Performance Evaluation of the ForenSeq mtDNA Control Region Solution

Paulina Walichiewicz, Justin Eagles, Anthony Daulo, Meghan Didier, Chris Edwards, Keenan Fleming, Yonmee Han, Timothy Hill, Sanja Li, Anthony Rensfield, Dan Sa, Julian Husbands, Cydne Holt, Kathryn Stephens

Verogen, Inc., 11111 Flintkote Ave., San Diego, CA 92121, USA

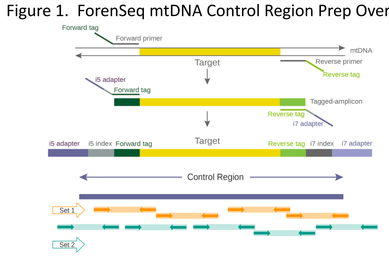
Introduction

Massively parallel sequencing (MPS) of human mitochondrial DNA surpasses Sanger sequencing and capillary electrophoresis with regard to labor intensity, reagent quality control and data quantity and quality. These improvements assist forensic samples in the context of research studies, criminal and missing persons casework and disaster victim identification.

We present a brief overview of recent advances in mtDNA genome knowledge and resultant design improvements now achieved in forensic mtDNA analysis. These include more comprehensive variant detection, heteroplasmy determination, and sensitivity using the MiSeq FGx sequencer and ForenSeq® Universal Analysis Software (UAS) v2. We show initial validation data that were generated using the ForenSeq mtDNA Control Region Kit and analyzed in ForenSeq Universal Analysis Software (UAS) v2.

ForenSeq mtDNA Control Region Kit library preparation uses the same basic workflow as the ForenSeq® DNA Signature prep kit (Fig 1) with the exception that two PCRs are prepared for each sample, each with a different Primer Mix (Set 1, Set 2). This approach supports a tiled approach to the primer design, which helps to ensure coverage (reads) across the entire control region (Fig 1).

Figure 1. ForenSeq mtDNA Control Region Prep Overview



- *Overlapping primary amplicons (< 150 bp) for complete coverage under primer binding sites*
- *3 bp spacing between primary amplicons to mitigate end of read trimming during analysis*
- *18 primer pairs comprised of 122 primers to efficiently amplify degenerate bases in mtGenome*

Materials and Methods

Human mtDNA libraries were prepared from different input gDNA concentrations (1 pg – 100 pg) using the Verogen ForenSeq mtDNA Control Region Kit. 12 or 48 libraries (HL-60 at varying inputs with replicates, other DNA samples at varying inputs, and 2-4 negative controls, as indicated) were sequenced together on the MiSeq FGx using the MiSeq FGx Regent Micro Kit following the User Guide (2 x 151 bp; run time = 18 hours). gDNA from matched sets of hair and buccal samples from volunteers was extracted[1]. DNA was quantified using DNA One assay on the Quantifluor[2]. Results were analyzed in the ForenSeq UAS 2.0. Samples included HL-60 Control DNA[3], five extracted DNAs from teeth[4], twelve extracted DNAs from bones[5,6], extracted matched sets of buccal and hair DNAs from eight volunteers, 2800M[2] and six high quality purchased DNAs[7,8].

Results & Discussion

Sensitivity studies were conducted to illustrate the ideal DNA input range and limit of detection of the ForenSeq mtDNA Control Region kit, under specific parameters. High quality DNA samples were serially diluted to input 1 to 100 pg gDNA and split between the two amplifications with the two primer mix sets (set 1, Set 2). The ForenSeq mtDNA Control Region kit supports two options for normalization prior to pooling and sequencing:

The Bead-Based Normalization (BBN) method is best for large numbers of high-quality samples (8-48) and especially when automated platform(s) are desired to conduct library normalization, prior to sequencing.

Libraries from low DNA input (<20 pg gDNA) or lower quality DNA samples benefit from the Manual Quantification and Normalization method as illustrated in Figure 2.

