Evaluation of performance of gDNA from saliva collected with Oragene®•DNA for the purpose of CNV analysis on the Agilent Human Genome CGH array 244K

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Introduction

Array-based comparative genomic hybridization (aCGH) is an efficient, high-resolution tool for detecting and defining genome-wide copy number variation (CNV). CNVs are gaining interest in recent years, as specific sequence changes have become linked with human pathologies. The Agilent Human Genome CGH Array 244K, which contains over 236,000 distinct probes representing both coding and non-coding sequences, is useful for CNV analysis.

Typically, genomic DNA from blood or tissue is used in aCGH studies. However, the use of saliva DNA is increasingly being considered as a viable alternative. Oragene•DNA simplifies sample collection by providing an alternative to blood, eliminating phlebotomy cost and complexity while facilitating collection from subjects dispersed throughout the general population. Oragene•DNA is a non-invasive, self-collection device intended for collection of large quantities of high-molecular weight genomic DNA (gDNA) from saliva. DNA is stabilized at ambient temperature for extended periods enabling sample collection and transport via regular mail and flexibility to process in batches. The current study evaluates the performance of gDNA from saliva on the Agilent Human Genome CGH Array 244K.

Materials and methods

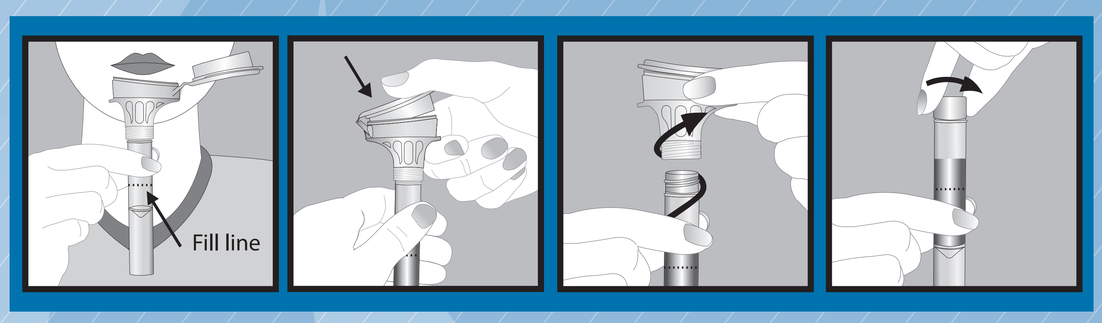

Figure 1: Collection of saliva using Oragene•DNA

- Saliva was collected according to DNA Genotek protocol PD-PR-061.
- 2 saliva samples were collected from 4 donors.
- Each saliva sample was collected on a different day.
- Blood samples were collected from the same 4 donors that donated saliva.
- 8 mL of whole blood was collected using EDTA tubes.
- Blood samples were centrifuged and the buffy coat was collected.
- DNA from buffy coat was purified using the Qiagen QIAamp Blood mini kit.

