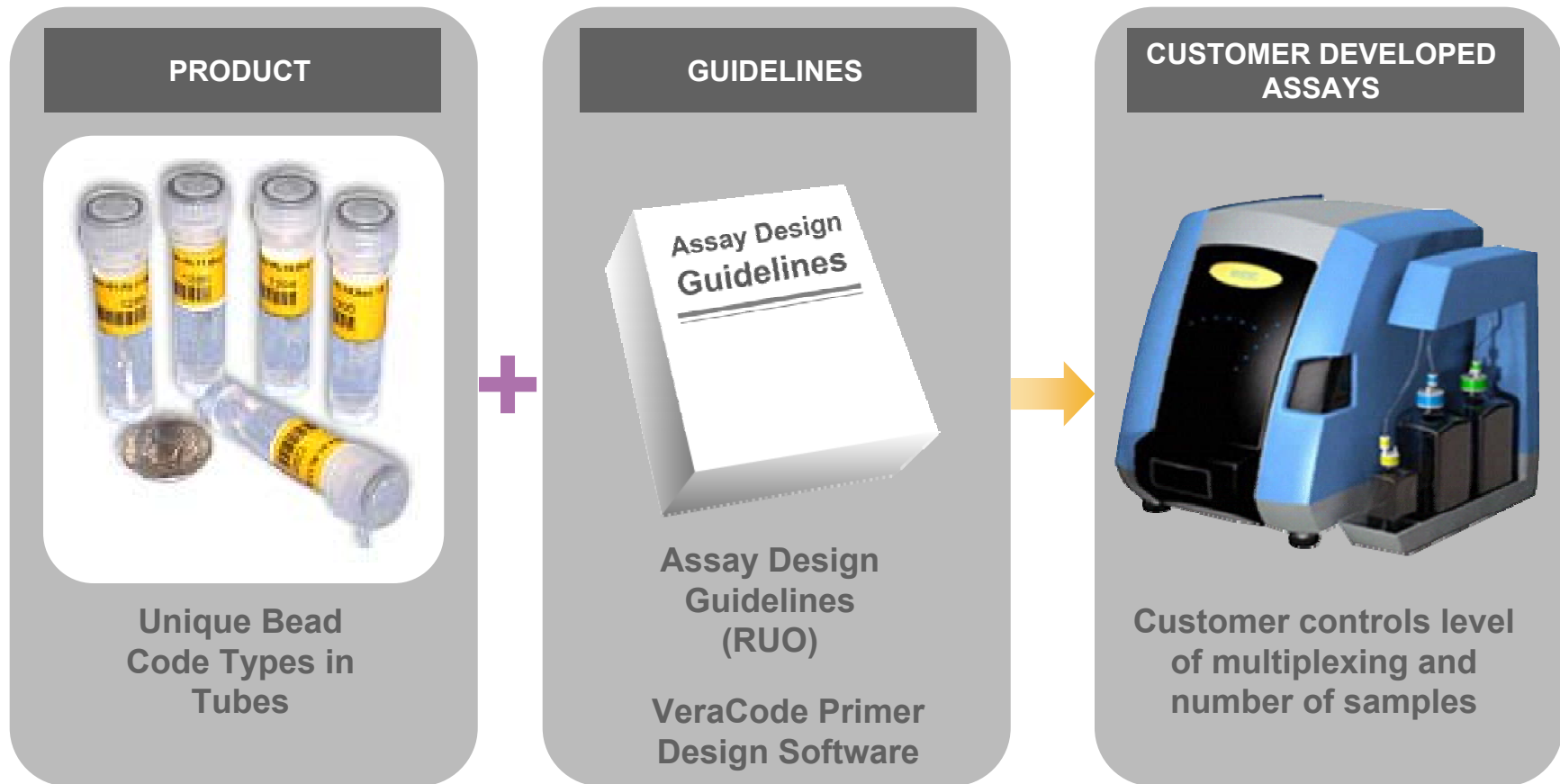
Nucleic
Acids

Cytokine Sandwich Assay Overview

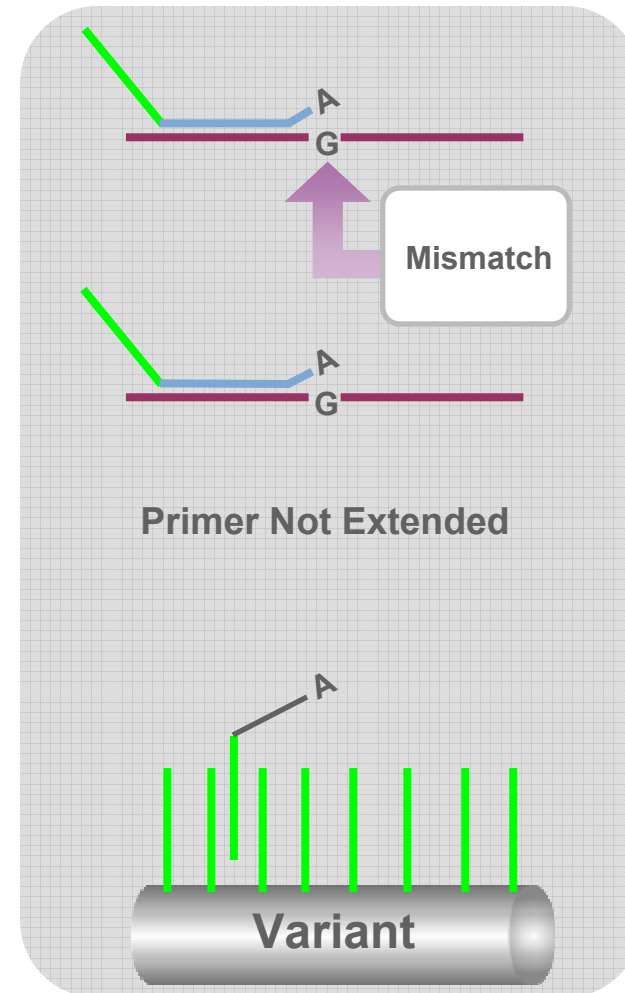
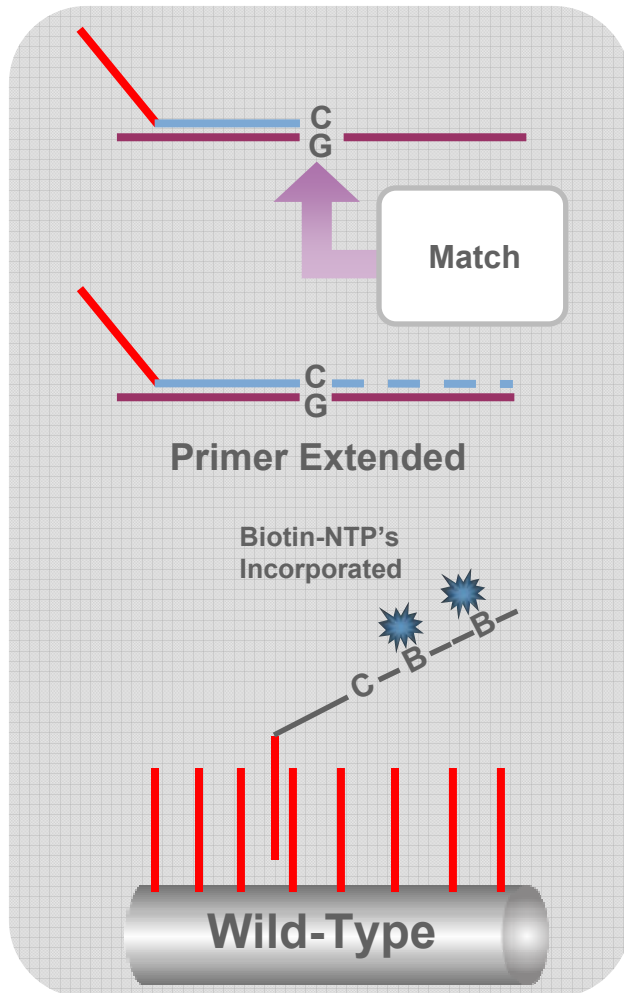


