prepIT®•C2D

Comparison of prepIT®•C2D vs competitor 'Q' in the extraction of DNA from Oragene® and ORAcollect®•DNA

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Saliva samples collected with Oragene® and oral samples collected with ORAcollect®•DNA may be purified with the prepIT®•C2D (PT-C2D) purification columns from DNA Genotek. When compared to competitor 'Q', samples purified with PT-C2D had equivalent DNA yields, ratios (A_{260}/A_{280}) and high molecular weight. Additionally these samples performed equivalently on downstream applications such as long-range and real-time PCR.

Introduction

The purpose of this technical bulletin is to compare the extraction performance of PT-C2D to a leading sample preparation kit and evaluate the DNA yield, purity, molecular weight and performance on downstream applications. This comparison was performed using samples collected with Oragene and ORAcollect•DNA self-collection kits.

Materials and methods

Sample collection

Twenty five saliva and oral samples were collected using the standard collection instructions (PD-PR-00213 and PD-PR-00188)[1, 2] for each of the Oragene and ORAcollect•DNA self-collection kits. After collection, the samples were mixed and heated at 50°C for 1 hour before purification.

Purification

A 250 µL sample aliquot was purified from each sample collected as this is the maximum input volume for competitor 'Q'. (Note: The maximum input volume of PT-C2D is 500 µL). For PT-C2D purifications a 250 µL aliquot from each donor was purified according to the PT-C2D purification protocol[3]. In brief, 350 µL of PT Buffer was added to each aliquot and then the sample was transferred to a spin column and centrifuged. The filtrate was then discarded and the column was washed once with Wash Buffer 1, followed by two washes with Wash Buffer 2. The DNA was then eluted in 100 µL of Elution Buffer. In order to demonstrate the maximum input volume of PT-C2D some samples were additionally purified using a 500 µL aliquot.

The same samples were then purified according to the competitor 'Q' protocol for blood and body fluid purification. In brief, the 250 µL aliquot was mixed with a binding buffer followed by a series of centrifugation and washing steps. The DNA was then eluted in 100 µL of Elution Buffer.

DNA analysis

The samples were quantified using fluorescence (Picogreen) and the A_{260}/A_{280} ratios were determined via absorbance. The absorbance scan of the samples was also performed in order to detect any potential contaminant carry-over from the purification process. To evaluate the molecular weight of the DNA, a 0.8% agarose gel was run and the samples were compared to a Lambda-Hind III digest ladder. A long-range PCR was performed using primers for CYP 2D beta and the product was run on an agarose gel to ensure that proper sized PCR product was amplified (2.6 kb). Finally real-time PCR was performed using primers for TS 143.

Results

The average yields and A_{260}/A_{280} ratios are summarized in Table 1 for comparison of PT-C2D and competitor 'Q'. There was no significant difference between the average yield and the average A_{260}/A_{280} ratios obtained from the two purification protocols. Table 2 demonstrates the advantage of increased yield in a single prep given the larger input volume of the PT-C2D kit.

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Purification protocol	Collection device	Aliquot volume (µL)	Average total yield per aliquot (µg)	A_{260}/A_{280}
PT-C2D	ORAcollect•DNA	250	0.58 ± 0.26	1.9 ± 0.1
	Oragene	250	1.19 ± 0.80	1.8 ± 0.1
Competitor 'Q'	Oragene	250	1.15 ± 0.76	1.8 ± 0.1

***Table 1**: Average DNA yield and A_{260}/A_{280} ratios.*

Purification protocol	Collection device	Aliquot volume (µL)	Average total yield per aliquot (µg)	A_{260}/A_{280}
PT-C2D	Oragene	500	2.12 ± 1.55	1.8 ± 0.1
Competitor 'Q'	Oragene	250	1.15 ± 0.76	1.8 ± 0.1

***Table 2**: Average DNA yields and A_{260}/A_{280} ratios when comparing the input volumes for both purification protocols.*

A representative absorbance scan from 230 nm to 350 nm was performed on all OG samples purified with both PT-C2D and competitor 'Q'. Figure 1 shows an example absorbance profile from donor #10, which indicates that samples purified with competitor 'Q' may have contaminant carry-over from the purification process, while the sample purified with PT-C2D shows no sign of carry-over.

