Final Summary Overview for NIJ-2013-MU-MU-K044 (submitted March 31st, 2017)
Development of Probe Capture Next-Generation Sequencing Assays for Degraded DNA
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Project Purpose and Goals

Samples with degraded or limited DNA are often encountered in mass disaster, missing person, and forensic cases, and can present major interpretation challenges for standard STR markers. Alternative markers such as nuclear SNPs (nSNPs) or mitochondrial DNA (mtDNA) can be used for increased genotyping success in these cases.

We proposed to 1) develop and optimize a probe capture enrichment assay for deep sequencing the entire mitochondrial genome, 2) analyze highly degraded and limited forensically relevant samples using the mtDNA probe capture NGS assay, 3) develop a custom software for analyzing mtDNA mixture data, and 4) design and test a nuclear SNP probe capture assay to show proof-of-principle of analysis of limited, degraded, and mixed DNA samples.

Our studies over the granting period show that Next Generation Sequencing (NGS) coupled with a probe capture enrichment system can be used for deep sequencing of the entire mitochondrial genome and nSNP markers, which can be successfully applied to both nuclear and mtDNA analysis of degraded and limited samples. A library preparation method, and a probe capture enrichment and massively parallel sequencing method for sequencing the entire mitochondrial genome has been successfully developed and optimized for reference samples including population samples from different haplogroups, low DNA amounts (1-100 pg) and highly degraded samples including bone specimens. Therefore, a single method can be used for both degraded as well as reference samples. The proof-of-principle using probe capture targeting nuclear SNPs to analyze degraded and limited DNA was also highly successful.

These assays have the potential to greatly improve the discrimination power compared to current mitochondrial HVI/HVII sequence analysis as well as to increase the success rate of genotyping of degraded samples. These methods would have broad applications to any field with low DNA starting amounts and critical to forensic applications.

1) Develop and Optimize a Probe Capture assay for sequencing the mitochondrial genome

A. Covaris Optimization:

One of our goals was to optimize the Covaris M220 shearing parameters for illumina sequencing and test the optimized parameters on both degraded and reference samples. Based on the sample size range obtained from fragmentation for 200, 250 and 300 bp (using Covaris M220 suggested manufactures parameters), we determined that the 250 bp parameter produced the most consistent and narrow fragment size range within the 250-500 bp requirement for Illumina sequencing.

The optimized fragmentation method was applied to various specimens including DNA extracted from bones (50 to 1500 years old), mock degraded samples, and blood derived DNA. These samples were successfully sequenced, showing that Covaris shearing is independent of sample quantity and quality.

B. Optimize a rapid DNA library preparation method:

One primary aim of the grant was to optimize a generic rapid DNA library preparation that can be applied to forensic samples for sequencing on any NGS platform (Illumina, 454, or IonTorrent) by changing the adapter sequence. Each mechanically sheared DNA sample is ligated with unique barcoded dual matched index adapter in order to be able to pool samples prior to probe capture. We use IDT's HT Dual Matched Index adapters rather than combinatorial indexes to be able to trace Jumping PCR/Template switching.

Early findings showed that our initial approach for library preparation lacked robustness and reproducibility. Based on the literature, we identified and tested an alternative library preparation method with manufacturer claims of improved ligation efficiency, higher PCR fidelity, higher sensitivity (optimized for as low as 1 ng), increased robustness for challenging DNA samples, and minimal hands on time (<3 hours). This library preparation method, which uses the KAPA Hyper Prep kit, was further optimized and modified for limited and degraded DNA samples often encountered in forensic cases over the course of this grant. A series of experiments were conducted in order to determine the optimum adapter concentration and PCR cycle number for optimum DNA amplification yields and minimal adapter and adapter-dimer concentrations prior to probe capture. We also optimized the SPRI select small fragment removal step.

The KAPA Hyper Prep Kit protocol has no recommendations on adapter concentrations for sample amounts <1ng, although it is noted that adapter to sample DNA molar ratios >100:1 may be beneficial for low DNA amounts. Adapter concentrations from 100nM to 400nM were tested with 100pg input sample amount of the control genomic DNA K562 (~700 copies of mtDNA). The bioanalyzer results showed that the 400nM adapter concentration resulted in the highest DNA yield, however the dimer quantity was also the highest. We further tested a higher range of adapter concentrations (300nM to 800nM) concentrations to determine if high PCR product yield was maintained. The bioanalyzer results revealed that higher adapter concentrations produced increasingly larger DNA fragments (artifacts) along with an increase in dimer formation. Based on these results, 300nM adapter concentration is recommended for 100pg DNA as artifacts are greatly reduced without compromising DNA yields.

