Evaluation of oral samples and tracheal fluid collection from premature newborns as a source of DNA for genotyping

Jacques Niles[1], Valerie Biran[2] and Rafal M. Iwasiow[1]

[1] DNA Genotek, Inc. Ottawa, Ontario, Canada
[2] Service de Pédiatrie et Réanimation NéonatalesHôpital Robert Debré, APHP, Université Paris 7, France

Introduction

Newborn screening is an important procedure in which newborn infants are screened for a list of genetic diseases. The standard procedure is to sample whole blood from the heel of the infant and blot it on a specially designed filter paper. Recently it has been suggested that screening dried blood spots for some infections, such as congenital cytomegalovirus (CMV), may not be suitable and that other specimen types, such as oral samples and tracheal fluids, should be explored.

In the current study, oral sample and tracheal fluid, both non-invasive collection methods, were evaluated for suitability of DNA collection from premature newborns for genotyping purposes. The oral samples were collected using sterile rayon swabs to collect sample from the mouth of the premature newborn and cutting the heads of the swab into an Oragene®•DNA kit containing preservation solution. Paired tracheal fluid was also collected by rinsing a tracheal tube with saline (between 300 and 500 µL) and adding the rinsed fluid to the Oragene•DNA collection kit. The samples were taken from premature newborns as young as 25.7 weeks (gestational age).

Oragene•DNA is a non-invasive collection device that is intended for collection and stabilization of DNA from saliva for extended periods at ambient temperature, thus enabling transport through regular mail. The performance of the extracted gDNA was assessed by evaluating different markers using PCR based genotyping methods. The results indicate good quality and quantity of DNA can be extracted from both tracheal fluids and oral samples collected into Oragene•DNA kit.

Materials and methods

Sample collection

For this study samples were collected from 29 donors, where paired samples of tracheal fluid and swabs were collected. The swab samples were collected using 3 sterile rayon swabs (Copan, Italy catalogue # 155C). The swab heads were cut and placed in an OG-250 collection device. For the tracheal samples a volume between 300 and 500 µL of fluid was collected into an OG-250 collection device.

DNA extraction

Both sample types were incubated at 50°C overnight upon arrival into the lab. After the incubation step, a 0.5 mL aliquot was purified using the DNA Genotek manual purification protocol (PD-PR-006). The rehydration volume was reduced to 50 µL of 1x TE instead of 100 µL in order to increase the purified concentration of each sample. The samples were then left at room temperature overnight in order to fully dissolve the DNA. The following day the gDNA was quantified using the Invitrogen Picogreen® Quant-IT™ and checked for purity using absorbance.

Data analysis

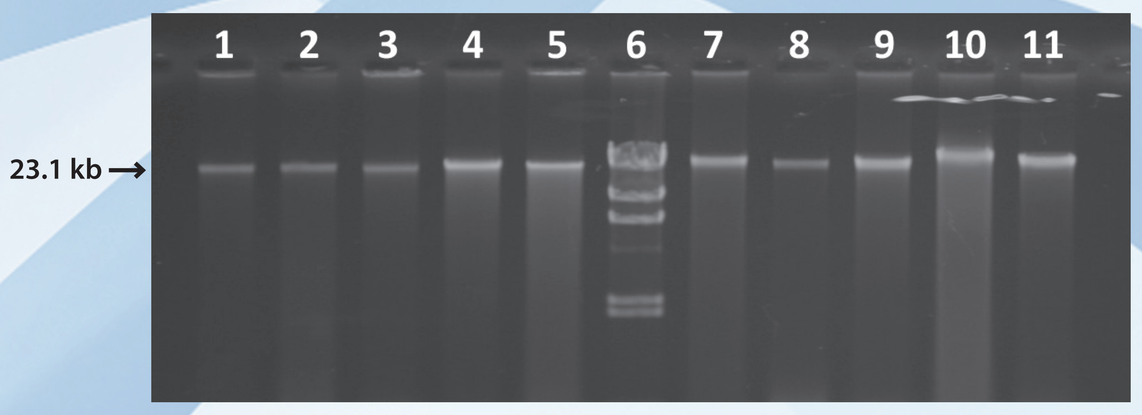
After purification, the gDNA was tested for performance on some downstream assays, such as sex typing (conventional PCR) and was also genotyped using several SNPs including drug response: CYP450 2C9 430 C>T (rs1799853), CYP450 2C9 1075 A>C (rs1057910), VKORC1 -1639G>A (rs9923231), disease carrier status SNPs such as BCKDHB 615 G>C (rs79761867) as well as traits such as MCM6 136325116 G>A (rs4988235). The genomic DNA was run on an agarose gel in order to check for quality.

