MicroGEM

*forensic*GEM® Sperm Lysis

Upper Wright Valley, Ross Sea Region - Antarctica
Source of ***forensic***GEM®

*forensic*GEM® Sperm Lysis: enzymatic method achieves better results than chemical method

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Introduction

The *forensic*GEM sperm lysis kit takes advantage of ZyGEM's (now MicroGEM) Acrosolv reagent to lyse sperm without the use of qPCR and STR inhibiting chemicals, such as SDS, mercaptoethanol and DTT. The method is 20 minutes or less when used with a thermal cycler, heat blocks, or the MicroGEM PDQeX nucleic acid extractor. There are no transfer steps, so yield is maximized and the opportunities for mistakes and contamination are minimized. This app note demonstrates the power of *forensic*GEM when compared to a commonly used competitor's method for post-differential sperm lysis. Additionally, data shows that this method is highly effective as Y screening chemistry.

Materials and Methods

Competitor comparison: liquid semen was diluted 1:10, 1:100, 1:1000 and 1:10000 in nuclease free water. Six 100 µL replicates were made for each dilution with three of each for ZyGEM and three for a competitor's bead-based extraction chemistry. The replicates were centrifuged at maximum angular velocity for 5 minutes in a microcentrifuge. 80 µL of supernatant was removed from each sample and discarded.

The competitor samples were processed following the manufacturer's protocol for liquid sperm samples. This followed a typical protocol of incubating in a lysis buffer of DTT and chaotropic salts, binding DNA to paramagnetic beads, wash steps, and finally elution of the DNA off the beads.

For the *forensic*GEM method, the ZyGEM sexcrime protocol was followed.

DNA Quantitation

After performing the *forensic*GEM and competitor's methods, the samples were quantified using the Promega Plexor® HY System on an Applied Biosystems 7500 Fast Real Time PCR System.

STR Profiling

Using the quantification data, all of the 1:1000 and 1:10000 replicates were amplified using the Promega Powerplex® Fusion 5C on an Applied Biosystems 9700. Separations were performed on an Applied Biosystems 3130xl. 1:10 and 1:100 replicates were not amplified since these were not considered challenging.

MicroGEM Application Note 013

Competitor Challenge Results

As shown in Figure 1, the qPCR data demonstrated that ZyGEM had much higher concentrations of DNA across all replicates and dilutions.

