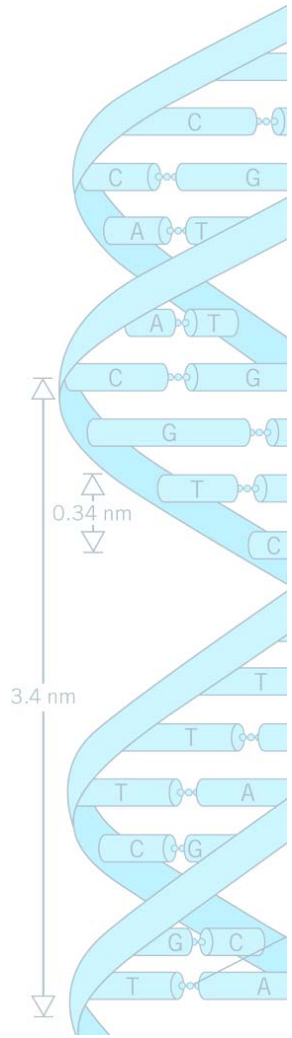
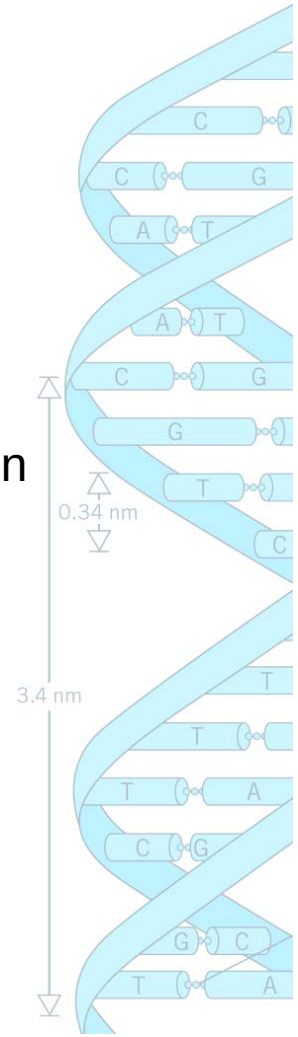
Utilization of the GoldenGate Assay for Veracode in Quality Control and Driving Innovation

Diane Leong, Genentech, Inc.



Presentation Outline

- Sample Repository
  - DNA collection in Genentech clinical trials
- Use of GoldenGate Assay
  - QC of extraction process and sample identity
  - Driving innovation
- Setting up the Platform
  - Adaptation to Biomek FXP and subsequent platform validation
- Case Study (e.g. DAWN clinical trial)
  - Background on AMD and DAWN
  - DNA isolation and SNP genotyping
  - “Confirming” sample identity
  - Plate controls
  - The genotype – call rate and repeatability
  - Presentations and posters

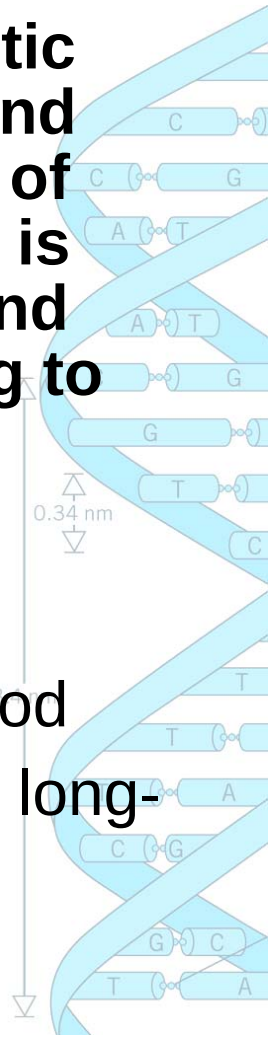


Sample Repository

- Mission Statement
  - The Sample Repository houses an unparalleled collection of biological samples that is the key to vastly increasing our understanding of the biological basis of disease, supporting Genentech's mission of transforming innovative science into breakthrough therapies for patients.
- Logo
  - "Unlock the Potential"

