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Recovery of Human DNA from Maggots

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Forensic Entomology

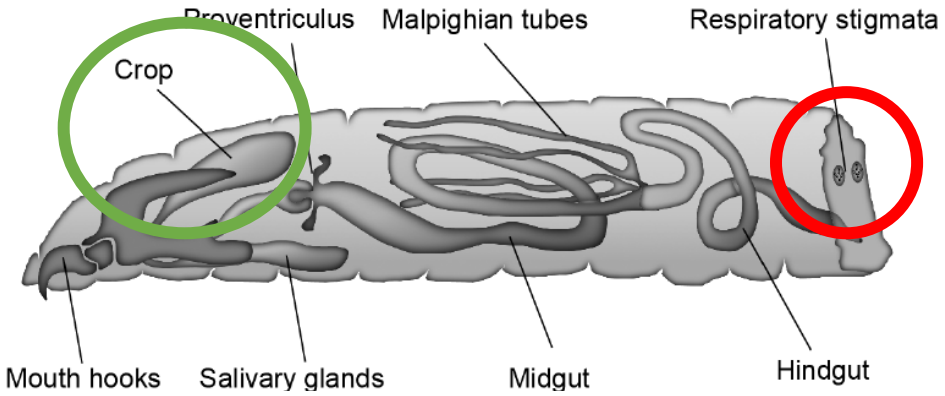
- Application of insects to criminal and civil investigations
- Post-mortem Interval (PMI)
  - Insect Succession
  - Accumulated Degree Hour (ADH)
- DNA Analysis
  - Insect Species Identification
  - **Human DNA**[1,2]

Forensic Entomology (Student Magazine Illustration). Oguchi Anyaele. (n.d.). https://oguchiart.artstation.com/projects/3oLm22 (accessed February 5, 2021).

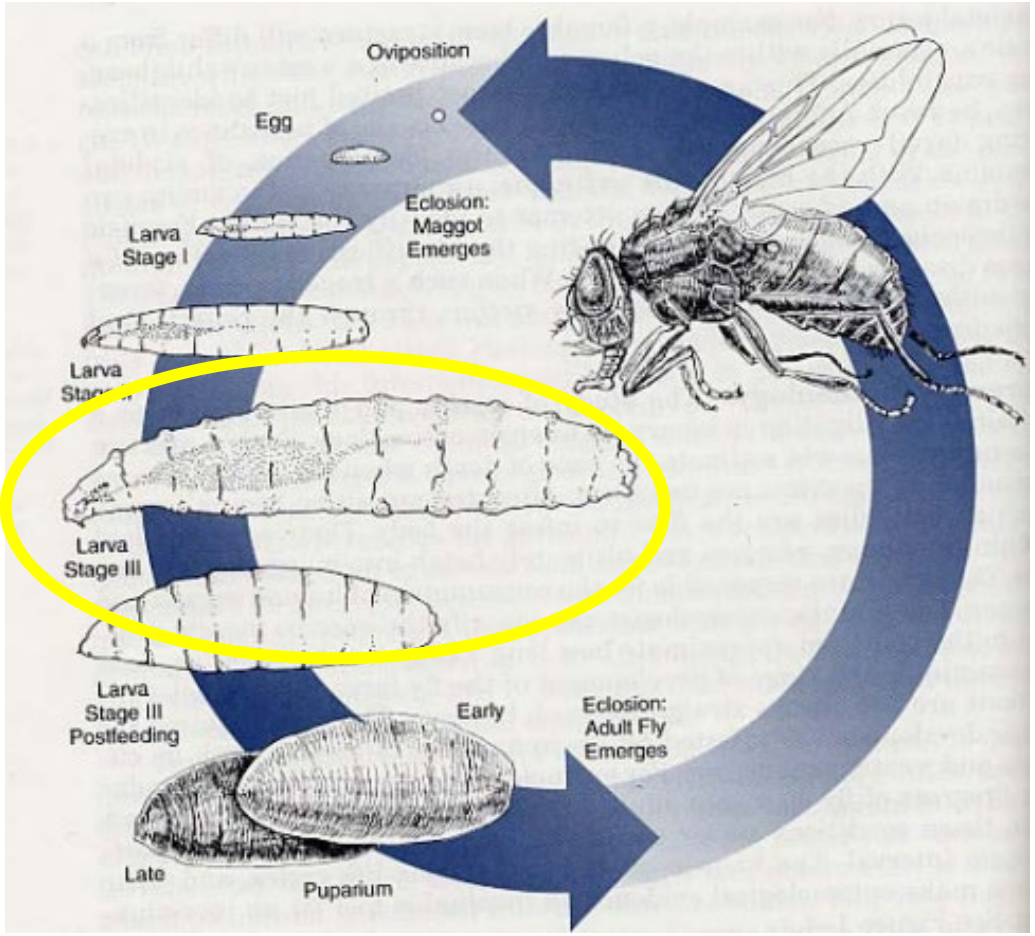
O'Brien, Jennifer. "Smell That? It's Forensic Entomology At The Body Farm." Science Friday, https://www.sciencefriday.com/educational-resources/forensic-entomology-body-farm/. Accessed 3 Nov. 2020.

Fly Larvae

- Family Calliphoridae
  - Blow flies
  - Forensically relevant
- Morphological Identification
  - Posterior spiracles
- Crop: food storage with little to no digestion



A. Baumann, New tools for maggot debridement therapy research: From the establishment of qRT-PCR to the characterization of Lucilia sericata Urate Oxidase, (n.d.) 90.