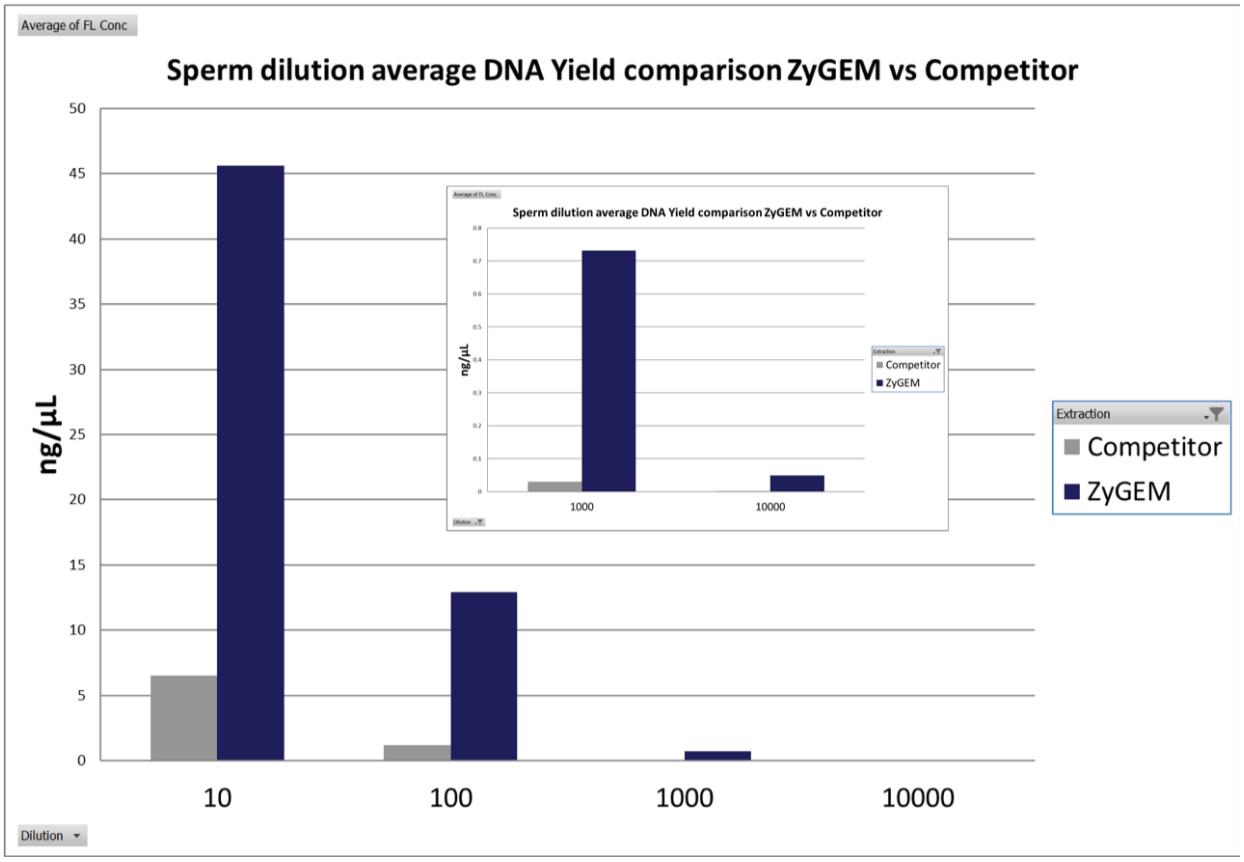

FIGURE 1

Based on the qPCR data, the competitor chemistry is less efficiently lysing the sperm cells and/or losing DNA during the purification process. Figure 2 shows the percent loss of DNA calculated by subtracting the competitor quant values from the ZyGEM quant values, dividing by the ZyGEM values and multiplying by 100.