DNA Collection from Clinical Trials

- **Purpose - Collecting DNA from clinical trial patients will allow Genentech to conduct genetic research - linking disease, patient response, and observed adverse events. This understanding of the implications of patients' genetic variability is critical to realizing our vision of discovering and delivering medicines that target "the right drug to the right patient."**
- Ensure informed consent
- Ensure patient confidentiality maintained
- Actual sample collected/stored – EDTA whole blood
- Genomic DNA extracted upon request and stored long-term



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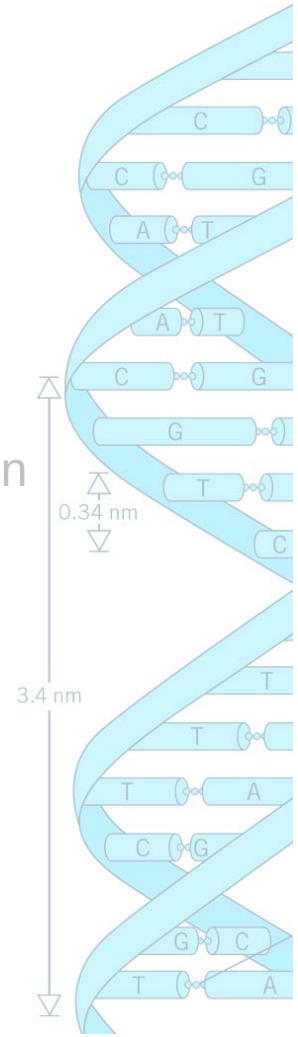

GoldenGate Assay Applications in the Sample Repository

- Quality control of DNA extraction process (QIAGEN Autopure LS) and sample identity
  - Utilize various graphs generated in BeadStudio software
    - E.g. p10GC vs. Index
  - Utilize controls dashboard
    - E.g. Gender
- Driving Innovation – investigating genetic markers for Age-related Macular Degeneration (AMD)

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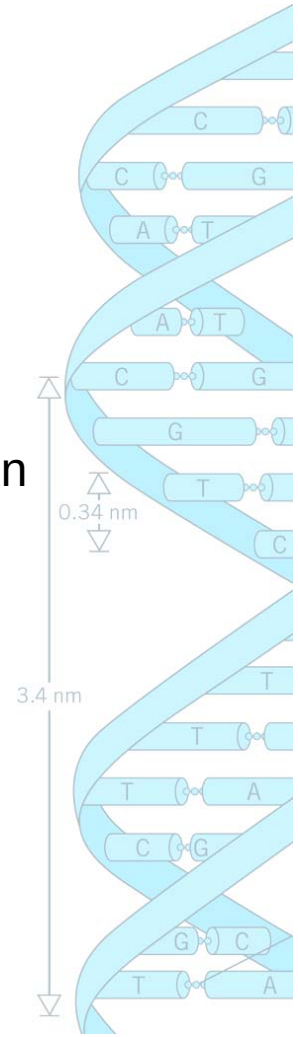


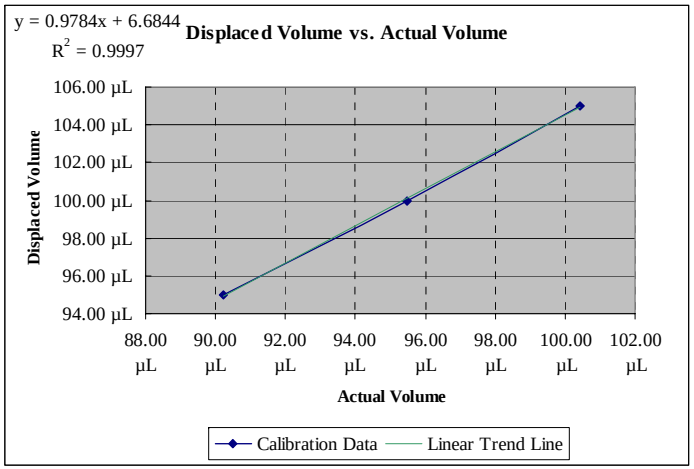
Adaptation of GoldenGate pre-PCR protocols to the Biomek FXP

