A method for DNA and RNA co-extraction for use on forensic sample using the Promega DNA IQ™ system

นำเสนอโดย

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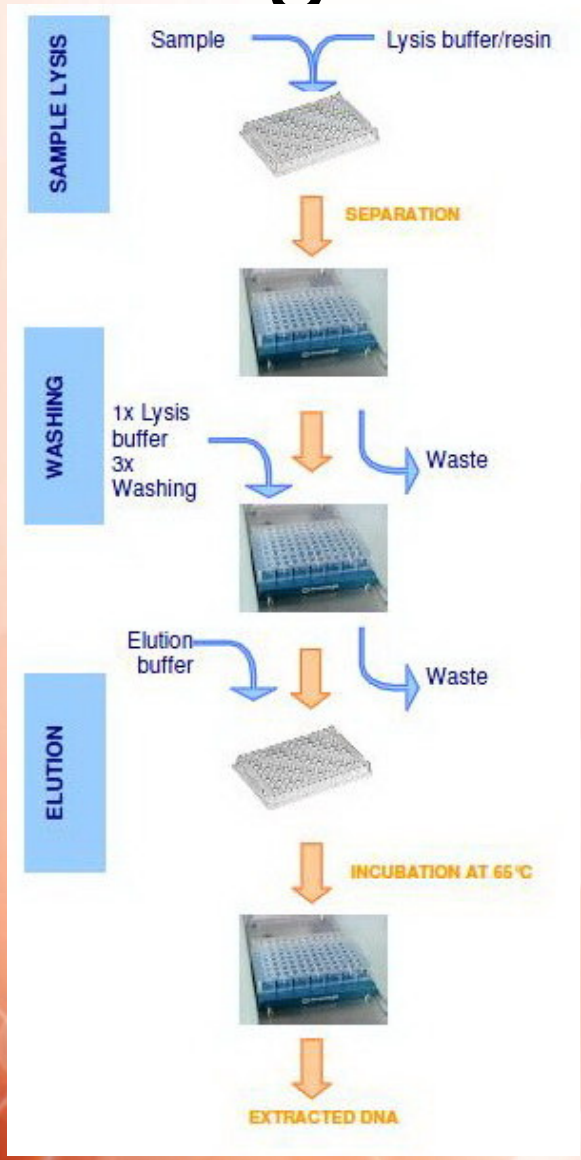
Introduction

- mRNA → body fluid identification
- Increased specificity
- the Promega DNA IQ™ system

the Promega DNA IQ™ system

- Uses magnetic beads & resin to extract DNA
- Manual & automated process
- High capacity, 96 samples within 70 min
- Low tip throughput
- Low cost solution

the Promega DNA IQ™ system



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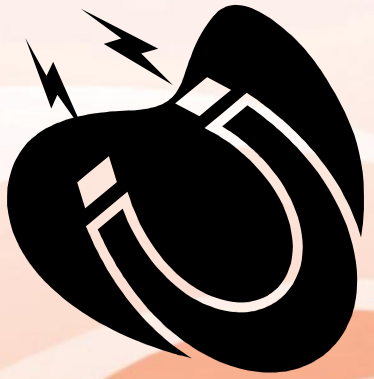
Methods

