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Urine as a high-quality source of host genomic DNA from wild populations

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Abstract

The ability to generate genomic data from wild animal populations has the potential to give unprecedented insight into the population history and dynamics of species in their natural habitats. However, in the case of many species, it is impossible legally, ethically, or logistically to obtain tissues samples of high-quality necessary for genomic analyses. In this study we evaluate the success of multiple sources of genetic material (feces, urine, dentin, and dental calculus) and several capture methods (shotgun, whole-genome, exome) in generating genome-scale data in wild eastern chimpanzees (*Pan troglodytes schweinfurthii*) from Gombe National Park, Tanzania. We found that urine harbors significantly more host DNA than other sources, leading to broader and deeper coverage across the genome. Urine also exhibited a lower rate of allelic dropout. We found exome sequencing to be far more successful than both shotgun sequencing and whole-genome capture at generating usable data from low-quality samples such as feces and dental calculus. These results highlight urine as a promising and untapped source of DNA that can be noninvasively collected from wild populations of many species.

Introduction

The development of methods to generate genetic data from noninvasively collected samples revolutionized the study of wild animal populations, allowing for DNA research without the capture or even observation of species of interest (Kohn & Wayne, 1997; Waits & Paetkau, 2005). While studies of individual DNA markers improved our understanding of behavior, ecology, and evolution, recent advances in massively parallel sequencing strategies make it possible to incorporate information from across the entire genome, giving unprecedented insight into the evolution and population history of non-model species (Ellegren, 2014). However, for many species, it is impossible legally, ethically, or logistically to obtain high-quality tissue samples required for large-scale genomic analyses. It is therefore critically important to develop and evaluate methods for sampling and capturing genome-scale data from noninvasive and alternative sources.

While a variety of noninvasively collected biological materials have been used in DNA analyses, feces have been the primary target of recent attempts to generate genomic data. Rich in gut epithelial cells and often the most abundant, easiest to collect source of DNA in the environment, feces have long played a role in noninvasive genetic analyses (Constable, Ashley, Goodall, & Pusey, 2001; Hoss, Kohn, Paabo, Knauer, & Schroder, 1992; Kohn & Wayne, 1997). However, the retrieval of DNA from feces presents a number of difficulties. Challenges, including low DNA yields, DNA fragmentation and degradation (Deagle, Eveson, & Jarman, 2006) and the presence of PCR inhibitors, can lead to genotyping errors (Taberlet, Waits, & Luikart, 1999). Moreover, DNA recovered from fecal material is dominated by microbes (>95% exogenous DNA), which further complicates genotyping (Perry, Marioni, Melsted, & Gilad, 2010). For genetic analyses involving small number of markers, these challenges are well

78 understood and can be overcome. However, these problems are exacerbated in massively parallel
79 sequencing, which typically requires higher quantities and qualities of input DNA, and generates
80 almost entirely microbial data due to the very low levels of host DNA in samples.

81 The main strategy that has been employed to combat these problems is enrichment of
82 host DNA. In this vein, there have been three major methodological developments. Perry and
83 colleagues (2010) first enriched DNA from feces on a genomic scale by using custom
84 chimpanzee baits designed to capture approximately 1.5 Mb of sequence across six western
85 chimpanzees (*Pan troglodytes verus*). While successful, this method required a reference
86 genome to design baits and was cost prohibitive for producing genome-scale datasets. To address
87 these challenges, Snyder-Mackler and colleagues developed a protocol to create RNA baits from
88 high-quality host DNA and improve post-capture enrichment (2016). However, for this method,
89 bait requirements—notably high-quality host DNA—and low sequencing coverage of host DNA
90 remain barriers for some study systems and questions. Recently, Chiou and Bergey introduced a
91 method that exploits differences in CpG-methylation densities between vertebrate and bacterial
92 genomes to capture host DNA, alleviating the need for high-quality host material or reference
93 genome to design baits (2018). However, CpG content varies substantially across the genomes of
94 primates and other mammals (Han, Su, Li, & Zhao, 2008), thus targeting these regions
95 specifically may bias the regions captured.