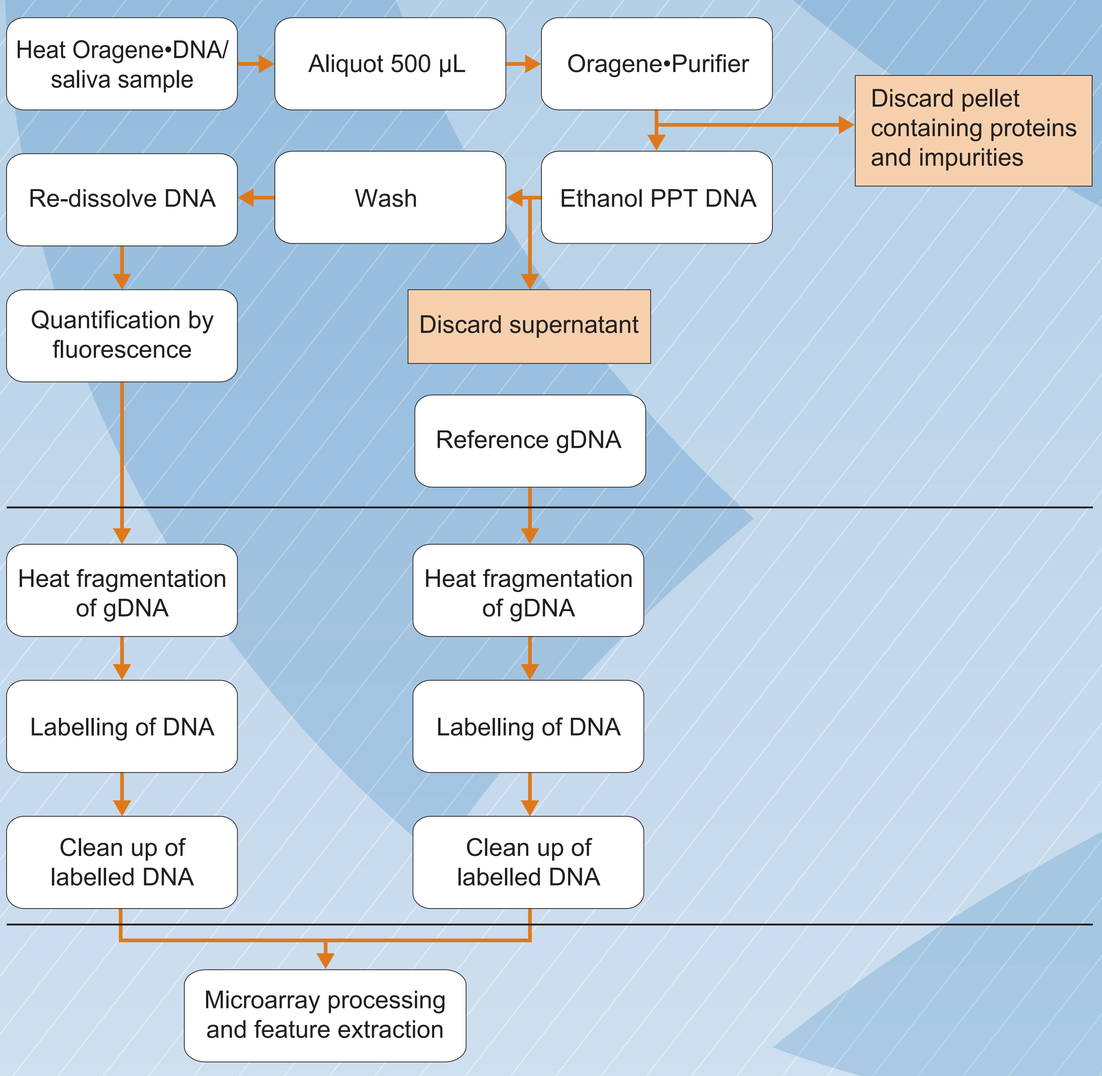

Figure 2: Schematic representation of processing flow

- Oragene•DNA/saliva samples were purified according to DNA Genotek protocol PD-PR-006. DNA was quanitfied using the Invitrogen Picogreen® Quant-iT™.
- Copy number variation analysis was performed using the DNA Analytics 5.0 software (Genomics Workbench Standard Edition 5.0) provided by Agilent Technologies.
- The Aberration Detection Method 2 (ADM-2) alogrithm was used to analyze the data.
- The ADM-2 algorithm identifies all aberrant intervals in a given sample with consistently high or low log ratios based on the Z statistical score implemented in the software. The ADM-2 algorithm searches for intervals in which a statistical score based on the average quality weighted log ratio of the sample and reference channels exceeds a user specified threshold (default 5.0 used). ADM-2 reports contiguous genomic regions of arbitrary size as aberrant regions.
- ADM-2 scores may identify extended aberrant segments with low absolute mean ratios. Often such aberrations represent noise, and are detected because of a high number of probes in the region. Fuzzy zero algorithm was applied to correct for the reliance on segment probe number.

