DNA genotek

Impact of population and laboratory methods on DNA yield and variability

Total DNA yield from Oragene®/saliva samples† can vary due to intra- and inter-donor variability, as well as the DNA extraction and quantification methods chosen. A comparative analysis of total DNA yield from peer-reviewed publications and in-house experiments is presented in this white paper to highlight the impact of population and laboratory method selection on final interpretation of data. Using comparative data, the average DNA yield from 2 mL of saliva extracted with prepIT®•L2P is 56 µg when quantified using fluorescence, and the average total nucleic acid yield is 129 µg when quantified by UV spectroscopy.

Introduction

In order to reduce costs and maximize donor compliance, researchers are turning to non-invasive, easy self-collection methods for obtaining genetic samples. Traditionally blood has been considered the standard sample for genetic testing, however the invasiveness and cost associated with this sample type has been a barrier for widespread use in various applications. Oral samples have been proven to be a reliable and accessible source of DNA for use in clinical diagnostics, genetic research and personalized medicine. On average, the amount of DNA per millilitre of sample is similar between blood and saliva. The amount and quality of DNA recovered from oral samples can vary depending on the collection method used as well as the extraction and quantification protocols. The Oragene self-collection kit is a non-invasive, simple to use device which makes it suitable for collecting samples in the field, in clinics and at home. This paper summarizes the total DNA yield from Oragene/saliva samples as reported in peer-reviewed publications, conference presentations and technical literature and discusses possible explanations for the variations seen between studies.

DNA quantification methodology

The most important factor to consider when comparing total DNA yield from different studies is the DNA quantification method used to determine sample yield. Different methods of quantification result in dramatic differences in reported yields. UV spectroscopy is perhaps the most commonly used method to quantify DNA. While inexpensive and convenient, it suffers from a lack of specificity in the nucleic acids it quantifies. UV spectroscopy cannot distinguish between the DNA and RNA content of the purified extracted sample, therefore the total yield reported will represent the total nucleic acid content, not just the DNA content. Comparatively, fluorescence-based methods of quantification, such as PicoGreen®, quantify only the double-stranded DNA resulting in a more accurate and specific measurement of DNA in a sample.

Several studies have compared UV spectroscopy to PicoGreen and reported significant differences between the DNA yields reported by the two methods. Data from these studies show that DNA quantification by absorbance are approximately 2-3 fold higher than those reported by PicoGreen quantification (Table 1: Studies 5,11,17,18 and 21; Table 2). Table 2 demonstrates the average DNA yield, regardless of purification method, is overestimated by 2 fold when relying on UV spectroscopy for quantification. The data underscores the importance of complete RNA removal from a sample if UV spectroscopy is to be used for accurate DNA quantification. However, since many downstream applications use only double-stranded DNA as a starting source, removal of RNA is an unnecessary additional step that can actually introduce variability and result in a loss of DNA. A simpler and more practical approach to accurately quantifying DNA yield, as opposed to total nucleic acid content, is quantification by fluorescence.

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

DNA extraction methodology

The protocol methodology used to extract the DNA from a raw Oragene/saliva sample has a direct impact on the DNA yield. The most common methods of DNA extraction include ethanol precipitation, spin columns and magnetic bead-based systems. Each method has advantages and disadvantages with respect to simplicity, quality and quantity of DNA obtained.

Magnetic bead-based and spin column-based methods are subject to the same limitations: binding capacity and binding efficiency. Binding capacity is the maximum amount of DNA that can be absorbed and is dependent upon the physical properties of the beads or filters used. Binding efficiency describes how well DNA will bind to the beads/filters and is affected by factors such as pH, salt concentration and the presence of competing macromolecules. DNA in a sample that is in excess of the binding capacity of the matrix being used, or that is unable to bind due to poor binding efficiency, will be lost during purification. Oragene/saliva samples are compatible with these purification methods (Studies 16-29). The average DNA yield, as measured using fluorescence, using magnetic bead-based or spin column-based methods is 31.2 µg (Table 2). This represents a 1.8 fold decrease in comparison to samples purified using the ethanol-based prepIT•L2P.

Ethanol precipitation-based extraction methods (i.e., prepIT•L2P or Puregene®) are not subject to binding capacities and efficiencies; virtually all high molecular weight DNA in the sample will be precipitated and collected by centrifugation. As a result, precipitation-based methods tend to provide higher recoveries than column- or bead-based systems. In Table 2 the summary of study results shows the average yield of total nucleic acids, by fluorescence (UV absorbance), from precipitation-based extractions (Studies 1-18, 21-24) was 56.3 µg (128.7 µg) purified using prepIT•L2P and 40.3 µg (101.2 µg) purified using Autopure LS®/Puregene. This compared to an average yield of 31.2 µg (58.4 µg) using bead/column methods (Studies 16-27). PrepIT•L2P is optimized for purification of Oragene/saliva samples and is not subject to loss of DNA pellets during automation.

