



From turbidity to clarity: Simple methods to improve the A_{260}/A_{280} ratio of Oragene®-purified DNA samples

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In general, DNA extracted from Oragene®/saliva samples[†] has a A_{260}/A_{280} ratio of >1.60 . Occasionally, the ratio is lower than this, presumably due to failure to remove food particles from the mouth before delivering saliva. DNA samples with a lower A_{260}/A_{280} ratio regularly perform well in downstream applications such as conventional PCR. Despite the fact that sample turbidity (i.e., absorbance at A_{320}) does not affect down-stream applications, some users have enquired about modifications to our standard protocol that will improve this ratio. In this document, we describe 3 alternative methods that can be used to obtain high A_{260}/A_{280} ratios from all samples.

Introduction

The Oragene self-collection kit is a non-invasive method for collecting large amounts of high quality DNA. Since Oragene/saliva samples are stable at ambient temperature for long time periods, there is no urgency to send samples to the laboratory to purify the DNA. Once in the laboratory, DNA can be easily and quickly extracted without a requirement for specialized kits or equipment. The Oragene/saliva samples need only to be heated, treated with the prepIT™•L2P and ethanol precipitated. The resulting DNA has an A_{260}/A_{280} ratio >1.6 . Occasionally, Oragene/saliva samples may contain foodstuffs from individuals who did not abstain from eating or drinking 30 minutes prior to delivering the saliva. Some foodstuffs may contribute particulates resulting in turbid samples, which can then lead to an increased apparent absorbance at 260 nm and 280 nm, and a lower A_{260}/A_{280} ratio. In this report, we describe how to correct the A_{260}/A_{280} ratio for turbid samples. Furthermore, we describe additional steps that can be used to eliminate turbid material and thereby improve the A_{260}/A_{280} ratio.

Materials and methods

Saliva collection

Two donors provided 2 mL of saliva using the Oragene self-collection kit. Sample 1 was collected from an individual immediately after eating lunch while Sample 2 was collected from an individual immediately after drinking coffee. Both individuals were specifically asked not to rinse their mouth prior to delivering saliva. The Oragene kit was then capped and the entire sample was mixed by inversion, allowing the DNA to be released and stabilized.

Sample treatment

As described in the standard prepIT•L2P protocol¹, samples were incubated in a water bath for 1 hour at 50°C. A 500 μ L aliquot was immediately removed from each sample.

DNA purification

The standard prepIT•L2P purification protocol was followed. In brief, 20 μ L (1/25th volume) of prepIT•L2P was added to each 500 μ L sample. Each tube was mixed well and incubated on ice for 10 minutes. The tubes were centrifuged at $15,000 \times g$ (13,000 rpm) for 5 minutes. The supernatant was removed and transferred to a fresh tube; an equal volume of 95% ethanol at room temperature was added. The sample was mixed by inversion and allowed to stand for 10 minutes at room temperature. The sample was centrifuged at $15,000 \times g$ (13,000 rpm) for 2 minutes and the supernatant was removed and discarded. The DNA pellet was dissolved in 100 μ L of TE buffer.

[†] Saliva samples were collected with Oragene®•DNA or Oragene®•DISCOVER.

DNA analysis

DNA from the purified samples was quantified using a fluorescent dye, SYBR® Green I. In brief, DNA samples were diluted and 5 µL was transferred to a PCR tube containing 1.25 µL of 20× buffer (0.1 M MgCl₂, 0.02 M CaCl₂ and 0.4 M Tris, pH 8.0), 5 µL of 5 mg/mL BSA, 0.125 µL SYBR Green (Sigma:S-9430, 100×), and 13.63 µL of water. The unknown samples were run with a standard curve using known amounts of reference DNA, ranging from 0 ng to 12.5 ng per assay. The samples were analyzed on a Corbett Research Rotor-Gene RG-3000A. The absorbance spectrum from 220 nm to 320 nm was measured using a Cary 1E Spectrophotometer. The A₂₆₀/A₂₈₀ ratio was corrected by subtracting the A₃₂₀ value from the A₂₆₀ and A₂₈₀ values. The molecular weight of the DNA and PCR products was determined by electrophoresis on a 0.8% agarose gel.

Results and discussion

Improving the A₂₆₀/A₂₈₀ ratio by subtraction of the A₃₂₀ value:

Purified DNA samples that contain turbid material can be assessed by determining their apparent absorbance at 320 nm; this value is due to light scattering and not due to true absorbance. As seen in Figure 1, the spectrum of Sample 1 demonstrates a turbid sample with an A₃₂₀ value of 0.234. When this value is subtracted from all values across the spectrum according to $g(\chi) = f(\chi) - A_{320}$, the resulting normalized spectrum in Figure 1 provides us with “corrected” A₂₆₀ and A₂₈₀ values. Corrected A₂₆₀ and A₂₈₀ values eliminate the contribution of turbid material to the absorbance spectrum and allow a more precise assessment of the true A₂₆₀/A₂₈₀ ratio. Since the same A₃₂₀ value is subtracted across the entire spectrum, the percent contribution varies at each point along the curve. For example, in Sample 1 at A₂₈₀ (0.576), the A₃₂₀ (0.234) contributed 40.6% to the value. However, at A₂₆₀ (0.877), the A₃₂₀ (0.234) only contributed 26.7% to the value.

