

Evaluation of OpiumPlex for Genetic Profiling of Opium Poppy

Greta Hundtofte, BS, Sara Willer, MS, Rachel Houston, PhD

Department of Forensic Science, Sam Houston State University, Huntsville, TX 77340

INTRODUCTION

Papaver somniferum L. (opium poppy) produces narcotic alkaloids including morphine, codeine, and thebaine, which are used medicinally but also serve as precursors for illicit drugs such as heroin. Although synthetic opioids now dominate drug markets, restrictions on precursor chemicals have contributed to renewed heroin production, emphasizing the need for reliable source attribution of natural opiates. Conventional analytical methods, such as GC-MS and HPLC-MS, are effective for chemical identification but have limited utility for source attribution due to environmental and processing effects. Genetic profiling provides improved resolution for differentiating plant varieties. Early DNA-based approaches using mitochondrial and chloroplast barcodes lacked sufficient intra- and inter-species discrimination, while the publication of the opium poppy genome in 2018 enabled the development of STR-based markers [2]. In 2021, Vašek *et al.* introduced OpiumPlex, a 27-locus STR system that successfully discriminated 30 of 36 *P. somniferum* varieties [1]. While promising, this system requires validation prior to forensic implementation. This study evaluates and optimizes one OpiumPlex multiplex following SWGDAM guidelines for internal STR validation [3], supporting the application of genetic profiling for source attribution and tracking of illicit opium poppy material.

MATERIALS & METHODS

SAMPLE COLLECTION: POPPY SEEDS FROM 20 BRANDS

DNA EXTRACTION: DNeasy Plant Mini Kit

QUANTIFICATION: Qubit™ 1x dsDNA HS Assay Kit

RESULTS & DISCUSSION

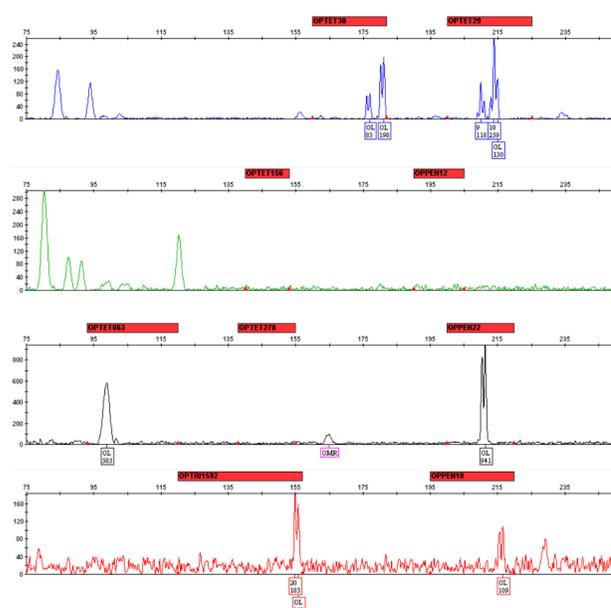


Figure 1. Example EPG using the original OpiumPlex parameters by Vašek *et al.* [1]

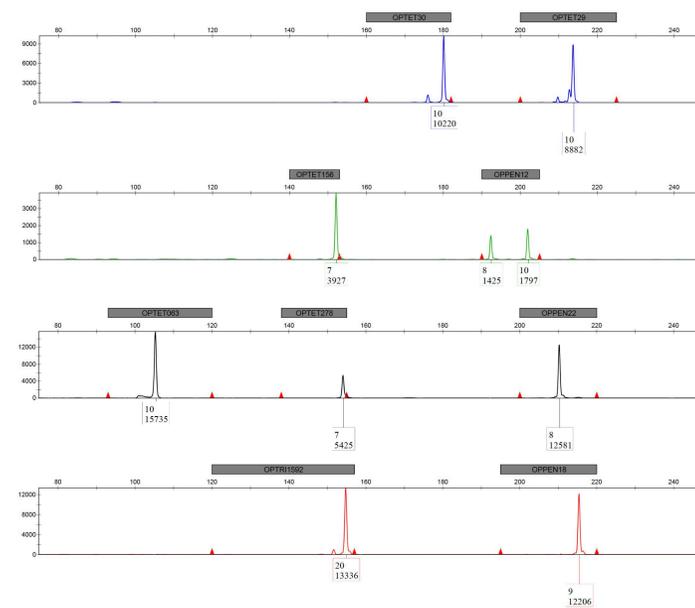


Figure 2. Multiplex profile of the first nine OpiumPlex loci using 0.5 ng of template DNA, using optimized PCR and CE parameters.