Available Products

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[™]
BY ILLUMINA[®]

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
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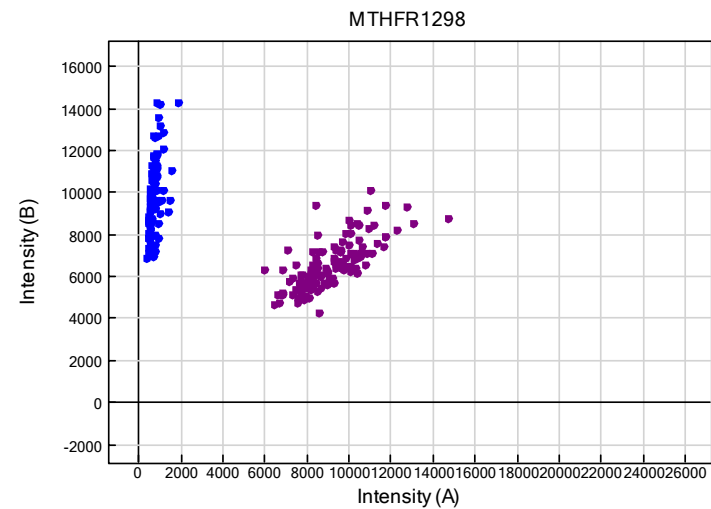
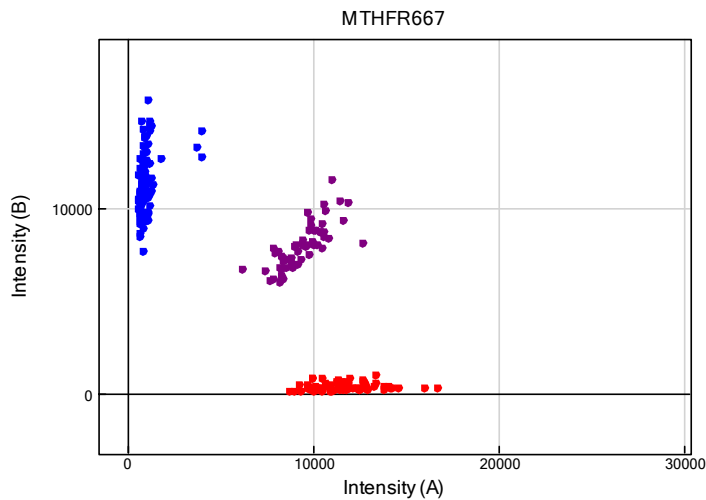
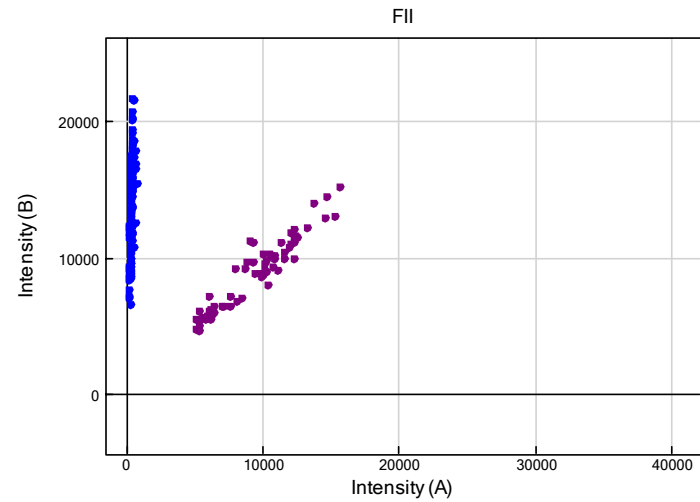
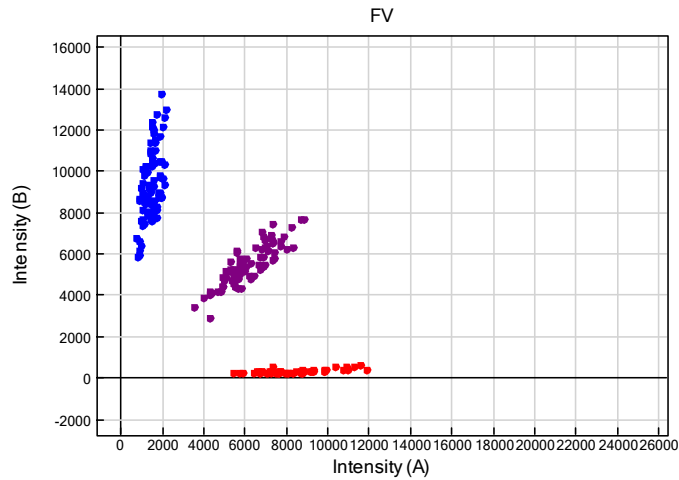
<< Back File Upload Multiplex >> Next >>