Compared to BBN, increased read coverage was observed from the lower input DNA samples when the Manual Quantitation and Normalization method was used. Coverage levels and variant calls concordance, analyzed in the UAS, showed reproducibility across the input ranges tested independent of the normalization method (Fig 3A,B). Coverage levels depend upon the numbers of samples sequenced together (sequencing multiplexy), sample type, quality, and the degree of heteroplasmy detection desired. 48 libraries were sequenced together for in this study for variant calling >10%. If lower level detection is desired, sequencing fewer (<48) samples together can provide much higher coverage to assist in detection of low-level variants. To obtain deeper coverage, 12 of the 48 libraries described here were also sequenced independently on the MiSeq FGx. Four times higher coverage per sample was seen for the 12-plex run compared to the same samples on the 48-plex run (Fig 4). The higher coverage seen in the 12 samples run did not adversely impact variant calling (Fig 5).

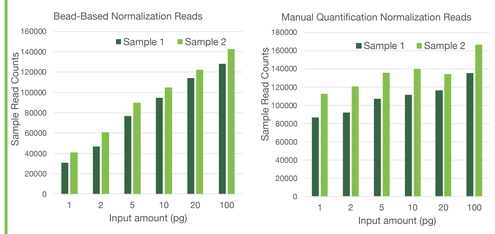
Control DNAs, that had Sanger-based variant calls available, were analyzed in a concordance study. SRM 2392[8] samples were sequenced in triplicate at 100 pg. All calls were concordant with the Certificate of Analysis from NIST with the exception of heteroplasmy seen at position 64 in sample CHR (Table 1). Heteroplasmy was detected at this position using ForenSeq and went undetected using Sanger sequencing[9].

ForenSeq mtDNA Control Region library prep's inhibitor resistance was studied using calcium ions, humic acid and bacterial DNA (Fig 6). Coverage similar to the control samples (no inhibitor) was observed. All calls were made across the concentrations of inhibitors tested.

Mixture samples were analyzed to determine the limit of detection of low-level variants in 48-plex runs on MiSeq FGx micro flow cells. Using a threshold setting of 2.5% for the AT and the IT, all variants from the minor contributor were detected with 100 pg gDNA as input template amount. The majority of variants from the minor contributor were detected in the mixture samples with 5 pg of gDNA. Once occurrence of drop-in was observed in the 1:5 mixture 5 pg gDNA sample that was also observed in the 5 pg gDNA HL-60 control library.

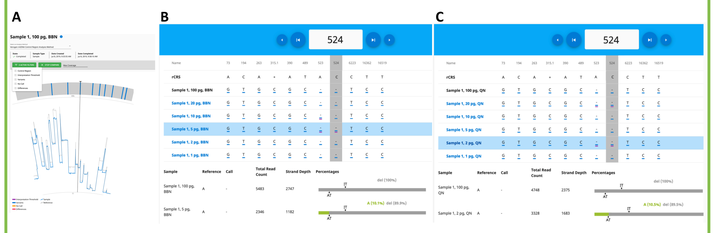
Performance of the ForenSeq library prep and software on mock casework samples was studied using DNA extracts from hair shafts (0.5 cm or 2 cm in length; no roots) and from buccal swabs, from the same individuals. 6 µL of hair and buccal extract was used in each of the ForenSeq amplifications, and from bone and teeth samples. 92% and 90% concordance were observed for the 2 cm and 0.5 cm hair extracts, respectively, relative to buccal samples for the 8 individuals tested. Seven of eight of the discordant calls were in the C-stretch of hVII. Results for two of the individuals are shown in Tables 3 and 4. Subject 2 (Table 3) shows an example of the C-stretch discordance. EMPOP reports were generated for each of these samples in the ForenSeq UAS; haplogroups were determined on the EMPOP website (https://empop.online/haplotypes). Results are shown in Table 5. Libraries were prepared from 100 pg gDNA extracted from a tooth from five different individuals[4] and from seven DNA extracts from burned or cremated bones[6]. Complete coverage across the control region was observed (Figs 7 and 8), and all variants were correctly called (Table 6). Performance was also assessed on DNA extracts from interred bone [5]. Libraries were generated from approximately 2000-4000 mitochondrial DNA copies [1] (data not shown) per ForenSeq PCR (Fig 9, coverage plot). Variants observed for these samples are shown in Table 6. Sample "Interred Bone 2" had lower coverage and some loss of coverage which may be the result of DNA damage (Fig 9).

