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### DNA Extraction and Analysis of Bone Samples from the Orton Quarry Ossuary

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# Ancient DNA Analysis of Forgotten Ossuary



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## Project Description

- The Orton Quarry site (36ER243) is a late prehistoric ossuary along the coast of Lake Erie in northwestern Pennsylvania. In March 1991, heavy-equipment operators accidentally exposed the site.
- Due to a personal interest in ossuaries, an extensive literature review was conducted and discovered that very little had been published on the site's importance or its original inhabitants.
- One of the primary objectives of this project is to expand the knowledge of the site by sequencing the aDNA of the individuals from the site while using the contamination avoidance protocols standard in the Snow lab. **We will obtain valuable data on the inhabitant's genetic ancestry.**

## Materials and Methods

In order to extract the ancient DNA from the bone samples, the modified Dabney et al. (2013) protocol was followed as outlined below:

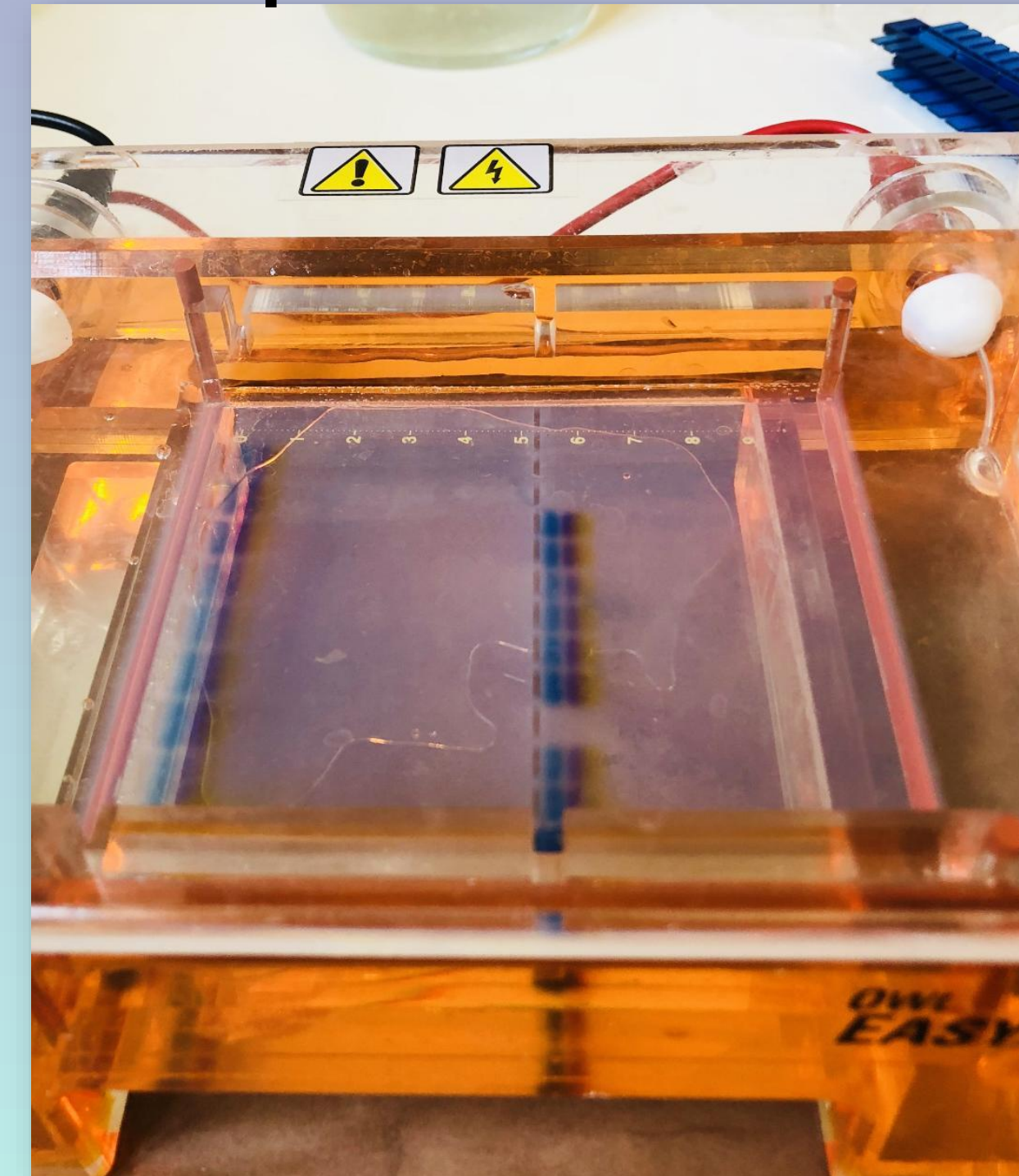
- 1.85-120 mg of material into 1 mL of EDTA, ProtK mix and incubated overnight at 55°C
2. Centrifuge at a high speed (16,100x g) for 2 minutes
3. Supernatant added to 15 mL tube with 13 mL of Binding Buffer
4. Spun at 1,500x g until buffer passes through Qiagen MinElute spin column
5. 750 uL of PE buffer added to spin column and washed through 1 min at 6k rpm
6. Added 12.5 uL of water and incubated for 2-5 minutes, then spun into final tubes

Extraction was followed by PCR amplification for mtDNA haplogroups and Hypervariable Region I and checked on 2% agarose gel. Sequences will be compared to others from the region to look for shared haplogroups and haplotypes.