Multiplex ASPE Genotyping Protocol



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

Low Plex ASPE Genotyping Results



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

Customer Success

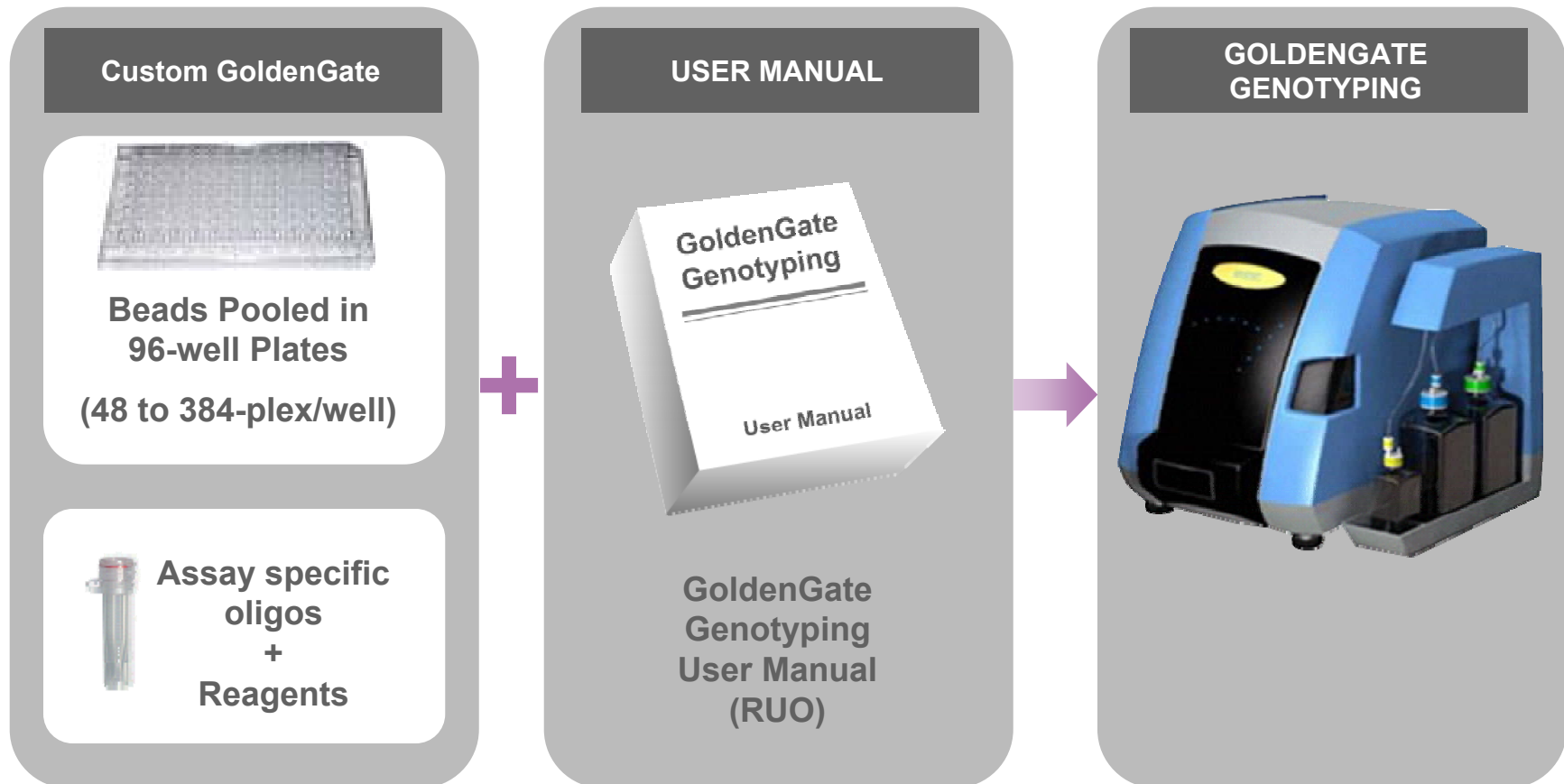
- Sciona – Health and Wellness company offering genetic testing and personalized exercise, diet and lifestyle recommendations
 - Validated assays presented in October 28, 2008 Webinar¹
 - GSTM1: Deletion of GSTM1 associated with compromised detoxification
 - GSTT1: Deletion of GSTT1 associated with compromised 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 Series²
 - 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

