An automated, high throughput methodology optimized for quantitative cell-free mitochondrial and nuclear DNA isolation from plasma

Sarah A. Ware¹, Nikita Desai¹, Mabel Lopez¹, Daniel Leach², Yingze Zhang³, Luca Giordano¹, Mehdi Nouraie³, Martin Picard⁴, and Brett A. Kaufman¹*

Supporting Information

Page S-1: Title page
Page S-2: Table S1. qPCR assay sequences
Page S-3: Figure S1. ccf-DNA variation
Page S-4: Figure S2. Raw data corresponding to Fig. 6a-dii
Page S-5: Figure S3. Applying 30 °C to extraction plates
Page S-6: Figure S4. Variable dispense of ProK and SDS by the LiHa
| Probe | Forward Primer | Reverse Primer |
|-------|----------------|----------------|
| ND1   | 5'-/5HEX/CCA TCA CCC/ZEN/TCT ACA TCA CCG CCC /3IABkFQ/-3' | 5'- GAG CGA TGG TGA GAG CTA AGG T -3' |
|       | 5'- CCC TAA AAC CCG CCA CAT CT -3' | |
| B2M   | 5'-/56-FAM/ ATG TGT CTG /ZEN/GGT TTC ATC CAT CCG ACA /3IABkFQ/-3' | 5'- TCT CTC TCC ATT CTT CAG TAA GTC AAC T -3' |
|       | 5'- CCA GCA GAG AAT GGA AAG TCA A -3' | |
| ND4   | 5'- /5HEX/ CCG ACA TCA /ZEN/TTA CCG GGT TTT CCT CTG G /3IABkFQ/-3' | 5'- ACA ATC TGA TGT TTT GGT TAA ACT ATG TTT -3' |
|       | 5'- CCA TTC TCCTCC TAT CCC TCA AC -3' | |
| PPIA  | 5'- /56-FAM/ AAT TCA CGC /ZEN/AGA AGGA ACC AGA CAG T /3IABkFQ/-3' | 5'- GTG GCG GAT TTG ATC ATT TGG -3' |
|       | 5'- CAA GAC TGA GAT GCA CAA GTG -3' | |

Table S1. qPCR assay sequences. Two sets of duplex qPCR assays were used in this study. The first targeted ND1 and B2M while the second targeted ND4 and PPIA.
Figure S1. ccf-DNA variation. When several days of ccf-DNA isolations were quantified at once by duplex qPCR, (a) both mtDNA and nDNA yields were significantly variable between days. Statistical analysis was performed using ordinary one-way ANOVA (p-values: * < 0.05, ** < 0.01, *** < 0.001, **** < 0.0001). (b–d) Raw data corresponding to Fig. 5a–cii with mtDNA and nDNA presented as ∆CT (plate median – experimental values).
Figure S2. ΔCT (plate median – experimental values) have been added to the plate depictions corresponding to those in Fig. 6a-dii.
Figure S3. Applying 30 °C across all extraction plates did not improve standard deviation or edge effects. For this experiment, which is a continuation of Fig. 5, both extractions were performed using Script 1. However, the temperature setting of the MPP was either (a) off (ambient) or (b) at 30°C. (a) is presented as a control in both Fig. 5a and Fig. S3a.
Figure S4. Volumes of ProK and SDS dispensed by the LiHa were variable. Fluorescein diluted in (a) water, (b) ProK, and (c) SDS was dispensed by the LiHa and fluorescence was measured (n = 64 per group). ∆Fluorescence (plate median – exp) was calculated for each dispense. (d) Violin plots were used to compare the distribution of variation of the three conditions and statistical analysis was performed using multiple F tests where water was used as the reference condition (p-values: * < 0.05, ** < 0.01, *** < 0.001, **** < 0.0001).