- Illumina optimized pre-PCR liquid handling on Tecan
- SR endeavored to optimize pre-PCR liquid handling on Biomek FXP
  - Challenges:
    - Reservoir creation and determination of dead volume
    - Pipetting accuracy and precision
    - Magnetic bead capture/wash steps

Determining Pipetting Accuracy and Precision

1. Used fluorescent calibration solution
2. For a determined volume (e.g. 100ul) had Biomek pipet 100ul into UV plate (using a 2x tiptouch technique, scaling factor of 1.0 and offset of 0) with enough TE to bring total volume to 200ul (wet dispense)
3. Created triplicate plates
4. Read ODs, subtracted column 1 (TE only) from "experimentals," and calculated %CVs (want %CV < 3)
5. Repeated steps 2-4 with 95 and 105ul
6. Manually pipetted 200ul calibration solution into separate plate, read OD, subtracted column 1 (TE only) from "experimentals," and calculated %CV

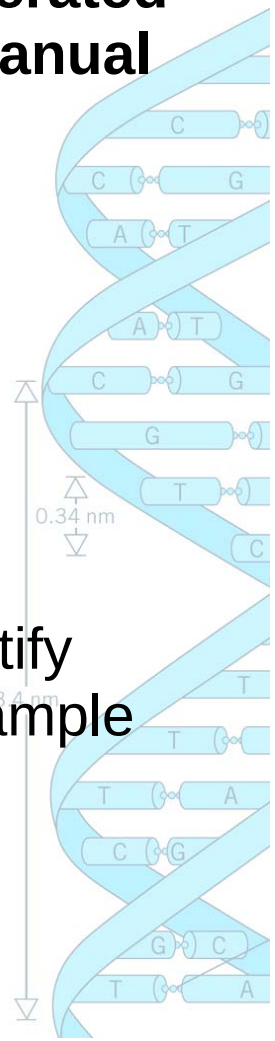
Determining Pipetting Accuracy and Precision continued



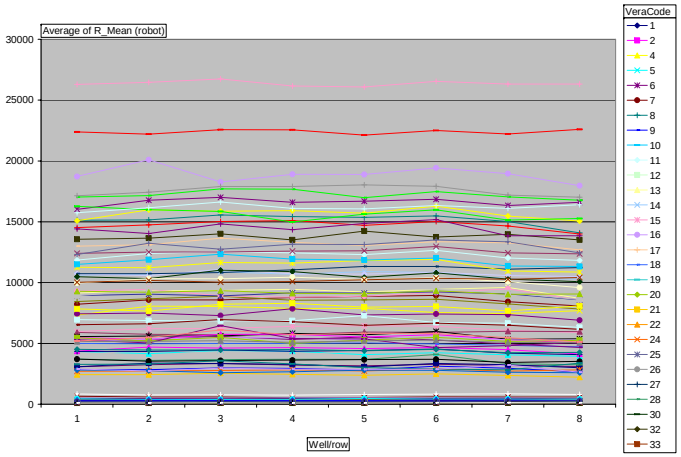
- Calibration curve generated and scaling factor and offset values were calculated
- 2x tiptouch technique modified with new scaling factor and offset
- Used Biomek and modified technique to create three new plates (100ul calibration solution, 100ul TE)
- Manually created separate 100ul calibration solution, 100ul TE plate
- %CVs and % Difference calculated
- Technique passed if %CVs < 3 and % Difference ± 5

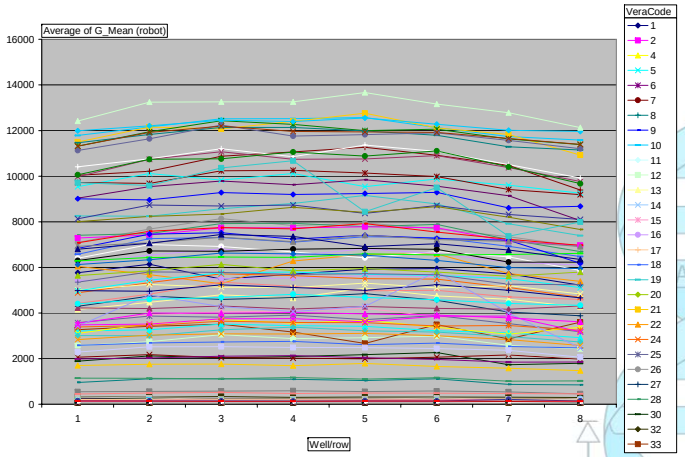
Platform Validation – Manual vs. Automated