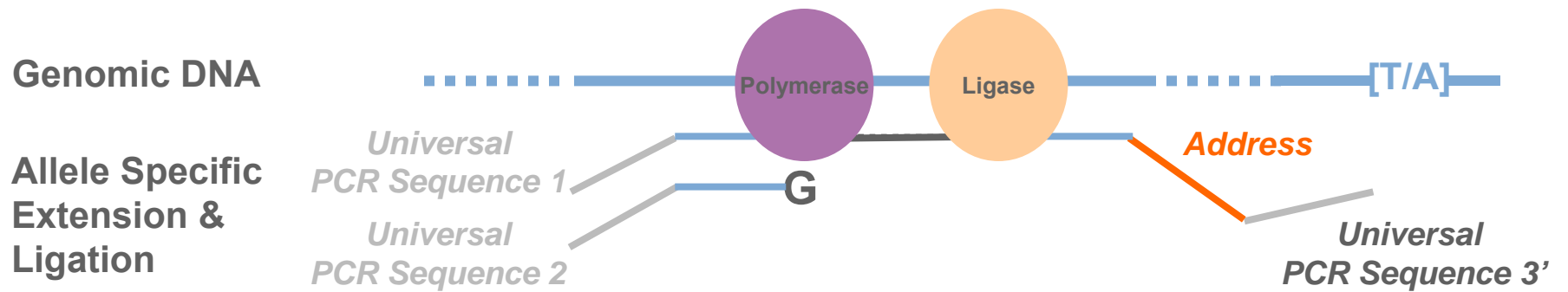
Available Products

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



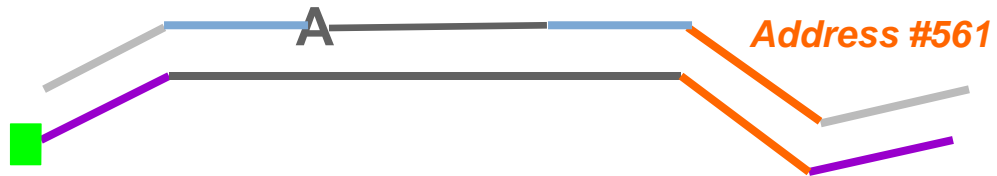
GoldenGate Genotyping Assay

Allele Specific Extension and Ligation

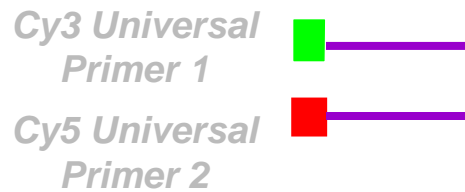


GoldenGate Genotyping Assay Amplification

Amplification
Template

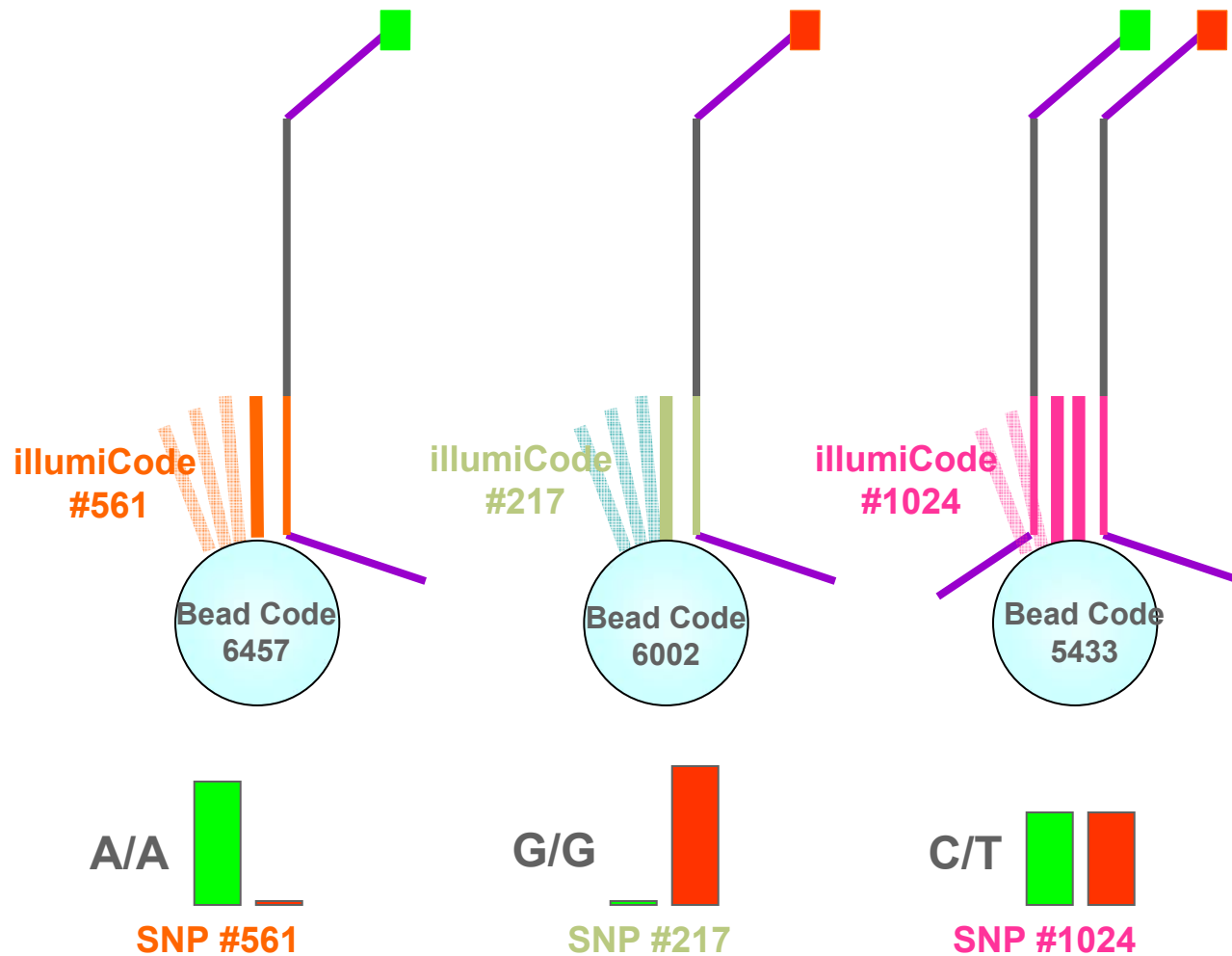


