

# Fragment Analysis Results from the Joint-RG Genotyping Pilot Study: The FARG 2007 Poster

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## ABSTRACT

The 2007 ABRF Joint-RG pilot study was set up to compare the various methods and platforms currently being used for SNP or STR genotyping studies. The study looked at more than 7 different methods, using multiple platforms, which were validated by 20 participating RG members. This study has generated a large amount of data even with the study's current limited scale. While the Joint-RG presentation will cover the study as a whole, some of the finer details found in the data will not be addressed because of time constraints. Specific aspects of these finer details as they relate to each of the individual ABRF Research Groups (RG's) discipline will be presented by the participating RG's and FARG is no exception. Every FARG member was involved in the STR analysis as well as other technologies for the Joint-RG pilot study. This poster will present the issues that are relevant to fragment analysis. Platform and analysis methods will be reviewed. This will include detailed data from the STR, pyrosequencing and DHPLC studies. Allele calls as reported by the participants will be examined along with the data analysis methods used.

## INTRODUCTION

The DNA polymorphisms found in the genomes of higher plants and mammals have long been the focus of genetic studies. Their association with a particular phenotype along with their power for biologic classification and/or identification make them ideal for scientific application in today's laboratories. Typical examples of DNA polymorphisms include single nucleotide polymorphisms (SNPs), microsatellite short tandem repeats (STRs), and insertion/deletion polymorphisms. Both STR's and SNPs are routinely used as biomarkers for population genetics, genotyping studies for medical diagnostics, forensic studies and/or clinical analysis of mixed DNA samples. For decades STR-genotyping has been the preferred genotyping method of laboratories performing fragment analysis. However, the FARG 2006 survey revealed that SNP-analysis is quickly becoming a more prominent approach for genotyping laboratories. What the survey did not address was which methods or platforms are best suited for SNP and STR genotyping. The 2007 ABRF Joint-RG pilot study was set up to compare the various methods and platforms currently being used for SNP and STR genotyping studies. This poster will present the issues that are relevant to fragment analysis. Platform and analysis methods will be reviewed.

## Methods & Study Design

**Study's Preparation:** To ensure that all participants of the pilot study would receive appropriate amounts of templates to genotype, genomic DNA of known genotype were subjected to whole genome amplifications (WGA) using a kit from GE Healthcare. The genotypes of the WGA samples were validated by pyrosequencing (for SNPs) and microsatellite analysis (for STR) prior to shipping to participants. One of the WGA was subjected to additional rounds of amplification (sample 8 in Table below). Ready-made STR-PCR were prepared in bulk using ABI profiler plus and distributed to participants for genotyping. Pyrosequencing, Sequenom, and Wave assays were developed in house by RG members.

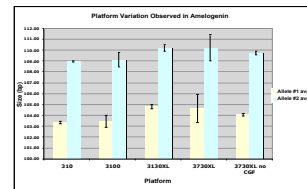
**Sample's Preparation:** A set of 12 (for SNP) or 14 (for STR) samples were shipped to participants (see Table for genotype calls).

**Data reporting and analysis:** A web based survey was posted on line to collect specific information on the genotyping methods used. Genotype calls were collected on an ftp site hosted by the university of Utah for analysis by the joint-RG members.

**Table 1: Study Design**

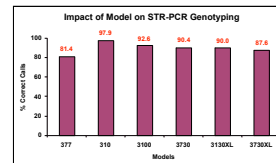
ID	MTNFR	TYMS	AR	TNF	Comment: Gender
Sample 1	T	C	AG	G	Pure DNA sample: Female
Sample 2	CT	C/T	G	G	Pure DNA sample: Female
Sample 3	C	C/T	G	G	Pure DNA sample: Female
Sample 4	C/T	C	G	G	Pure DNA sample: Male
Sample 5	C/T	C/T	G	AG	Pure DNA sample: Male
Sample 6	C/T	T	G	G	Pure DNA sample: Female
Sample 7	C/T	C/T	G	AG	Pure DNA sample: Male
Sample 8	C/T	C/T	G	AG	Sample 7 Genotyped 5.8 X
Sample 9	C/T	C/T	AG	AG	Mix 90% sample 13, 10% sample 14
Sample 10	C/T	C/T	AG	AG	Mix 75% sample 13, 25% sample 14
Sample 11	C/T	C/T	AG	AG	Mix 50% sample 13, 50% sample 14
Sample 12	C/T	C/T	AG	AG	Mix 25% sample 13, 75% sample 14
Sample 13	C	C	G	G	Pure DNA sample: Male
Sample 14	CT	CT	A	G/A	Pure DNA sample: Female

## RESULTS (STR Genotyping Platforms Comparison)

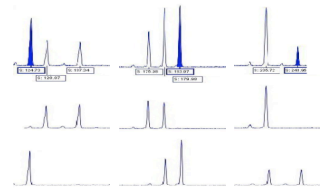


**Figure 1: Allele sizing variation observed between ABI genetic analysis platforms for the Amelogenin marker.** Columns indicate average allele size called among platforms. Error bars indicate standard deviation of allele sizes called among platforms. "3730XL no CGF" shows the effect on standard deviation and average sizes when the data for one group (CGF) is removed from the equation. While the data for CGF is consistent with the other platforms regarding size variance between allele #1 and allele #2, the average allele sizes were approximately 3.5 bp larger than for the rest of the 3730XL group. This can be accounted by the fact that the lab used an in-house reference with these samples. Amelogenin is an XY chromosome-linked marker.