96 Despite these improvements to both capture and enrichment, DNA capture from feces is
97 still far less efficient than from high-quality tissues. This leads to a tradeoff: attempting to
98 capture large genomic regions leads to very low sequence coverage; however, targeting a subset
99 of the genome can lead to biases. A compromise would be to target a small subset of the genome
100 that is biologically important. One potential option is exome sequencing, a capture-based method

that targets the entire coding region of the genome, comprising approximately 1.5% of the total length of the genome. Coding regions are among the best understood in the genome and are of great evolutionary and conservation interest (Bataillon et al., 2015; George et al., 2011; Hvilsom et al., 2012). Because exome sequencing is so widely used in human genomics, many commercial kits are available and much cheaper than custom alternatives. Human exome baits have been successfully used in a number of nonhuman primate studies (George et al., 2011; Jin et al., 2012; Vallender, 2011) and have been shown to work in primate species as distantly related from humans as Strepsirrhines (Webster, Guevara, Lawler, & Bradley, 2018). Moreover, recent work has shown that exome capture successfully enriches host DNA in chimpanzee fecal samples (Hernandez-Rodriguez et al., 2018; White et al., 2019). However, some of this work involves first screening for endogenous content using quantitative PCR (qPCR), which although successful, can be a limiting factor for smaller labs at the scales for population genomics. For example, after screening 1,780 fecal samples, White and colleagues estimated 101 samples contained enough endogenous DNA for sequencing (>1%) (White et al., 2019).

In addition to methodological development, turning to other sources of biological material might improve sequencing success in wild populations. Efforts up to this point have focused almost exclusively on feces, and many other noninvasive alternatives remain to be explored. Urine, in particular, is abundant for many large-bodied species, and has been used, albeit infrequently, as a source of DNA collected noninvasively from the environment (Hedmark et al., 2004; Sastre et al., 2009; Valiere & Taberlet, 2000). Although difficult to obtain in certain field conditions, urine contains far fewer microbes than feces, does not contain traces of dietary DNA, and lacks many inhibiting compounds commonly found in feces that impact PCR success (Hausknecht, Gula, Pirga, & Kuehn, 2007; Inoue, Inoue-Murayama, Takenaka, & Nishida, 2007;

Thomas-White, Brady, Wolfe, & Mueller, 2016). Another source of interest is skeletal material, which is often found at field sites and in museum collections. Dentin and dental calculus, in particular, are both capable of yielding host nuclear DNA (Ziesemer et al., 2018). Combining data from historic populations with those from contemporary populations has the potential to provide genomic insight into wild populations on a scale not yet fully realized.

In this study, we evaluate the success of several sources of host DNA and capture methods in generating genome-scale data in a population of wild, endangered animals. Specifically, we extracted and captured endogenous DNA from feces, dental calculus, dentin, and urine recovered from wild chimpanzees (*P. t. schweinfurthii*) from Gombe National Park, Tanzania. From these data we compared the success of whole-genome capture and targeted exome capture. We demonstrate that urine harbors the highest concentration of endogenous DNA of the materials sampled in this study. For other sources, whole-genome sequencing appears possible, but not cost-effective. Employing a targeted approach, such as exome capture, reduces the amount of sequence obtained in the genome, but it may result in increased sequencing efficiency. Finally, we show that genotypes generated from fecal and urine samples exhibit high levels of concordance and argue that genotypes from urine are less subject to contamination. Together, our results demonstrate that, while further methodological advances might improve host DNA extraction in feces, dentin, and dental calculus, urine is a promising source of noninvasive DNA from which genome-scale data can be easily generated. We anticipate the ability to generate genomic data from urine to be broadly useful across study systems, including many protected species.

Materials and Methods

147 *Sample Collection and Extraction*

148 We collected fecal samples in RNAlater from four wild chimpanzees as described (Stone
149 et al., 2010) (7069, 7150, 7365, and 7507) from Gombe National Park between August 2011 and
150 January 2014 and shipped them to the University of Pennsylvania for storage at -80°C. Using a
151 sterile cut pipette tip, we removed roughly 200 µL of the fecal slurry and extracted DNA using
152 QIAamp DNA Stool Minikit (Qiagen) according to manufacturer's protocol. To obtain enough
153 DNA, we repeated this process 8-12 times for each sample, then pooled and desiccated each
154 sample down to 50-100 µL. We combined a total of 2 µg of DNA and molecular grade H_2O into
155 a 50 µl tube and then sheared DNA using a Covaris Sonicator for 4min at 150 bp according to
156 manufacturer specifications.