Sample

* Semen
* Blood
* Saliva
* Vaginal fluid

Methods

- Organic extraction method
- The Promega DNA IQ™ system



Methods

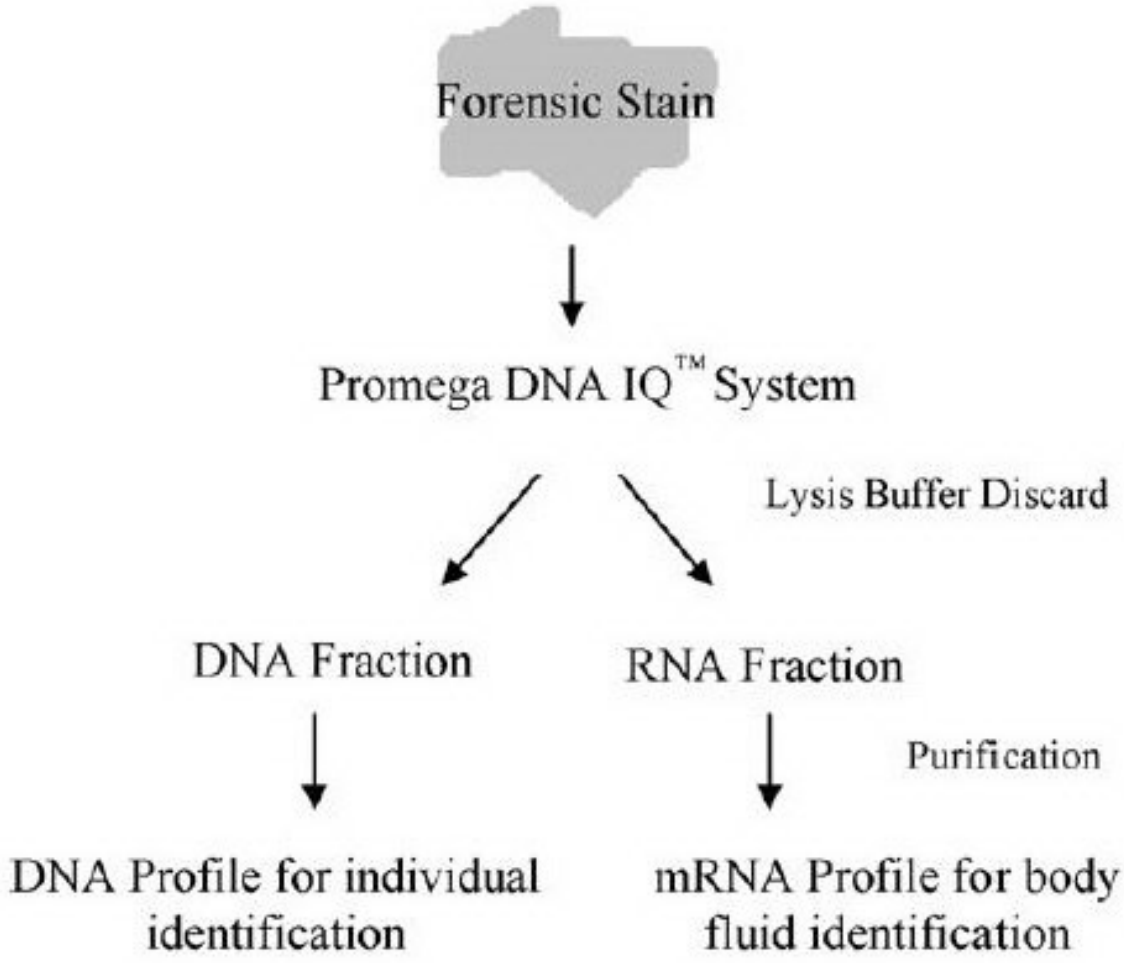
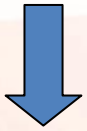


Fig. 1. Simplified schematic of overall Promega DNA IQ™ method with purification using the Zymo Research Mini RNA Isolation Kit™ II.

Methods

RNA

synthesising



cDNA

Table 1
Primer concentrations used in the mRNA multiplex.

Primer	Body fluid	Concentration (μM)
Glycophorin A (GlycoA)	Blood	0.4
Matrix metalloproteinase 11 (MMP11)	Menstrual blood	0.2
Histatin 3 (HIS)	Saliva	0.5
Statherin (STATH)	Saliva	0.05
Protamine 2 (PRM2)	Spermatozoa	0.05
Transglutaminase 4 (TGM4)	Semen	0.05
Transcription elongation factor (TEF)	Housekeeping gene	0.05
Glucose-6-phosphate dehydrogenase (G6PDH)	Housekeeping gene	0.4
Ubiquitin conjugating enzyme (UCE)	Housekeeping gene	0.25
L. crispatus (CRIS)	Vaginal fluid	0.2
L. gasseri (GASS)	Vaginal fluid	0.05

Results

Table 2
Concentration of DNA and RNA extracted from each body fluid in duplicate using the modified DNA IQ™ manual method compared to those obtained with the organic method (average values).