Cannon K, Cannon M. Analysis of Entomological Specimens Recovered Under Ancient Bison Carcasses and Recent Dead Large Animal Carrion from the Salmon River Bison Jump Area in Eastern Idaho June, 2007. 2015.

Recovery of human DNA from maggots

- Possible to recover human DNA from maggots[1-9]
  - Criminal cases in China and Mexico[4,10]
- Uses:
  - Identification of a victim
  - Link deceased to a scene[11]
- Studies have shown:
  1. Crop preferred[2]
  2. Preservation method[5]
  3. Third instar maggots[2]
  4. Purification of extract[12]

Goal: Determine best extraction method and forensic genotyping methods for analysis of human DNA recovered from a maggot

Methods

Collection and Dissection

Southeast Texas Applied Forensic Science (STAFS) Facility

DNA Extraction

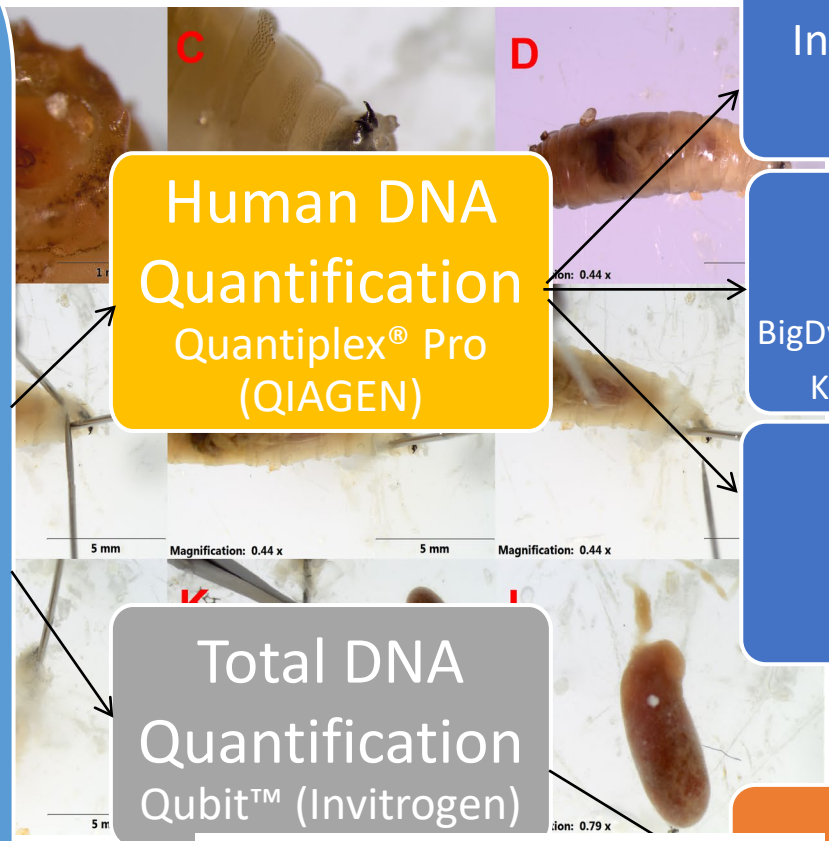
DNeasy® Powersoil® Pro (QIAGEN) (n=10)
Crops and Whole Fly Larvae

EZ2™ DNA Investigator® (QIAGEN) (n=10)

QIAcube® Connect (QIAGEN) (n=10)

Postfeeding (n=5)
Whole Fly Larvae

Burned (n=5)



Human DNA Quantification

Quantiplex® Pro (QIAGEN)

Total DNA Quantification

Qubit™ (Invitrogen)

STR

Investigator® 24plex (QIAGEN)

mtDNA

HV1 and HV2
BigDye Direct Cycle Sequencing Kit (Applied Biosystems)