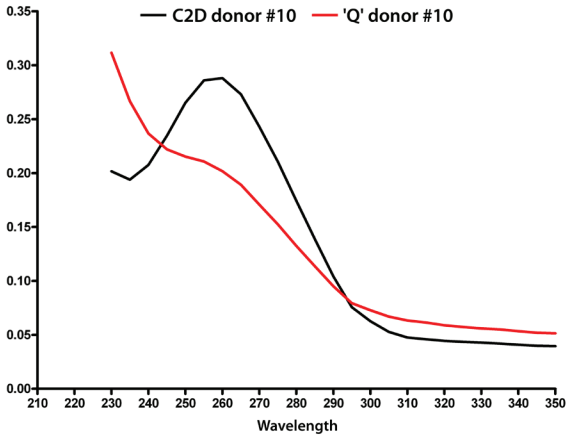
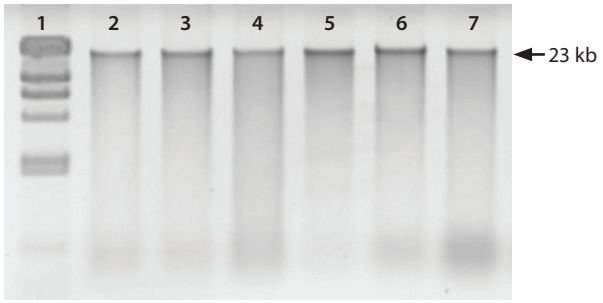


***Figure 1**: Absorbance profile of donor #10 purified with both PT-C2D (black) and competitor 'Q' (red).*

Figure 2 shows the molecular weight of a selected 6 samples from PT-C2D (A) and competitor 'Q' (B).

A: PT-C2D



B: Competitor 'Q'

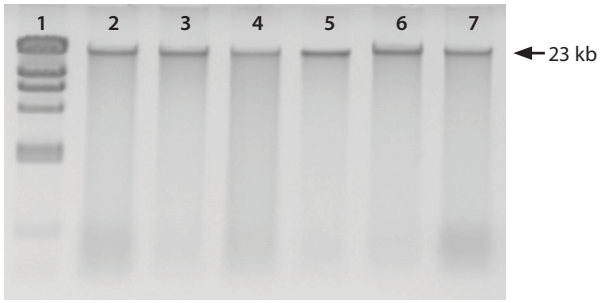


***Figure 2**: Agarose gel electrophoresis of PT-C2D purified DNA (A) and with competitor 'Q' (B). A Lambda-Hind III digest was used as the marker in lane 1, lanes 2 to 7 are samples 1 to 6 respectively.*

Lastly the purified DNA was tested for performance in downstream applications. Long-range PCR was performed to amplify a 2.6 kb fragment, in figure 3 it can be observed that a single product has been amplified using a subset of 4 samples from each purification method. In figure 4 the real-time PCR amplification profile of TS 143 product from DNA obtained from PT-C2D purifications of both Oragene (A) and ORAcollect•DNA (B) samples as well as Oragene samples purified with competitor 'Q' is presented.