157 We retrieved dental calculus from two skeletons (individuals 7057 and 7433; less than ten
158 mg per sample) and dentin from one skeleton (individual 7057; less than 50 mg per sample) at
159 the University of Minnesota using a sterile dental scaler. We decontaminated calculus using
160 exposure to UV irradiation for five min. This was followed by an initial 0.5M EDTA (Ambion)
161 wash in a 2.0 mL tube for 15 min. We subjected samples to a two day 0.5 EDTA and proteinase
162 K (10 mg/mL; Qiagen) digestion, at which point we combined the resulting solution with 12 mL
163 of PB buffer and followed standard MinElute PCR Purification Kit (Qiagen) protocol. Our dentin
164 protocol followed previously published methods (Nieves-Colón et al., 2018). We did not shear
165 dental calculus and dentin samples prior to shotgun library builds.

166 We collected urine from seven wild Gombe chimpanzees—three with matched fecal
167 samples (7150, 7365, and 7507) and four others (7072, 7323, 7535, and 7650)—in the early
168 morning using fresh plastic bags attached to sticks suspended below chimpanzee nests. We
169 immediately transferred between 10 mL and 30 mL of urine to a 50 mL tube and centrifuged the

material for ten min at 3k rpm. We removed supernatant and covered the resulting pellet with 5 mL of RNAlater for storage in the field. In the lab, we extracted samples using the Urine DNA Isolation Kit (Abcam) according to manufacturer protocols. We sheared the resulting elution using the Covaris sonicator as previously described and desiccated the resulting solution down to 20 µL.

Shotgun Build and Amplification

We built shotgun libraries using the resulting elutions from feces, urine, dentin, and calculus extractions. For initial blunt end repair, we added a total of 20 µL (~800 ng) of DNA to 5.0 µL NEB Buffer, 0.50 µL dNTP mix (2.5mM), 4.0 µL BSA (10 mg/mL), 5.0 µL ATP (10mM), 2.0 µL T4 PNK, 0.40 µL T4 Polymerase, and 13.10 µL ddH_2O. We incubated this solution at 15°C for 15 min followed by 25°C for 15 min. We then cleaned the solution using PCR MinElute Purification Kit according to manufacturer protocol before eluting into 18 µL EB buffer. For adapter ligation, we added 18 µL of template DNA to 20 µL Quick Ligase Buffer, 1.0 µL Solexa Mix (Meyer & Kircher, 2010), and 1.0 µL Quick Ligase and incubated the solution at room temperature for 20 min. We then cleaned again using PCR MinElute Purification (Qiagen) according to manufacturer protocol and eluted the solution into 20 µL EB buffer. For the final fill in portion of the shotgun build, we added 20 µL of template DNA to 4.0 µL Thermo pol buffer, 0.50 µL dNTP mix (2.5mM), 2.0 µL Bst polymerase, and 13.50 µL ddH_2O. We incubated the solution at 37°C for 20 min followed by 80°C for 20 min. We amplified shotgun libraries using Amplitaq Gold before splitting libraries into four identical PCR reactions which contained 9.0 µL of DNA, 9.27 µL PCR Buffer II (10x), 9.27 µL $MgCl_2$ (25mM), 3.68 µL dNTP mix (10nM), 2.21 µL BSA (10 mg/mL), 2.0 µL P5 primer, 2.0 µL P7 primer, 61.09 µL of ddH_2O,

and 1.48 µL of Amplitaq Gold enzyme. We used the following PCR conditions: initial denaturation at 95°C for 15 min, followed by cycling of 95°C for 30 sec, 58°C for 30 sec, and 72°C for 45 sec, with a final elongation of 72°C for ten min. We amplified each sample between 8 and 13 cycles (Table S1) using Illumina adapter primers with unique forward and reverse barcodes. We then purified samples using the Minelute PCR Purification Kit according to manufacturer protocol before eluting into 30 µL of EB buffer. We used a total of 7 µL of amplified calculus, dentin, and fecal DNA for each of the capture sets. For urine, we desiccated amplified material from 30 µL down to 7 µL before undergoing a single exome capture.