## Microsatellite STR-PCR

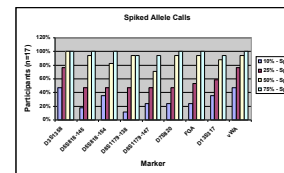
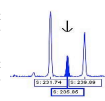


**Figure 2: Percentage of correctly called alleles across 6 ABI genetic analysis platforms.** Variation in allele calling over the different platforms may be due to platform to platform variations such as amount of background and peak height. The variation may also be due to the level of analysis software automation and the experience of the user making the calls. Participants using ABI Genescan and Genotyper must make all calls manually, whereas ABI Genemapper is more automated.

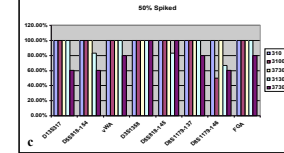
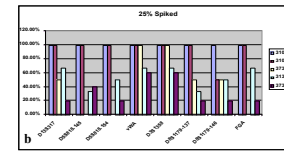
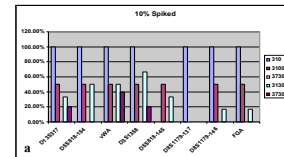


**Figure 3: Addition of Spike sample to the Reference results in the addition of 8 peaks.** The appearance of the 3 peaks (solid blue color) in the 5-FAM label at a 50/50 mixture are shown

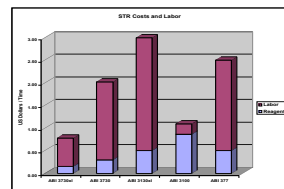
**Figure 4:** illustrates a prominent stutter peak which was called by several participants as a Spike peak. Such large stutter peaks are not common when amplifying tetra-nucleotide repeats.



**Figure 5:** Fewer than half of the participants correctly identified the additional peaks when the sample contained 10% spike. As expected performance increased as the spike level increased with most participants recognizing the 75% spiked peaks for all of the 8 loci.



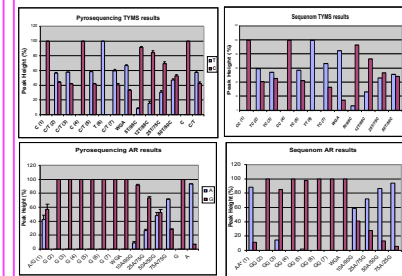
**Figure 6 (a) 10% spike, (b) 25% spike (c) 50% spike.** Identification of low level spike peaks varied widely depending on the analysis platform used with the ABI 310 and 3100 performing best. These results may reflect to experience of the operator in interpreting STR results.



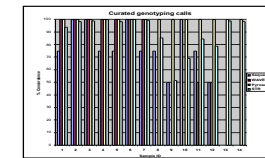
**Figure 7:** Reagent and Labor trends seem to be emerging but caution needs to be stressed as there was insufficient data reported to make any real conclusions. Discounted reagents along with different methods used between laboratories make a true platform-to-platform comparisons difficult at best.

## RESULTS (Genotyping Methods Compare and Contrast)

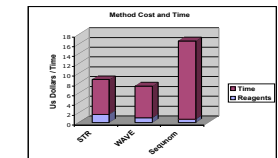
### Pyrosequencing and Sequenom Effect of Alleles and Loci on Genotyping Calls



**Figure 8- Allelic quantitation (AQ) obtained by participants using pyrosequencing and sequenom:** 12 or 14 samples (sequenom and pyrosequencing, respectively) were genotyped using assays designed to genotype the 4 SNP included in this study. Samples ID and genotypes are as indicated on the X axis. In cases of mixed samples some of the genotyping calls submitted by the participants were discordant in particular for sample 9 and 12 (see Table 2). However, the AQ recorded by both sequenom and pyrosequencing were 100% concordant in picking up the correct allelic mixture even for samples 9 and 12. The figure shows the similarity in data for 2 SNPs across both platforms, as well as illustrating quantitatively the allelic imbalance found in sample 8 which was generated by multiple rounds of WGA of sample 7. This allelic imbalance is also observed in STR-PCR as depicted in Figure 2.



**Figure 9:** Concordance of data across 3 SNP genotyping platforms and STR genotyping. Best performing platforms in allele calling are Pyrosequencing and Wave for unspiked samples



**Figure 10:** Reagent cost appear to be decreasing with the new genotyping methods. However as only one WAVE and Sequenom were used in this study, more participants data will be used to get more accurate cost and time data.

## DISCUSSION

### STR platform comparisons

Inter-platform allele size variance between allele #1 and allele #2 was found to be minimal. Similarity in technology/capillaries of ABI capillary sequencers (ABI 3130, 3730 and 3730XL) and the same analysis software supplied with the instruments may account for the allele concordance seen.

- Lower than expected correct allele calls can be contributed to the presence of the spiked samples artificially skewing the results.
- User experience in allele calling and with this STR kit, and not platform variability, had a greater influence on the percentage of correct calls made.
- Stutter peaks are uncharacteristic of tetranucleotide repeats and more commonly seen with dinucleotide repeats.

### SNP genotyping comparisons

- Pyrosequencing and Sequenom SNP genotyping show very similar patterns when comparing peak height generated using both platforms, but the percentage of correct allele calls by the two techniques is very different.
- Within the unspiked samples, WAVE and Pyrosequencing showed the best concordance of data.
- Within the spiked samples, Pyrosequencing correctly identified all alleles. Sequenom perform better on the low percentage spiked samples.
- The methods of allele calling for all techniques used are different and have their advantages and disadvantages depending on the samples being tested. For example, Pyrosequencing and Snapshot use raw peak height data while the Sequenom relies on a clustering algorithm. The mixed samples caused lower than expected correct allele calling using the clustering method.
- Many of the apparent trends seen within this poster may not hold true when tested over a larger sample size for all of the technologies used. A larger study is planned in the next year that will allow a better understanding of the technologies used for both STR and SNP genotyping.