JACOBS PUBLISHERS

OPEN ACCESS

Jacobs Journal of Forensic Science

*Research Article*

# Differential Extraction of Sperm Mixtures Deconvolutes the Mixture

Lay-Hong Seah[1*], Boon-Hui Wee[2]

[1]*Department of Chemistry Malaysia, Kuching Branch, Lot 3148, Block 14, Jalan Sultan Tengah, 93050 Petra Jaya, Kuching, Malaysia*

**Corresponding author: Dr. Lay-Hong Seah, Department of Chemistry Malaysia, Kuching Branch, Lot 3148, Block 14, Jalan Sultan Tengah, 93050 Petra Jaya, Kuching, Malaysia, Tel: (60)19-6684813; Email: lhseah@kimia.gov.my*

*Received:* 05-18-2016

*Accepted:* 09-09-2016

*Published:* 10-06-2016



## Abstract

Differential extraction of semen stains is intended to separate the epithelial DNA from the sperm DNA into the non-sperm and sperm extracts respectively. However, the separation profiles of stains containing entirely of sperm mixtures (as usually encountered in gang-rape) are determined by the proportion of the sperm DNA contributed by the respective sources in the mixtures. Experiments with controlled amounts of sperm mixtures on cloth and FTA cards demonstrated the DNA of the major sperm contributor persisted in the sperm extract and the DNA of the minor sperm contributor(s) is usually detected only in the non-sperm extract. This phenomenon was not observed in mixtures of other cell types. Differential extraction of sperm mixtures thus results in deconvolution of the mixture allowing the minor sperm contributor to be identified in the non-sperm extract. The interpretation of results from differential extraction of semen mixtures particularly in inferring cell-origin thus requires a careful consideration of the derived separation profiles. The relevance of the evidence and the probative value of the DNA profile are important issues in the evaluation of forensic evidence from sperm mixtures.

**Keywords:** Differential Extraction; Sperm Mixtures; Deconvolute; Gang-Rape; DNA

## Introduction

The differential extraction procedure first described by Gill et al. [1] refers to the process by which the DNA from epithelial cells and sperm cells can be separated into different fractions. Compared with other methods such as flow cytometry [2] and laser microdissection [3, 4], differential extraction is relatively quick, effective and low-cost [5,6,7].

In the investigation of cases of sexual assault, semen stains on vaginal swabs and clothing or bedding items provide the most incriminating evidence. The elucidation of the perpetrator or contributor by DNA analysis is most often straightforward in a single-rape scenario. However, in a gang-rape situation the elucidation process becomes more complex due to the presence of multiple DNA contributors in the semen stains [8,9,10]. Semen stains on clothing or bedding items in a gang-rape could invariably consist entirely of sperm DNA from multiple perpetrators.

When differential extraction is performed on these stains, the DNA differential profiles in both epithelial and sperm fractions would hence be of sperm origin.

In this study, semen from different sources was separately dried on cloth and FTA cards to mimic casework stains. FTA cards are essentially cellulose paper with protective matrix to archive DNA [11]. Differential extraction was carried out on imbalanced mixtures of sperm with an excess of DNA from one source, purportedly the major contributor. The differential profiles were analyzed to determine the separation patterns.

In addition, liquid blood and buccal cells from different sources were collected and dried in similar manner for comparison of separation patterns from other cell types.

## Materials and Methods

The differential extraction protocol was carried out using a one-tenth volume of 10 mg/ml Proteinase K for the non-sperm extract and a one-tenth volume of 0.39 M DTT (dithiothreitol) for the sperm extract.

Semen from three voluntary donors were stained separately on white polyester-viscose cloth and FTA cards. Organic extraction was carried out on 1.2-mm and 2-mm disc punches

of the semen-stained cloth and FTA cards. The DNA was then quantified using the ABI Quantifiler® Human DNA Quantification Kit.

Six different sperm mixtures were then set up (see Table 1) comprising of semen on cloth as well as FTA cards. Each mixture is represented by a major and minor(s) contributors where DNA estimates were from quantification of discs punched from the semen-stained cloth and FTA cards.