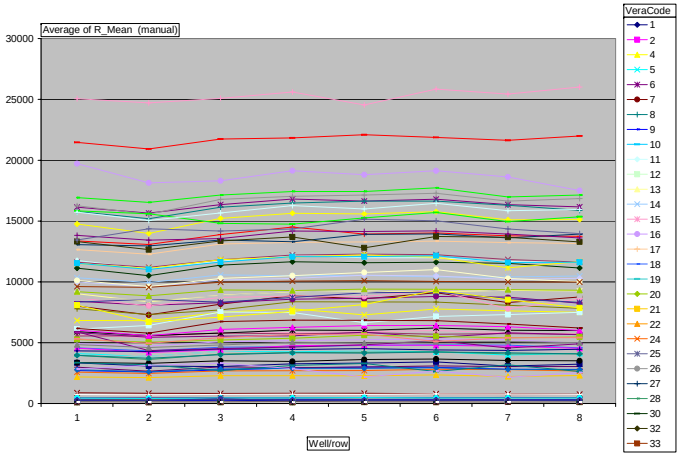
- **Purpose: To compare GoldenGate assay data generated by employing the Biomek vs. data generated by manual means**
- Used same Coriell DNA sample in all 96 wells
- Used DNA test panel OPA
- Used same lot reagents
- Pre-PCR protocols run back to back (Mon/Tues)
- Post-PCR protocols for both plates run on same day
- Average G and R means by column and rows (to identify possible problematic plate positions), call rates and sample performance was compared (amongst other criteria)

