



Development of QC Methods to Monitor Cross-Contamination from Fixed-tip Automation used to Extract DNA from Clinical Blood Samples

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DNA Extraction Robotics at Pfizer's BioBank



- **Tecan Freedom EVO 200**
- **Chemistry:** Promega Sciences; standard silica\ alcohol affinity extraction. Cells are lysed in presence of magnetic particles. DNA is attracted to silica in the presence of alcohol. Magnetic beads are repeatedly washed. DNA is finally eluted with buffer solution and heat.
- **Software:** Gemini\ User interface and Pfizer custom application
- **Input:** Whole blood – 1 to 96 vacutainer tubes per 9 hr shift
- **Output:** Variable volume and concentration of DNA in elution buffer collected in a standard 48 deep well plate



Experimental Design: Evaluation of BioBank's DNA Extraction Process



- Rationale:** Develop QC procedures to evaluate whether the re-usable tips on the Tecan robotic system introduces detectable cross-contamination
- TaqMan genotyping assays used initially
 - ◆ Functional assay
 - ◆ High specificity and sensitivity
 - ◆ PG labs routinely use these assays
 - ◆ Limitation: difficult to quantitate
 - ◆ Modifications made to DNA extraction process that reduced observed contamination from 20% to <2% of test wells



Initial QC Procedure for the DNA Extraction Process

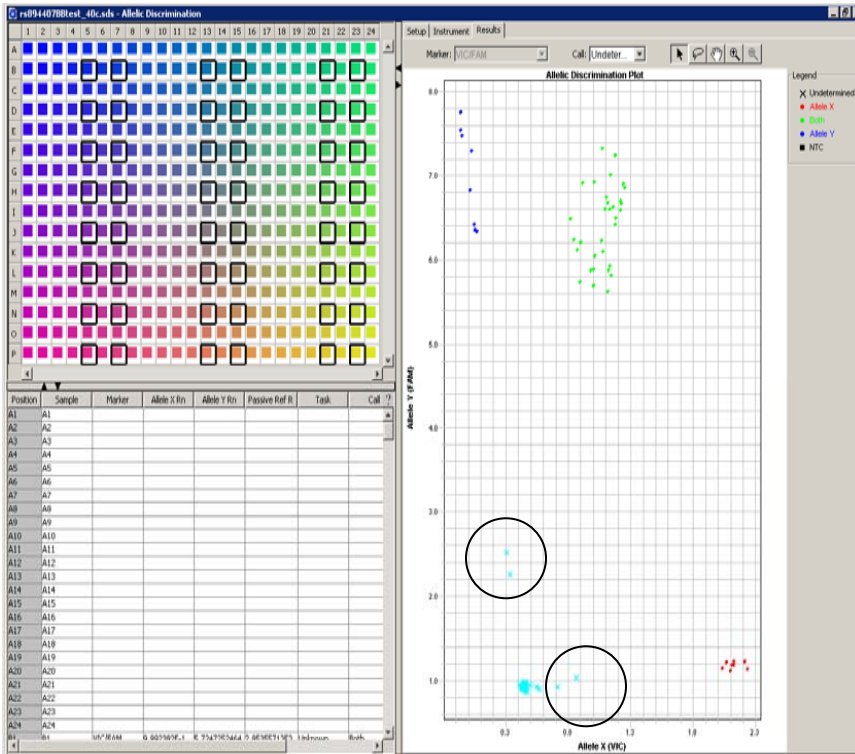
- 96 well plates were received from the BioBank with alternating columns of DNA and water
 - ◆ 48 DNA samples and 48 water samples on each plate
- 3 TaqMan genotyping assays performed for each plate
 - ◆ TaqMan assays for SNPs with high heterozygosity used to increase detection of SNP variation
 - ◆ Increased stringency for the detection of potential contamination was applied by evaluating genotyping assays at both 40 and 60 cycles of PCR
 - ◆ Additional NTCs (dH₂O) included for each genotyping assay plate to ensure that contamination wasn't introduced by sample handling in the lab