Figure 2. Sensitivity and Reproducibility Studies for Both BBN and Manual Quant Normalization of mtDNA Libraries



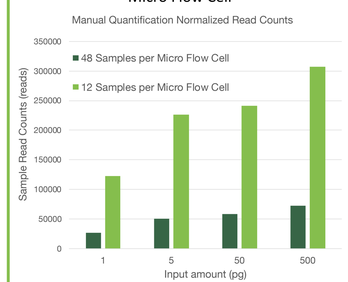
ForenSeq libraries were prepared from two high quality DNAs across a range of gDNA input amounts. The same libraries were either normalized using the Bead-Based or the Manual Quantification Normalization methods following the user guide. The pools were sequenced independently and the results compared.

Figure 3. Sensitivity and Reproducibility Studies: Coverage and Variant Calling from Two Normalization Methods



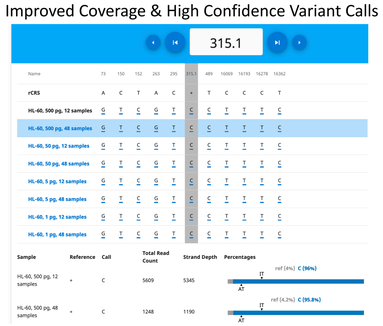
Read coverage and variant calls for ForenSeq libraries in Fig 2: A) ForenSeq UAS 2.0 screenshot of the mtDNA Navigator showing coverage for two, overlaid samples across the Control Region. B) ForenSeq UAS 2.0 screenshot showing Sample Compare feature of the software, as employed among seven samples (rCRS, Sample 1 @100pg and 5 other samples in the dilution series), that were normalized using BBN. C) ForenSeq UAS 2.0 screenshot showing Sample Compare feature of the software, as employed among the same samples as 3B) but normalized using Manual Quantification and Normalization (QN). The ForenSeq UAS 2.0 Analytical Threshold was 10%; heteroplasmy proportions for Sample 1 at positions 523/524 hovered at ~10%, leading to call rate variation for the 5pg sample.

Figure 4. Coverage Relative to Sample Multiplexy: 12 vs. 48 Libraries per MiSeq FGx Micro Flow Cell



Reducing the sample multiplexy on a MiSeq FGx micro flow cell by 4-fold (48 samples and 12 samples) increases read coverage 4.1 - 4.6 fold for the 4 ForenSeq libraries represented above (1, 5, 50 and 500 pg gDNA), with a range of 4.1 - 5.0 across all 12 libraries.

Figure 5. Coverage Relative to Sample Multiplexy: Improved Coverage & High Confidence Variant Calls



ForenSeq UAS 2.0 Sample Compare feature employed across 8 samples (HL-60 500, 50, 5, and 1 pg on MiSeq FGx micro flow cell runs of 12 and 48 samples). All mtDNA control region variants called correctly at the varying DNA inputs in the 48-plex sequencing run and in the 12-plex sequencing run.