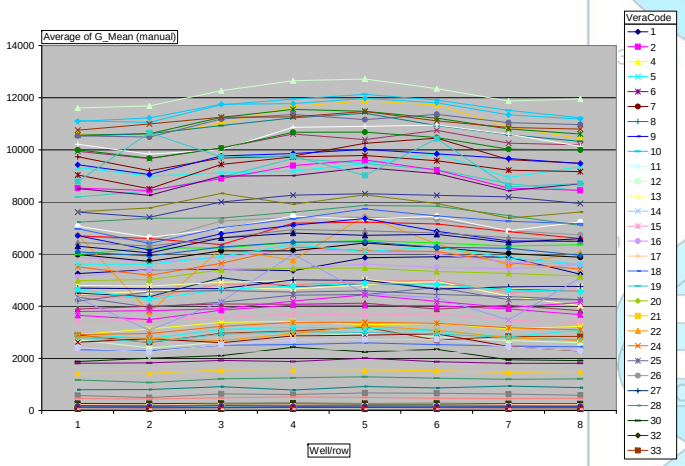


Average G and R means by SNP and well

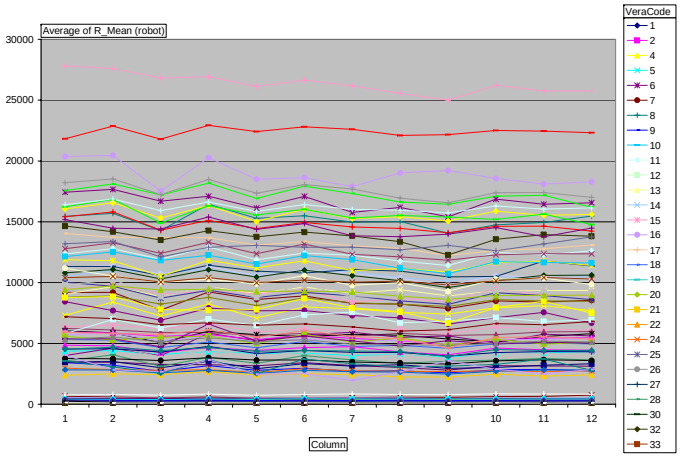


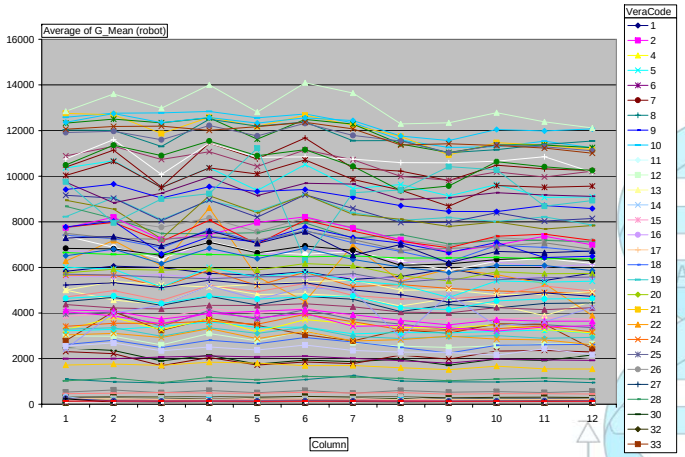


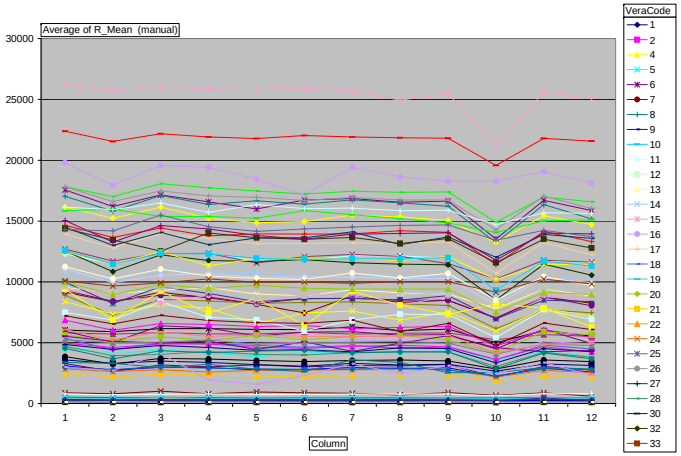


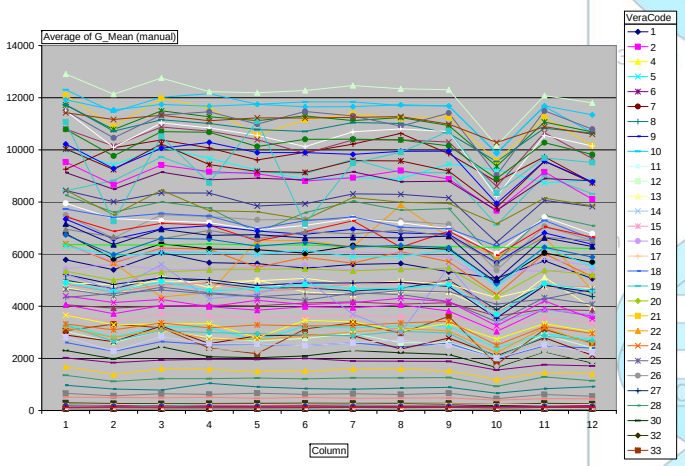


Average G and R mean by SNP and column



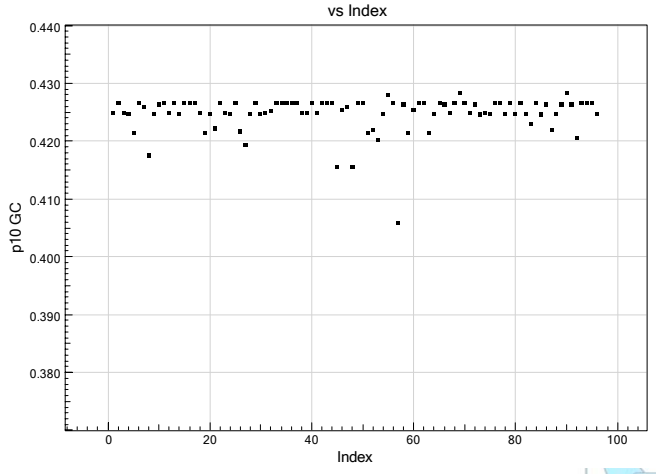


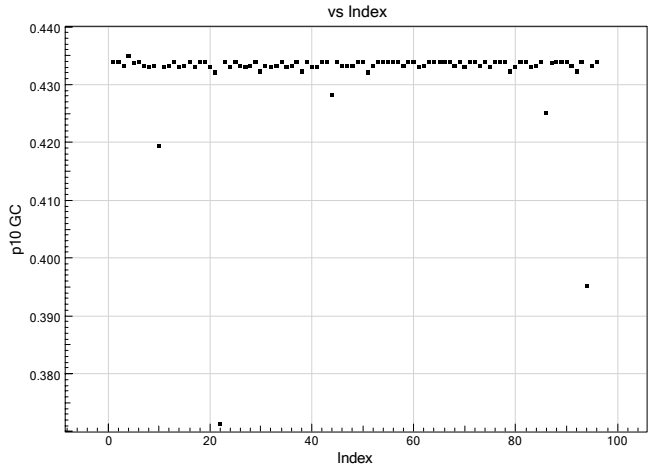




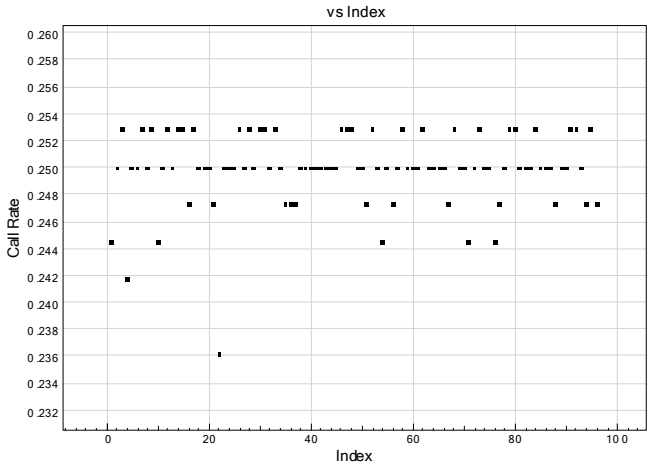
Sample Performance – p10GC vs. Index

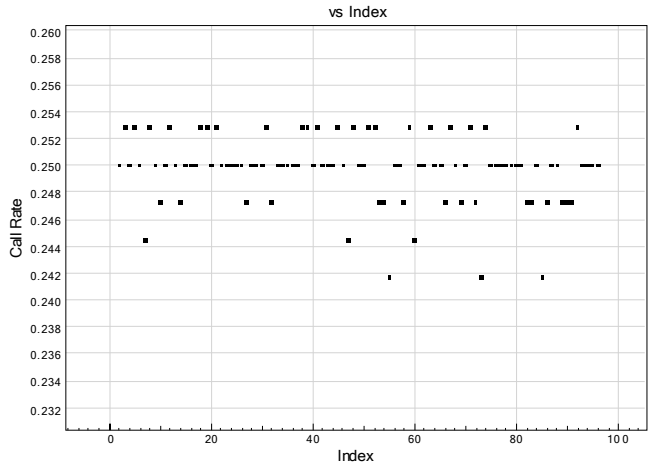
- p10GC slightly lower for plate run on Biomek
- Graph a bit “noisier” in plate run on Biomek
- Manual plate has a couple significant outliers





Sample Performance – Call Rate vs. Index





- No significant difference between Call Rates
- Corroborated by determining the number of no calls
  - Biomek – Call Rate of 99.41%
  - Manual – Call Rate of 99.50%
- Qualitatively, genotype calls were concordant and repeatable
  - Three or less “wrong calls” for all samples, all SNPs (for both plates)