The KAPA Hyper Prep Protocol also does not suggest PCR cycle numbers for input amounts <1ng. To test the feasibility of the assay for DNA amounts ranging from 0.1ng – 1ng, we determined the optimal PCR cycle number to generate 0.5 µg - 1 µg of amplified PCR product, while minimizing over amplification artifacts to be 17 cycles for 1 ng; 18 cycles for 0.5 ng; and 19-21 cycles for 0.1 ng. Since this grant we have further optimized the PCR cycle number for lower DNA amounts.

Agencourt AMPure® XP (Beckman Coulter, Brea, CA, USA) bead system was used for purification and size selection of the adapter-ligated products to remove excess adapters as well as unincorporated dNTPs, primers, primer dimers, and salts. The beads work well for low concentration DNA cleanup, which is optimal for forensic-type samples with low DNA quantity. Results from our experiments show that for complete removal of small unwanted adapter products, a double AMPure bead clean-up is recommended post-adapter ligation and post-PCR. We also show the 1:1 bead to sample volume ratio ensures that adapter dimer (~140bp), and primer dimer are removed before sequencing for all sample types (degraded and reference). We showed that increased AMPure XP® bead to sample volume ratios (1.2X to 1.4X) increasingly captured smaller fragments, including adapter dimers. We recommend using the standard AMPure XP® bead to sample volume ratios 1.0X for all types of samples post-adapter ligation and post-PCR steps.

To improve the workflow efficiency of the probe capture method, a reduced hybridization time was tested: reduced from the recommended 24 hours to an overnight incubation (14-16 hours). The mtDNA genome coverage maps show no significant differences in probe capture efficiency between the 24 hours and overnight incubation times or specificity (~96%).

We optimized our DNA library insert sizes for Illumina sequencing using methods such as a Dual-SPRI AMPure XP beads and the Pippin Prep for size selection of our library pool insert. Using these methods, we were able to narrow the distribution of insert sizes of our library pool (eliminating any dimer artifacts or fragments larger than 1000bp) to the optimal size range for sequence cluster generation.

C. Illumina Sequencing Optimization:

Illumina, Miseq (Illumina, San Diego, CA, USA) sequencing improvements were achieved in order to reliably obtain higher yields of data. We found that increasing the amount of PhiX v3 spike-in from 1% to 5% improved our sequencing results for highly degraded and limited samples.

We have optimized the library pool input concentration for sequencing. We found that 11pM-12.5pM is a good range for optimal cluster density on the flow-cell and that ≥14pM results in over-clustering of the flow-cell. We also determined that changing the paired end read length configurations from 2x250bp to 2x150bp or 100bp is optimal for highly degraded samples where fragment insert sizes are much shorter. With these changes, we are able increased the yield of data generated with reduce cost.

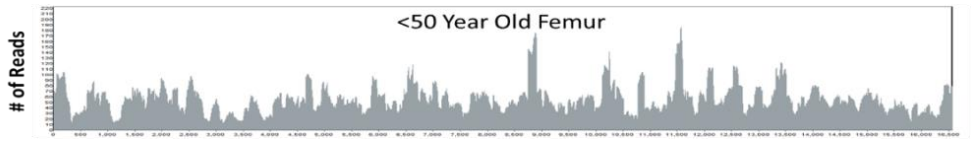
2) Analyze highly degraded and limited forensically relevant samples

The mitochondrial probe capture NGS assay was tested for sensitivity and specificity, and performance with population samples, mock degradation samples, mixtures, and bone specimens. The specificity and sensitivity of the capture assay was tested by preparing DNA libraries of the K562 control DNA at a range of DNA input amounts 100ng-10pg followed by capture and sequencing. All limiting dilutions led to full (100%) coverage of the whole mitochondrial genome with ~5000 read depth and 96% on target rate (specificity). We further tested the limit of sensitivity of the assay by testing K562 samples at starting amounts of 10pg, 5pg and 1pg (1,000 mtDNA copies). All dilutions led to 100% coverage of the mitochondrial genome with average coverage of 11,775 per base 95.85% average on target rate

(specificity). Therefore, the capture assay with optimized KAPA Hyper Prep and Illumina methods was shown to be more sensitive, and allowed us to lower the initial DNA input to 1 pg with successful full mitochondrial genome coverage. We tested 20 DNA samples from 4 different population groups (Caucasian, African-American, Hispanic and Japanese) to determine if mutations present in different haplotypes impact the probe capture process. All samples led to 100% coverage of the whole mitochondrial genome with a ~95% average on target rate, demonstrating the mitochondrial probe capture NGS assay can be used on varying sequence haplotypes. Additionally, the population samples were compared to HVI/HVII Sanger sequencing data and showed to be concordant for all samples.