| Marking | Semen Mixture |
|---|---|
| SSM1_1 | 1.2 mm disc [M1(a)] + 2 mm disc [M2(a)] |
| SSM1_1 | 107 ng DNA [M1(a)]+ 9 ng DNA [M2(a)]<br>107 ng DNA [M1(a)]+ 9 ng DNA [M2(a)]<br>Major [M1(a)] 12 : Minor [M2(a)] 1 |
| SSM1_2 | 1.2 mm disc [M1(a)] + Two 2 mm disc [M2(a)] |
| SSM1_2 | 107 ng DNA [M1(a)] + 18 ng DNA [M2(a)]<br>107 ng DNA [M1(a)] + 18 ng DNA [M2(a)]<br>Major [M1(a)] 6 : Minor [M2(a)] 1 |
| SSM1_3 | 1.2 mm disc [M1(a)] + 2 mm disc [M3(a)] |
| SSM1_3 | 107 ng DNA [M1(a)] + 8 ng DNA [M3(a)]<br>107 ng DNA [M1(a)] + 8 ng DNA [M3(a)]<br>Major [M1(a)]13 : Minor [M3(a)] 1 |
| SSM1_4 | 1.2 mm disc [M1(a)] + Two 2 mm disc [M3(a)] |
| SSM1_4 | 107 ng DNA [M1(a)] + 16 ng DNA [M3(a)]<br>107 ng DNA [M1(a)] + 16 ng DNA [M3(a)]<br>Major [M1(a)] 7 : Minor [M3(a)] 1 |
| SSM1_5 | 1.2 mm disc [M1] + 1.2 mm disc [M2(b)] + 1.2 mm disc [M3] |
| SSM1_5 | 280 ng DNA [M1] + 57 ng DNA [M2(b)] + 9 ng DNA [M3]<br>280 ng DNA [M1] + 57 ng DNA [M2(b)] + 9 ng DNA [M3]<br>Major [M1] 31 + Minor I[M2(b)] 6.3 + Minor II[M3] 1 |
| SSM1_6 | 1.2 mm disc [M1] + 1.2 mm disc [M2(b)] + 2 mm disc [M3] |
| SSM1_6 | 280 ng DNA [M1] + 57 ng [M2(b)] + 25 ng [M3]<br>280 ng DNA [M1] + 57 ng [M2(b)] + 25 ng [M3]<br>Major [M1] 11 + Minor I [M2(b)] 2.3 + Minor II [M3] 1 |

**Table 1.** Sperm mixtures comprising of a two-source major-minor on cloth in SSM1_1 to SSM1_4 and a three source major-minors on FTA cards in SSM1_5 to SSM1_6. DNA amounts are estimates only.

Differential extraction was carried out on the six mixtures. Template DNA (1 ng estimate) was amplified with AmpFlSTR®Identifiler® Plus amplification kit for 28 PCR cycles according to manufacturer's instructions. The amplicons were separated on an Applied Biosystems 3130xl Genetic Analyzer with CE injection at 3 kV/10 s and analyzed using GeneMapper ID v3.2.1 software. A detection threshold limit of 50 rfu was used to designate alleles. The experiment was repeated by another analyst.

| Marking | Semen mixture | Non-sperm extract | Sperm extract |
|---|---|---|---|
| SSM1_1 | [M1(a)]:[M2(a)] 12:1 | [M1(a)]+ [M2(a)]<br>[M1(a)] = major | [M1(a)] dominant<br>[M2(a)] not reportable |
| SSM1_2 | [M1(a)]:[M2(a)] 6:1 | [M1(a)]+ [M2(a)]<br>[M1(a)] = minor | [M1(a)] dominant<br>[M2(a)] not reportable |
| SSM1_3 | [M1(a)] :[M3(a)] 13:1 | [M1(a)]+ [M3(a)]<br>[M1(a)] = minor | [M1(a)] dominant<br>[M3(a)] not reportable |
| SSM1_4 | [M1(a)] :[M3(a)] 7:1 | [M1(a)]+ [M3(a)]<br>[M1(a)] = minor | [M1(a)] dominant<br>[M3(a)] not reportable |
| SSM1_5 | [M1] :[M2(b)] :[M3] 13 :6.3 :1 | [M1] +[M2(b)] +[M3] | [M1] major<br>[M2(b)] minor<br>[M3] not reportable |
| SSM1_6 | [M1] :[M2(b)] :[M3] 11 : 2.3 : 1 | [M1] +[M2(b)] +[M3]<br>[M3] = Major | [M1] major<br>[M2(b)] not reportable<br>[M3] not reportable |

**Table 2.** Results from differential extraction of semen mixtures

In a supplementary experiment, liquid blood from seven donors was stained separately on FTA cards. Organic extraction was carried out on 1.2-mm disc punches of the blood-stained FTA cards. The DNA was then quantified using the ABI Quantifiler® Human DNA Quantification Kit. Six different two-source mixtures were then set up comprising of a major and a minor contributor (see Table 3) from the blood-stained FTA cards. Differential extraction was then carried out on these mixtures. Amplification and Genetic Analyzer run conditions were as described above.