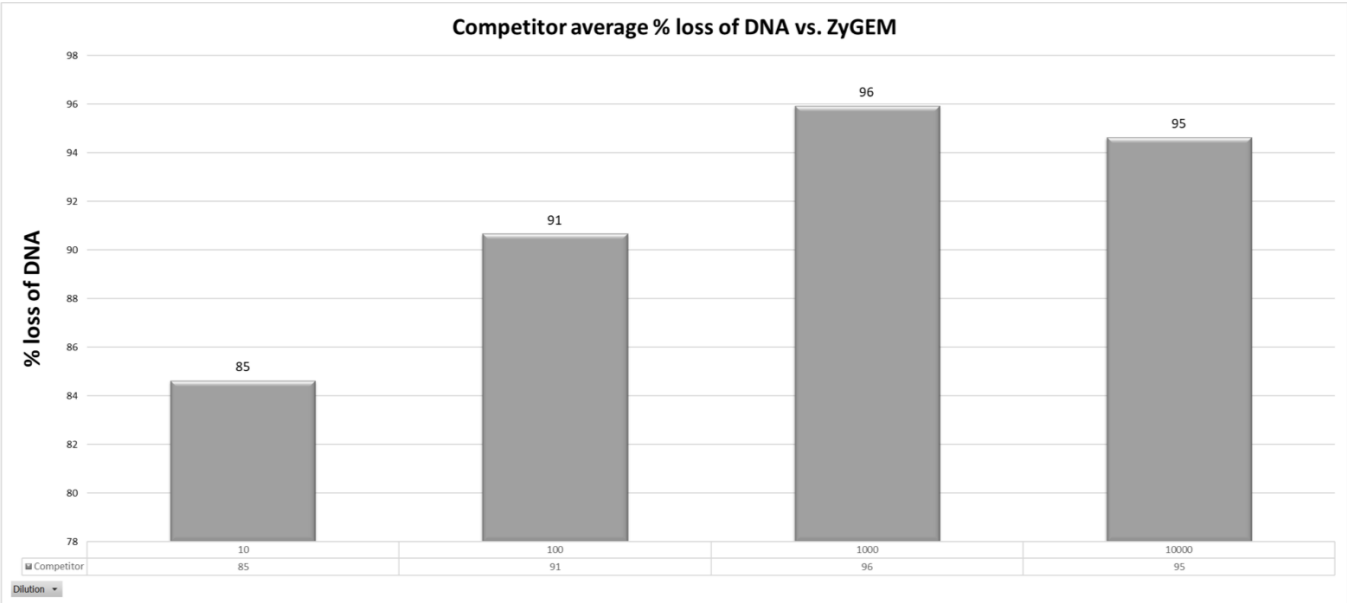

FIGURE 2

FIGURE 3 shows the STR data from the comparison of the ZyGEM and the competitor kit was consistent with the qPCR data, showing that the ZyGEM chemistry could achieve useful DNA profiles down to a 1:10000 dilution, while the competitor had no peaks above 100 rfus at this level of dilution.

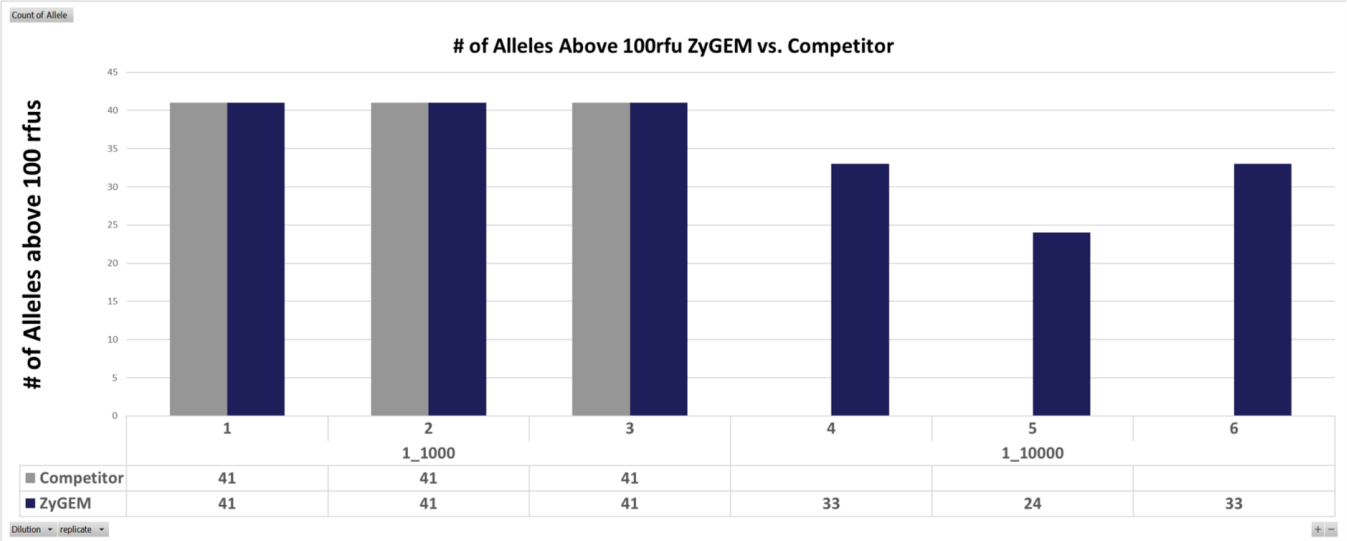

FIGURE 3

Figure 4 shows a ZyGEM profile at 1:1000.