PCR with
Common
Primers

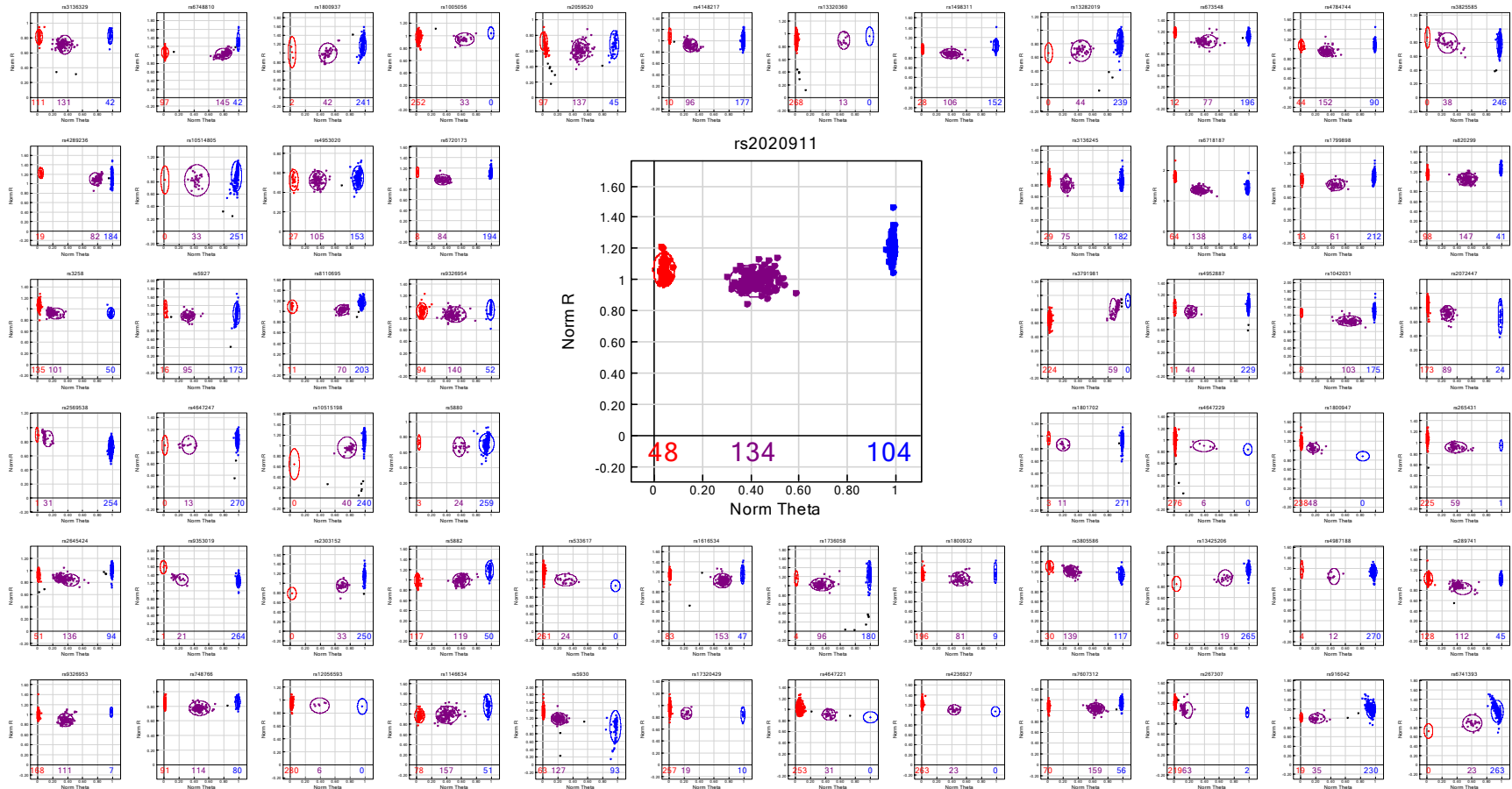


GoldenGate Assay – two color detection

- Hybridization to VeraCode Beads – one SNP per bead type



GoldenGate Assay Results

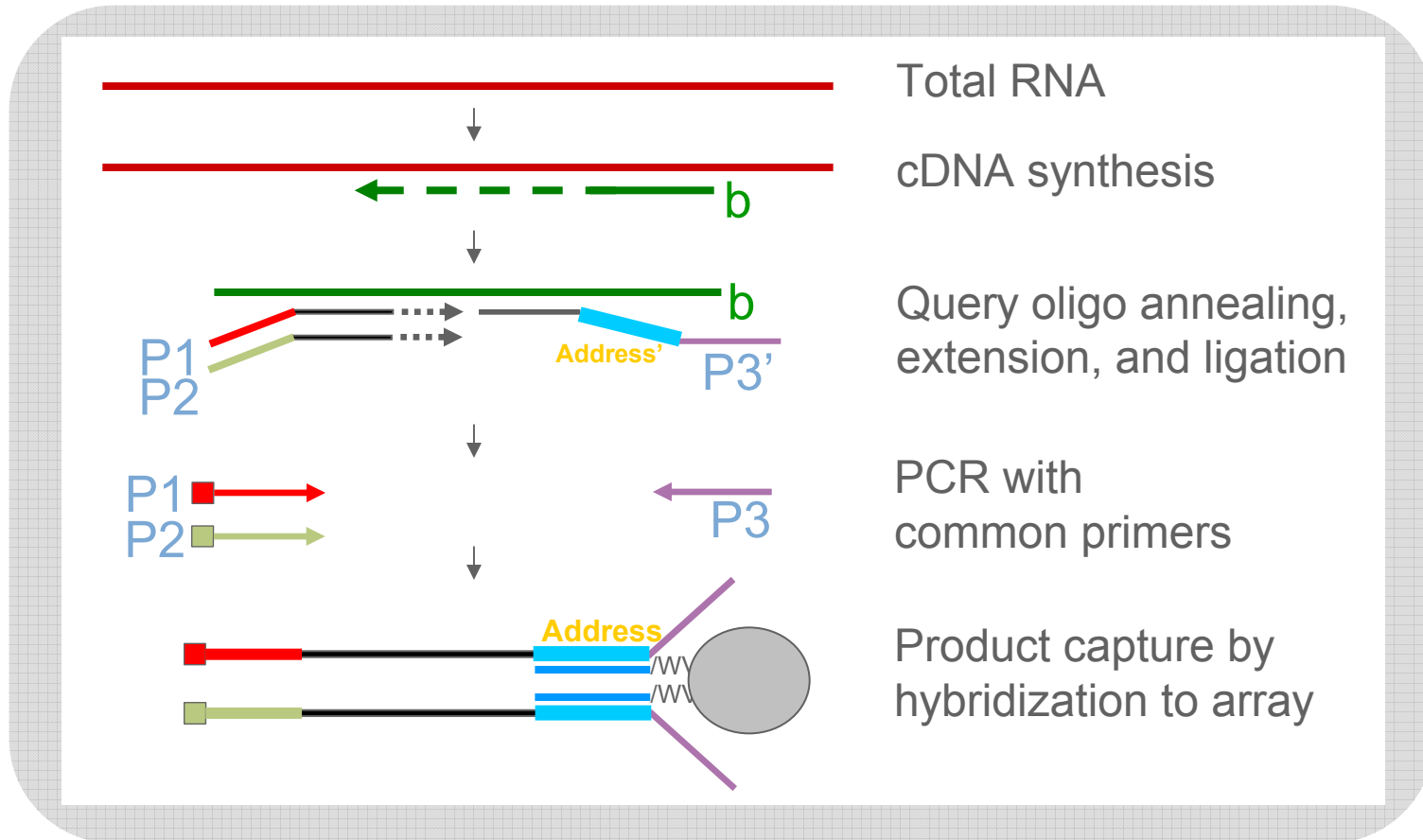


Reaction Multiplex	Samples Tested	Call Rate	Total # of Data Points	Reproducibility	Heritability
96	286	99.9%	41,184	100%	100%

