

ForensicGEM Sperm Lysis on the ZyGEM PDQeX: validation of a rapid sperm lysis protocol for Y screening using real time PCR

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The *forensicGEM* sperm lysis kit takes advantage of ZyGEM's new Acrosolv reagent to lyse sperm without the use of chemicals that inhibit qPCR, such as SDS, mercaptoethanol and DTT. When used with ZyGEM's new PDQeX instrument, the reagents allow comprehensive lysis of sperm cells in 20 minutes. Since there are no transfer steps, the yield is maximized and the opportunities for mistakes and contamination are minimized. The protocol described in this note can be used to screen potential sexual assault evidence by following lysis with a Y chromosome-specific real time PCR assay, or used directly for sperm lysis as part of a differential extraction procedure.

Materials and Methods

Small cuttings from vaginal swabs or cloth can be cut and placed in PDQeX tubes. The sperm lysis reagents are mixed as shown below:

Mastermix (1X)

73 μ L Acrosolv
2 μ L *forensicGEM* enzyme
25 μ L *forensicGEM* RED Plus Buffer

PDQeX setup

100 μ L of the mastermix was pipetted into each PDQeX tube. Tubes were flicked to dislodge any bubbles and to fully immerse the material. Up to 24 PDQeX tubes can be loaded into the PDQeX simultaneously. Collection tubes were placed in the lower drawer and then the instrument was loaded with PDQeX tubes.

The following program is run for sperm lysis:

52°C for 5 mins
75°C for 3 mins
95°C for 3 mins
110°C for 2 mins

DNA Quantitation

After extraction, the substrate remains in the PDQeX tube, and liquid DNA extract is passed into the collection tube ready for qPCR or STR profiling. For the purpose of Y screening, qPCR was performed using a Promega Plexor® HY System on an Applied Biosystems 7500 Fast Real Time PCR System.

STR Profiling

For samples amplified for STR loci, the Promega Powerplex® Fusion System was used. Approximately 1ng of template DNA was amplified for 28 cycles on an Applied Biosystems 9700. Separations were performed on an Applied Biosystems 3130xl.

Dilution Series Preparation

A dilution series of sperm cells mixed with female epithelial cells was created using previously counted spermatozoa (see Table 1). Epithelial cell count was based on prior qPCR data. The dilution series was extracted on the PDQeX in triplicate and then quantified using real time PCR.

Sperm (per μ L)	Epithelial cells (per μ L)
400	370
50	370
5	370
1	370

Table 1: Liquid dilution series of sperm mixed with female epithelial cells.

Mock Casework Sample Preparation

The dilution series described above was also used to create mock sexual assault samples. 100 μ L samples from each of the dilution series were deposited onto sterile cotton swabs and dried overnight to mimic a vaginal swab. Small cuttings (the tip of the cotton swab \sim 3mm²) were taken from each swab and extracted in the PDQeX.

To mimic a typical forensic casework sample, semen of unknown cell count was deposited on denim jeans with garden soil stains. The jeans were then stored at room temperature for one month. Two cuttings (\sim 3mm² each) were taken from a portion of the jeans with obvious soil. An additional two cuttings were taken from an unsoiled area of the jeans.

Results:

Sensitivity Study Results

The dilution series was extracted in triplicate and the average autosomal and Y quantifications are reported in Table 2.

Sperm Count	Average of Auto (ng/μL)	Average of Y (ng/μL)
400	4.424	3.736
50	3.550	0.534
5	2.204	0.025
1	2.243	0.008

Table 2: Real time PCR results from dilution series of sperm and epithelial cells from Table 1. The average concentration of DNA from three replicate extractions at each sperm cell count.

The qPCR data in Table 2 demonstrates that the PDQeX lyses sperm and epithelial cells in mixtures that can then be quantified using real time PCR to detect male DNA. The method is sensitive and the ease of use makes it ideal for rapid evidence screening. This process also provided a good estimate of the male DNA to total genomic DNA actually in the sample.

Mock Casework Results

The data from the mock vaginal swabs (Table 3) created from the dilution series in Table 1, demonstrated that the PDQeX can extract DNA from mixtures of epithelial and sperm cells dried on cotton swabs.