To mimic highly degraded DNA often encountered in forensic cases, a mock degradation experiment was performed for samples at 1ng and 100pg that were first mechanically sheared to 150 bp on average and then processed using the standard 250 bp protocol fragmentation protocol. 100% coverage of the whole mitochondrial genome was obtained for both samples with ~83.13% average on target rate. The average read length was ~142 bp for the samples mimicking degraded DNA compared to control samples with an average of ~250 bp demonstrating proof-of-concept of probe capture and NGS sequencing of degraded DNA samples.

A <50 year-old femur was also tested to demonstrate proof-of-concept for analysis of degraded DNA from bone. This analysis resulted in a 100% coverage of the mitochondrial genome with average read depth of 54 as shown in Figure 1. A lower than normal on-target rate of 44% was observed and likely attributed to a higher amount of exogenous DNA from environmental exposure as would be expected for bone specimens compared to blood.



Sample	Ave Read Length (bp)	Total Number of Reads	Percent Aligned Reads	Average Coverage per Base	Percent Coverage of mt genome
<50 Year Old Femur	153	14,139	44.03%	54	100.00%

Figure 1. 100% coverage of the whole mitochondrial genome of a Modern Bone

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Throughout the course of the grant project, three sets of bones samples, dating to 100, 2000, and 4000 years old respectively, were tested. The seven highly degraded 100 year-old bone samples recovered from a comingled tomb in Rijeka, Croatia were successfully sequenced with mitochondrial genome coverage ranging from ~52% to 98%. Haplogroups were determined for six of these samples based on the sequenced variants present in the entire mitochondrial genome. Additionally, six prehistoric bone samples (dating ~2000 years ago) were sequenced with coverage of the mitochondrial genome ranging from 26% to 100%. The last set of samples, dating to 4000 years old, was recovered from a necropolis on the island of Korčula, Croatia. These six samples were successfully sequenced with coverage of the mitochondrial genome ranging from 26% to 100%. We determined coverage and read depth can be increased by combining data across multiple runs. Four DNA libraries were each sequenced three separate times. When the sequence data of all three runs were aligned to the rCRS simultaneously, the coverage of the mtGenome increased for each sample. For example, the lowest coverage of 52.79% for sample K37.2 increased to 88.38% when the sequencing data of all three runs was combined. These data show that different parts of the mtGenome were sequenced during each run and that there was not a systematic failure to capture one part of the genome. The average coverage per base also increased for each sample when data was combined across the three runs. Reads per base added across all three runs, resulting in an increased read depth. By increasing the coverage and depth of coverage, we also increase our confidence in the sequencing results of difficult, degraded samples.

3) Develop custom software for analyzing NGS data for forensic applications and markers

Massively-parallel, clonal sequencing provides a powerful method to analyze DNA samples from many sources. It is now routine to recover and sequence minute traces of DNA in forensics settings. A common challenge, however, is that many forensic samples are often known or suspected DNA mixtures from multiple individuals. Haploid lineage markers, such as mtDNA, are useful for analysis of mixtures because, unlike nuclear genetic markers (except Y chromosomal markers), each individual contributes a single sequence to the mixture. De-convolution of these mixtures into the constituent mitochondrial haplotypes is a challenge since sequence read lengths are typically short and human mitochondrial haplotypes may be

similar. In collaboration with University of California, Santa Cruz (UCSC), we have tested two software tools for analyzing mtDNA data from potentially mixed DNA forensic samples.

The first tool is called **hap-summary.pl.** This PERL based program takes as input aligned next-generation sequence data generated via PCR amplification of defined regions of the mtDNA genome, usually the HVI/II. The program works in stages, first analyzing all the sequence reads to identify positions that are variant, i.e., where some reads carry a different DNA base than other reads. Because these amplicon data are all full-length representations of single molecules, it is then possible to analyze the reads that cover the variant positions to define *haplotypes*, i.e., linked variant sites. **hap-summary.pl** then generates a summary of the observed haplotypes in the input dataset. The program has the following features for ease of use and analysis: 1) Allows the user to define a PCR primer region to ignore when finding variant sites; 2) Allow the user to define a black list of sites to ignore (recurrent mutations, known heteroplasmy, etc.); 3) Shows the coverage for each variant site of each observed haplotype on both strands for quality control; 4) Includes indel positions; and 5) Reports all variants in the rCRS coordinates in standard nomenclature. We found that this program is sensitive in detecting and quantifying the mixture components when the minor component is at least 10% of the overall sample and accurately identifies the sites that differ between the contributors in these mixed samples.