Table 1. Characteristics of the first nine OpiumPlex STR markers.

Marker	Dye	Repeat Motif	Observed Alleles	Annealing Temperature (°C)	1X Primer Concentration (µM)
OPTET030	6FAM	TCTA	6,10,10del	61.0	0.2
OPTET029	6FAM	AATG	8,9,10	63.1	0.2
OPTET156	VIC	AAAT	5,6,7	55.8	0.2
OPPEN12	VIC	AAGAA	8,10	63.1	0.1
OPTET063	NED	(CTTT) _x (CTTC) ₁	10,11,12	63.1	0.3
OPTET278	NED	TTCA	7	58.5	0.3
OPPEN22	NED	TTTGT	8,9	64.0	0.1
OPTR1592	PET	(CTT) _x (CTC) ₂ (CTT) _x (CTC) ₁ (CTT) ₂ (CTC) ₁	14,18,20	64.0	0.3
OPPEN18	PET	(CAAAA) _x (TAAAA) ₁	7,9	64.0	0.3

Table 2. Sample origins of twenty *P. somniferum* samples grouped by distinct genotype.

Genotype	A	B	C	D	E	F
Origin	Spain	UK	Holland	Turkey	Canada	South Asia
	Spain	UK	Holland	Turkey		
	Tasmania	UK	USA (Ohio)			
	Afghanistan	England	Unknown			
	Afghanistan	Unknown				
	Unknown					
	Unknown					

- * Electropherograms using the published OpiumPlex parameters exhibited suboptimal morphology (Fig. 1).
- * Electropherogram quality improved using PCR parameters from the Type-it Microsatellite PCR kit with a 60-minute extension time (Fig. 2).
- * Six distinct genotypes were observed across twenty different *P. somniferum* L. samples (Table 2).

REFERENCES

1. Vašek J, Čilová D, Melounová M, Svoboda P, Zdeňková K, Čermáková E, *et al.* OpiumPlex is a novel microsatellite system for profiling opium poppy (*Papaver somniferum* L.). *Scientific Reports*. 2021;11:12799. <https://doi.org/10.1038/s41598-021-91962-1>.
2. Guo L, Winzer T, Yang X, Li Y, Ning Z, He Z, *et al.* The opium poppy genome and morphinan production. *Science*. 2018;362(6412):343–7. <https://doi.org/10.1126/science.aat4096>.
3. SWGDAM. Validation Guidelines for DNA Analysis Methods. 2016.

MATERIALS & METHODS

MARKER SELECTION: OpiumPlex [1] Multiplex 1

SINGLEPLEX ANNEALING TEMPERATURE DETERMINATION

MULTIPLEX ASSAY OPTIMIZATION

PCR PARAMETERS

CE PARAMETERS

ALLELE SEQUENCING

ALLELIC LADDER DESIGN

INTERNAL VALIDATION

PRECISION & ACCURACY

POPULATION STUDIES

SPECIFICITY

SENSITIVITY

CONCLUSIONS

- * The parameters by Vašek *et al.* were not replicable (Fig. 1) and not suitable for use in forensic laboratories.
- * Optimized parameters for multiplex 1 of OpiumPlex provided full STR profiles for opium poppy seed DNA extracts (Fig. 2).
- * 22 alleles across 9 STR loci in Multiplex 1 were shown in 20 poppy seed samples (Table 1).
- * Six different genotypes were identified for 20 poppy seed samples, showing the ability to differentiate by origin country (Table 2).

ACKNOWLEDGEMENTS

I would like to extend my appreciation to Sara Willer, Dr. Rachel Houston, and the Department of Forensic Science at Sam Houston State University.



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