NGS

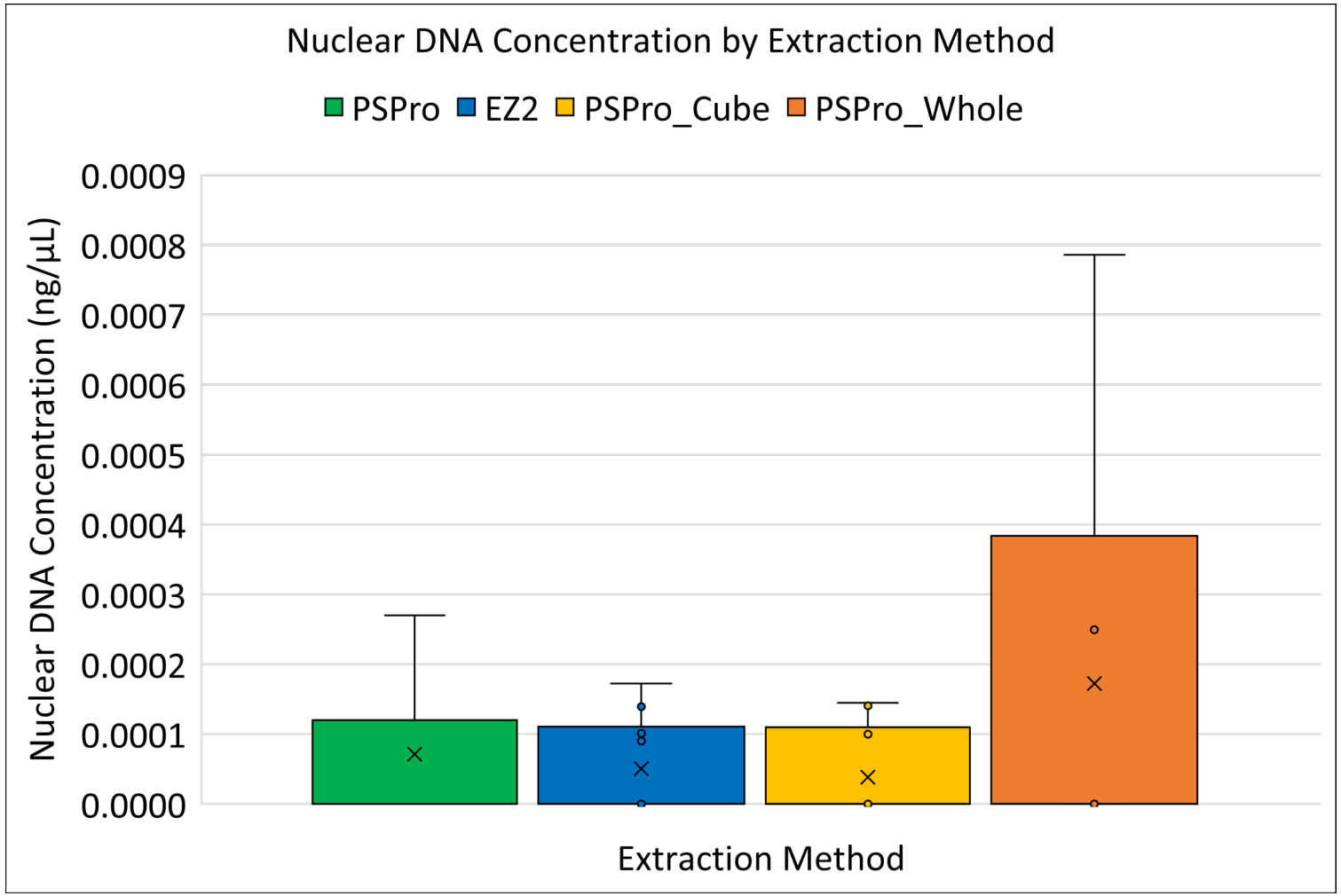
Mainstay SE (Verogen)

DNA Barcoding

COI

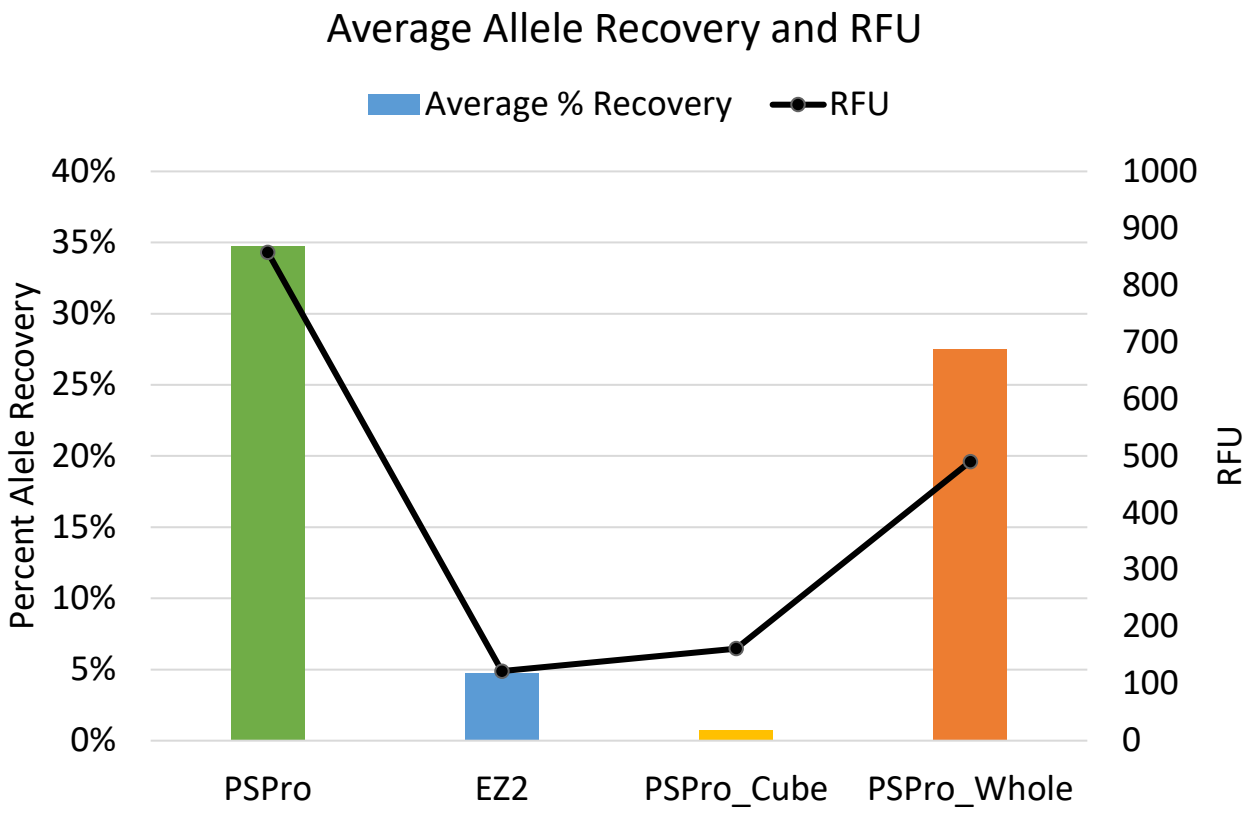
Cantu C, Bucheli S, Houston R. Comparison of DNA extraction techniques for the recovery of bovine DNA from fly larvae crops. JFS 2022;67(4):1651–9. https://doi.org/10.1111/1556-4029.15010.