Whole-Genome and Exome Capture Kits

We used two whole-genome kits (chimpanzee and human baits) and one human exome kit to capture host DNA from the variety of samples. For the whole-genome chimpanzee kit, Arbor Biosciences produced a custom whole-genome capture MYBaits kit using *Pan troglodytes schweinfurthii* DNA. Genomic DNA extracted from the blood of a chimpanzee (Stone et al., 2010) was used as source material for baits. We pooled extractions for a total of 5 ug of DNA which Arbor Biosciences then used to produce the whole-genome capture baits. For the human whole-genome capture baits, we used a MYcroarray whole-genome human capture kit (using African/Masai male DNA). Finally, we also used the IDT xGen Exome Research Panel (v1.0), a commercially available human exome capture kit.

For feces, we used an input total of 7 uL of amplified material regardless of concentration for each of the three capture kits: the *P. t. schweinfurthii* MYBaits capture, the human MYBaits capture, and the IDT xGen Exome Research Panel. For the chimpanzee whole-genome capture MYBaits kit, we captured each sample according to MYbaits Kapa HiFi HotStart ReadyMix

protocol with a hybridization time of 24 hours and a final post-capture PCR amplification of 14 cycles. We purified all samples post-capture through removal of beads, cleanup using the MinElute PCR Purification Kit, and elution into 30 µL. We re-amplified a second time using identical PCR conditions, with the number of cycles dependent upon the outcome of quantification from a Bioanalyzer DNA 1000 chip (Agilent). We purified all samples post-capture using the MinElute PCR Purification Kit according to manufacturer specifications and eluted into 30 µL.

For the MYbaits human whole-genome kit, we captured each of the four amplified fecal samples in the same manner, using the same amount of starting amplified material. However, during the final phase of the MYBaits protocol, all samples were amplified 14 cycles instead of the usual 12 cycles. As such, no samples were re-amplified post-capture after we confirmed high concentrations using a Bioanalyzer DNA 1000 chip.

For the xGen Exome Research Panel, from IDT, the unique P5 and P7 7 nt barcodes used to identify the amplified samples necessitated custom xGen Universal Blocking oligos from IDT. We used a total of 7 ul of amplified material from each sample (greater than the suggested 500 ng input of DNA) for the capture in accordance with manufacturer protocol. The exception to this was for urine, which we desiccated from a starting volume of 30 µL, due to the initial low concentrations. We amplified each capture pool to 12 cycles using KAPA HiFi Hotstart ReadyMix, purified each using Agencourt AMPure beads, and eluted into 22 µL of EB Buffer (Qiagen) as suggested by the protocol. Lastly, we quantified the samples using a Bioanalyzer High Sensitivity DNA chip and amplified each for six more cycles.

Samples were then pooled (see Table 1 for breakdown) before being sent for sequencing at the Yale Center for Genome Analysis. Samples were sequenced on four different Illumina

239 HiSeq2500 Rapid runs (2x100 paired-end) and an Illumina HiSeq2500 standard run (2x150
240 paired-end).
241
242 *Read Processing, Read Mapping, Variant Calling, and Depth of Coverage*
243 Before mapping reads, we examined read quality using FastQC (v0.11.7;
244 http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and MultiQC (v1.5.dev0; (Ewels,
245 Magnusson, Lundin, & Käller, 2016)), and trimmed adapters and low-quality sequence from
246 reads using BBDuk (v37.90; https://jgi.doe.gov/data-and-tools/bbtools/) with the following
247 parameters: "ktrim=r k=21 mink=11 hdist=2 tbo tpe qtrim=rl trimq=10 minlength=30". Using
248 default parameters, we mapped reads to the chimpanzee reference genome (panTro4; (Waterson,
249 Lander, Wilson, The Chimpanzee, & Analysis, 2005)) with BWA-MEM (v0.7.17-r1188; (Heng
250 Li, 2013). We then used SAMtools to fix mate pairings, and sort and index BAM files (v1.7;
251 (Heng Li & Durbin, 2009). Because we sequenced some of the samples across multiple lanes
252 (Table S1), we used Sambamba to merge BAM files from these samples using default
253 parameters (v0.6.6; (Tarasov, Vilella, Cuppen, Nijman, & Prins, 2015). Note that we only
254 merged BAM files within individual, biological material, and sequencing library (i.e., samples
255 from the same individual but different source material or capture method were left unmerged and
256 treated separately, as these were different units in our analyses). Finally, we marked duplicates
257 using Picard (v2.18.10; http://broadinstitute.github.io/picard).
258 We next called variants on each processed BAM file separately using Genome Analysis
259 Toolkit's (GATK's) HaplotypeCaller with default parameters (v4.0.8.1; (Van der Auwera et al.,
260 2013)). We then filtered each VCF using BCFTools (v1.6; (H. Li et al., 2009)) . We included
261 sites for which mapping quality >= 20, site quality (QUAL) >=30, and genotype quality >= 30.