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%

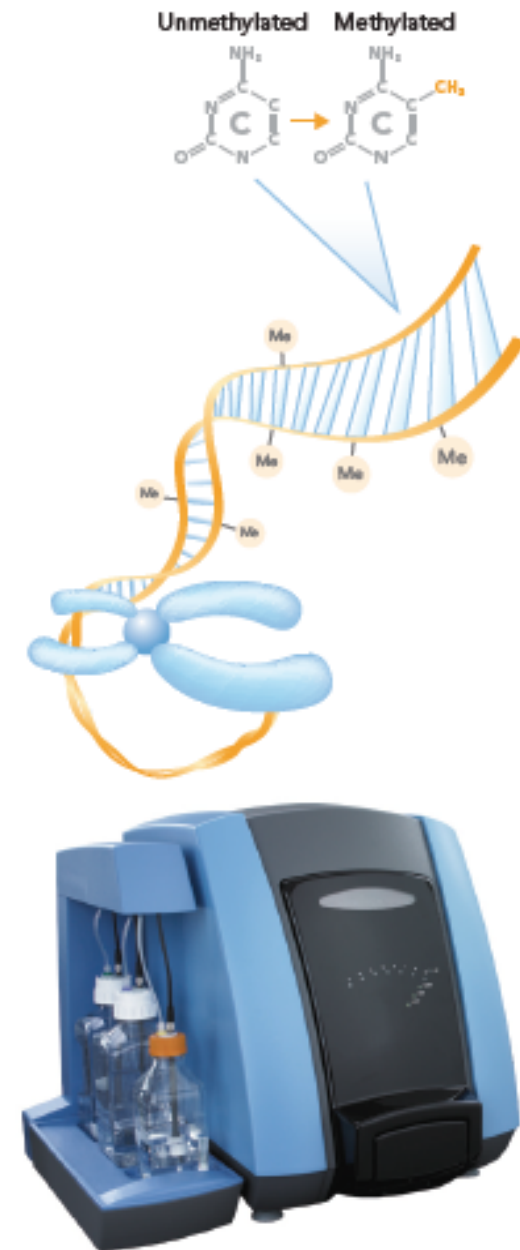
The DASL Assay



- 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.

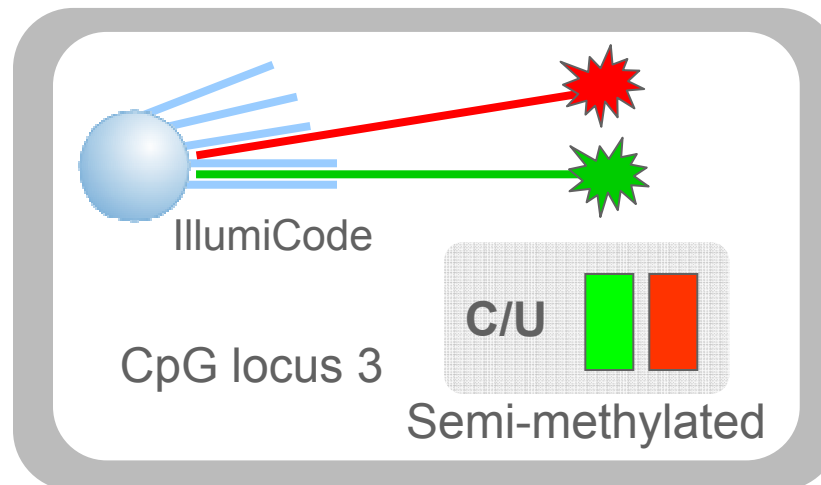
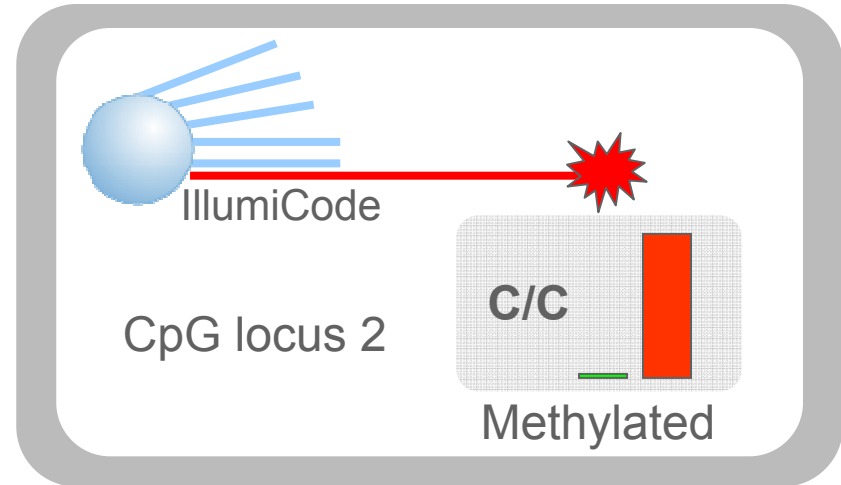
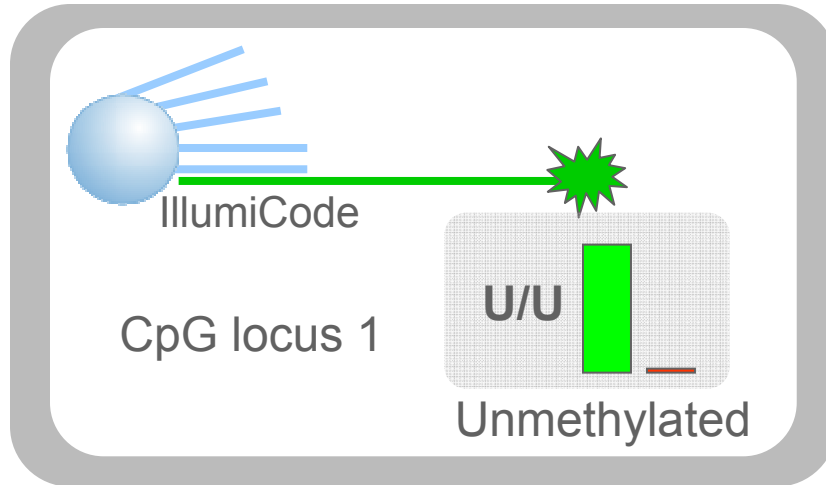
GoldenGate Methylation Assay