Body fluid	Promega DNA IQ™ system extraction		Organic extraction	
	RNA concentration (ng/μL)	DNA concentration (ng/μL)	RNA concentration (ng/μL)	DNA concentration (ng/μL)
Blood (1 μL)	0.045	0.225	0.032	0.030
Semen (1 μL)	3.625	5.335	3.025	7.995
Saliva (10 μL)	0.051	3.48	0.090	6.345

Results

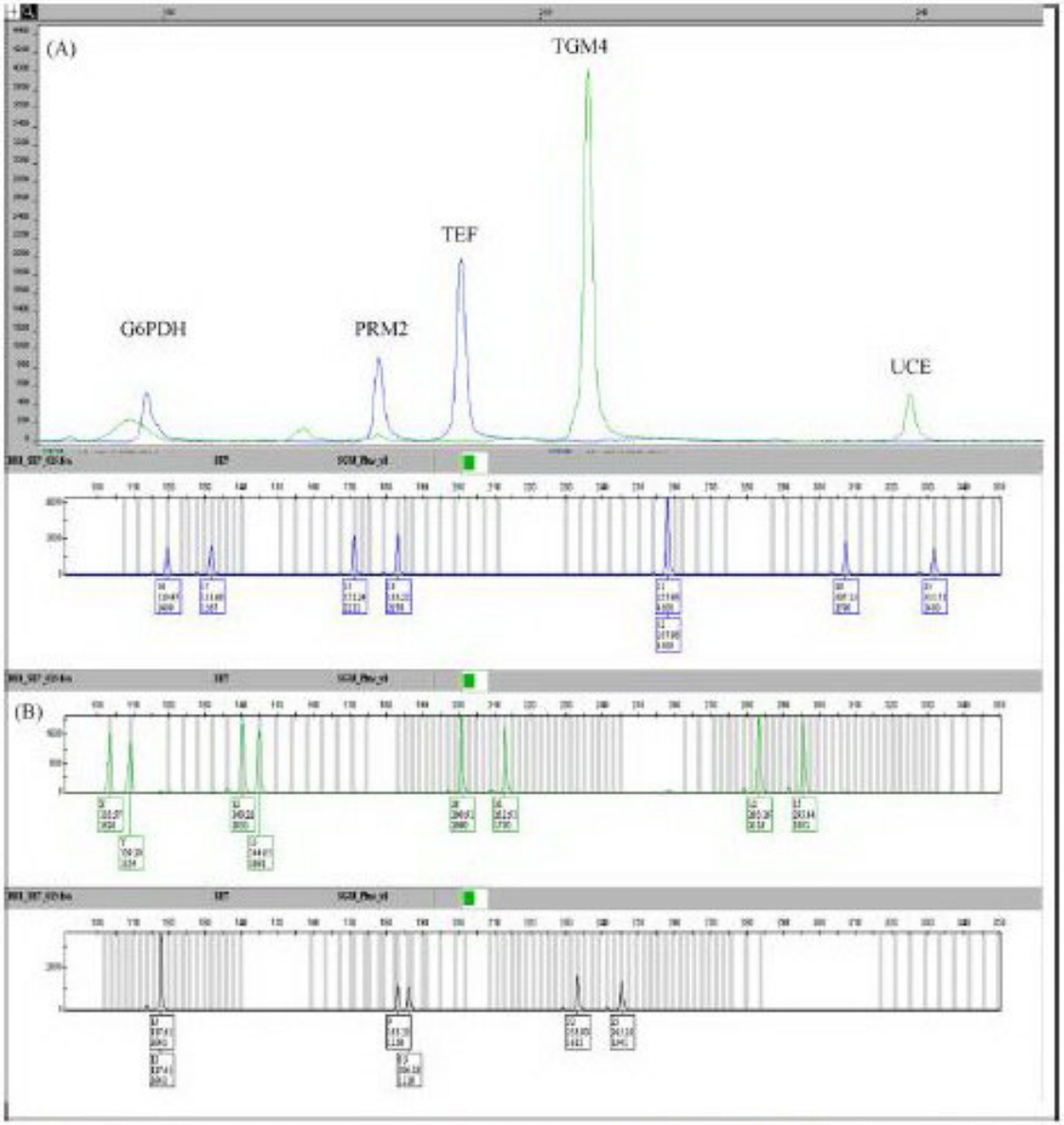


Fig. 2. (A) mRNA multiplex profile from a 10 μl semen sample. (B) Corresponding AmpFESTR® SGM Plus® DNA profile from the 10 μl semen sample.