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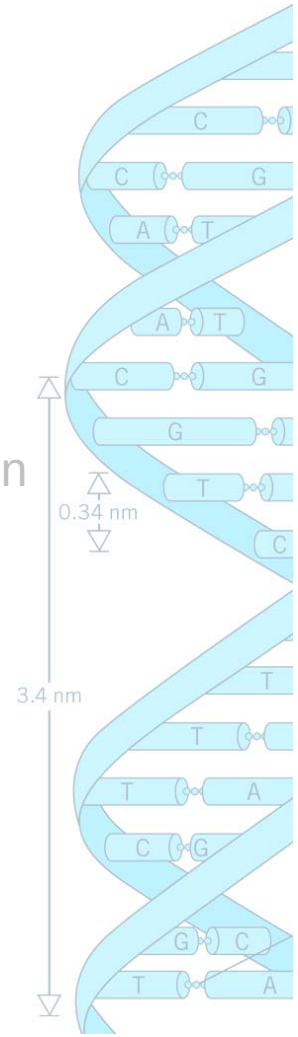
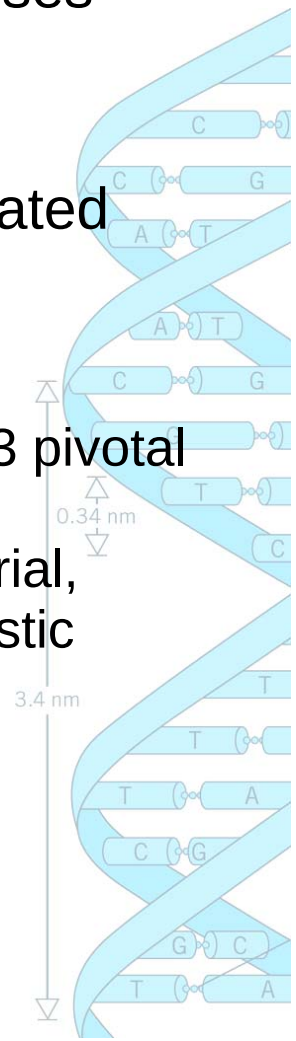


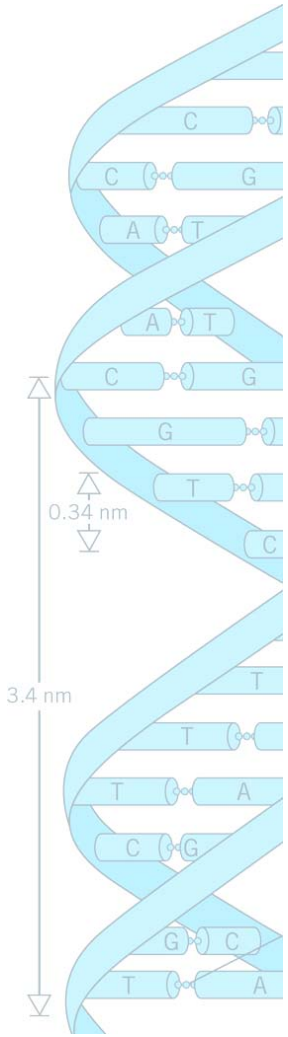
AMD and DAWN – A case study

- Genome-wide association studies and targeted analyses indicate a significant genetic component in AMD
- SNPs in and around complement factor H (CFH) and ARMS2/HTRA1 are associated with significantly elevated risk of late-stage AMD in Caucasians
- DAWN sub-study objectives
  - In patients with neovascular AMD who completed 1 of 3 pivotal trials (MARINA, ANCHOR, FOCUS) of Ranibizumab (Lucentis®) and continued in the HORIZON extension trial, investigate association between several known prognostic genetic variants for AMD as they relate to:
    - disease characteristics
    - response to Ranibizumab



DNA Isolation and SNP Genotyping

- Genomic DNA was isolated from the whole blood of 352 DAWN patients
- A customized OPA (oligo pool array) was designed utilizing known genetic variants associated with disease risk as well as candidate risk variants
- Four plates standardized to 50ng/ul were processed utilizing Illumina's GoldenGate Assay for VeraCode



Graphical Analysis and the BeadStudio Software – Utilizing Inherent Plate Controls (Gender) to "Confirm" Sample Identity

Control_Code	Sample_Name	Gender determined by GoldenGate Assay	Gender match between control assays	Actual Gender	Gender match GoldenGate and Coriell
1878	Sample 1	male	yes	MALE	yes
2911	Sample 1	male			
1878	Sample 2	female	yes	FEMALE	yes
2911	Sample 2	female			
1878	Sample 3	female	yes	FEMALE	yes
2911	Sample 3	female			
1878	Sample 4	male	yes	MALE	yes
2911	Sample 4	male			