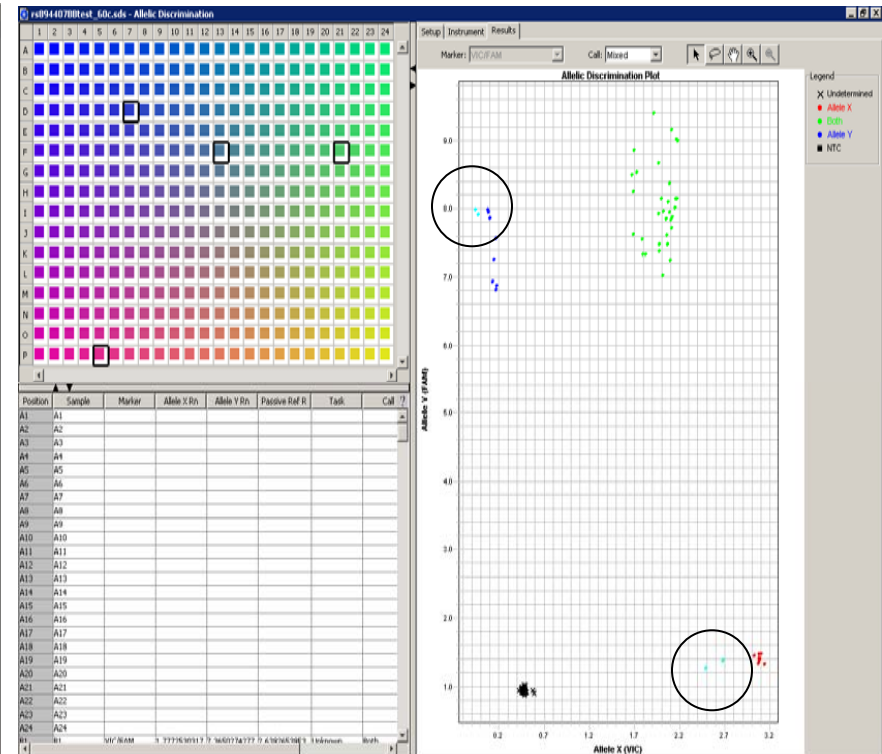
System Modifications Reduced Contamination Levels

- System washes were improved by adding a 10% bleach wash of tips prior to the transfer of concentrated DNA samples. Washes were also optimized after waste removal by flushing the lines with SDS and sodium hydroxide. This modification reduced the contamination level from 10-60% of water samples resulting in a genotype call to 2-4% of water samples resulting in a genotype call
- Introduction of a 20% bleach wash further reduced contamination levels to ~2%
- Low level contamination continues to be observed despite further attempts to isolate and reduce its presence