The second program we designed, implemented, and tested is called **mixemt.** This program is a powerful and fast approach for determining the constituent haplotypes in sequencing data from potentially mixed samples. At the heart of our approach is an Expectation-Maximization based algorithm that co-estimates the overall mixture proportions and the source haplogroup for each read individually. This approach, implemented in the software package Mixemt, correctly identifies haplogroups from mixed samples across a range of mixture proportions.

We tested **mixemt** on a wide variety of *in silico* and experimental mixtures of shotgun mtDNA sequence data. We used Illumina paired-end reads and limited these analyses to 3,000 input reads for each comparison. In two component mixtures, we found that **mixemt** reliably identifies the components of the mixtures across a wide range of mixture proportions. In every case with a minor contribution greater than

5%, an exact or close match for the major and minor haplogroup was made across various mixture proportions. It is possible to resolve mixtures with very low minor contributors (95/5) with additional sequence data.

We also evaluated **mixemt** on three component mixtures, mixed at ratios of 75%, 20%, and 5%. For this test, we sampled 10,000 total sequence reads. We correctly identify an exact match haplogroup for the 75% component in all 132 mixtures tested, an exact or close match for all 20% components and an exact or close match for 129 of 132. Therefore, **mixemt** is reliable even in cases of three person mixed samples.

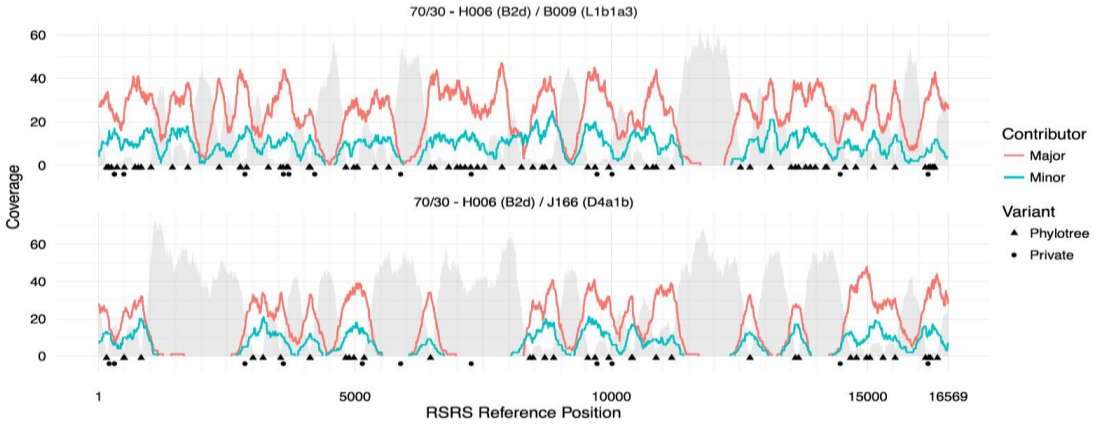

Figure *2*: Read coverage across reference for 2 mixtures, one with more diverged haplotypes (top) and less diverged haplotypes (bottom). Gray area represents reads that could not be assigned to either contributor.

4) Show proof-of-principle analysis using novel probe capture enrichment for nuclear SNPs

Highly degraded and limited nuclear DNA can be analyzed by SNP typing since a single base polymorphism is analyzed per template. To analyze SNPs without the need of intact primer binding sites, a custom nuclear SNP probe capture assay was developed for massively parallel sequencing and tested with high sample amounts (average depth of coverage), sensitivity samples, size selected and mock degraded samples, mixed DNA samples, and telogen hair samples. 451 SNPs were selected from literature for a custom probe assay. The SNPs included were Identity Informative, Ancestry Informative, Phenotypically Informative, Lineage Informative, Tri-allelic, Tetra-allelic, and Micro-haplotype. 448 out of 451 SNPs were successfully captured with average depth of coverage of ~900 reads across 16 samples at 25ng. The sensitivity test was carried out for sample amounts of 50ng, 25ng, 10ng, 5ng, 1ng, 0.5ng, 0.25ng, 0.1ng,

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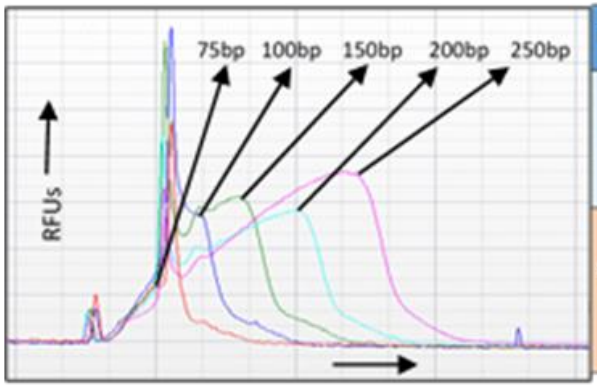
and 0.05ng. Sample at 5ng and higher exhibited complete allele calls for 100% of the 448 SNPs (100% full SNP call). The 0.1ng sample amount exhibited 23% full SNP call (103 out of 448 SNPs exhibited complete allele call) which is still more than the minimum number of SNPs (50-60) required for useful investigative purposes.