Table 1. Control DNAs, Concordance: ForenSeq mtDNA Control Region Kit and Software

NIST 2392/2392-I Control Evaluation *

Sample	*Input*	*Reps*	*Concordance*	*Expected Variants*	*Observed Variants*
CHR	100 pg	3	93%	73G 195C 204C 207A 263G 309.1C 315.1C 16183C 16189C 16193.1C 16223T 16278T 16519C	**64Y†** 73G 195C 204C 207A 263G 309.1C 315.1C 16183C 16189C **16193.1c‡** 16223T 16278T 16519C
9947A	100 pg	3	100%	93G 195C 214G 263G 309.1C 309.2C 315.1C 16311C 16519C	93G 195C 214G 263G 309.1C 309.2C 315.1C 16311C 16519C
HL-60	100 pg	3	100%	73G 150T 152C 263G 295T 315.1C 489C 16069T 16193T 16278T 16362C	73G 150T 152C 263G 295T 315.1C 489C 16069T 16193T 16278T 16362C
GM03798	100 pg	3	100%	263G 315.1C 16357C 16519C	263G 315.1C 16357C 16519C
GM10472A	100 pg	3	100%	73G 185A 228A 263G 295T 315.1C 462T 482C 489C 16069T 16126C 16292T	73G 185A 228A 263G 295T 315.1C 462T 482C 489C 16069T 16126C 16292T

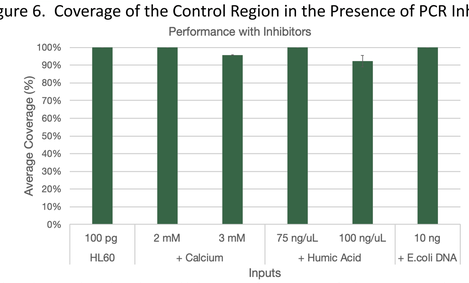
** Analysis Settings used were Analytical Threshold (AT) = 10% , Interpretation Threshold (IT) = 25%, Minimum Quality Score = Q30; Minimum Read Count = 10*

† Heteroplasmy observed in MiSeq FGx data [9]

‡ Insertion called as minor allele (43.2% avg, n = 3)

ForenSeq libraries were prepared from 100 pg of five NIST SRM gDNAs, split across the two mtDNA control region PCRs, and in triplicate. The resulting 15 libraries were sequenced on a MiSeq FGx with 27 other libraries, including negative controls, for a 42-sample plex run.

Figure 6. Coverage of the Control Region in the Presence of PCR Inhibitors



Inhibitors (calcium, humic acid, E. coli DNA) were added to 100 pg of HL-60 gDNA, to final concentrations indicated on the x-axis, in ForenSeq PCR1. Coverage (read counts) was normalized to the control sample with no inhibitor (sample at far left). All control region bases were called correctly. The sample containing E. coli DNA was also analyzed for presence of microbial DNA in the sequencing results. No bacterial sequence was detected as analyzed using BWA alignment to the E. coli genome (data not shown).

Table 2. Detection of Minor Contributor Variants in Mixture Samples

2800M:HL-60 Mixtures, 100 pg Input, 2.5% AT/IT*

Mix Ratio	*Expected VAF*	*Minor Component Range*	*Major Component Range*	*Expected Variants*	*Observed Minor Component Variants*
1:3	33 / 67 %	22 - 36 %	64 - 78 %	10	10
1:5	16 / 84 %	10 - 17 %	82 - 90 %	10	10
1:15	6 / 94 %	4 - 7 %	93 - 96 %	10	10

2800M:HL-60 Mixtures, 5 pg Input, 2.5% AT/IT*

Mix Ratio	*Expected VAF*	*Minor Component Range*	*Major Component Range*	*Expected Variants*	*Observed Minor Component Variants*
1:3	33 / 67 %	24 - 36 %	64 - 76 %	10	10
1:5	16 / 84 %	4 - 19 %	81 - 96 %	10	11†
1:15	6 / 94 %	3 - 7 %	93 - 97 %	10	8

** 10% AT/IT for C-stretches and AC repeat region*

† Drop-in 501Y 4.4%

Table 3. Variant Reports For Buccal, 2 cm and 0.5 cm Hair Shaft Extracts from Subject 2

