Next Generation PCR Microfluidic Systems

Angela Morris†, Damian Curtin†, David McGuire†, Bryan Witherbee††, Brian Barrett†, David Kinahan†, Michael Sayers†, Noel Sirr†, Xiaona Hou†, Mauro Aguanno†, Damien King†, Conor McCarthy†, Brian Chawke†, David Doolan†, Sinéad O'Doherty†, Jim Walsh*, Kieran Curran**, Mark Davies†, Tara Dalton†, Chris Bauer††, Thomas Le††.

† Stokes Bio, Lonsdale Rd, National Technology Park, Limerick, Ireland.
†† Monsanto Company, Breeding Technology, 800 North Lindbergh BLVD, St. Louis, Missouri 63167, USA.
* Former Director of Stokes Bio, Lonsdale Rd, National Technology Park, Limerick, Ireland.
** Curran Scientific, Raheen Industrial Estate, Raheen, Limerick, Ireland.

ABSTRACT

Stokes Bio, founded in 2005, develops innovative microfluidic technologies. In 2008 in collaboration with Monsanto, an application driven development for a high-throughput instrument in the detection and characterisation of Single Nucleotide Polymorphisms (SNPs) in agricultural crops was initiated.

Stokes technology is designed to generate aqueous nanolitre scale droplets of reagents and samples, wrapped in a carrier fluid from standard microtitre plates and to mix them using Stokes Bio's proprietary liquid bridge mixers. Following mixing the complete assay is transferred in the carrier fluid through the use of a continuous flow system, to a flow through thermal cycler and an optical reading station.

This poster summarises results collated using the Stokes Bio genotyping platform currently based in Monsanto. Data will be presented to illustrate the dynamic capabilities of the instrument, highlighting the enhanced sensitivity and reproducibility of PCR in droplet format compared to well-based technologies.

Stokes Bio Technology

Stokes Bio technology is unique in a number of ways:

- The reaction vessel is a droplet, not a well.
- High reaction throughput capabilities.
- Highly efficient nanoscale reaction volume.
- Exceptional arraying capability and flexibility.
- Low reagent consumption.

Technology Background

➢ The reaction vessel is a droplet, which flows through teflon tubing in a continuous oil flow format thus resulting in trains of aqueous droplets as shown in Figure 1. These aqueous droplets are approximately 300nl in volume and are separated by the carrier oil. The droplets are completely wrapped by this carrier oil thus generating a micron scale film between the droplets and the tubing wall. The droplet volume and separation is controllable. As the system is modular, parallel lines enable high throughput capability.

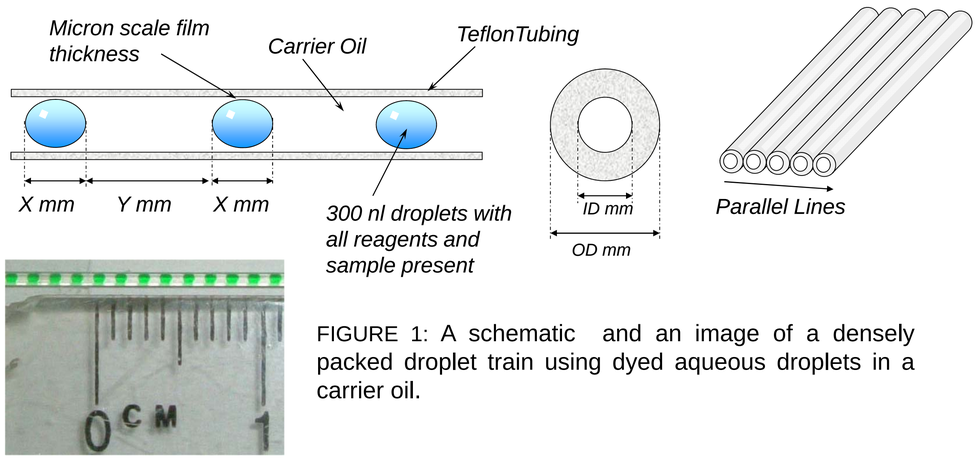


FIGURE 1: A schematic and an image of a densely packed droplet train using dyed aqueous droplets in a carrier oil.

➢ Droplets are mixed in Stokes Bio's proprietary liquid bridge. A schematic of a liquid bridge tower is shown in Figure 2. These bridges facilitate the mixing of nanoliter-scale droplets, containing master mix, reagents and DNA sample, in a precise and controlled fashion.

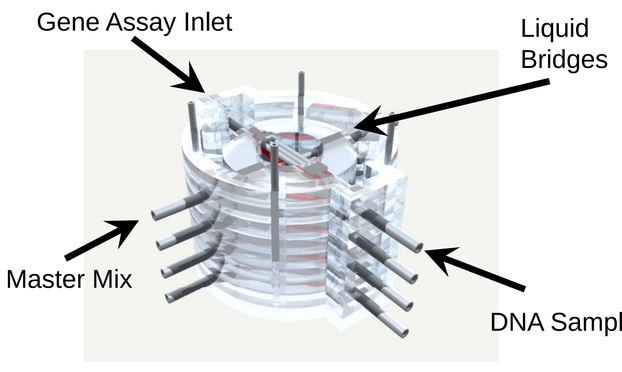


FIGURE 2: Schematic of Stokes Bio liquid bridge technology. This figure shows a partially assembled three way 6X bridge stacked in a tower format. This can be increased to a 96-Bridge format (24 x 4 towers) allowing an increase in mixing throughput.