The size selection experiment was carried out to determine the efficiency of capture for highly fragmented and limited DNA samples as may be found in archaeological bone samples. Samples fragmented to an average of 175bp were size selected for ≤75bp, ≤100bp, ≤150bp, ≤200bp, and ≤250bp. All samples were tested at 1ng and the ≤75bp and ≤150bp size selections were also tested at 10ng and 0.5ng. All samples yielded at least 96% SNPs with coverage ≥10X and 95% full SNP calls (Figure 2). We also tested the capture efficiency with mock degraded samples where the samples were fragmented to an



Experiment	Sample DNA size (bp)	Amount (ng)	Average Coverage (%)	Average SNP call (%)
Size Selection	<75	1	97.1 ± 1.2	94.9 ± 1.6
	<100			
	<150			
	<200			
	<250			
Limited and degraded samples	<75	10	97.3 ± 0.6	96.5 ± 1.2
		1		
		0.5		
	<150	10	96.2 ± 0.5	92.3 ± 3.0
		1		
		0.5		

Figure 3: Bioanalyzer results of Size Selections and subsequent sequencing results

average of 150bp at 10ng, 1ng, and 0.5ng amounts. Over 90% full SNP call was obtained for samples amounts ≥1ng. Therefore, SNPs can be successfully analyzed for samples fragmented to ≤75bp and as limited as 0.5ng when implementing a capture enrichment-NGS system. For the mixture test two-person male-male mixtures were tested at ratios of 60:40, 70:30, 80:20, 90:10, 95:5, and 97.5:2.5. The mixtures were detected at all ratios. Out of 131 unique minor contributor alleles, the lowest detection was 38 for the 95:5 sample. Over 85% of unique minor contributor alleles were detected for ratios between 60:40 and 90:10. Finally, a single telogen hair was analyzed from 3 female individuals with telogen hair nuclei content of >60 nuclei, 20-60 nuclei, and <20 nuclei. Because these were single-source female samples, only the 370 autosomal and X SNPs were analyzed. The percent of SNPs with coverage ≥10X was: 99.1% for >60

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nuclei, 65.1% for 20-60 nuclei, and 3% for <20 nuclei. Based on these results, the custom probe panel was highly successful in capturing SNP regions in degraded and mixed DNA samples, and has the potential to be applied to forensic samples.

Impact to Criminal Justice System, Forensic Science and Other Fields of Science:

Next Generation Sequencing (NGS) coupled with a probe capture system can be used for deep sequencing of the entire mitochondrial genome and nSNP markers and has the potential to greatly improve the discrimination power compared to current mitochondrial HVI/HVII sequence analysis as well as to increase the success rate of successfully genotyped degraded samples. The probe capture mitochondrial genome enrichment and NGS assay was evaluated by the California Department of Justice (CA DOJ), Richmond to assess its capability to enrich and sequence the mitochondrial genome from forensically relevant samples and is now validating the method for implementation into their missing persons program. The CA DOJ reported, "The probe capture/MPS assay demonstrated its capability to successfully sequence full mitochondrial genomes from DNA in different levels of quality and quantity. Based on this study, it is our opinion that the implementation of this powerful tool would allow for more efficient and discriminating mitochondrial analysis".

The proof-of-principle using probe capture targeting nuclear SNPs to analyze degraded and limited DNA was highly successful. The mtDNA and nuclear SNP probe capture enrichment and NGS assays can be used to supplement STR data or as stand-alone assays. The capture enrichment coupled with NGS is an ideal system for analysis of compromised samples, such as missing persons, mass disasters, forensic hair analysis, and low template DNA as well as mixed DNA samples. More affordable NGS platforms are now available making access to NGS more attainable to forensics laboratories and implementation feasible.

The NGS software, Mixemt developed for this project will be customized for forensic applications and will be made publicly available. The tools we have developed should be broadly useful for the forensics community in the case where samples may be of mixed origin. In addition, a collaboration with SoftGenetics led to development and inclusion of features for forensic applications for NGS mtDNA sequencing in the NExtGene commercial software.