Because some of the downstream coverage analyses are affected by differing number of raw reads across samples, we downsampled merged BAM files (without duplicate marking) to 40 million reads. To do so, we used SAMtools view (v1.7; (H. Li et al., 2009)) with the flag "-s *downsample_fraction*", where *downsample_fraction* is equal to 40 million divided by the sample's total number of raw reads. Note that for analyses requiring downsampling, we only included samples with 40 million or more reads. We next marked duplicates, as above, using Picard (v2.18.10; http://broadinstitute.github.io/picard). We used downsampled BAM files for coverage analyses, but not endogenous content estimates or variant calling.

To calculate depth of coverage from BAM files, we first used SAMTOOLS view (v1.7; (H. Li et al., 2009) with the flags '-F 1024 -q 20' to remove duplicates and only retain reads with the minimum mapping quality of 20. We then used Bedtools GenomeCov (v2.27.1; (Quinlan & Hall, 2010)) with the flag -bg to output a bedgraph file with coverage statistics. Next, again using Bedtools, we intersected bedgraph files with Ensembl coding sequences (CDS) for the panTro4 genome downloaded from the UCSC Table Browser (Karolchik et al., 2004). Finally, using a custom python script, "Compute_histogram_from_bed.py" (see Data Accessibility), we calculated histograms of CDS depth.

Analysis

We used the SAMtools stats tool to calculate basic metrics related to fraction of reads mapping, duplicates, etc. (v1.7; (H. Li et al., 2009)) across all sample types. We calculated these metrics both with and without duplicates, and for primary and downsampled BAM files. To remove duplicates, we first used SAMtools view with the '-F 1024' flag, before piping output to SAMtools stats. From these metrics, we estimated post-capture endogenous content as the

fraction of reads mapping to the reference genome. To test for statistical differences in post-capture endogenous content among sample sources we used an ANCOVA test in R (R Development Core Team, 2014).

Within R, we generated "reverse cumulative" plots (Reed, Meade, & Steinhoff, 1995) of coverage across CDS for feces vs. urine and exome vs. whole-genome for feces ("plot_coverage.R"; see Data Accessibility). These plots display the proportion of total panTro4 CDS (Y-axis) covered by X or more reads (where X is a value on the X-axis).

Using exome data, we examined genotype concordance between paired urine and fecal samples for three individuals (7150, 7507, 7365), and paired calculus and dentin samples for one individual (7057). To estimate concordance, we ensured that variant calls were made at identical sites in the paired samples. We did this by first using BCFtools merge (v1.6; (H. Li et al., 2009)) with the flag "-m all" to merge the paired (urine and feces, or calculus and dentin) exome VCF files for each individual. We then conducted a second round of variant calling using GATK's HaplotypeCaller (v4.0.8.1; (Van der Auwera et al., 2013)) as described above, with the addition of the flag "-ERC BP_RESOLUTION" and the merged VCF as an interval file via the "-L" flag. These flags force HaplotypeCaller to call genotypes at the same sites—any site called in either the urine or fecal sample (or calculus or dentin sample) from a given individual—in both samples. We then, for each site, compiled genotype, depth, mapping quality, and genotype quality measures from the newly generated VCFs using the custom Python script "Compare_vcfs.py". From this compiled dataframe, we removed "random" (containing "_random") and unplaced (containing "chrUn") scaffolds. We then used the Python script "Process_dropout.py" (see Data Accessibility) to estimate genotype concordance for paired samples at four different minimum depths (4x, 6x, 8x, and 10x). The script finds all sites passing

minimum quality thresholds (minimum depth >= value described previously, mapping quality >= 30, and genotype quality >= 30) in both samples, and from those sites counts the number of sites with shared genotypes, genotypes consistent with allelic dropout, and ambiguous genotypes. We considered genotypes consistent with dropout if one of the two samples was heterozygous, while the other was homozygous for one of the alleles in the first sample's genotype (e.g., "0/1" in urine and "1/1" in feces would be counted as dropout in feces). Genotypes were classified as ambiguous if they were not shared and did not fit a pattern consistent with dropout; for example, if the urine sample had a genotype of "1/1" while the fecal sample had a genotype of "0/2".