SNP rs894407 PCR: 40 cycles



SNP rs894407 PCR: 60 cycles



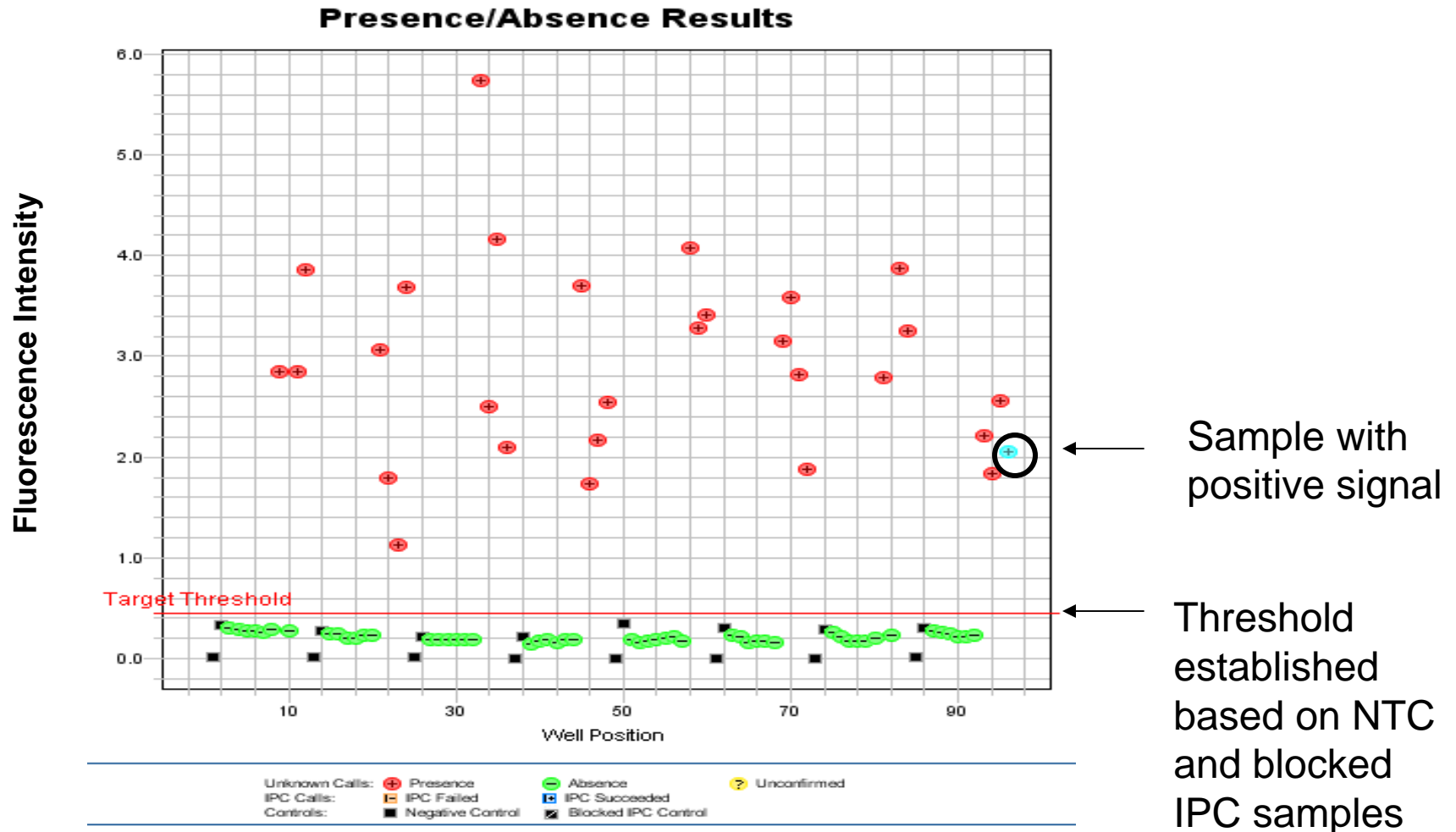
Limitation: Does not provide absolute quantitation of contamination levels

Copy Number Variation (CNV) and Plus/Minus Combination Assay

- CNV assays used to characterize each sample
 - ◆ 5 human CNV assays: CYP2D6, CYP2A6, CYP2E1, GSTT1 and GSTM1
 - We evaluated 3 CNV assays: CYP2D6, CYP2A6 & GSTM1
 - ◆ Copy number is an important polymorphism in the genome that is associated with diseases such as cancer, etc.
 - ◆ Assays report copy number for gene of interest

