Ultra-Low Volume TaqMan® Genotyping Reactions on the Douglas Scientific® Nexar® System

ABSTRACT
Douglas Scientific has developed automated, inline instruments for high throughput end-point DNA amplification. These instruments have well established performance characteristics when used with TaqMan and other PCR assay chemistries. This paper describes a proof-of-concept experiment to establish the effect of further miniaturization of human SNP genotyping assays.

- The Nexar is an inline liquid handler enabling submicroliter dispensing of DNA samples and assay chemistry into Array Tape®
- The Soellex® is a high capacity PCR waterbath for reactions performed in Array Tape
- The Araya® enables rapid inline detection of end-point PCR fluorescence
- These instruments allow researchers to perform high quality end-point PCR for a fraction of the cost by reducing total chemistry use

INTRODUCTION
Douglas Scientific has developed a fast, cost effective solution for high-throughput genetic analysis that significantly reduces cost per reaction.

Douglas Scientific Instrumentation
The Nexar, Soellex, and Araya instruments were used in the human SNP genotyping experiments described below. All instruments utilize Array Tape in place of a standard microtiter plate and are fully automated for inline processing of low reaction volumes.

- **Nexar Liquid Handling and Assay Processing System** (Figure 1)
The Nexar System supports high throughput sample and reagent liquid handling with dual head dispensing. Each array is processed and sealed in less than 70 seconds.

![Nexar System Image](image-url)
• **Soellex Thermal Cycler** (Figure 2)  
The Soellex is an ultra-high capacity PCR thermal cycling station consisting of a three chamber water bath. The system enables researchers to thermal cycle up to 600 arrays in a single run.

• **Araya Detection Instrument** (Figure 3)  
The Araya is an inline detection instrument for end-point PCR performed in Array Tape. The system can scan each array for up to three multiplexed dyes in 28 seconds.

• **Array Tape** (Figure 4)  
Array Tape is a continuous polymer strip, serially embossed with reaction wells in customized volumes and formats. It is a flexible microplate replacement for streamlined lab processes such as PCR.

• **Intellics® Software Analysis** (Figure 5 - Figure 10)  
The Douglas Scientific Intellics Software Suite and Intelliscore® software were used to analyze and score this data.

**MATERIALS AND METHODS**

**Materials**  
GTxpress™ Master Mix and the three genotyping assays were supplied by Life Technologies. All assay components were otherwise prepared and handled according to the manufacturer’s recommendations.

Ten human whole blood samples were processed using Sample-to-SNPTM kit (Life Technologies) and diluted in TE buffer to produce a 2:5 blood lysate for PCR.

**Methods**  
Varying reaction volumes (Table 1) consisting of either 2X (undiluted) or 1X (diluted with nuclease free water) TaqMan® GTxpress Master Mix and blood lysate were processed on the Nexar using Array Tape. A standard PCR protocol was performed on the Soellex:

Initial hold at 95 °C for 20 seconds followed by 40 cycles at 95 °C for 15 seconds and 60 °C for 60 seconds.

Following PCR, the end-point fluorescence was detected using the Araya. All data analysis and scoring was performed using Intellics.

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<table>
<thead>
<tr>
<th>TEST SET</th>
<th>LYSATE SAMPLE (nL)</th>
<th>MASTER MIX (nL)</th>
<th>TOTAL REACTION VOLUME (nL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEST SET 1</td>
<td>800</td>
<td>800 (2X)</td>
<td>1,600</td>
</tr>
<tr>
<td>TEST SET 2</td>
<td>500</td>
<td>500 (2X)</td>
<td>1,000</td>
</tr>
<tr>
<td>TEST SET 3</td>
<td>500 (dried)</td>
<td>500 (1X)</td>
<td>500</td>
</tr>
<tr>
<td>TEST SET 4</td>
<td>500 (dried)</td>
<td>250 (1X)</td>
<td>250</td>
</tr>
</tbody>
</table>
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Table 1. Reaction volumes tested
RESULTS

All three genotyping assays tested produced discernible clusters for each genotype without respect to miniaturization of the reaction volumes. Although the reporter dye intensity decreased with lower reaction volumes, there was clear separation between NTCs and samples in every test set. Additionally, there was 100% concordance of genotype calls between all test sets performed. Figures 5-10 show the allelic discrimination plots for each assay.

CONCLUSION

The Nexar, Soellex, and Araya system for end-point SNP genotyping is a very powerful tool for laboratories and researchers wishing to produce rapid, high-throughput results. Laboratories will realize significantly reduced chemistry costs as a direct result of the lower reaction volumes reported here, without sacrificing data quality.

‘For research use only. The products of Douglas Scientific, LLC are not FDA-approved for use in human diagnostic procedures.