Complete List of Deliverables

1. **Protocols:**

   Name: Library Preparation and Capture Protocol for Limited and High Quantity DNA Samples v1.0, October 2016.

   Description: Combined protocol for library preparation based on the KAPA Hyper Prep Protocol (1.14) and the NimbleGen SeqCap EZ Library User SR (v5.1) Optimized single protocol for preparing DNA libraries from pristine references/controls as well as casework samples (degraded/mixed/limited) with starting amounts ranging from 1ng to 100pg. Up to 24 samples can then be captured enriched

   Accessibility: Protocol can be obtained from PI, Dr. Calloway (scalloway@chori.org) upon request.

2. **Probe Capture Panel:**

   Name: Custom whole mitochondrial genome SeqCap EZ Choice DNA probe solution (Calloway Lab Design)

   Description: DNA probes with high on target capture redundancy for efficient capture of up to 24 samples at equal amounts.

   Accessibility: Available from Roche NimbleGen with prior permission from Dr. Cassandra Calloway (scalloway@chori.org).

3. **Software Programs:**

   A. Name: Hap-summary.pl

      Description: Hap-summary.pl is a PERL based program used to detect mixture proportions from mtDNA amplicon NGS data based on linked variant sites in sequenced reads.

      Accessibility: Available upon request from Richard Green, UC Santa Cruz (ed@soe.ucsc.edu)

   B. Name: mixemt

      Description: mixemt is a software program helpful in determining variant mixture proportions and observed haplotypes in multiple contributor mixtures.

      Accessibility: Currently available as freeware in the following link: https://github.com/svohr/mixemt

Complete List of Disseminated Information:

I. **Publications**

A. Peer Reviewed Publications:

1. Shih, Shelly, Nikhil Bose, Anna Beatriz R. Gonçalves, Henry A. Erlich, and Cassandra D. Calloway. Applications of Probe Capture Enrichment Next Generation Sequencing for Whole Mitochondrial Genome and 426 Nuclear SNPs for Forensically Challenging Samples. (2018) Genes, 9(1):49. PMID: 29361782.

2. Bose, Nikhil, Katie Carlberg, George Sensabaugh, Henry Erlich, and Cassandra Calloway. Target Capture Enrichment of Nuclear SNP Markers for Massively Parallel Sequencing of Degraded and Mixed Samples. (2018) FSI Genetics, 34:186196.

3. Vohr, Samuel, Rachel Gordon, Jordan M. Eizenga, Henry A. Erlich, Cassandra D. Calloway, and Richard E. Green. A fast method for haplotype analysis of sequence data from complex mitochondrial mixtures. (2017) FSI Genetics, 30:93-105. PMID: 28667863

4. Kim, Hanna, Henry Erlich and Cassandra Calloway. (2015) Analysis of mixtures using next generation sequencing of mitochondrial DNA hypervariable regions. Croat Med J, 56(3): 208–217. PMCID: PMC4500979

B. Application Notes

1. McGuigan, J., LeVan, K., McCluskey, M., Liu, J., Ni, S (2014, September). Human Identity Analysis using NextGENe® Software. SoftGenetics Application Note. Cassandra Calloway and Hanna Kim contributed to the SoftGenetics NextGENe® developmental modifications

C. Theses/Dissertations

1. Nikhil Bose. (2016, December). *Development of a Nuclear SNP probe Capture Assay for Massively Parallel Sequencing of Degraded and Mixed DNA Samples* (UC Davis Master's Thesis). Retrieved from ProQuest Dissertations and Theses.

2. Cassandra Taylor. (2016, December). *Analysis of Highly Degraded DNA from Bone Samples Using Probe Capture Enrichment of the Entire Mitochondrial Genome and Next Generation Sequencing*. (UC Davis Master's Thesis). Retrieved from ProQuest Dissertations and Theses.

3. Sarah Copeland. (2015, December). *Mitochondrial DNA Analysis of Highly Degraded Bone Samples using Next-Generation Sequencing*. (UC Davis Master's Thesis). Retrieved from ProQuest Dissertations and Theses.

4. Daniela Cuenca. (2013, June). *Optimization and validation of a probe capture/NGS assay for sequencing the whole mitochondrial genome on forensically relevant samples*. (UC Davis Master's Thesis). Retrieved from ProQuest Dissertations and Theses.