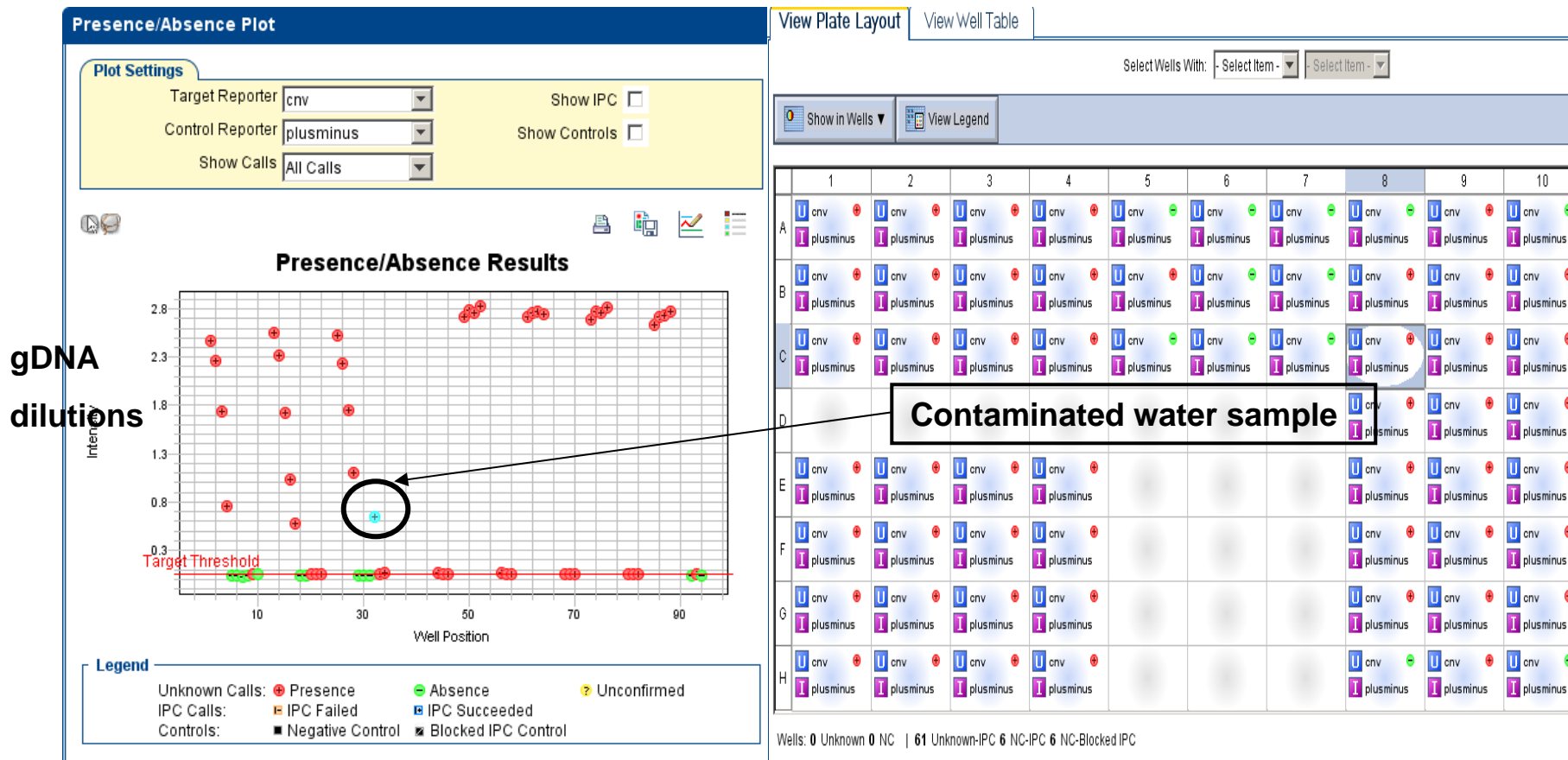
- Plus/Minus assay
 - ◆ A commercial genomic DNA sample is used to generate a standard curve by serial dilution of known DNA concentrations (10 ng/ul-0.01pg/ul). This provides the basis for the quantification of detected contamination
 - ◆ A blocked DNA template is spiked into some control samples to establish a true negative reaction to set the threshold level in combination with the no template control samples

Plus/Minus Assay: Graphical Output



Human CYP2D6 Assay with Plus/Minus Assay

Detected one water sample with low level contamination: ~1pg/ul
 Similar results observed for the 2 additional CNV assays