Subject 2:			*Buccal*							*2 cm Hair*							*0.5 cm Hair*						
Variant	*Ref*		*Total Read Count*	*Strand Depth*	*% A*	*% C*	*% G*	*% T*	*% Del*	*% Ref*	*Total Read Count*	*Strand Depth*	*% A*	*% C*	*% G*	*% T*	*% Del*	*% Ref*	*Total Read Count*	*Strand Depth*	*% A*	*% C*	*% G*	*% T*	*% Del*	*% Ref*
73G	A		9364	5071	0%	0%	100%	0%	0%	0%	9310	4792	0%	0%	100%	0%	0%	0%	7409	3845	0%	0%	100%	0%	0%	0%
146C	T		3477	2048	0%	100%	0%	0%	0%	0%	3807	2019	0%	100%	0%	0%	0%	0%	3471	1845	0%	100%	0%	0%	0%	0%
150T	C		6457	3466	0%	0%	0%	100%	0%	0%	7053	3581	0%	0%	0%	100%	0%	0%	6211	3153	0%	0%	0%	100%	0%	0%
263G	A		8992	4559	0%	0%	100%	0%	0%	0%	6359	3202	0%	0%	100%	0%	0%	0%	5456	2734	0%	0%	100%	0%	0%	0%
309.1c	+		158	125	0%	80%	0%	0%	0%	20%	122	32	0%	28%	0%	0%	0%	72%	113	61	0%	58%	0%	0%	0%	43%
315.1c	+		186	165	0%	90%	0%	0%	0%	10%	138	120	0%	89%	0%	0%	0%	11%	*127*	*119*	*0%*	*94%*	*0%*	*0%*	*0%*	*6%*
523a	A		2455	1253	11%	0%	0%	100%	0%	0%	1106	575	14%	0%	0%	86%	0%	0%	853	439	12%	0%	0%	88%	0%	0%
524c	C		2448	1244	0%	11%	0%	0%	89%	0%	1102	572	0%	14%	0%	0%	86%	0%	848	437	0%	12%	0%	0%	88%	0%
16129C	T		22911	11547	0%	100%	0%	0%	0%	0%	19488	9760	0%	100%	0%	0%	0%	0%	14733	7391	0%	100%	0%	0%	0%	0%
16292T	C		18508	9618	0%	0%	0%	100%	0%	0%	29602	15115	0%	0%	0%	100%	0%	0%	21035	10771	0%	0%	0%	100%	0%	0%
16294T	C		19534	10145	0%	0%	0%	100%	0%	0%	29976	15224	0%	0%	0%	100%	0%	0%	21586	11005	0%	0%	0%	100%	0%	0%
16296T	C		19673	10175	0%	0%	0%	100%	0%	0%	30060	15346	0%	0%	0%	100%	0%	0%	21696	11071	0%	0%	0%	100%	0%	0%
16519C	T		5325	3080	0%	100%	0%	0%	0%	0%	7519	3899	0%	100%	0%	0%	0%	0%	5679	2983	0%	100%	0%	0%	0%	0%

Italics indicate a call in 0.5 cm hair extract that was below the 10% AT

Table 4. Variant Reports For Buccal, 2 cm and 0.5 cm Hair Shaft Extracts from Subject 3