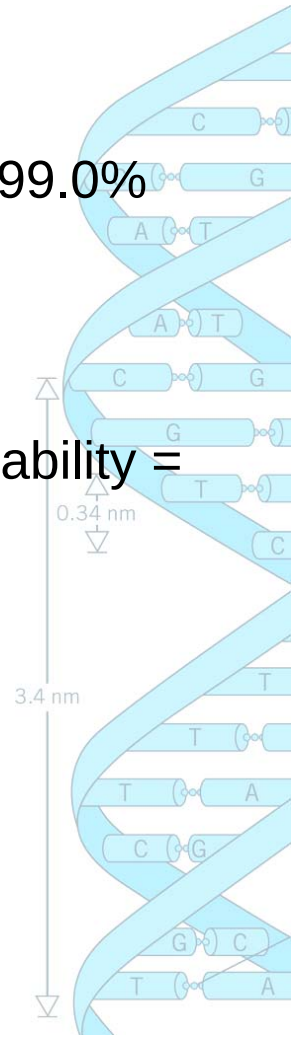
- Qualitatively analyzed gender control graph in BeadStudio
- Exported *ControlsDashboard.csv* file
- Theta > 0.2 = male
- Determined gender for each of the two control beads (per sample) and compared
- Obtained actual gender (from clinical information) and compared to gender determined by assay

Positive and Negative Plate Controls

- Column 12 reserved for 7 Coriell DNA samples and one well of TE (negative control)
- Coriell samples genetically diverse and have a high percentage of heterozygosity
- Repeatability of genotype calls compared amongst plates
- Negative control not recommended by Illumina due to primer-dimer formation and the possibility of false-positive genotype calls
  - Generally see NC for all 96 SNPs
  - If SNP returns genotype call, plate is scrutinized for possible sample cross-contamination

The Genotype - Call Rate and Repeatability

- Experimentals - no replicates
  - Sample success determined by call rate
    - Excluding “poor” SNPs, but no samples, call rate = 99.0%
- Coriell controls
  - Highly repeatable across 4 plates
    - Excluding “poor” SNPs and 1 “poor” sample, repeatability = 98.47%



DAWN Presentations and Posters

- Data generated from this study included in:
  - Presentation presented at the annual meeting of the American Academy of Ophthalmology
  - Poster presented at the annual meeting of the Association for Research in Vision and Ophthalmology

AMD-Associated Genetic Variants and Correlation with Response to the Anti-VEGF Inhibitor Ranibizumab (Lucentis®)

Tsontcho (Sean) Ianchulev, MD
Joseph Arron, MD, PhD
Robert R. Graham, PhD
Howard Shapiro, PhD
Genentech, Inc.
South San Francisco, California

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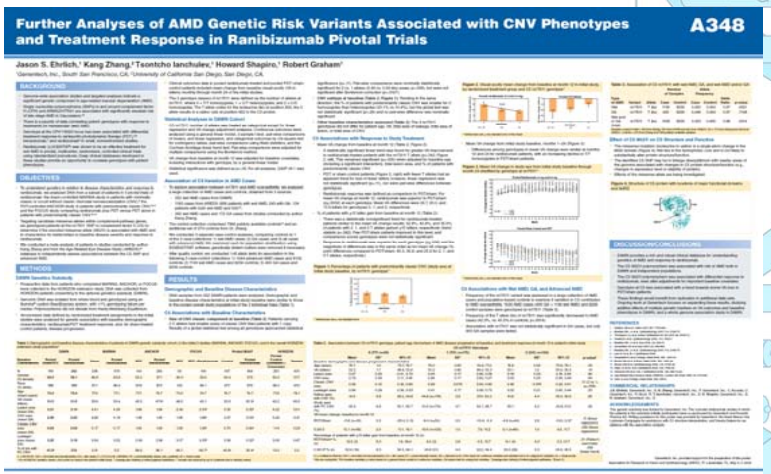
Nov 8 - 11, 2008



Acknowledgements and Questions

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