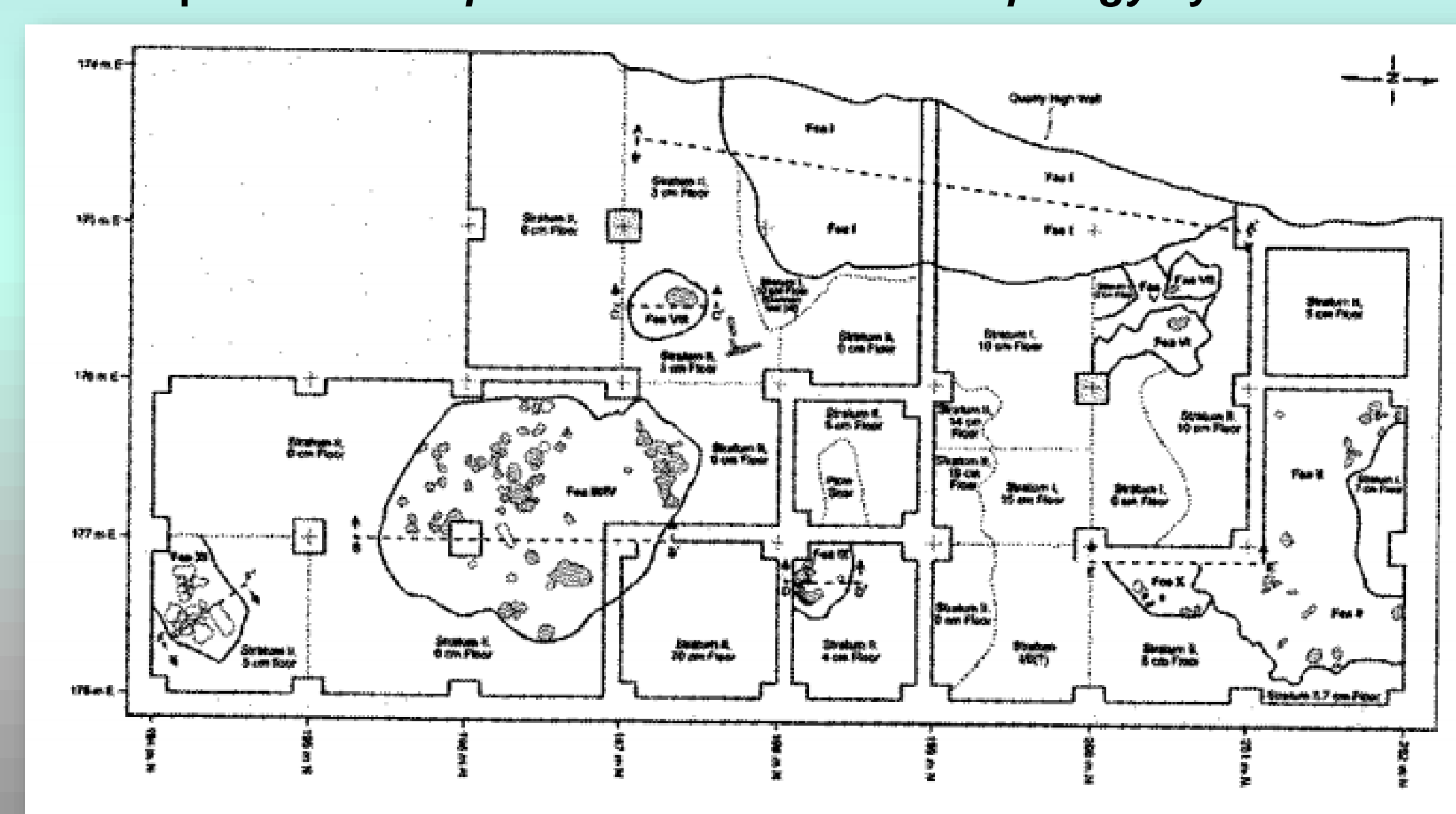
## Qubit results: DNA Concentration of Samples



Gel electrophoresis for PCR results



Site Map from *A Companion to Forensic Anthropology* by D. Dirkmaat



## Acknowledgments

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## What We Have Thus Far

### & What's Next

- Thus far the DNA from the first five samples have been isolated, and are being amplified for regions of the mtDNA that carry diagnostic haplogroup SNPs. RFLP analysis is ongoing to check for the presence/absence of these markers to assign haplogroups to the samples.
- In the future, sequences of the HVRI will be completed and reviewed in Sequencer software. Any SNPs that are identified in comparison with the revised Cambridge Reference Sequence will then be compared with the Haplogrep software in order to confirm haplogroup assignment.
- The aspiration of this project is to analyze the data and compare the results to other ancient and modern DNA data from the Great Lakes region, using haplogroup and haplotype comparisons, as seen in Pfeiffer et al. (2014).
- **Ultimately all of these results will then be written and presented on, adding to both the knowledge of the Orton Quarry Ossuary at well as the genetic data for the Great Lakes region.**

## In Conclusion...

**We are attempting to better understand who these individuals were and whom they were related to, both prehistorically and in modern day. This ossuary's remains have not been repatriated and the descendants are unknown, therefore the only way we can potentially attribute descendants correctly is through genetic analysis.**

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