Subject 3:			*Buccal*							*2 cm Hair*							*0.5 cm Hair*						
Variant	*Ref*		*Total Read Count*	*Strand Depth*	*% A*	*% C*	*% G*	*% T*	*% Del*	*% Ref*	*Total Read Count*	*Strand Depth*	*% A*	*% C*	*% G*	*% T*	*% Del*	*% Ref*	*Total Read Count*	*Strand Depth*	*% A*	*% C*	*% G*	*% T*	*% Del*	*% Ref*
73G	A		12063	6480	0%	0%	100%	0%	0%	0%	9935	5149	0%	0%	100%	0%	0%	0%	9015	4682	0%	0%	100%	0%	0%	0%
146C	T		6119	3533	0%	100%	0%	0%	0%	0%	6019	3162	0%	100%	0%	0%	0%	0%	4821	2560	0%	100%	0%	0%	0%	0%
154C	T		8670	4839	0%	100%	0%	0%	0%	0%	8371	4363	0%	100%	0%	0%	0%	0%	7124	3719	0%	100%	0%	0%	0%	0%
200G	A		3283	1752	0%	0%	100%	0%	0%	0%	2563	1309	0%	0%	100%	0%	0%	0%	2458	1259	0%	0%	100%	0%	0%	0%
215G	A		3320	1729	0%	0%	100%	0%	0%	0%	1081	552	0%	0%	100%	0%	0%	0%	629	325	0%	0%	100%	0%	0%	0%
263G	A		3133	1791	0%	0%	100%	0%	0%	0%	993	557	0%	0%	100%	0%	0%	0%	571	328	0%	0%	100%	0%	0%	0%
309.1c	+		72	59	0%	83%	0%	0%	0%	17%	17	16	0%	94%	0%	0%	0%	*6%*	19	14	0%	79%	0%	0%	0%	21%
315.1C	+		85	84	0%	99%	0%	0%	0%	*1%*	18	16	0%	89%	0%	0%	0%	11%	21	17	0%	86%	0%	0%	0%	14%
319C	T		81	81	0%	100%	0%	0%	0%	0%	20	20	0%	100%	0%	0%	0%	0%	19	17	0%	100%	0%	0%	0%	0%
326G	A		83	83	0%	0%	100%	0%	0%	0%	16	16	0%	0%	100%	0%	0%	0%	20	18	0%	0%	100%	0%	0%	0%
489C	T		1453	755	0%	100%	0%	0%	0%	0%	191	100	0%	100%	0%	0%	0%	0%	106	56	0%	100%	0%	0%	0%	0%
16223T	C		12088	6210	0%	0%	0%	100%	0%	0%	21925	11299	0%	0%	0%	100%	0%	0%	16979	8761	0%	0%	0%	100%	0%	0%
16311Y	**T**		**40703**	**20529**	**0%**	**20%**	**0%**	**80%**	**0%**	**0%**	*55808*	*28115*	*0%*	*0%*	*0%*	*100%*	*0%*	*0%*	*42448*	*21398*	*0%*	*0%*	*0%*	*100%*	*0%*	*0%*

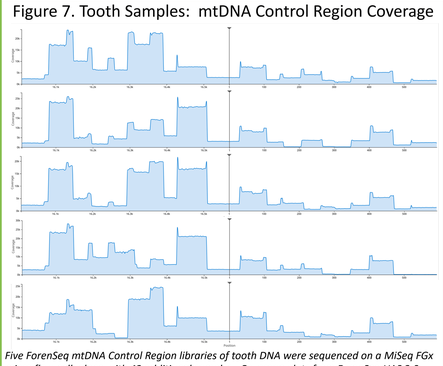
Table 5. Haplogroups Determined from Sequencing Results of Each of the DNA Extracts