Extraction Method Results



- No significant difference between human DNA recovered
- Powersoil Pro with manual purification was more consistent in recovering human DNA
- No trend observed between crop weight (g) and DNA concentration[2]

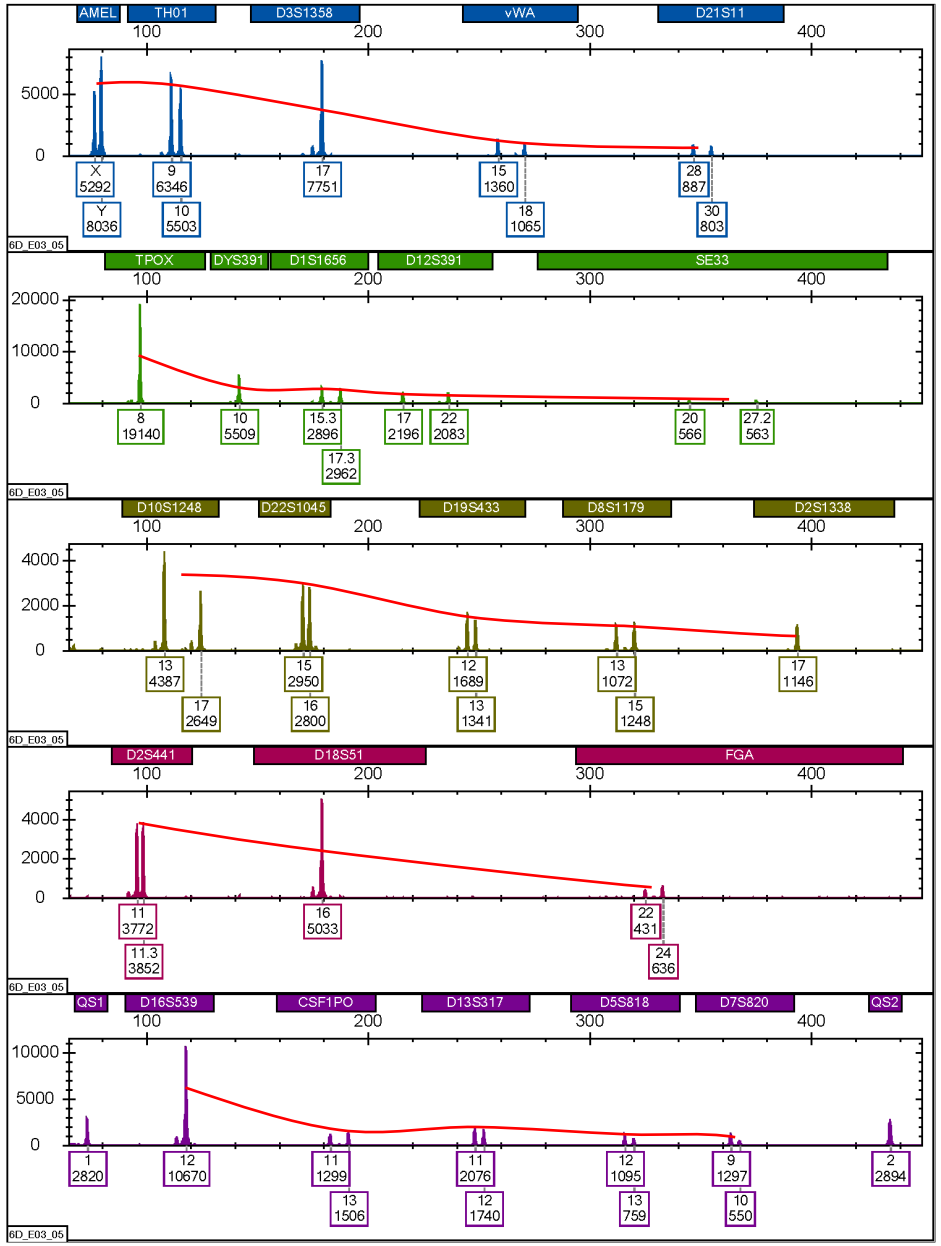
STR Profiling Success

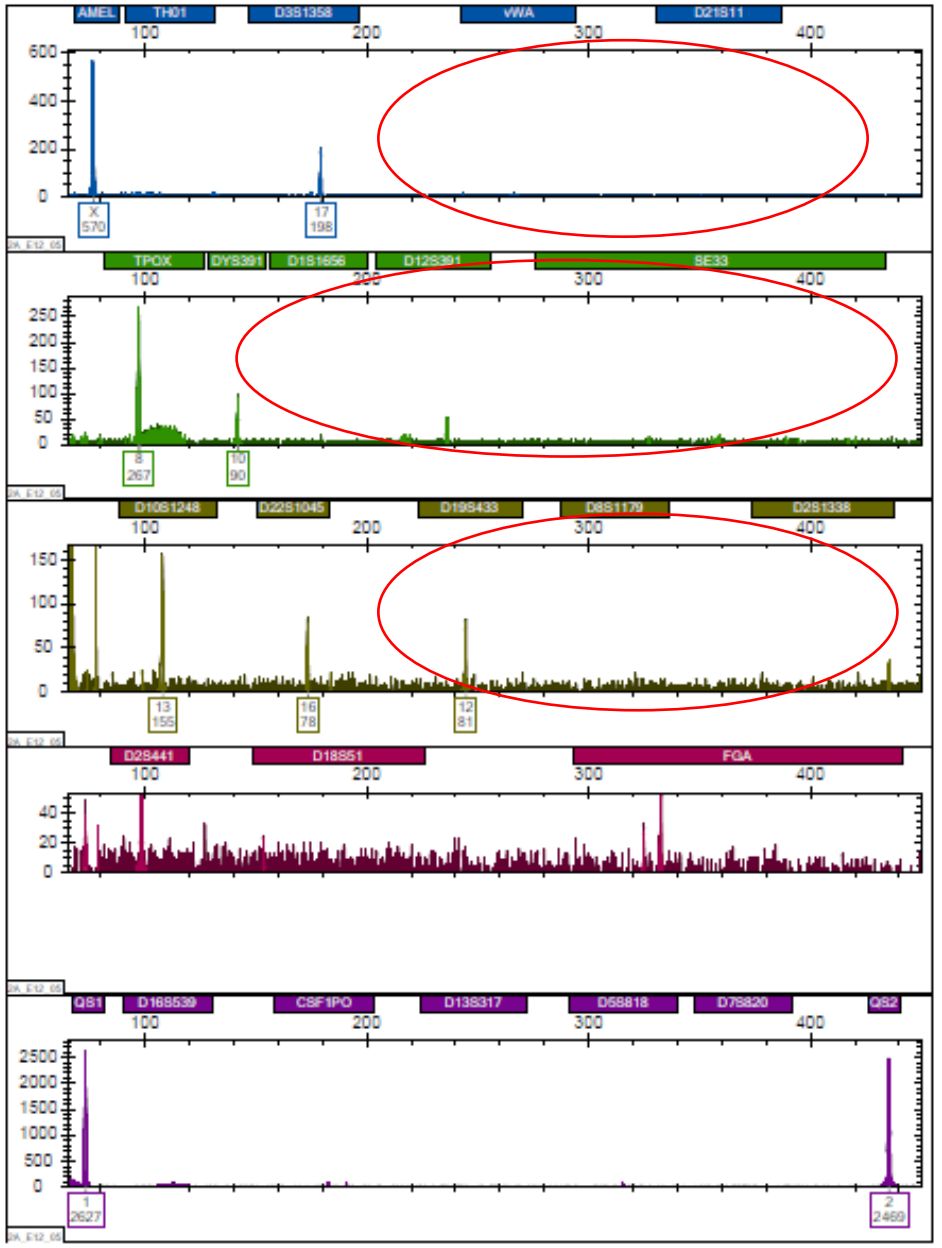


- CODIS eligible samples produced by PowerSoil Pro extractions
  - Crop, Whole, and Post-feeding samples
- Automation could be detrimental to human DNA recovery
- Degradation
  - Consumption by fly larvae and decomposition