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

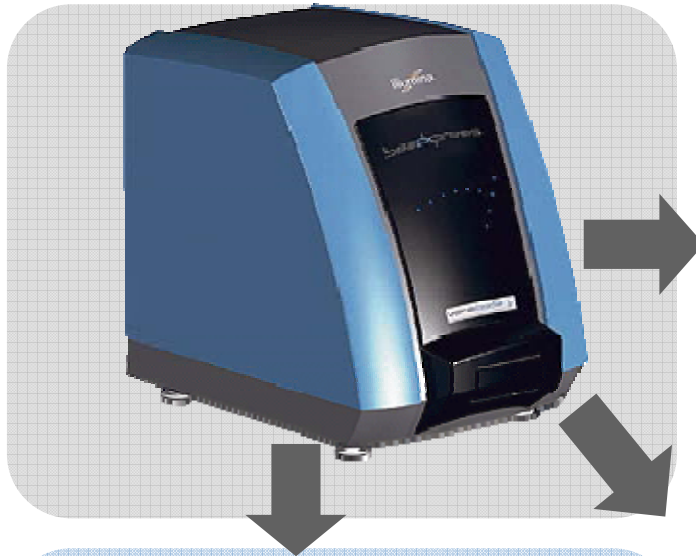


GoldenGate Methylation Assay

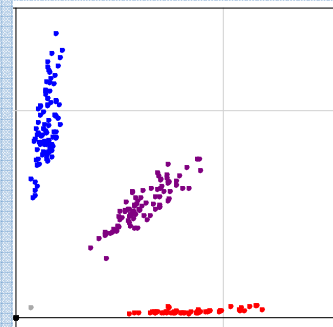
Hybridization & Detection



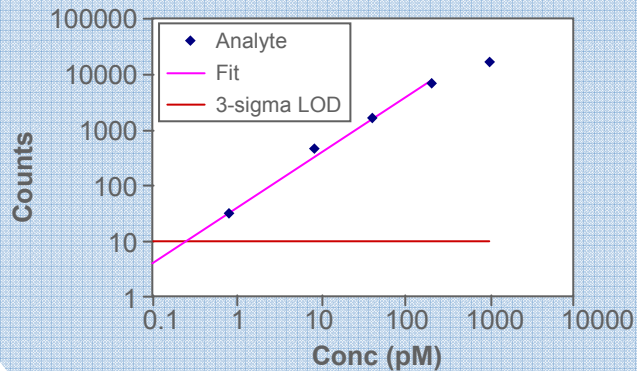
BeadXpress – Application Versatility



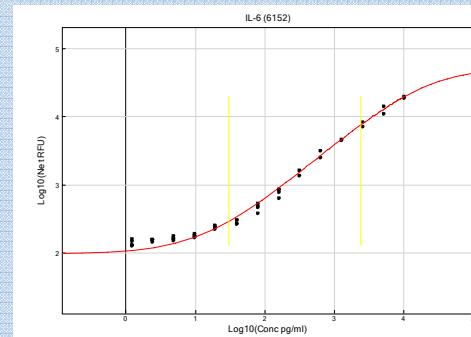
GENOTYPING

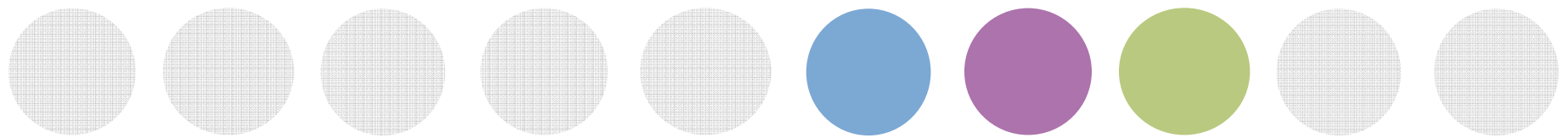


GENE REGULATION



PROTEIN EXPRESSION





The road to diagnostics

illumina*Dx*TM

Bridging from the lab to the clinic

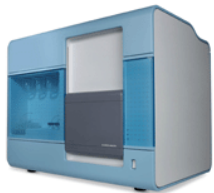
Laboratory



Translate



Clinic



Genome Analyzer



iScan

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



Services



BeadXpress



Applications in pharmacogenetics

A genome-wide scan for common genetic variants with a large influence on warfarin maintenance dose

Cooper GM, Johnson JA, Langaee TY et al. (2008) Blood 112: 1022-1027

- Whole Genome Association study using Illumina's HumanHap550
- Replication analysis with VeraCode GoldenGate 384-plex on loci associated with Warfarin dose at P value $\leq 10^{-4}$ in the index patient population
- Conclusions: Common SNPs with large effects on warfarin dose are unlikely to be discovered outside of the *CYP2C9* and *VKORC1* genes. Randomized clinical trials that account for these 2 genes should therefore produce results that are definitive and broadly applicable.

Human Hap550 (>550K SNPS)

VeraCode 384plex GGGT



iScan

From Whole Genome Association
To
Focused analysis and Validation



BeadXpress

illumina Diagnostics Vision

To Be the Leader in
Cancer Translational Diagnostics

Focus Core Technologies on Cancer

- Next Generation Sequencing
- Whole Transcriptome Analysis
- Methylation Profiling



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

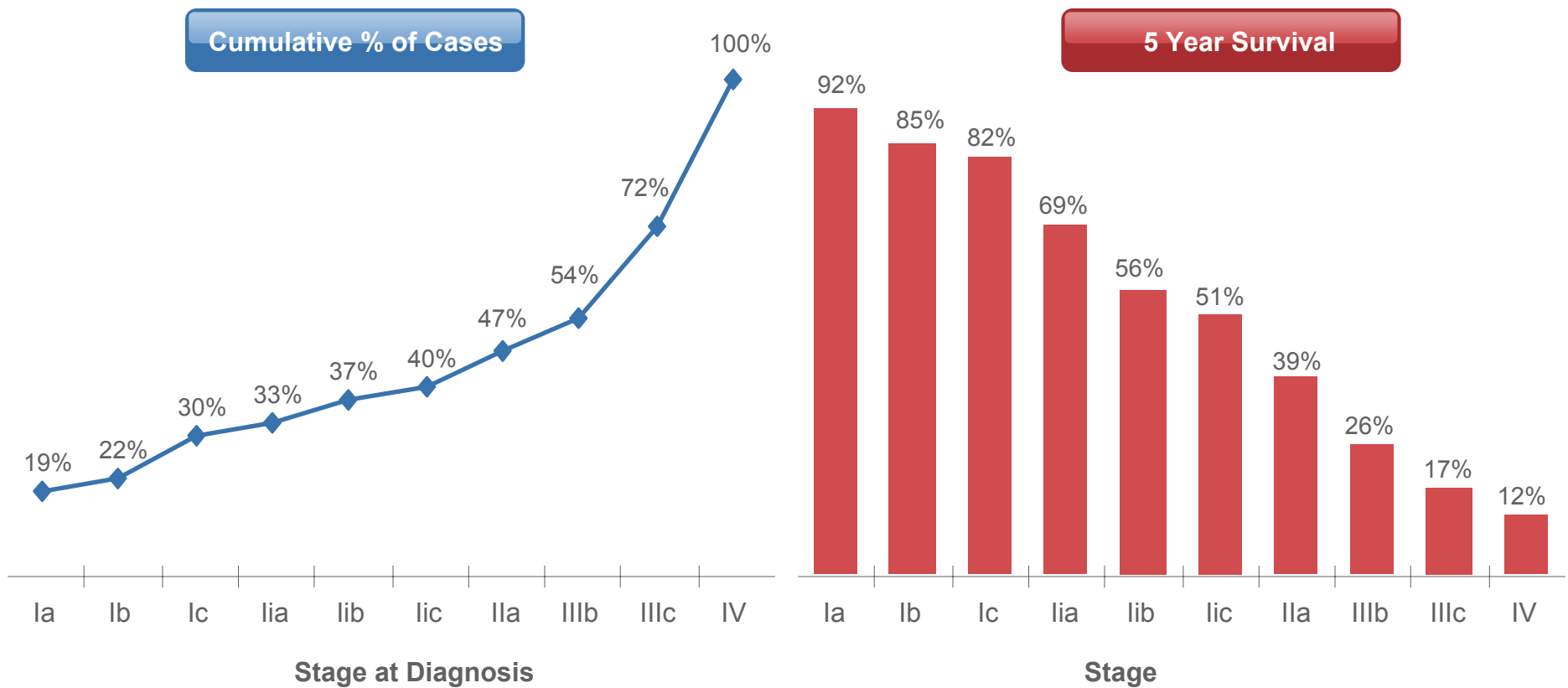
- ▶ Abdominal/pelvic exam, Ultrasound, CT, CA-125
- ▶ Patients present with a suspicious/palpable mass
- ▶ BRCA 1/2 positive in a limited number of cases