Without correction, the A₂₆₀/A₂₈₀ ratio of Sample 1 would be 1.52, a low value. When corrected by taking into account the turbidity $(A_{260}-A_{320})/(A_{280}-A_{320})$, the ratio is 1.88, which is a more precise assessment

of the low amount of protein or other soluble material in the purified sample that could absorb at 280 nm. Some spectrophotometers allow the user to automatically subtract the A₃₂₀ value from their A₂₆₀ and A₂₈₀ readings, while others require the user to take the A₃₂₀ reading and manually subtract it from the A₂₆₀ and A₂₈₀ values. Regardless of the spectrophotometer used or the turbidity of sample, subtracting the A₃₂₀ value is good practice and will result in a more precise assessment of the purified sample.

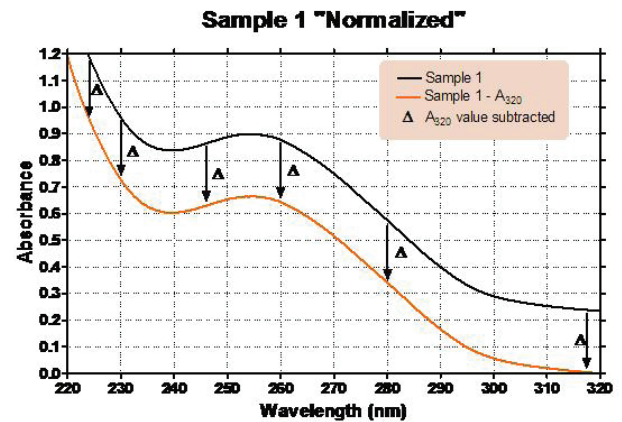


Figure 1: Absorbance spectrum of uncorrected and normalized Sample 1 DNA.

In our experience, very few samples have a A₂₆₀/A₂₈₀ ratio of <1.6. In order to simulate such a sample, Sample 1 was collected *immediately* after the donor ate lunch and Sample 2 was collected *immediately* after the donor drank coffee. The uncorrected A₂₆₀/A₂₈₀ ratios of the two samples were 1.52 and 1.45, respectively. When the A₃₂₀ values were subtracted, the corrected A₂₆₀/A₂₈₀ ratios were 1.88 and 1.80, respectively (Table 1).

Example:

Sample 1

$$A_{260} = 0.877; A_{280} = 0.576; A_{320} = 0.234$$

Not corrected A₂₆₀/A₂₈₀ ratio =

$$A_{260} \div A_{280} = 0.877 \div 0.576 = 1.52$$

Corrected A₂₆₀/A₂₈₀ ratio =

$$(A_{260}-A_{320}) \div (A_{280}-A_{320}) = (0.877-0.234) \div (0.576-0.234) = 0.643 \div 0.342 = 1.88$$

Methods to decrease the amount of turbid material in purified DNA samples

As discussed, subtracting the A_{320} value from A_{260} and A_{280} will result in a more accurate A_{260}/A_{280} ratio. However, some users may wish to remove as much of the turbid material as possible. The following describes approaches that can be used after standard purification to clarify the samples further.

1. Additional centrifugation

Following purification and proper re-hydration of the DNA in TE or similar buffer, if a sample appears to be turbid, the user can centrifuge the sample for up to 15 minutes at maximum speed. The DNA (still in solution in the supernatant) can be transferred to a fresh tube, carefully avoiding contamination with the material in the pellet. The turbid material is composed of very fine particulate or colloidal material, so the amount removed will depend upon the speed and length of centrifugation. In our experiments, we found that a 5 minute centrifugation at maximum speed (13,000 rpm, in a micro-centrifuge) was sufficient to remove about 1/2 of the turbid material (reduction of about 50% of the A_{320} reading) (Figures 2 and 3). Extending the length of centrifugation to 15 minutes removed a small amount more of the turbid material. The additional centrifugation step did not affect the DNA concentration but did improve the uncorrected A_{260}/A_{280} ratio (Table 1).

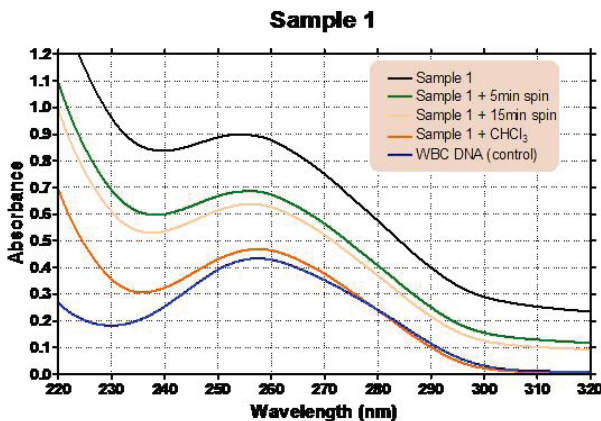


Figure 2: Absorbance spectra of purified DNA from Sample 1.