5. Sam Vohr. (2016, Dec). *Identification and Mixture Deconvolution of Ancient and Forensic DNA using Population Genomic Data*. (UC Santa Cruz Doctoral Dissertation). Retrieved from http://escholarship.org/uc/item/8zh708h9

II. **Presentations**

A. Webinars

1. Cassandra Calloway. (2017, March). Development of a Probe Capture NGS System for Forensics. FTCOE Webinar. **https://rticqpub1.connectsolutions.com/content/connect/c1/7/en/events/event/shared/1178106013/event_landing.html?sco-id=1208236424&_charset_=utf-8**

B. CCI Courses

1. Mitochondrial DNA Analysis for Forensic Applications using Sanger Sequencing, Linear Arrays and Next Generation Sequencing. December 3-5, 2013; January 22-24; January 27-31, 2014 (84 instruction hours). CCI Course at CA DOJ Richmond lab. Instructor: Cassandra Calloway; Assistant instructor: Hanna Kim. This lecture and lab course was taught to 10 criminalists at the CA DOJ and covered mtDNA genetics and forensic applications, Sanger sequencing, and NGS sequencing, including 8 days of lab insctruction on 454 NGS mtDNA sequencing.

C. Abstracts

1. Nikhil Bose, Rachel Gordon, Cassandra Taylor, Shelly Shih, Samuel Vohr, Guillermina Almada, Anna Beatriz Gonscalves, George Sensabaugh, Richard Green, Henry Erlich, and Cassandra Calloway. (2017, September) Mitochondrial Genome and Nuclear SNP Probe Capture Next-Generation Sequencing System for Analyzing Degraded and Mixed DNA Samples. Poster Presentation at ISFG. Seoul, North Korea.

2. Henry Erlich, Rachel Gordon, Sam Vohr, Richard Green, Cassandra Calloway. (2017, May). De-Convolution of Forensic Mixtures Using NGS Analysis of Mitochondrial Genome Sequences. Oral Workshop Presentation at the

Sequencing, Finishing, and Analysis in the Future (SFAF) Meeting. Santa Fe, New Mexico.

3. Nikhil Bose, Katie Carlberg, George Sensabaugh, Henry Erlich, and Cassandra Calloway. (2017, February). Development of a Nuclear SNP probe Capture Assay for Massively Parallel Sequencing of Degraded and Mixed DNA Samples. Oral Presentation at AAFS Meeting. New Orleans, LA.

4. Cassandra Taylor, George Sensabaugh, Henry Erlich, and Cassandra Calloway. (2017, February). Analysis of Highly Degraded DNA from Bone Samples Using Probe Capture Enrichment of the Entire Mitochondrial Genome and Next Generation Sequencing. Oral Presentation at the AAFS Scientific Meeting. New Orleans, LA. February 17th, 2017.

5. Nikhil Bose, Katie Carlberg, George Sensabaugh, Henry Erlich, Cassandra Calloway. (2016, October). Development of a Nuclear SNP Probe Capture Assay for Massively Parallel Sequencing of Degraded and Mixed DNA Samples. Oral Presentation at the CAC Northern DNA Study Group. Sacramento, California.

6. Nikhil Bose, Katie Carlberg, George Sensabaugh, Henry Erlich, Cassandra Calloway. Development of a Nuclear SNP probe Capture Assay for Massively Parallel Sequencing of Degraded and Mixed DNA Samples. Poster Presentation at 27th International Symposium on Human Identification. Minnesota, Minneapolis. September 27th, 2016.

7. Anna Beatriz R. Gonçalves, Guillermina Almada, Shelly Shih, Samuel Vohr, Rachel Gordon, Henry Erlich, and Cassandra Calloway. (2016, August). Analysis of Mixtures by Mitochondrial DNA and Nuclear Markers for Generation of Forensic Profiles. Poster Presentation at Semana de Biomedicina (The Biomedical Science's Week). Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

8. Cassandra D. Calloway, Rachel Gordon, Henry Erlich. (2015, September). Whole Mitochondrial Genome Sequencing Using Probe Capture Enrichment and Illumina Next-Generation Sequencing for Analysis of Mixtures and Degraded DNA. Oral presentation at ISFG. Krakow, Poland.

9. Rachel Gordon, Sarah Copeland, George Sensabaugh,D.Crim., Henry Erlich, PhD, Cassandra Calloway, PhD. (2015, September) Whole Mitochondrial Genome Sequencing Method for Limited and Highly Degraded DNA Samples for Use in Forensic Casework.. Poster Presentation. CAC Conference. Burlingame, CA.

10. Cassandra D. Calloway, Rachel Gordon, Hanna Kim, Henry Erlich (2015, June). Probe capture enrichment and NGS of whole mitochondrial Genome. Oral

presentation. Oral Presentation at the 9th International Society Applied Biological Sciences. Bol, Croatia.

