Extraction Efficiency experiments were conducted to evaluate the percentage of DNA recovered through three extraction methods: a manual phenol-chloroform method, the use of the Qiagen EZ1 Advanced XL Extraction robot, and with Qiagen QIAamp DNA Mini extraction kit. Three DNA sources (cells, blood, extracted DNA) were tested at varying known concentrations. Extracted samples were quantified with the use of droplet digital PCR with nuclear DNA assays designed and optimized in-house. Results indicated that the observed recovery value range was lower than many reported extraction efficiency calculations using the number of full STR profiles produced.


### What is Extraction Efficiency?

Knowing the original amount of DNA in the extraction process allows for the comparison of extraction protocols and methods to accurately determine the efficiency of the extraction process. **Digital PCR** offers absolute quantitation without the need of a calibration standard curve for determining the amount of recovered DNA post extraction.

### Extraction Methods

#### Qiagen EZ1 Advanced XL

Materials were extracted according to manufacturer’s recommended protocols for the DNA Investigator kit [1]. This kit utilizes silica covered magnetic particles for DNA purification.

50 µL sample was collected from 140 µL of G2 Buffer and 10 µL of Proteinase K and incubated at 56°C in a thermomixer for ten minutes. Purification took place on the EZ1 Advanced XL instrument with the DNA Investigator Kit. Samples were eluted in 50 µL TE [2].

#### Qiagen QIAamp Spin Columns

Materials were extracted according to manufacturer’s recommended protocols for the QIAamp DNA Mini extraction kit following the Purification from Blood or Body Fluids Spin Protocol [2]. Samples were normalized to a volume of 200 µL and added to 205 µL Buffer AL and 20 µL Proteinase K. Samples were incubated at 56°C in a thermomixer for ten minutes. Purification took place in the QIAamp silica spin columns with Qiagen Buffers. Samples were eluted in 200 µL AE Buffer.

#### Phenol-Chloroform (Organic)

100 µL of sample was added to 400 µL Nucleic Lysis Buffer, 4 µL 10 % SDS, and 10 µL Proteinase K and incubated overnight at 37°C.

After incubation, an equal volume of phenol chloroform (514 µL) was added and vigorously mixed before adding solution to a phase lock light tube (Quantabio, WFR, 10847-800) for complete separation of the two phases. The phase lock light tubes were centrifuged at 14,000 g for 15 minutes to separate the layers. The aqueous layer was transferred to a fresh tube where 2x the volume of ethanol was added.

After gently mixing the aqueous layer with the ethanol, the tubes were centrifuged at 12,000 rpm for 1 minute. Proteinase K and incubated overnight at 37°C.

**DDPCR**

**ddPCR allows for absolute quantitation without the need of a standard curve or calibrator**

**ddPCR**

- **Sample/mastermix is placed in a droplet generator**
- **Individual droplets in an oil emulsion are formed**
- **PCR amplification is performed (end point)**
- **Droplets are read as being positive or negative**
- **Counts of positive droplets can be converted into sample concentration**

### Conclusion

- Additional operators (Repeatability and Reproducibility)
- Examination of alterations in extraction incubation times
- Addition of carrier RNA for lower DNA inputs
- Use of Microcon Centrifugal Filters for Organic extraction
- Altering elution buffer volume
- Examination of additional extraction chemistries, methodologies, kits, and techniques
- Additional of a modification to the extraction process

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