gDNA dilutions

Contaminated water sample



Mixing Experiments to Assess Impact of Contamination on Genotyping Results

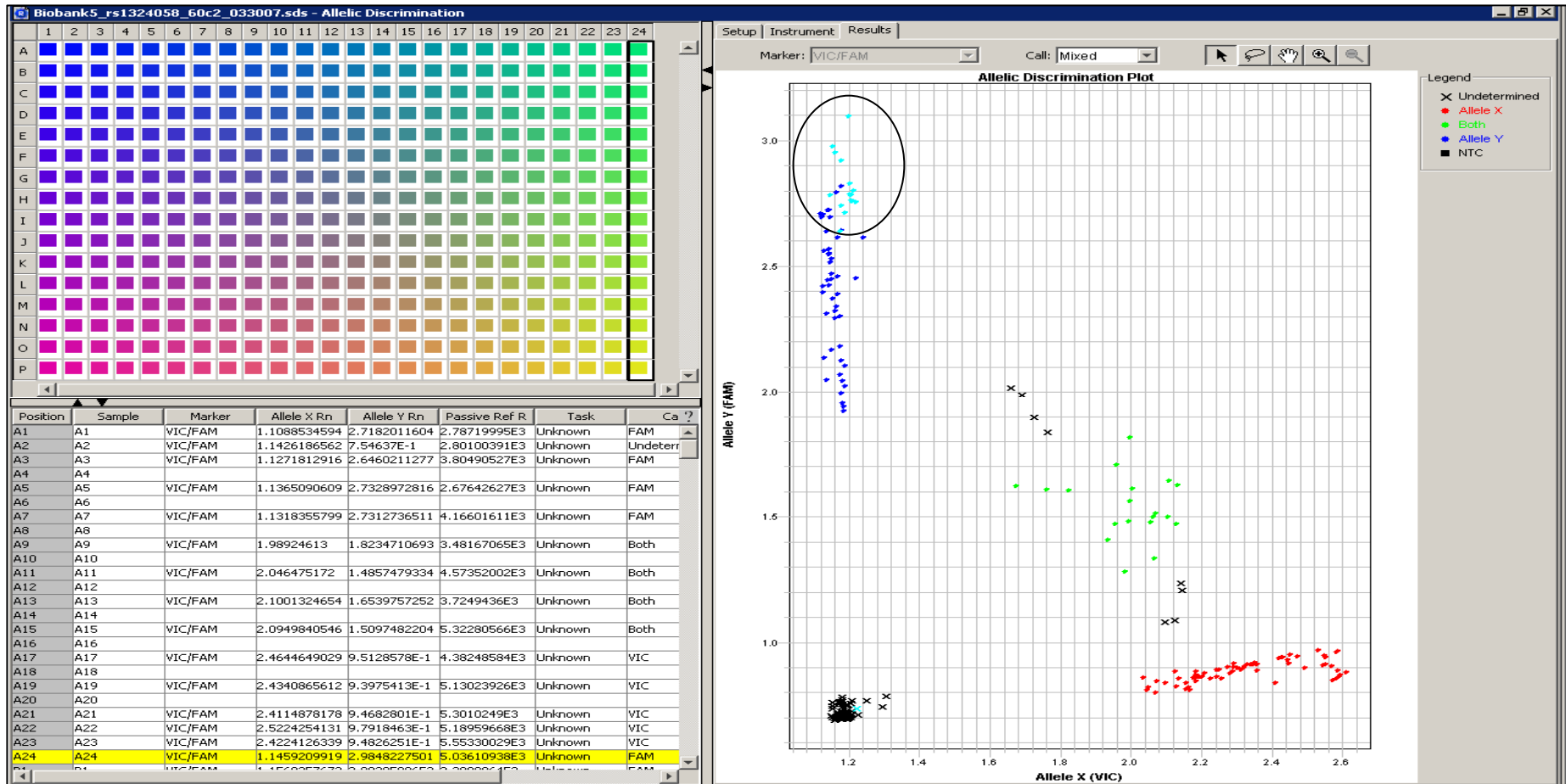


- Identified DNA samples homozygous for each allele of the SNP tested
- The homozygous DNA sample was mixed 50:50 with a water sample contaminated with the opposite homozygote allele (concentration= 5 ng)
- Genotyping assays subjected to 40 and 60 cycles of PCR amplification
- **No changes observed for genotype call even with a 50:50 mix. This was observed in 6 independent experiments.**