STR Profiling Success

Full Profile
Powersoil Pro





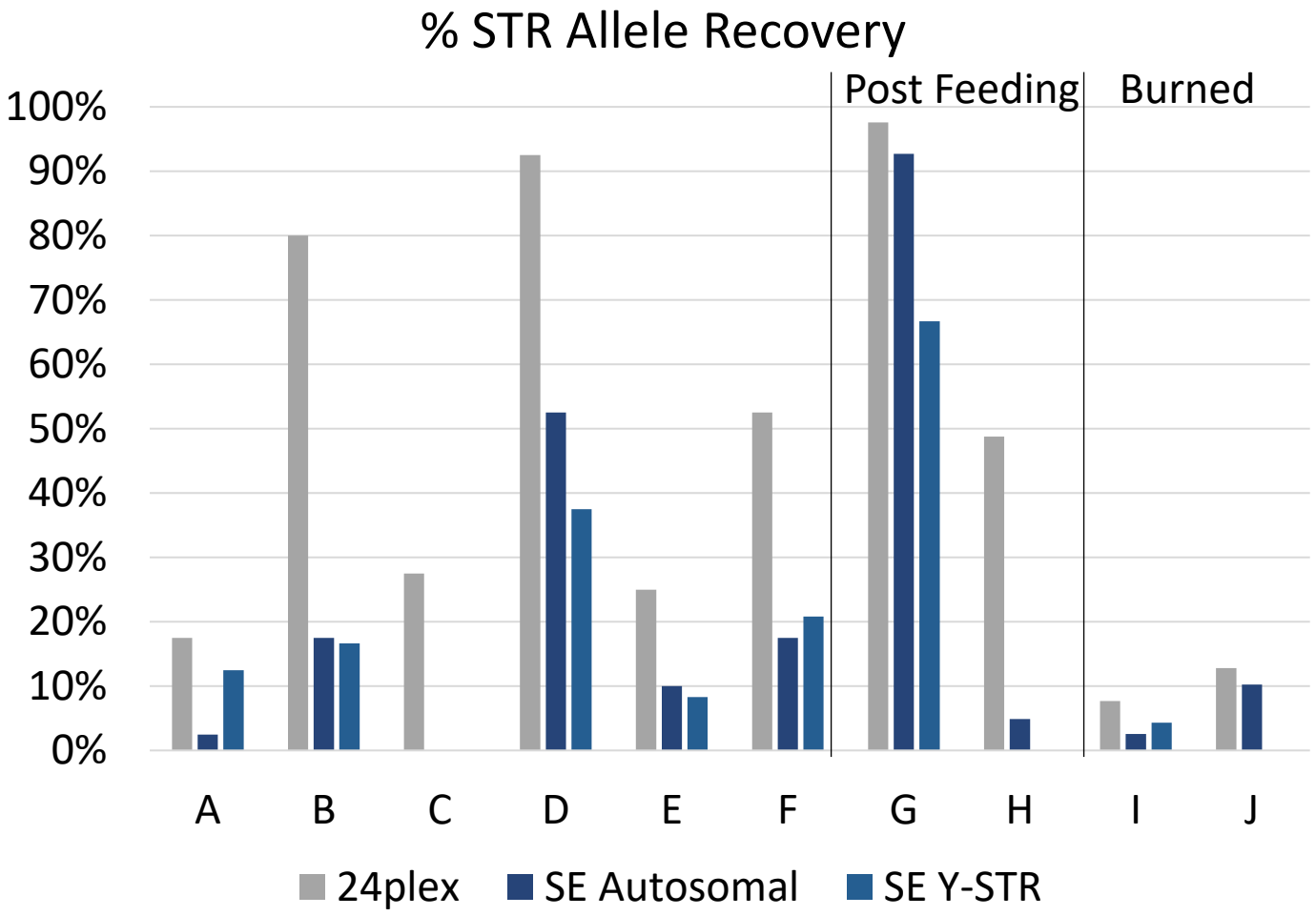
Partial Profile
Powersoil Pro

mtDNA

Sample	Extraction	Type	Small Target Quant	% STR Allele Recovery	Haplotype
1	PSPro	Crop	0.0001	2.5	U4b1+146+152
2	PSPro	Crop	0.0011	80	U4b1+146+152
3	PSPro	Crop	0.0003	27.5	U4b1+146+152
4	PSPro	Crop	0.0000	2.5	U4
5	EZ2	Crop	0.0001	0	U4b1+146+152
6	EZ2	Crop	0.0000	7.5	U4b1+146+152
7	EZ2	Crop	0.0002	27.5	U4b1+146+152
8	PSPro_Cube	Crop	0.0001	5.0	U4b1+146+152
9	PSPro_Cube	Crop	0.0001	0	R8
10	PSPro_Cube	Crop	0.0001	2.55	U4b1+146+152
11	PSPro	Whole	0.0000	2.5	U4b1+146+152
12	PSPro	Whole	0.0002	0	U4b1+146+152
13	PSPro	Postfeeding	-	4.9	V+@16298
14	PSPro	Postfeeding	-	0	H2a2a
15	PSPro	Postfeeding	0.0003	0	H2a2a
16	PSPro	Burned	0.0003	7.7	H7d2
17	PSPro	Burned	0.0001	12.8	H7d2
18	PSPro	Burned	0.0002	5.1	H7d2
19	PSPro	Burned	0.0003	12.8	H7d2

- mtDNA typing successful for samples with little to no quantifiable nuDNA
- Alternative method when STR typing yields no results