➢Stokes Bio technology utilises common laboratory consumables. The PCR reaction is prepared and aliquoted into two microtitre plates, the PCR gene assays are put into one plate and the DNA samples of interest are placed into the other. The master mix is placed in a separate reaction vessel. The aqueous solutions are then overlaid with a carrier oil as shown in Figure 3.

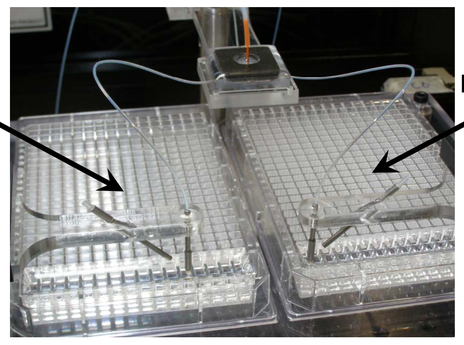


FIGURE 3: Stokes Bio platform set up. This figure shows the two microtitre plates and a single liquid bridge.

➢ Tubing extends from the liquid bridge and is dipped into each reaction plate using a robotic liquid handling system. Droplets of DNA, gene assay and master mix are formed and mixed in the liquid bridge. This segregation of the PCR components enables a truly flexible instrument as any DNA sample can be arrayed against any gene of interest in nanoliter volume reactions.

➢ Following mixing in the liquid bridge, the droplet train flows through the required thermal zones for amplification. A schematic of a Stokes Bio thermal cycler is shown in Figure 4. A laser, optical fibres and cameras form the optical system that detects fluorescence signal due to amplification. For real-time PCR, the droplet fluorescence is read at each cycle whereas in genotyping the fluorescence is only measured at the end point. The resulting signal from each droplet is then analysed by software and amplification curves or allelograms can be generated depending on the application.

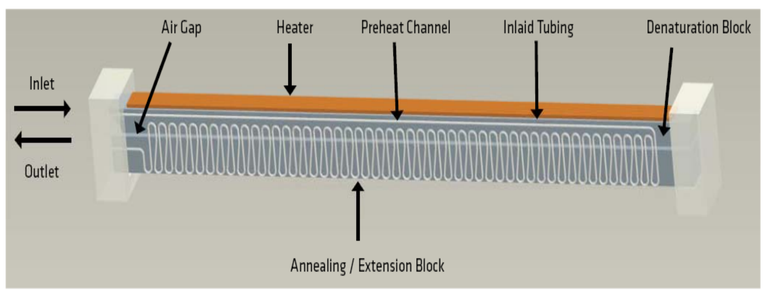


Figure 4: The thermal cycler is made up of two blocks that are heated to the required temperature for amplification. The tubing meanders in and out of these thermal zones thus resulting in PCR cycles.

Genotyping at Monsanto

Monsanto aims to double its year 2000 yields in core crops of corn, cotton and soybeans by 2030 while simultaneously reducing by a third the amount of inputs, such as water, energy and fertilizer, needed per unit produced. To assist in achieving this goal Monsanto requires a significant increase in their genotyping throughput capacity while reducing cost. The Stokes Bio instrument is beneficial to Monsanto in three ways. Firstly, the throughput can be increased by parallelising the liquid bridges and the thermal cyclers. The Stokes Bio instrument can achieve tens of thousands of data points per hour. Secondly, the cost per reaction is also reduced due to the nanoliter volumes used. Finally, the unique sampling technique enables exceptional flexibility so that any DNA versus gene combination can be performed.

The following experiments were performed to demonstrate the enhanced sensitivity and reproducibility of PCR in droplet format compared to well-based technologies. Extracted DNA from soy seed was amplified on Monsanto's current high throughput genotyping process. The same extracted soy DNA was amplified on the Stokes Bio genotyping instrument at Monsanto shown in Figure 5.

Figure 5: Stokes Bio genotyping Instrument

In both cases three polymorphic soy SNP markers were investigated and the resulting genotyping data was examined and visualized in allelograms. Allelograms A-C in Figure 6 represent individual markers ran on Monsanto's current high throughput genotyping process. Allelogram D represents 96 DNA samples ran on the Stokes Bio instrument against all three polymorphic SNP markers.