Results

Table 1: Summary data from both swab and tracheal collections

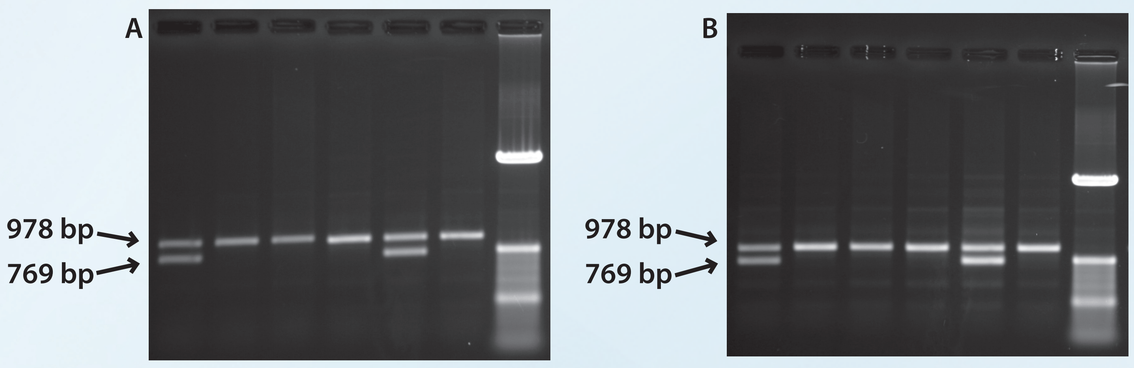
Sex		Gestational age (weeks)	Volume of tracheal fluid (mL)	DNA Yield per sample (ug)		Ratio (260/280)	
				Swab	Tracheal	Swab	Tracheal
17 Males	Average	29.9	0.3	1.02	13.82	1.6	1.6
12 Females	Min	25.7	0.2	0.31	0.11	1.2	1.4
	Max	40.6	0.5	1.87	85.44	1.9	1.8
	Median	29.6	0.3	0.91	4.76	1.6	1.7
	Std Dev	N/A	N/A	0.50	18.75	0.2	0.1

Figure 1: Representative agarose gel of 5 paired swab (Lanes 1 to 5) and tracheal (Lanes 7 to 11) samples. 50 ng of DNA was loaded onto a 0.8% agarose gel and run for 45 minutes at 80 volts.



All samples had high molecular weight genomic DNA

Figure 2: Representative agarose gel of sex typing PCR (Amelogenin). Double bands (978 bp and 769 bp) indicate a male donor, while a single band (978 bp) indicates a female donor. Figure A are swab samples while figure B are the paired tracheal samples.



There was a 100% concordance between the swab and tracheal samples.

Figure 3: Graphical representation of genotyping results for swab samples.

	CYP450 2C9 430 C>T	CYP450 2C9 1075 A>C	VKORC1 -1639G>A	BCKDHB 615 G>C	MCM6 136325116 G>A
Wild type	1 2 3 4 5 6 7 8 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 27 28 29	1 2 3 4 5 6 7 8 9 12 14 15 16 17 18 19 20 21 24 27 28 29	1 3 4 5 6 7 9 10 13 18 20 22 23 24 25 27 28	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29	N/A
Heterozygous	9 26	10 11 13 22 23 25 26	8 11 12 14 17 21 29	N/A	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
Mutant	N/A	N/A	2 15 16 19 26	N/A	N/A

All tracheal samples demonstrated 100% concordance with the data obtained with swab samples.

Conclusion

- Collection of both swab and tracheal fluids using Oragene•DNA yields high quality genomic DNA.
- The purified DNA from both sample types is suitable for downstream applications such as SNP genotyping assays.
- There is a 100% concordance between the swab and tracheal fluid samples.
- The gender of each donor was correctly identified using a conventional PCR sex typing method.

U.S. Patent No. 7,482,116;
European Patent No. 1 513 952 and patent pending
Canadian Design No. 113861
U.S. D562,462 S
Community Design No. 522339-0001

DNA genotek

Superior samples
Proven performance

www.dnagenotek.com • support@dnagenotek.com