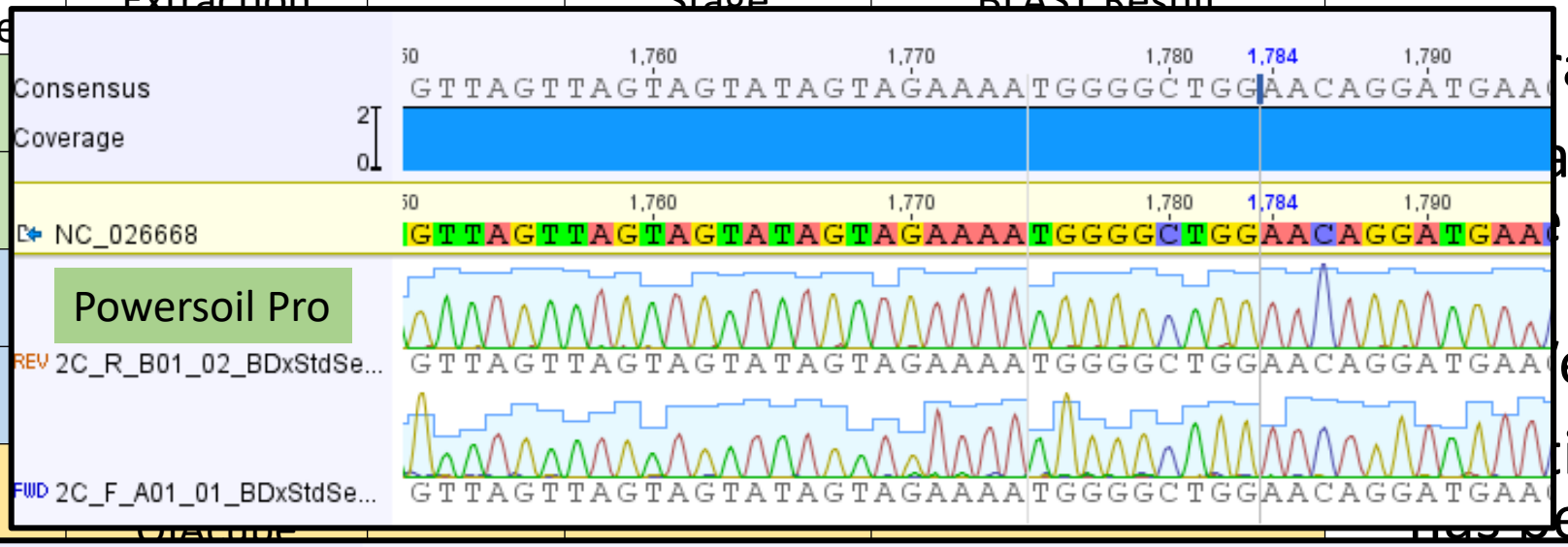
NGS-Mainstay SE

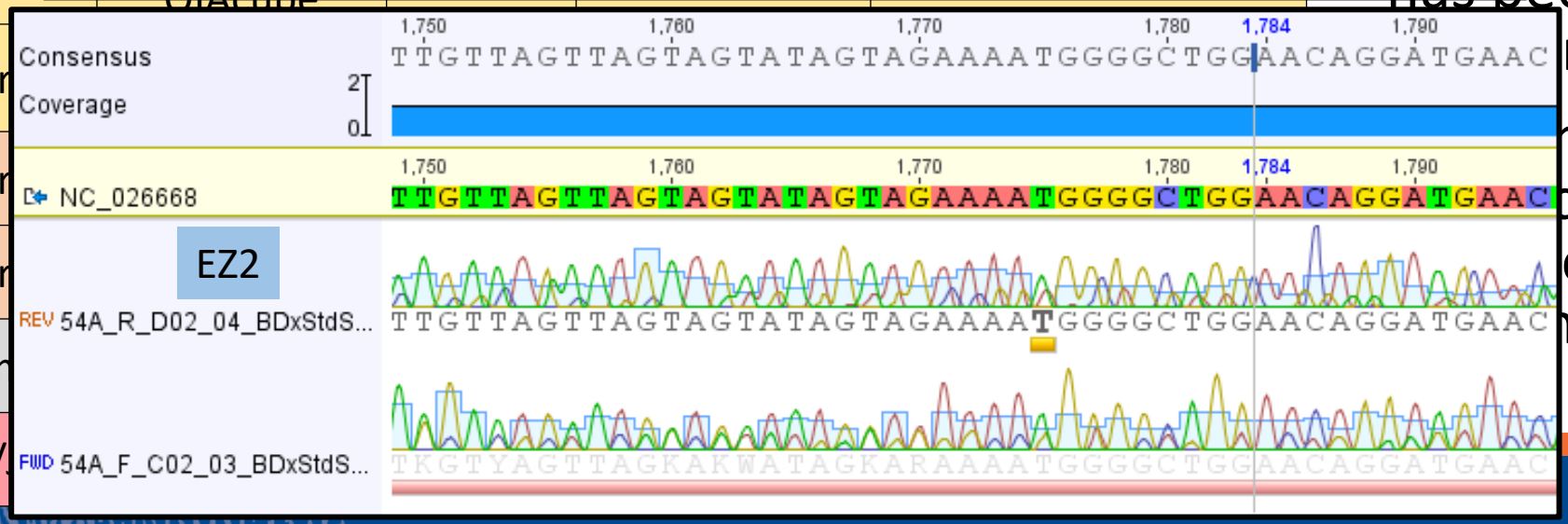


- Significant difference between autosomal STR recovery ($p<0.05$)
- Required minimum DNA input could not be met for NGS

Species ID Barcoding

Sample	Month Collecte	Extraction	Sample	Stage	BLAST Result
20	April				
21	April				
22	April				
23	April				
24	April				
25	Apr				
26	Apr				
27	Apr				
15	Septer				
17	June/				





ate species ID

an mtDNA did not any interference

concordance with ells et al. (2001)

time *Phormia regina* een used for the ry of human DNA

mples resulted in quality sequencing equence was still nible

Post-mortem Interval Estimation

- Species specific development rates can be used for PMI estimation
  - *Phormia regina*
  - Minimum PMI estimation of 5-9 days
  - Actual PMI= 11 days
    - Improve PMI estimation with more data

Stage	ADH Byrd et al.(2001)+		ADH Núñez-Vázquez et al. (2013)*
First Instar	380	800	615
Second Instar	720	1,480	1,282.7
Third Instar	1,240	2,040	1950.5
Prepupa^	1,600	3,880	-
Pupa	3,760	6,000	4,269.9
Adult	7,160	10,520	8,170.7

+ Byrd et al. reported a range in hours
^Prepupa include postfeeding/wandering fly larvae
*Prepupa combined with third instar

Byrd et al.

Núñez-Vázquez et al.

	Date	Average Temperature (°C)	ADH
1	4/1/2022	17.50	180.00
2	3/31/2022	15.56	313.33
3	3/30/2022	19.17	533.33
4	3/29/2022	25.00	893.33
5	3/28/2022	22.78	1,200.00
6	3/27/2022	23.06	1,513.33