Early Detection for Ovarian Cancer is Critical

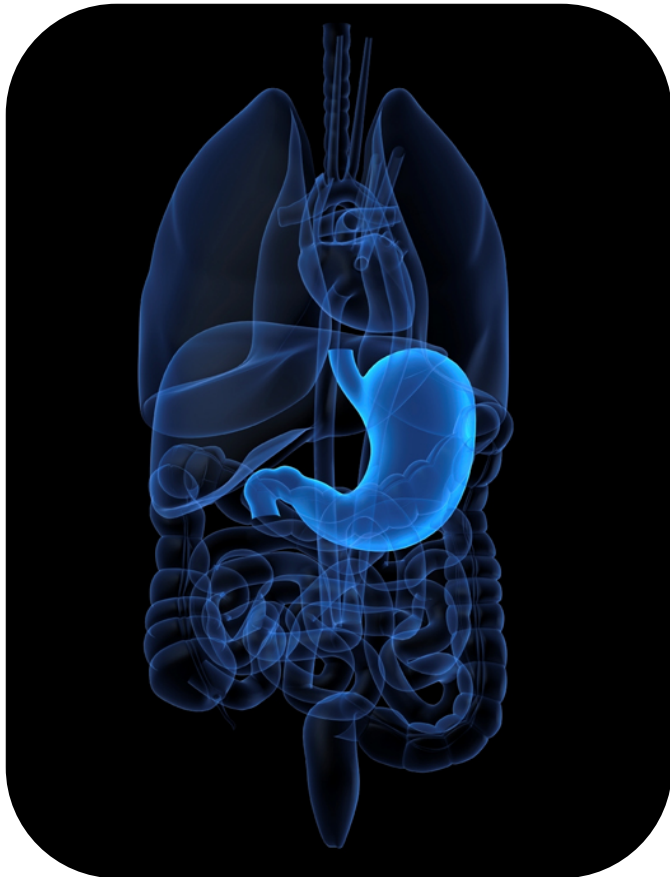
Late stage diagnosis ...

... results in poor prognosis

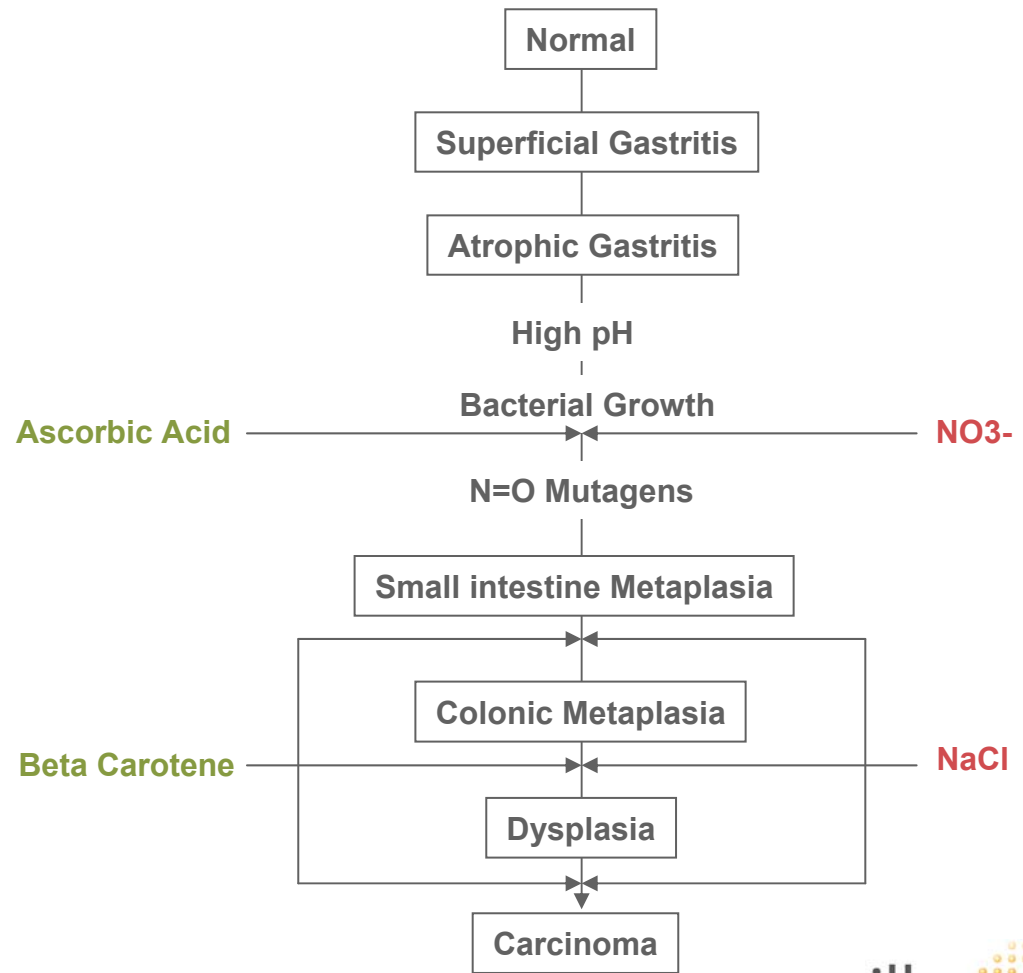


Gastric Cancer will be Our Second Target

Cardia 15% of Gastric Cancers
Increasing due to obesity



Gastric Carcinogenesis



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 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