Sample	Auto(ng/μL)	Y Conc (ng/μL)
Swab_400	20.78	8.33
Swab_50	8.513	0.81
Swab_5	13.16	0.14
Swab_1	6.390	0.025

Table 3: Real time PCR quantification data from dilutions of sperm in female epithelial cells dried onto cotton swabs.

The real time PCR from the semen deposited on both soiled and unsoiled denim showed that the DNA extraction provides DNA ready for qPCR (see Fig. 1). The qPCR data from these samples was used to determine the amount of template added for STR amplification.

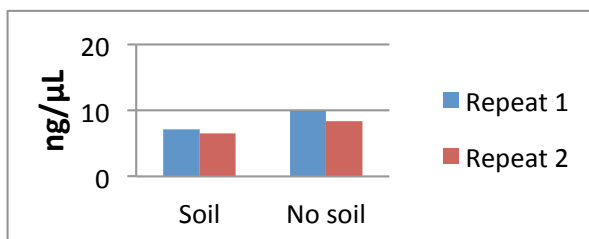


Figure 1: Y qPCR data from semen from two replicates at soiled and non-soiled areas of denim jeans.

STR Data

The qPCR data from Figure 1, was used to calculate the template DNA input for STR amplification of the semen samples dried on denim. All four samples yielded full profiles upon amplification, indicating the qPCR data was indicative of DNA quantity and quality. Figures 2 and 3 below are STR profiles from two of those samples.

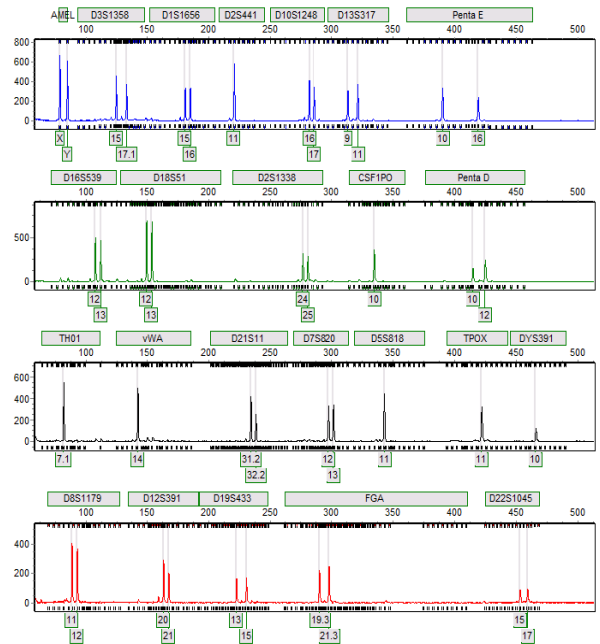


Figure 2. STR profile from semen deposited on soiled area of denim jeans.

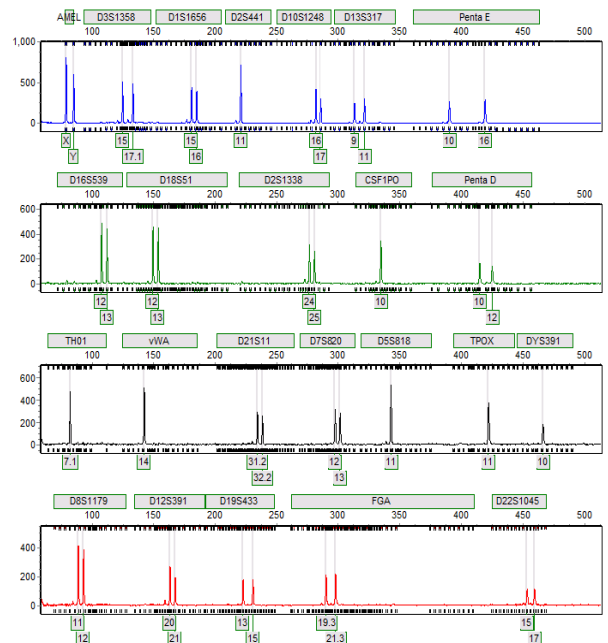


Figure 3. STR profile from semen deposited on unsoiled area of denim jeans.