7	3/26/2022	20.28	1,760.00
8	3/25/2022	18.06	1,953.33
9	3/24/2022	13.61	2,040.00
10	3/23/2022	14.17	2,140.00
11	3/22/2022	15.83	2,280.00

Conclusion

- Use of crops and whole fly larvae can yield investigative information
  - Whole fly larvae benefits: gross dissection and post-feeding fly larvae
- STR and mtDNA results in agreement with other studies[4-7,9,10,15,16]
  - Recovery of full and CODIS eligible partial profiles only from Powersoil Pro
- First time in which NGS has been attempted on human DNA recovered from fly larvae
  - Hindered due to low DNA input

Conclusion

- First report of *Phormia regina* in the recovery of human DNA from fly larvae
- Post-feeding fly larvae can be used, even if no crop is visible
  - Digestive tract could have human DNA that can be recovered
- Future work includes reattempting NGS with better samples

HUMAN

- Identity- STR or NGS
- Ancestry- mtDNA or NGS
- Phenotype- SNPs

SPECIES IDENTIFICATION

- Aid Post-mortem Interval Estimation
- Geographical Origin

Acknowledgements

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SOUTHEAST TEXAS APPLIED FORENSIC SCIENCE FACILITY

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References

1. Schal C, Czado N, Gamble R, Barrett A, Weathers K, Lodhi KM. Isolation, identification, and time course of human DNA typing from bed bugs, *Cimex lectularius*. Forensic Science International 2018;293:1–6. https://doi.org/10.1016/j.forsciint.2018.10.008.