2. Chloroform treatment

A small amount of chloroform added to the re-hydrated DNA was found to efficiently and quickly remove nearly all particulates that scatter light at 320 nm (Figures 2 and 3). To remove the turbid material, we added 1/20th volume of chloroform (but no less than 2.5 μL) to the DNA dissolved in TE and vortexed the sample vigorously for a few seconds. Chloroform has very little solubility in water. The “coagulated” turbid material and residual chloroform were removed by a 10 minute centrifugation step at maximum speed. The upper aqueous layer (containing the DNA) was transferred to a fresh tube. DNA is not precipitated by chloroform treatment.

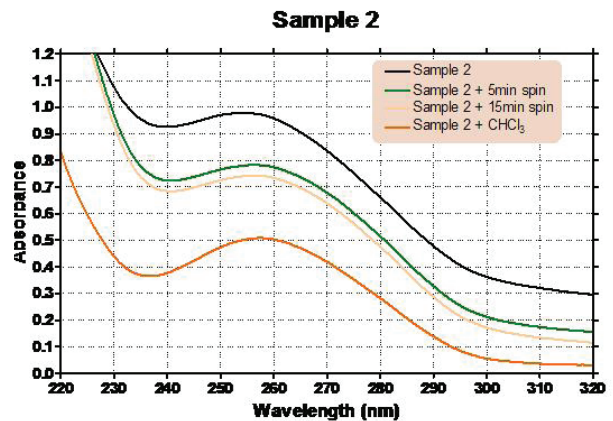


Figure 3: Absorbance spectra of purified DNA from Sample 2.

Care should be taken not to disturb the chloroform layer or the pellet. When small amounts of chloroform are used (e.g., 2.5 μL), the chloroform phase may not be seen; only a small pellet of impurities may be visible.

Treatments	Sample 1				Sample 2			
	-	5 min. spin	15 min. spin	CHCl ₃	-	5 min. spin	15 min. spin	CHCl ₃
A ₂₆₀ /A ₂₈₀	1.52	1.65	1.69	1.93	1.45	1.50	1.57	1.78
(A ₂₆₀ -A ₃₂₀)/(A ₂₈₀ -A ₃₂₀)	1.88	1.91	1.92	1.96	1.80	1.72	1.72	1.88
Concentration (ng/μL)	91.70	86.96	92.93	91.66	145.73	140.45	146.11	146.62

Table 1: Summary of A₂₆₀/A₂₈₀ ratios and DNA concentration for purified samples.

Despite the turbid nature of the samples described, the purified DNA was both of high quality (as assessed by the corrected A₂₆₀/A₂₈₀ ratios, Table 1) and of high molecular weight, >23kb (Figure 4A). Furthermore, the treatments described did not have any detrimental effect on the downstream processing as assessed by PCR (Figure 4B).

Conclusions

In order to accurately evaluate the quality of each purified DNA sample, we strongly recommend that the A₃₂₀ value be subtracted prior to calculating the A₂₆₀/A₂₈₀ ratio regardless of whether additional purification steps were executed or if the samples did not appear turbid to begin with. The turbidity does not appear to affect DNA quality or downstream processing. For users who wish to remove these particulates, we recommend first up to a 15 minute centrifugation of the re-hydrated DNA samples. If an even higher level of clarity of the samples is desired, then a chloroform purification step of the rehydrated DNA samples can be added.

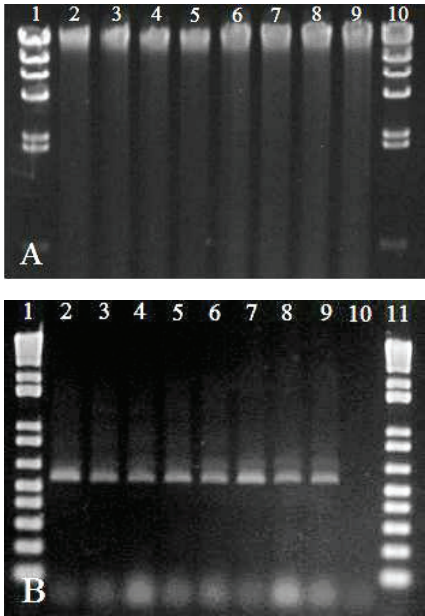


Figure 4: Visualization of purified and PCR amplified DNA. **A:** prepIT-L2P-purified DNA, Lanes 1,10: Lambda-HindIII marker; **B:** 550 bp PCR amplicon of the human TS gene, Lanes 1,11: 1kb+ DNA ladder; Lane 10: no template control; Lanes 2-9 in both images correspond to samples as follows: Lane 2: Sample 1; Lane 3: Sample 1 + 5min spin; Lane 4: Sample 1 + 15min spin; Lane 5: Sample 1 + CHCl₃; Lane 6: Sample 2; Lane 7: Sample 2 + 5min spin; Lane 8: Sample 2 + 15min spin; Lane 9: Sample 2 + CHCl₃.

References

¹ Laboratory protocol for manual purification of DNA from 0.5 mL of sample. DNA Genotek. PD-PR-006.

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