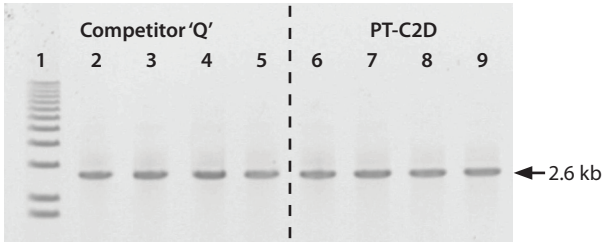
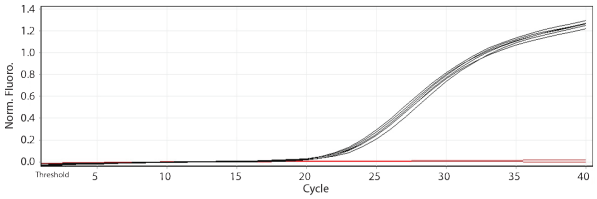
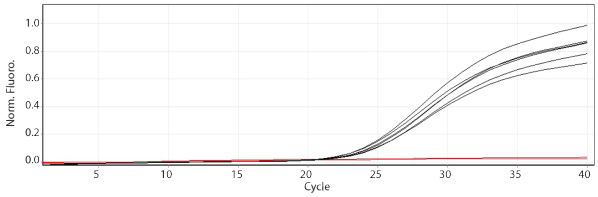


***Figure 3**: Agarose gel electrophoresis of samples using CYP 2D6 beta PCR primers (2.6 kb) for samples #1 to 4. A 1 kb plus ladder was used as a marker in lane 1, lanes 2 to 5 are samples # 1 to 4 purified with competitor 'Q' and lanes 6 to 9 are samples # 1 to 4 purified with PT-C2D.*

A: Oragene



B: ORAcollect•DNA



C: Competitor 'Q'

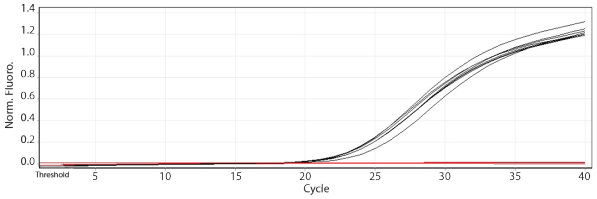


***Figure 4**: Amplification profile of the TS 143 product amplified from both Oragene (A) and ORAcollect•DNA (B) samples purified with PT-C2D as well as Oragene purified with competitor 'Q' (C) for samples #1 to 6. The red line on each graph represents the no template control.*

Discussion and conclusion

The yields and ratios obtained using these purification protocols were not statistically different, meaning that both were equally efficient at recovering DNA from saliva samples. The samples purified with PT-C2D showed a high molecular weight band >23 kb and the DNA performed equally well to the DNA obtained using the competitor 'Q' protocol in both long range and real-time PCR reactions demonstrating that the DNA recovered was of both high quality and integrity. According to the absorbance scans samples extracted with PT-C2D appear to be free of any carry-over of potential inhibitors whereas samples extracted with competitor 'Q' may not be. In summary the PT-C2D purification process performs equivalently to a leading column-based purification kit for the extraction of samples collected using Oragene and ORAcollect•DNA kits.

A major advantage of the PT-C2D kit offers is that it has the added capability of processing larger sample input volumes compared to competitor 'Q'. The option to process 2 times as much saliva sample on a single miniprep column leads to an opportunity to obtain a higher yield per prep and utilize fewer disposable reagents to process each Oragene or ORAcollect•DNA sample.

References

1 OG-500 collection instructions. DNA Genotek. PD-PR-00213-ML.
2 OCR-100 collection instructions. DNA Genotek. PD-PR-00188.
3 prepIT•C2D Genomic DNA MiniPrep Kit Saliva and oral sample protocol. DNA Genotek. PD-PR-00211.

Disclaimer

The prepIT•C2D Genomic DNA MiniPrep Kit is for research use only, not for use in diagnostic procedures.
DNA Genotek Inc. makes no claims regarding the performance of these kits for clinical or diagnostic applications.

Oragene®•DNA is not available for sale in the United States.
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Some DNA Genotek products may not be available in all geographic regions.

All DNA Genotek protocols, white papers and application notes, are available in the support section of our website at www.dnagenotek.com.