Results

Sample ID	Design No.	QC Status	DLRSpread	SignalToNoise Green	SignalToNoise Red	SignalIntensity Green	SignalIntensity Red	BGNoise Green	BGNoise Red	Reproducibility Green	Reproducibility Red
Donor 1 - Blood	14693	Pass	0.15	117.93	106.41	414.33	503.72	3.51	4.73	0.07	0.11
Donor 1 - Saliva rep. 1	14693	Pass	0.18	128.19	106.69	335.25	354.04	2.62	3.32	0.09	0.11
Donor 1 - Saliva rep. 2	14693	Pass	0.15	123.01	82.81	334.19	319.87	2.72	3.86	0.07	0.08
Donor 2 - Blood	14693	Pass	0.19	109.72	140.52	324.33	387.53	2.96	2.76	0.09	0.15
Donor 2 - Saliva rep. 1	14693	Pass	0.18	143.98	105.21	327.20	302.76	2.27	2.88	0.09	0.13
Donor 2 - Saliva rep. 2	14693	Pass	0.13	150.99	131.75	430.80	503.83	2.85	3.82	0.08	0.09
Donor 3 - Saliva rep. 1	14693	Pass	0.16	146.85	102.21	422.50	434.26	2.88	4.25	0.08	0.09
Donor 3 - Saliva rep. 2	14693	Pass	0.17	142.66	106.22	436.00	501.76	3.06	4.72	0.06	0.07
Donor 3 - Blood	14693	Pass	0.19	101.25	143.50	308.84	407.10	3.05	2.84	0.09	0.13
Donor 4 - Saliva rep. 1	14693	Pass	0.15	119.95	120.73	304.77	335.47	2.54	2.78	0.11	0.16
Donor 4 - Saliva rep. 2	14693	Pass	0.14	129.40	92.10	358.12	358.80	2.77	3.90	0.11	0.14
Donor 4 - Blood	14693	Pass	0.20	92.64	108.37	245.58	286.63	2.65	2.64	0.13	0.19
Agilent acceptance criteria:											
Poor		>0.3	<30		<50		>10		>0.2		
Good		0.2-0.3	30-100		50-150		5-10		0.05-0.2		
Excellent		<0.2	>100		>150		<5		<0.05		

Table 1: Quality control metrics for both blood and saliva samples

- All samples were observed to have good or excellent QC parameters as determined by the Agilent DNA Analytics software.

CNV analysis:

CNV Call Criterions where set as follows:

- Minimum number of probes: 5
- Minimum average of absolute log ratio: 0.25
- Maximum number of aberrations per sample: 100

Donor sample	Total CNVs		Common CNVs between replicates
	Replicate 1	Replicate 2	
1	28	24	20
2	16	15	12
3	12	15	9
4	19	18	17

Table 2: CNV reproducibility analysis in each donor saliva sample

Donor sample	Total CNVs		Common CNVs between saliva and blood
	Saliva replicates (1+2)	Blood	
1	20	20	16
2	12	20	12
3	9	15	9
4	17	30	17

Table 3: CNV reproducibility analysis in saliva versus blood samples

- Less than 29 CNVs per genome were detected in individual saliva samples.
- There was a high degree of concordance between replicate samples from the same donor (Table 2).
- Excess of 80% of the CNVs found in saliva were present in the corresponding blood samples (Table 3).

Conclusions

- Saliva collected using the Oragene•DNA self-collection kit provides genomic DNA of sufficient quality for CNV analysis on the Agilent Human Genome CGH array 244K.
- DNA from saliva does not vary over time as demonstrated through intra-donor CNV reproducibility of samples taken from the same donor on two different days.
- DNA from saliva is a suitable substitute to the use of DNA from blood for analysis of CNVs on the Agilent Human Genome CGH array 244K.

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