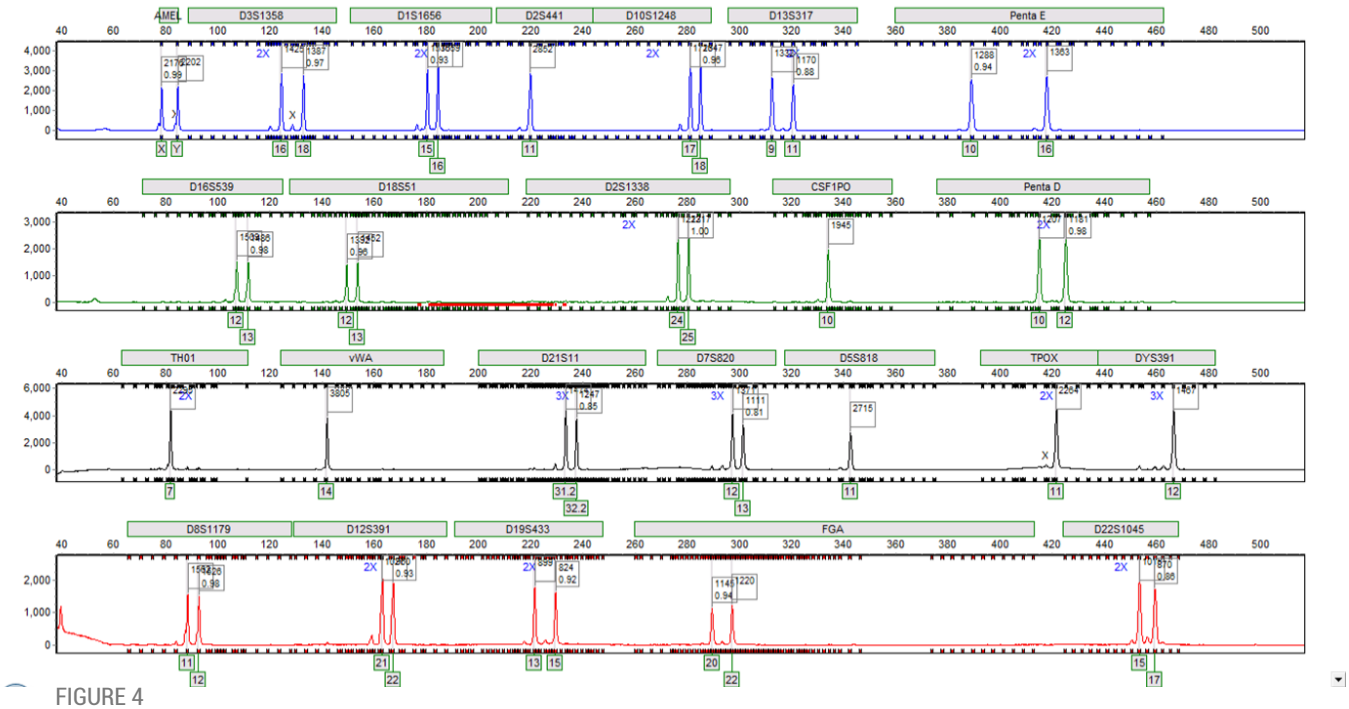

FIGURE 4

Additionally, the ZyGEM chemistry had better quality profiles at the 1:1000 dilution as measured by average heterozygote peak balance (figure 5) and intracolor balance (figure 6).