2. Campobasso CP, Linville JG, Wells JD, Introna F. Forensic genetic analysis of insect gut contents. American Journal of Forensic Medicine and Pathology 2005;26(2):161–5. https://doi.org/10.1097/01.paf.0000163832.05939.59.

3. 1. Chamoun CA, Couri MS, Garrido RG, Moura-Neto RS, Oliveira-Costa J. Recovery & identification of human Y-STR DNA from immatures of *Chrysomya albiceps* (Diptera: Calliphoridae). Simulation of sexual crime investigation involving victim corpse in state of decay. Forensic Science International 2020;310. https://doi.org/10.1016/j.forsciint.2020.110239.

4. de Lourdes Chávez-Briones M, Hernández-Cortés R, Díaz-Torres P, Niderhauser-García A, Ancer-Rodríguez J, Jaramillo-Rangel G, et al. Identification of Human Remains by DNA Analysis of the Gastrointestinal Contents of Fly Larvae. Journal of Forensic Sciences 2013;58(1):248–50. https://doi.org/10.1111/j.1556-4029.2012.02279.x.

5. Di Luise E, Magni P, Staiti N, Spitaleri S, Romano C. Genotyping of human nuclear DNA recovered from the gut of fly larvae. Forensic Science International: Genetics Supplement Series 2008;1(1):591–2. https://doi.org/10.1016/j.fsigss.2007.10.075.

6. Njau DG, Muge EK, Kinyanjui PW, Omwandho COA, Mukwana S. STR analysis of human DNA from maggots fed on decomposing bodies: Assessment of the time period for successful analysis. Egyptian Journal of Forensic Sciences 2016;6(3):261–9. https://doi.org/10.1016/j.ejfs.2015.04.002.

7. Kondakci GO, Bulbul O, Shahzad MS, Polat E, Cakan H, Altuncul H, et al. STR and SNP analysis of human DNA from *Lucilia sericata* larvae's gut contents. Forensic Science International: Genetics Supplement Series 2009;2(1):178–9. https://doi.org/10.1016/j.fsigss.2009.08.147.

8. Wells JD, Introna F, Di Vella G, Campobasso CP, Hayes J, Sperling FAH. Human and Insect Mitochondrial DNA Analysis from Maggots. Journal of Forensic Sciences 2001;46(3):685–685. https://doi.org/10.1520/JFS15022J.

9. Zehner R, Amendt J, Krettek R. STR Typing of Human DNA from Fly Larvae Fed on Decomposing Bodies. Journal of Forensic Sciences 2004;49(2):337–337. https://doi.org/10.1520/JFS2003248.

10. Li X, Cai JF, Guo YD, Xiong F, Zhang L, Feng H, et al. Mitochondrial DNA and STR analyses for human DNA from maggots crop contents: a forensic entomology case from central-southern China. Trop Biomed 2011;28(2):333–8.

11. Durdle A. Insects as vectors of DNA in a forensic context. WIREs Forensic Science 2020;2(2). https://doi.org/10.1002/wfs2.1355.

12. Cantu C, Bucheli S, Houston R. Comparison of DNA extraction techniques for the recovery of bovine DNA from fly larvae crops. JFS 2022;67(4):1651–9. https://doi.org/10.1111/1556-4029.15010

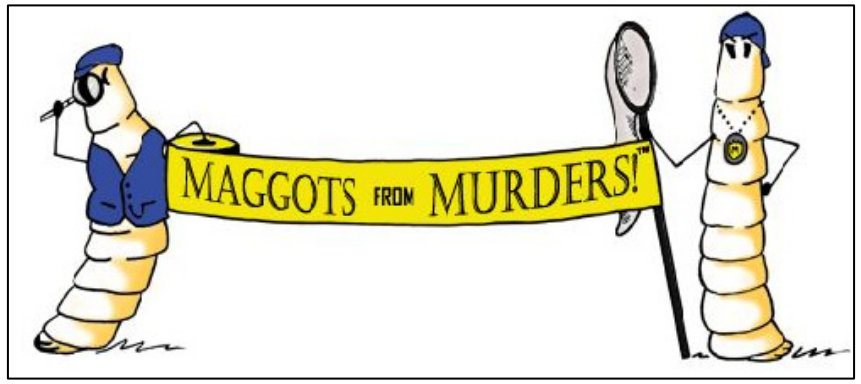
13. 1. Byrd JH, Allen JC. The development of the black blow fly, Phormia regina (Meigen). Forensic Sci Int 2001;120(1–2):79–88. https://doi.org/10.1016/s0379-0738(01)00431-5.

14. Núñez-Vázquez C, Tomberlin JK, Cantú-Sifuentes M, García-Martínez O. Laboratory Development and Field Validation of *Phormia regina* (Diptera: Calliphoridae). Jnl Med Entom 2013;50(2):252–60.

15. Linville JG, Hayes J, Wells JD. Mitochondrial DNA and STR Analyses of Maggot Crop Contents: Effect of Specimen Preservation Technique. J Forensic Sci 2004;49(2):14. https://doi.org/10.1520/JFS2003266.

16. Oliveira TC, Santos ABR, Rabelo KCN, Souza CA, Santos SM, Crovella S. Human autosomal dna and X chromosome STR profiles obtained from *Chrysomya albiceps* (Diptera: Calliphoridae) larvae used as a biological trace. Genetics and Molecular Research 2016;15(4). https://doi.org/10.4238/gmr15047622.

Questions?



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