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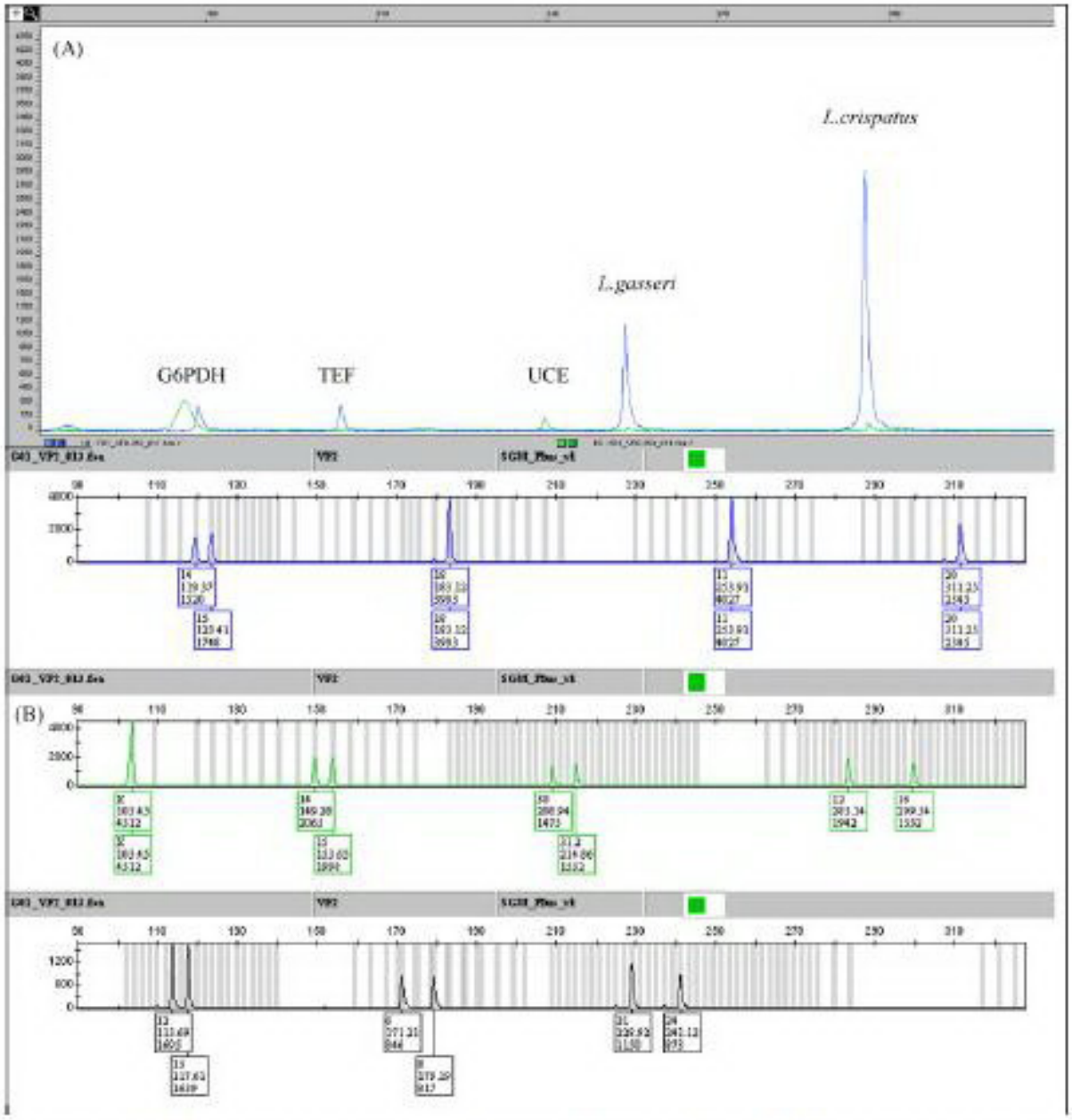
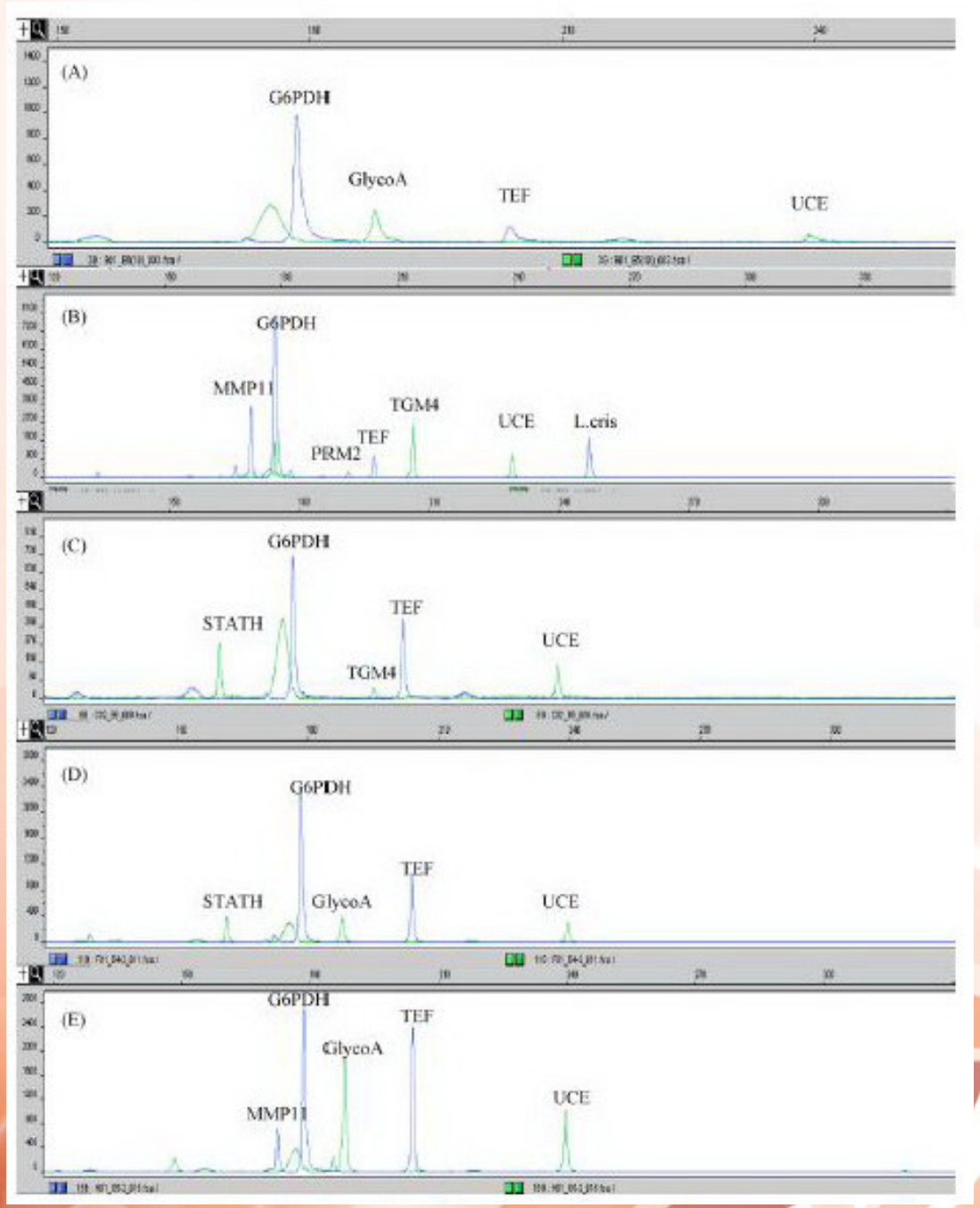


Fig. 3. (A) mRNA multiplex profile from a vaginal swab. (B) Corresponding AmpFISTR® SGM Plus® DNA profile from a vaginal swab.

Results


(A)
G6PDH
GlycoA
TEF
UCE
(B)
G6PDH
MMP11
TGM4
UCE
L.cris
PRM2
TEF
(C)
G6PDH
STATH
TEF
UCE
TGM4
(D)
G6PDH
TEF
STATH
GlycoA
UCE
(E)
G6PDH
TEF
GlycoA
UCE
MMP11

Conclusion

- มีประสิทธิภาพไม่แตกต่างกับ organic extraction method
- ไม่ต้องแบ่งตัวอย่างที่เก็บมาได้ แยกกันวิเคราะห์ระหว่าง RNA และ DNA เหมือนกับ organic extraction method
- พัฒนาการใช้ mRNA ที่ได้จากการสกัด DNA มาประยุกต์ใช้ในงานด้านนิติวิทยาศาสตร์


Thank you