Data Accessibility

We deposited raw reads in NCBI's Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) under Bioproject PRJNA508503. We implemented the full assembly and analysis pipeline in Snakemake (Köster & Rahmann, 2012), and managed software using Bioconda (Grüning et al., 2018). All code, scripts, and software environments are available on Github (https://github.com/thw17/Gombe_noninvasive_genomic_methods).

Results

We processed a total of 14 samples from ten different chimpanzees in Gombe National Park, Tanzania from urine (n=7), feces (n=4), dental calculus (n=2), and dentin (n=1) (Table S1). We then captured and sequenced samples using at least one of the following: undirected shotgun amplification (n=2), MYBaits *Pan troglodytes schweinfurthii* capture (Arbor Biosciences; n=4), MYBaits *Homo sapien*s capture (Arbor Biosciences; n=6), xGen (human) Exome Research Panel

(IDT; n=26) (Table S1). In total, we analyzed 38 different combinations of individual, source, and sequencing protocol (Table S1).

Concentrations of extracted DNA varied widely across samples (Table S1). Initially, concentrations ranged from 0.11 ng/μL to 65.6 ng/μL with a single urine sample from 7365 too low to be measured. Sequencing success was similarly variable (Table S2). After merging BAM files from the same sample across multiple runs, we generated between 6.9 and 169.5 million reads per sample and while we successfully produced data for the problematic urine sample (individual 7365), it produced the fewest reads (Table S2). We observed high duplication rates likely resulting from PCR amplification during library construction and capture in most, but not all samples (range from 0.05% to 89.4%; Table S2). In general, exome capture had higher duplication rates than whole-genome capture, which, in turn, had higher duplication rates than shotgun sequencing (Table S2; Figure S1). We also observed a linear increase in duplication rate with an increasing number of mapped reads for whole-genome capture, but not exome capture or shotgun sequencing (Figure S1). After filtration and duplicate removal, we were left with between 1.4 and 26.2 million passing reads per sample (Table S2).

Interestingly, we found that samples ranged in the amount of post-capture endogenous DNA (i.e., DNA from the host after sequence capture, as opposed to other sources) from 33.9% to 99.1% (Figure 1; Table S2). We discovered that this effect was driven by the source of the sample (Figure 1; ANCOVA: $F(3,18) = 125.493$; $p < 0.001$). Upon further investigation, a post hoc Tukey test revealed that urine (n =7; mean endogenous percentage = 96.4%) had significantly more endogenous DNA than dentin ($p = 0.03$; n=1; mean=75.9%), feces ($p < 0.001$; n= 12; mean= 44.9%), and calculus ($p < 0.001$; n=6 ; mean=38.3%).

We evaluated capture success using reverse cumulative plots to assess the proportion of CDS in PanTro4 (i.e., the fraction of sequence in PanTro4 annotated as coding sequence) sequenced at different depths. For all samples, we started with a fixed 40 million reads before duplicate removal. We first used fecal samples to compare exome capture with whole-genome capture, and found that exome capture, despite its higher duplication rate, led to broader and deeper coverage across CDS than whole-genome capture (Figure 2). In addition, when comparing urine and fecal samples (exome capture), urine outperformed feces (Figure 3). Across all urine samples, more than 90% of CDS was captured, while only two fecal samples generated data covering more than 50% of CDS (Figure 3). This pattern became even more pronounced as depth increased; for example, at a minimum depth of 8x, more than 75% of CDS was captured in all urine samples, while all fecal samples fell below 10% CDS covered (Figure 3). Finally, when comparing calculus and dentin, we found more than 85% of CDS was captured for the single dentin sample, with 20% of CDS captured at a minimum depth of 8x (Figure 4). However, less than 25% of CDS was captured in both analyzed calculus samples, which decreased to less than 1% at a minimum depth of 8x (Figure 4).