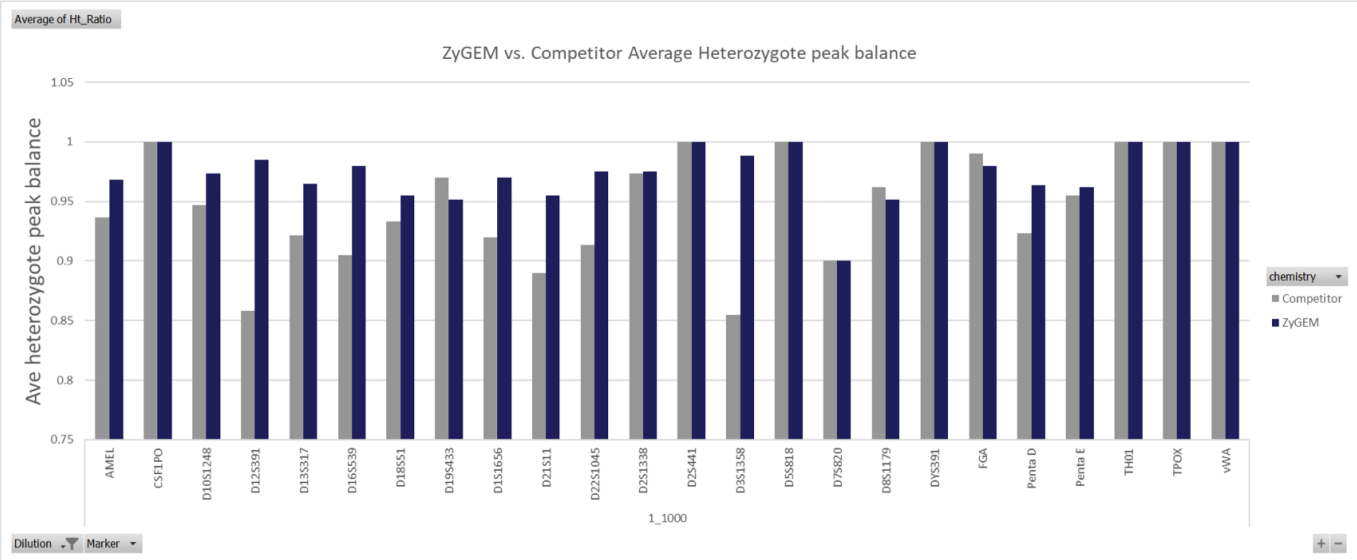

FIGURE 5

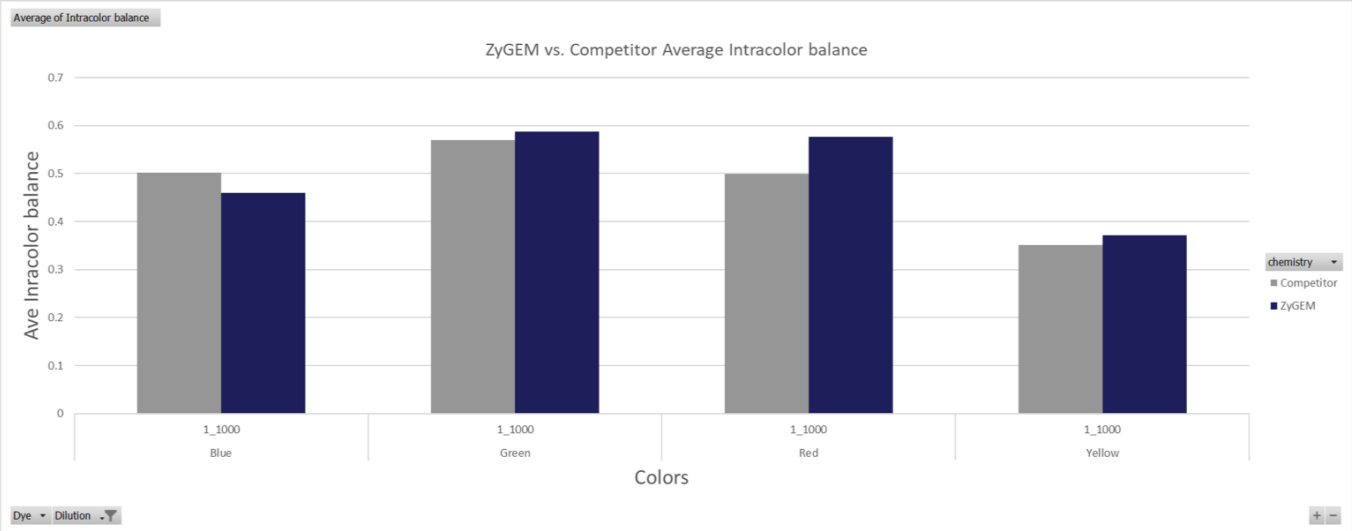

FIGURE 6

Conclusions

The MicroGEM ***forensic***GEM sperm lysis chemistry is faster, easier and produces more high-quality DNA than competitive chemistry. This method can easily be automated or manually performed and, based on the above data, may provide useful data from evidence that would normally fail with traditional methods.