EMPOP Results

Analytical Threshold: 10% *Interpretation Threshold: 10%*

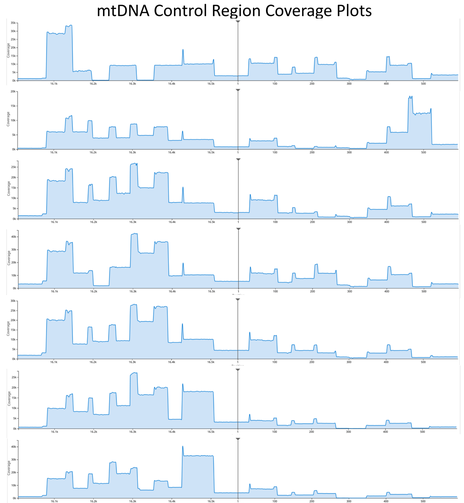
Subject	Type	HaploGroup	Subject	Type	HaploGroup
1	Buccal	B4b1a+207	*5*	Buccal	R0
	0.5 cm Hair	B4b1a+207		0.5 cm Hair	R0
	2.0 cm Hair	B4b1a+207		2.0 cm Hair	R0
2	Buccal	T2c1+146	*8*	Buccal	D4b2b
	0.5 cm Hair	T2c1+146		0.5 cm Hair	D4b2b
	2.0 cm Hair	T2c1+146		2.0 cm Hair	D4b2b
3	Buccal	M11b1a	*11*	Buccal	F1a1a
	0.5 cm Hair	M11b1a		0.5 cm Hair	F1a1a
	2.0 cm Hair	M11b1a		2.0 cm Hair	F1a1a
4	Buccal	HV	*12*	Buccal	R0
	0.5 cm Hair	HV		0.5 cm Hair	R0
	2.0 cm Hair	HV		2.0 cm Hair	R0

Figure 7. Tooth Samples: mtDNA Control Region Coverage



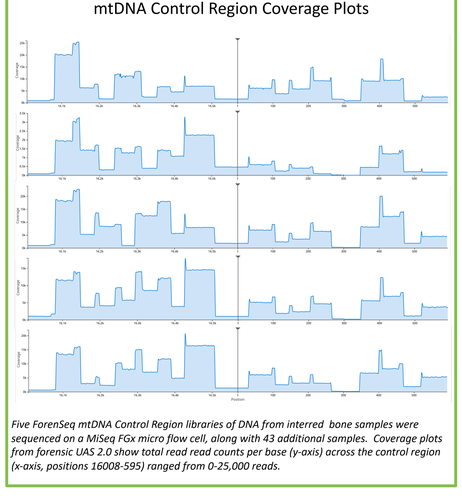
Five ForenSeq mtDNA Control Region libraries of tooth DNA were sequenced on a MiSeq FGx micro flow cell, along with 43 additional samples. Coverage plots from ForenSeq UAS 2.0 show total read read counts per base (y-axis) across the control region (x-axis, positions 16008-595) ranged from 100-28,000 reads.

Figure 8. Burned Bone Samples: mtDNA Control Region Coverage Plots



Seven ForenSeq mtDNA Control Region libraries of DNA from burned bone samples were sequenced on a MiSeq FGx micro flow cell, along with 41 additional samples. Coverage plots from ForenSeq UAS 2.0 show total read read counts per base (y-axis) across the control region (x-axis, positions 16008-595) ranged from 100-42,000 reads.

Figure 9. Interred Bone Samples: mtDNA Control Region Coverage Plots



Five ForenSeq mtDNA Control Region libraries of DNA from interred bone samples were sequenced on a MiSeq FGx micro flow cell, along with 43 additional samples. Coverage plots from forensic UAS 2.0 show total read read counts per base (y-axis) across the control region (x-axis, positions 16008-595) ranged from 0-25,000 reads.