We measured genotype concordance in the three individuals for which we sequenced at least 40 million reads each for paired fecal and urine samples (Table 1; 7150, 7365, 7507) and a single additional individual for paired dentin and calculus (7057). Likely due to the differences in endogenous DNA content and coverage described above, we obtained very few variant sites (i.e., sites with one or both alleles differing from the reference genome) passing quality and depth filters in feces compared to urine (Table 1). For example, at a minimum depth of 10x, we obtained 227, 368, and 2014 sites from the fecal samples from the three individuals, while we obtained 4952, 93,244, and 115,955 sites from the same individuals from urine samples. In total,

we were able to compare between 59 and 1,309 sites depending on the individual and depth threshold used (Table 1). Overall, genotypes were overwhelmingly concordant, with less than 11% of sites discordant across all comparisons. Most discordant sites were consistent with a pattern of allelic dropout—that is, one sample was heterozygous, while the other was homozygous for one of the two alleles present in the first sample. Among these dropout sites, at a minimum depth of 10x, feces exhibited higher rates of dropout than urine in two of our three comparisons (fecal dropout = 2-8% of all sites; urine dropout = 0.8-4% of all sites). We also observed "ambiguous" sites—discordant sites inconsistent with the dropout pattern described above—at 1-3% of all sites (Table 1). For calculus and dentin, we compared between 27 and 291 shared sites and observed calculus as having the highest dropout rates of any source of DNA at depth thresholds of 8x and 10x (17.86% and 18.42%, respectively). Although we observed less dropout in dentin, these rates are comparable to our highest observed dropout rates for feces (7.89% dropout at a depth of 10x in dentin, 7.86% dropout in feces at a depth of 10x for individual 7507).

Discussion

The development of noninvasive genomic methods is critically important for studying wild populations, particularly those that cannot otherwise be legally or ethically sampled. In this study, we evaluated four biological sources of DNA that can be sampled from wild populations of many taxa: feces, urine, dentin, and dental calculus. Feces and urine may be noninvasively sampled from contemporary living populations, while dentin and dental calculus can often be sampled from skeletal collections of wild populations present in collections at museums and field sites. We assessed the quality of these sources in three different ways. First, we determined post-

capture endogenous content, the amount of captured DNA is derived from the host. Next, we evaluated the breadth and depth of sequencing coverage across genomic targets. Finally, we measured the concordance of genotypes between pairs of samples captures from different sources from the same individual.

In regard to post-capture endogenous content, of the four sources, we found that urine samples contained the highest proportion of host DNA. While post-capture endogenous content was similar in calculus and feces (ranging from approximately 30-50%), all urine samples contained more than 95% host DNA. Previous studies have demonstrated both that host DNA is present in urine and can be successfully extracted and amplified (Hausknecht et al., 2007; Hayakawa & Takenaka, 1999; Hedmark et al., 2004; Nota & Takenaka, 1999; Valiere & Taberlet, 2000; Waits & Paetkau, 2005); however, our results show for the first time that urine in fact has an high fraction of host DNA compared to other sources of DNA, like feces, that are far more commonly used in genetic studies of wild animals, and thus is well-suited for genomic analysis. While we measured post-capture endogenous content in the same way across the sources of DNA that we tested, we are unable to determine for certain from this study whether the difference in endogenous content directly reflect raw differences in the fraction of host DNA across sources. We cannot easily envision a process that would cause sources of DNA to differ in endogenous content after capture but not before, but future work could aim to measure pre-capture differences to confirm our results.

We found that these differences in endogenous content meaningfully impact downstream sequencing success, as exome capture and sequencing of urine samples led both broader and deeper coverage across the coding sequence of the chimpanzee reference genome than any of the other sources of DNA. With the exception of a single problematic sample, all of the urine

421 samples captured more than 90% of coding sequence at a depth of 4x or greater (after duplicate
422 removal), despite extremely high duplication rates. This means that, without optimization or any
423 other methodological considerations, our urine samples produced sufficient data for most
424 evolutionary and population genetic analyses. In contrast, not a single fecal, calculus, or dentin
425 sample in our study produced enough data for downstream analyses (Figures 3 and 4). Rather
426 than suggest that any of these sources of DNA are more or less useful for genomic analyses, we
427 instead argue that these results indicate that urine might work well "out of the box" similar to
428 other high-quality sources like blood and other tissues, while the other sources that we tested
429 require additional methodological considerations for use, like the many developments for feces
430 (Chiou & Bergey, 2018; Perry et al., 2010; Snyder-Mackler et al., 2016).