11. Cassandra D. Calloway, Hanna Kim, Rachel Gordon, Daniela Cuenca, Samuel Vohr, Richard E. Green, PhD, George Sensabaugh, and Henry Erlich, PhD (2015, May). Analysis of mtDNA Mixtures Using Next-Generation Sequencing Technologies and Custom Software. Oral presentation at Sequencing, Finishing, and Analysis in the Future Meeting. Santa Fe, New Mexico
12. Cassandra D. Calloway, PhD, Hanna Kim, Daniela Cuenca, MS, Valarie McClain, Richard E. Green, PhD, George Sensabaugh, D. Crim, Henry Erlich, PhD (2014, October) Analysis of mtDNA Mixtures Using Next Generation Sequencing Technologies and Custom Software. Poster presentation at 25th Annual International Symposium of Human Identification. Phoenix, Arizona.

13. Daniela Cuenca, Valerie McClain, Hannah Kim, George Sensabaugh, Henry Erlich, Cassandra Calloway. (2014, February). Development of a Whole Mitochondrial Genome/Probe Capture NGS Method for Analysis of Limited and Degraded DNA Samples. Oral Presentation at the 66th Annual AAFS Meeting. Seattle, WA.

D. Invited Talks

1. Cassandra Calloway. (2017, June). Development of a Probe Capture NGS System for Forensics. Oral Presentation at the ISABS Conference on Forensic and Anthropologic Genetics and Mayo Clinic Lectures in Individualized Medicine. Dubrovnik, Croatia.

2. Cassandra Calloway. (2017, March). Targeted Massively Parallel Sequencing of mtDNA and Nuclear SNP Markers Using Probe Capture for Analysis of Mixtures and Degraded DNA. Oral Presentation at the 2nd Annual Genetics in Forensics Congress. London, UK.

3. Henry Erlich. (2016, September). Thirty Years of Forensics DNA Analysis. Illumina Workshop Presentation at 27th International Symposium on Human Identification. Minnesota, Minneapolis. September 28th, 2016.

4. Henry Erlich. (2015, October). Thirty Years of Forensics DNA Analysis. Founder's Lecture Presentation at 126th CAC Semi Annual Seminar. Burlingame, CA

5. Henry Erlich. (2015, June). Thirty Years of Forensics DNA Analysis. ISABS. Oral Presentation at ISABS Conference on Forensic and Anthropologic Genetics and Mayo Clinic Lectures in Individualized Medicine. Bol, Croatia.

E. Lectures

1. ETX20 Forensic Science Introduction. *DNA Techniques* (05/20/2015) (1 hour), University of California, Davis

2. FOR280 Forensic DNA Analysis. *Mitochondrial DNA Analysis* (02/23/2015), (03/11/2016), (02/27/207) (3 hours each), University of California, Davis

3. ETX/FOR278 Molecular Techniques. Forensic Science Graduate Group, UC Davis, (12/02/2015) (1hr 30min) Forensic Applications of Mitochondrial DNA Analysis Using Next-Generation Sequencing. Cassandra D. Calloway.

F. Student Research/Thesis Presentations

1. Anna Beatriz R. Gonçalves, Guillermina Almada, Shelly Shih, Samuel Vohr, Rachel Gordon, Henry Erlich, and Cassandra Calloway. (2016, August). Analysis of Mixtures by Mitochondrial DNA and Nuclear Markers for Generation of Forensic Profiles. Poster Presentation at CHORI 2016 Summer Student Research Symposium Children Hospital Oakland Research Institute, Oakland, CA. Awarded: 2nd place in the category of Best Poster Presentation.

2. Nikhil Bose. (2016, May). Development of a Nuclear SNP probe Capture Assay for Massively Parallel Sequencing of Degraded and Mixed DNA Samples. Oral Presentation at UC Davis Forensic Science Program: Thesis Presentation. Davis, CA

3. Cassandra Taylor. (2016, May). Analysis of Highly Degraded DNA from Bone Samples Using Probe Capture Enrichment of the Entire Mitochondrial Genome and Next Generation Sequencing. Oral Presentation at UC Davis Forensic Science Program: Thesis Presentation. Davis, CA.

4. Sarah Copeland. (2015, May). Mitochondrial DNA Analysis of Highly Degraded Bone Samples using Next-Generation Sequencing. Oral Presentation at UC Davis Forensic Science Program: Thesis Presentation. Davis, CA.

5. Daniela Cuenca. (2013, May). Optimization and validation of a probe capture/NGS assay for sequencing the whole mitochondrial genome on forensically relevant samples. Oral Presentation at UC Davis Forensic Science Program: Thesis Presentation. Davis, CA.