Genotype of gDNA=FAM Homozygote, Genotype of Contaminated Water=VIC Homozygote

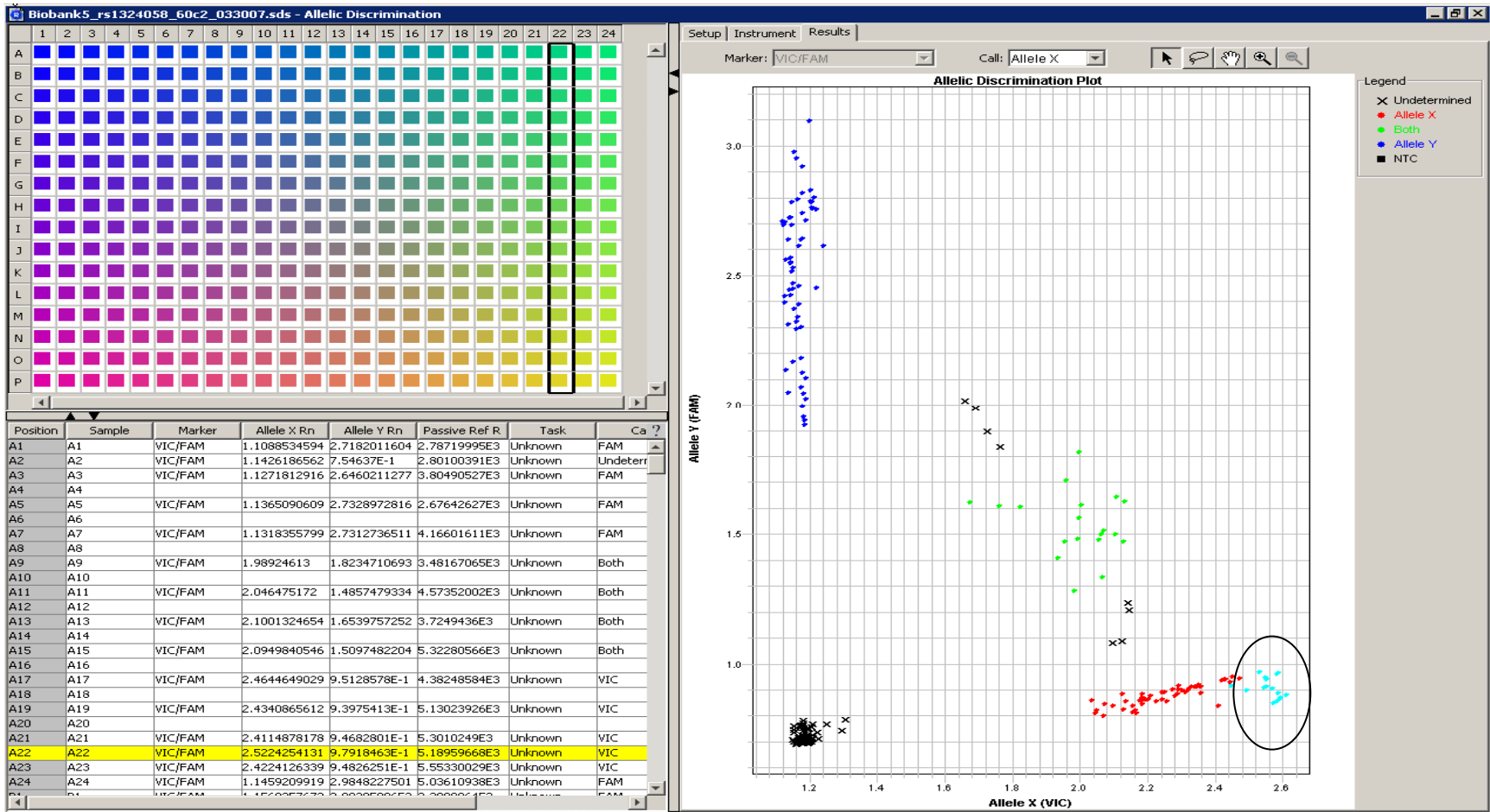
SNP=rs1324058; PCR cycles: 60





Genotype of gDNA= VIC Homozygote,
Genotype of Contaminated Water= FAM Homozygote

SNP=rs1324058; PCR cycles: 60





Summary

- TaqMan genotyping assays can be used to monitor DNA cross-contamination with fixed-tip automation and resulted in system modifications that reduced initial contamination levels from 20% to < 2% of test wells
- The CNV/Plus-Minus combination assay provides:
 - ◆ a functional, quantitative assay to monitor cross-contamination during the BioBank's automated DNA extraction process
 - ◆ System contamination detected at a level <1 pg/ul
- Mixing experiments demonstrated that despite detection of low level system contamination, genotyping results are not compromised