In spite of the differences in yield and cost when compared to ethanol precipitation, column- and bead-based extraction methods may provide sufficient yield for downstream analyses and have the added advantages of being simpler and quicker protocols. However, the impact on yield must be taken into consideration when comparing studies using oral samples and when determining the experimental design.

Intra-donor variability

The DNA content of the saliva of a particular individual may vary naturally over the course of a day and may be influenced by particular eating or drinking habits. For this reason, the instructions for collecting an Oragene/saliva sample using an self-collection kit include the precaution to wait 30 minutes after eating, drinking, smoking or chewing gum before giving a saliva sample.

Two internal studies (DNA Genotek, 2008, unpublished data) investigated the intra-donor variability of saliva samples. The first study (Table 1, Study 14) included 33 donors and 3 samples/donor. The average yield per donor was 62.3 µg, however variability within donors had an average standard deviation of ±21.7 (range ±1-75 SD). The second study (Table 1, Study 15) investigated the variation among 41 donors with 6 samples/donor for a total of 246 samples. Here the average yield was 49.9 µg with an average ±25.3 SD (range ±3-95 SD). The data highlights some considerations that need to be taken into account when comparing yield data between different collections.

Variation within a population

Total DNA yield from saliva varies between individuals making the study population an important consideration when assessing average yield of an entire study. The DNA yield from a population of healthy adults can vary from a few micrograms to almost 400 µg in 2 mL of saliva, with 95% of donors falling between 10 and 165 µg (Table 1, Study 13). When collecting from a more restricted population the average yield and yield range may vary. For example, in a study of transplant recipients conducted by Chakkera et al.[4], the average DNA yield from 2 mL of saliva was 13.3 µg compared to average yields of >50 µg with healthy populations (Studies 1-15). Population based factors must be taken into account when evaluating DNA yield from saliva.

Table 1: Summary of average DNA yield from Oragene self-collection kits by study

Study number	Number of samples	DNA extraction method	Mean yield for 2 mL saliva		
			UV Spectroscopy	Fluorescence	Reference number
1	2101	prepIT•L2P	167.6		1
2	291	prepIT•L2P	187.2		2
3	21	prepIT•L2P	95.2		3
4	91	prepIT•L2P	13.3		4
5	539	prepIT•L2P	155.2	82.2	5
6	72	prepIT•L2P	86.4		6
7	790	prepIT•L2P		NA	7
8	17	prepIT•L2P	154.9		8
9	66	prepIT•L2P	152		9
10	10	prepIT•L2P	NA		10
11	20	prepIT•L2P	139.30	63.65	11
12	208	prepIT•L2P		110*,**	12
13	450	prepIT•L2P		58.5	13
14	99	prepIT•L2P		62.28	14
15	246	prepIT•L2P		49.87	15
16	8	prepIT•L2P	224		16
		Qiagen QIAamp	82		
17	24	prepIT•L2P	40.4	32.8	17
		Promega	74	70.9	
18	60	prepIT•L2P		44.5	18
		GeneFind	75.5	64.7	
		DNAdvance	55.4	33.0	
19	20	prepIT•C2D	21.0	15.8	19
20	20	prepIT•C2D	17.7	13.85	20
21	NA	Gentra Puregene	NA		21
22	16	Gentra Puregene	85.5		22
23	90	Autopure LS (Puregene)	135.9	40.3	23
24	16	Autopure LS (Puregene)	82.3		24
25	10	AutoGenFlex	122.4		25
26	96	Promega Magazorb DNA Mini (Tecan Freedom EVO)	67.2		26
27	18	Qiagen BioRobot EZ1 Tissue Kit		64.0*,**	27
28	20	Magtration 12GC	76.8		28
29	16	Promega Maxwell 16 Tissue Kit	56 (NA)		29

NA = information not published by author
** Median, not mean*
*** F/D Assay*

Table 2: Overall DNA yield from Oragene self-collection kits

DNA extraction method	Mean yield for 2 mL saliva (µg)		Overestimation by UV spectroscopy (µg)	Fold overestimation by UV spectroscopy
	UV spectroscopy	Fluorescence		
prepIT•L2P	128.7	56.3	72.4	2.3
Autopure	101.2	40.3	60.9	2.5
Beads/Columns	58.4	31.2	27.2	1.9
All methods	96.1	42.6	53.5	2.3

Conclusion

When evaluating results from an Oragene/saliva collection, or when trying to compare data from multiple collections or studies, it is important to take the following parameters into consideration: the population being sampled (age, health status, demographics), the time of collection, adherence to collection instructions, purification and quantification methods. Highest yields are observed when purifying with prepIT•L2P. Quantification using fluorescence provides an accurate and DNA specific quantification while quantification by UV spectroscopy tends to overestimate the amount of DNA by 2-3 fold.

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Oragene®•DNA is not available for sale in the United States.

Oragene®•DISCOVER is for research use only, not for use in diagnostic procedures.

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