In another supplementary experiment, buccal cells collected from ten donors was stained separately on both cloth and FTA cards. The extraction and analysis procedures were as outlined for semen and blood. Eight different two-source mixtures were

| Marking | Blood cell mixture | Non-sperm extract | Sperm extract |
|---|---|---|---|
| BBM1_1 | [B2]:[B1] 13:1 | [B2] + [B1]<br>[B2] = major | [B2] + [B1]<br>[B2] = major<br>Stochastic |
| BBM1_2 | [B2]:[B3] 10:1 | [B2] + [B3]<br>[B2] = major | [B2] + [B3]<br>No clear major/minor<br>Stochastic |
| BBM1_3 | [B2]:[B4] 12:1 | [B2] + [B4]<br>[B2] = major | [B2] + [B4]<br>No clear major/minor<br>Stochastic |
| BBM1_4 | [B2]:[B5] 20:1 | [B2] + [B5]<br>[B2] = major | [B2] + [B5]<br>No clear major/minor<br>Stochastic |
| BBM1_5 | [B2]:[B6] 7:1 | [B2] + [B6]<br>No clear major/minor | Allele dropouts ++<br>Stochastic |
| BBM1_6 | [B2]:[B7] 5:1 | [B2] + [B7]<br>No clear major/minor | Allele dropouts ++<br>Stochastic |

**Table 3.** Results from differential extraction of two-source major-minor blood cell mixtures.

then set up comprising of a major and a minor contributor (see Table 4) from the buccal cells stained on cloth and FTA cards.

## Results and Discussion

This study was undertaken to assess the effect of differential DNA extraction on sperm mixtures and to determine the genotypes and sources of the DNA in the non-sperm and sperm extracts respectively.

In the first four two-source sperm mixtures on cloth comprising of one major and one minor contributor (see Table 2) with major:minor ratio ranging from 6:1 to 13:1, the differential DNA extraction results revealed the major contributor to persist in the sperm fraction. The DNA profile of the minor contributor was detected and could only be read in the non-sperm fraction. In the three-source sperm mixtures on FTA cards (SSM1_5 and SSM1_6 in Table 2), similar separation patterns was observed. The major contributor persists in the sperm fraction; the minor peaks in the sperm fraction are mostly below the reporting threshold of 100 rfu. The other two minor contributors could only be read in the non-sperm fraction. The experiment was duplicated with a different sperm source as the major contributor. The differential profiles revealed similar separation patterns (results not shown).

This is an interesting phenomenon. It is generally accepted that the autosomal DNA characteristics of the minor contributor of a DNA mixture cannot be detected when ratios exceed 1:10 to 1:20 [12,13]. Therefore, the minor male contributor in a sperm mixture could not have been distinguished without differential extraction. There appears to be preferential extraction of the minor contributor in the non-sperm extract leading to unmasking of the minor contributor.

To investigate whether the cause of this preferential extraction was purely quantitative, supplementary experiments were car

| Marking | Buccal mixture | Non-sperm extract | Sperm extract |
|---|---|---|---|
| UUM1_1 | [U1(a)]:[U2(a)] 300:1 | U1(a)<br>U2(a)=ND | U1(a)<br>U2(a)=ND |
| UUM1_2 | [U1(a)]:[U3(a)] 16:1 | U1(a)<br>U3(a)=ND | U1(a)<br>U3(a)=ND |
| UUM1_3 | [U1(a)]: [U4(a)] 9:1 | U1)a)<br>U4(a)=ND | U1(a)<br>U4(a)=ND |
| UUM1_4 | [U1(a)]:[U5(a)] 14:1 | U1(a)<br>U5(a)=ND | U1(a)<br>U5(a)=ND |
| UUM1_5 | [U6(b)]:[U7(b)] 2.6:1 | U6(b) + U7(b) | U6(b) + U7(b) |
| UUM1_6 | [U6(b)]:[U8(b)] 3.9:1 | U6(b) + U8(b) | U6(b) + U8(b) |
| UUM1_7 | [U6(b)]:[U9(b)] 4:1 | U6(b) + U9(b)<br>U6(b)=major | U6(b) + U9(b)<br>U6(b)=major |
| UUM1_8 | [U6(b)]:[U10(b)] 3.2:1 | U6(b) + U10(b)<br>U6(b)=major | U6(b) + U10(b)<br>U6(b)=major |

**Table 4.** Results from differential extraction of two-source major-minor epithelial (buccal) cell mixtures

ried out with two-source major-minor blood cell mixtures and epithelial (buccal) cell mixtures. After differential extraction (see Table 3), blood cell mixtures with major-minor ratio exceeding 10:1 exhibited mixed profiles in both the non-sperm and sperm extracts. The major contributor predominates only in the non-sperm extracts, but the sperm extracts showed no clear major-minor contributors, probably caused by loss of DNA from differential extraction [13]. Mixtures with major-minor ratios less than 10:1 display no distinct major contributor in either non-sperm or sperm extracts.

With the two-source epithelial (buccal) cell mixtures, the minor contributor was not detected in both sperm and non-sperm extracts with major-minor ratios exceeding 9:1 consistent with the observation in the study of Vuichard et al. [10,13]. With lower major-minor ratios (UUM1_5 to UUM1_8 in Table 4) mixed profiles were detected in both non-sperm and sperm extracts.