Table 6. Variants Observed for Tooth and Bone DNA Extract Samples

Unknown Extracted Tooth, Burned Bone, and Interred Bone Samples

Sample, Extraction Method	*Input*	*CR Coverage*	*Observed Variants*
Tooth 1661, InnoGenomics	100 pg	100%	73G 150T 152C 263G 315.1C 523C 524C 16124C 16223T 16311C 16399G
Tooth 1662, InnoGenomics	100 pg	100%	73G 153G 195C 225A 226C 263G 309.1c 315.1C 16189C 16193.1c 16223T 16278T 16519C
Tooth 1663, InnoGenomics	100 pg	100%	73G 150T 152C 195C 198T 263G 315.1C 16189c 16223T 16320T 16519C
Tooth 1664, InnoGenomics	100 pg	100%	73G 146C 152C 195C 263G 309.1C 315.1C 507C 16223T 16278T 16286T 16294T 16309G 16390A 16519C
Tooth 1665, InnoGenomics	100 pg	100%	64T 93G 185A 189G 200G 236C 247A 263G 315.1C 522DEL 523DEL 16129A 16148T 16168T 16172C 16187T 16188G 16189C 16223T 16230G 16311C 16320T 16325C 16362C
Cremated Bone, PrepFiler BTA	100 pg	100%	73G 150T 263G 315.1C 16189C 16193.1c 16270T 16398A
Embalmed Bone, Total Demin.	100 pg	100%	73G 150T 185A 228A 263G 295T 309.1C 315.1C 462T 489C 16069T 16126C
Embalmed Bone, PrepFiler BTA	100 pg	100%	73G 143A 146C 152C 189G 195C 263G 315.1C 16129A 16189C 16192T 16223T 16278T 16294T 16309G 16390A
Early Decomp. Bone, PrepFiler BTA	100 pg	100%	73G 152C 263G 315.1C 16093C 16256T 16270T 16399G
Burned Bone, Total Demin.	100 pg	100%	195C 263G 315.1C 523DEL 524DEL
Mildly Burned Bone, PrepFiler BTA	100 pg	100%	73G 263G 309.1c 315.1C 16126C 16294T 16296T 16519C
Mildly Burned Bone, PrepFiler BTA	100 pg	100%	263G 309.1C 315.1C 316A 16291T 16519C
Interred Bone 1, Unknown	8000 copies	100%	73G 263G 315.1C 16192T 16256T 16270T 16291T 16399G
Interred Bone 2, Unknown	4000 copies	96%*	73G 152Y 185K 189G 195Y 263G 357G 482Y 489Y 523DEL 524DEL 16126C 16187T 16189C 16223Y 16264T 16270T 16278T 16293G 16311C 16519C
Interred Bone 3, Unknown	8000 copies	100%	152C 263G 309.1C 315.1C 16234T
Interred Bone 4, Unknown	8000 copies	100%	257G 263G 315.1C 477C 16519C
Interred Bone 5, Unknown	8000 copies	100%	73G 153G 195C 263G 309.1C 309.2c 315.1C 489G 16189c 16223T 16278T 16294T 16519C

** Drop out region from 297 -347*

Conclusions

The ForenSeq mtDNA Control Region solution using a tiled, targeted amplicon approach for the analysis of the human mitochondrial control region.

The ForenSeq mtDNA Control Region Kit allows library preparation from up to 12 µL of sample volume to assist with low DNA concentration samples. The primary amplicons in the tiling design are <150 bp, facilitating degraded sample amplification.

MiSeq FGx sequencing allows flexibility with ability to choose between the Micro or Standard MiSeq FGx Reagent kits for increased coverage of degraded or low-level input samples and modulation of sample numbers, depending on project or casework needs. Two normalization methods are provided for library preparation to support processing of large numbers of high quality samples (bead based normalization) or small numbers of low input or low-quality samples (manual quantification and normalization). Sequencing at low or high sample multiplexy modulates the amount of coverage seen for each sample with no effect on variant calls. 48 high quality samples can be sequenced together to determine variant calls with the Micro MiSeq FGx Reagent kit, but if low level heteroplasmy detection, deeper coverage across the C-stretch regions or better coverage of low-quality/low input samples is desired, fewer samples can be sequenced together (<12).

ForenSeq UAS 2.0 enables accurate and confident base calls, examination of read depth and strandedness of coverage, user-adjustable threshold settings for Analytical and Interpretation Thresholds, Minimum Read Counts, and can compare samples (with and without filtering for variants). The software creates reports for export for samples, project, CODIS upload, or EMPOP queries.

The system accurately amplifies and sequences known, control DNA samples as illustrated in the concordance data. The library prep method is resistant to known inhibitors which improves performance on hair, bone and teeth DNA extracts.

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