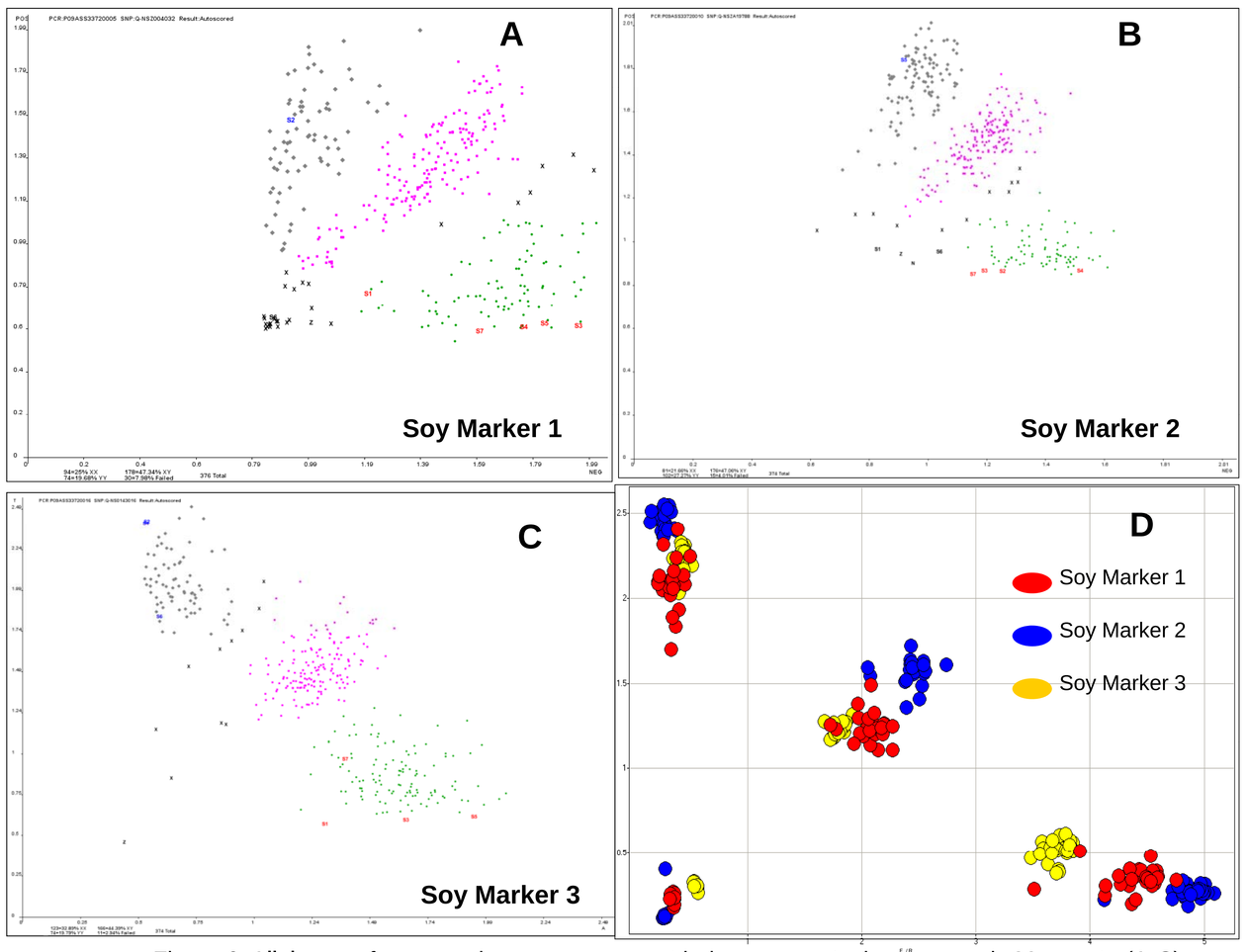
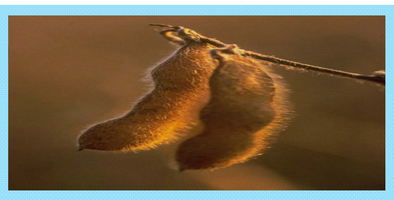


Figure 6: Allelogram for soy makers run on current in-house genotyping process in Monsanto (A-C) . The same soy markers were run on the Stokes Bio instrument and are presented in allelogram D.

The allelogram from the Monsanto PCR process (Figure 6 A-C) is very disperse which makes accurate genotyping calling difficult. The resulting allelogram from the Stokes Bio instrument (Figure 6 D) is more tightly clustered and thus easier to score. Scoring algorithms can be easily and more confidently applied to an allelogram that give distinctive clusters. Stokes Bio technology enables more accurate calling and facilitates a faster turn around time between seed analysis and planting.

Conclusion

Stokes Bio technology offers improved functionality over existing systems that suffer from high reagents costs and an inability to array multiple DNA samples against multiple gene assays in a flexible format. As presented in Figure 6, the Stokes Bio instrument yielded a superior clustering allelogram plot compared to the current process at Monsanto. This was also achieved at a reduced reagent cost per data point and at a higher throughput. Stokes Bio technology will have a major impact on genotyping at Monsanto and indeed our novel technology will be extended into the real-time PCR and digital PCR space also.

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