Clearly, the preferential extraction of DNA from the minor contributor in a DNA mixture during differential extraction is dependent on cell type. This phenomenon seen with sperm cells does not occur with DNA from blood cells as well as from epithelial buccal cells. It is thus postulated that the observed separation patterns for major-minor sperm mixtures is dependent on the relative proportions of sperm DNA contributed by the respective sources as well as on the biochemical properties of sperm cells. Further work is required to investigate the biochemical properties of semen to explain the separation patterns of major-minor sperm mixtures.

## Conclusion

Differential extraction could provide a useful means for separation of a two-source major-minor sperm mixture. The deconvolution of the sperm mixture by differential extraction offers an approach for 'capture' of the minor sperm source allowing identification. Forgoing the differential extraction technique for semen mixtures could result in either the minor contributor being masked up by the major contributor with high major-minor ratios or in obtaining mixed profiles leading to complexity in interpretation [14].

Interpretation of sperm mixtures commonly encountered in gang-rape thus requires a careful consideration of the separation profiles derived from differential extraction. The detection of the minor sperm contributor in the non-sperm extract impacts on inferring cell-origin of DNA in the non-sperm extract. Complementarily, methods such as high throughput DNA sequencing or analysis of SNPs panels [15], which allow access to the minor component of unbalanced DNA mixtures, may in the future help improve the success rate of DNA mixture analysis.

**Acknowledgement**

My special thanks to the staff of the Kuching DNA Laboratory for technical assistance and support. This project is funded by Ministry of Science, Technology and Innovation Malaysia ScienceFund Project No: 02-05-10-SF0009.

## References

1. Gill P, Jeffreys AJ, Werrett DJ. Forensic application of DNA 'fingerprints'. Nature. 1985, 318(6046): 577-579.

2. Schoell WM, Klintschar M, Mirhashemi R, Pertl B. Separation of sperm and vaginal cells with flow cytometry for DNA typing after sexual assault. Obstet Gynecol. 1999, 94(4): 623-627.

3. Murray C, McAlister C, Elliott K. Identification and isolation of male cells using fluorescence in situ hybridization and laser microdissection, for use in the investigation of sexual assault. Forensic Sci Int Genet. 2007, 1(3-4): 247-252.

4. Sanders CT, Sanchez N, Ballantyne J, Peterson DA. Laser microdissection separation of pure spermatozoa from epithelial cells for short tandem repeat analysis. J Forensic Sci. 2006, 51(4): 748-757.

5. Yoshida K, Sekiguchi K, Mizuno N, Kasai K, Sakai I. The modified method of two-step differential extraction of sperm and vaginal epithelial cell DNA from vaginal fluid mixed with semen. Forensic Sci Int. 1995, 72(1): 25-33.

6. Buoncristiani MR, Timken MD. Development of a procedure for Dielectrophoretic (DEP) Separation of sperm and epithelial cells for application to sexual assault evidence, (2009). Submitted to U.S. Department of Justice.

7. Butler JM. DNA Extraction, In Fundamentals of Forensic DNA Typing, San Diego, Academic Press (2010), 99-109.

8. Dror IE, Hampikian G. Subjectivity and bias in forensic DNA mixture interpretation. Sci Justice. 2011, 51(4): 204-208.

9. Rudin N, Inman K. An Introduction to Forensic DNA Analysis, 2nd ed. USA, CRC PRESS (2002), 87-98.

10. Montesino M, Salas A, Crespillo M, Albarrán C, Alonso A et al. Analysis of body fluid mixtures by mtDNA sequencing: An inter-laboratory study of the GEP-ISFG Working Group. Forensic Sci Int. 2007, 168(1): 42-56.

11. Seah LH, Burgoyne LA. DNA Archiving on FTA paper: Photosensitizer-initiated attacks as models of aging. Progress in Forensic Genetics. 2003, 9: 689-697.

12. Cerri N, Ricci U, Sani I, Verzeletti A, De Ferrari F. Mixed stains from sexual assault cases: autosomal or Y-chromosome short tandem repeats? Croat Med J. 2003, 44(3): 289-292.

13. Vuichard S, Borer U, Bottinelli M, Cossu C, Malik N. Differential DNA extraction of challenging simulated sexual-assault samples: a Swiss collaborative study. Investig Genet. 2011, 2: 11.

14. Thompson WC, Taroni F, Aitken CG. How the probability of a false positive affects the value of DNA evidence. J Forensic Sci. 2003, 48(1): 47-54.

15. Voskoboinik L, Darvasi A. Forensic identification of an individual in complex DNA mixtures. Forensic Sci Int Genet. 2011, 5(5): 428-435.