431 Our analyses revealed that genotypes generated from feces and urine from the same
432 individual were broadly concordant, especially when a minimum depth threshold of 10x was
433 used. Urine fared better generally, with fewer sites ambiguously discordant or consistent with
434 dropout. However, we only had paired fecal and urine samples for three individuals, so these
435 results must be taken as preliminary. Regardless of whether genotypes from urine are
436 comparable or better than those of feces, the low rates of allelic dropout underscore the quality of
437 urine as a source of DNA for genomic analyses. In addition, while we are unable to test it at this
438 time, we hypothesize that urine might be less susceptible to problematic contamination than
439 feces. As discussed above, estimates of the proportion of exogenous DNA in urine before capture
440 are unknown; however, it is well known that feces contain overwhelmingly exogenous DNA
441 (Chiou & Bergey, 2018; Perry et al., 2010; Qin et al., 2010). In addition to the microbiota that
442 dominate feces, fecal samples also contain dietary DNA from food items consumed by the host
443 (Bradley et al., 2007; Clayton et al., 2016). In the case of chimpanzees, food items include a

444 wide array of plant and animal items, including nonhuman primate prey (Gilby, 2006; Hobaiter,
445 Samuni, Mullins, Akankwasa, & Zuberbühler, 2017; Mitani, Watts, & Muller, 2002; Pruetz et
446 al.; Uehara, 1997). Because of the extremely high proportion of microbiota in feces, some sort of
447 DNA capture is required to target endogenous DNA (Chiou & Bergey, 2018; Hernandez-
448 Rodriguez et al., 2018; Perry et al., 2010; Snyder-Mackler et al., 2016; White et al., 2019).
449 However, baits can successfully capture sequence across more than 65 million years of
450 divergence (i.e., across the entire primate order) and much of this captured sequence will map to
451 a reference genome equivalently divergent (Webster et al., 2018). This means that the same baits
452 designed to capture host DNA in the feces will also likely successfully capture DNA from
453 primate prey species and that these contaminant sequences will successfully map to the host
454 reference genome, introducing artifacts into genotyping. This possibility needs to be studied
455 further, but if present in our samples, it would artificially increase our observed rates of allelic
456 dropout for urine (calculated as heterozygous sites in feces that are homozygous for one of the
457 alleles in urine). We thus consider our estimates of allelic dropout in urine to be conservative
458 overall.

459 Our analyses of genotype concordance in dentin and calculus were limited, as we only
460 had a single individual with data from both sources and we recovered very little usable data in
461 the calculus sample. However, in that comparison, we observed a rate of allelic dropout in
462 calculus more than double that of any other tissue. Our estimates for dentin were similar to feces
463 at our most rigorous depth threshold. These results are consistent with previous research showing
464 that yields and quality of host genetic material are lower in calculus compared to dentin (Mann et
465 al., 2018). Yet, calculus has been used to recover full mitochondrial and nuclear genomes from

466 human calculus samples (Ozga et al., 2016; Ziesemer et al., 2018). We therefore suggest that
467 more work is needed to explore and optimize DNA capture from calculus in wild populations.
468 Taken together, we suggest using urine as a primary source of noninvasive genomic
469 DNA. However, urine is not universally available in sufficient quantities for collection and
470 extraction, and its availability and collectable volume will vary by organism body size, study
471 habitat, and level of habituation. When using other noninvasive biological materials, our results
472 build on previous research (Chiou & Bergey, 2018; Hernandez-Rodriguez et al., 2018; White et
473 al., 2019) showing that targeting a smaller subset of the genome leads to an increase in usable
474 data. In particular, we argue that exome capture is an ideal option, as it targets a small subset of
475 the genome commonly used in evolutionary analyses and there are commercially available
476 human kits that can be used across the entire primate order (Webster et al., 2018). However, like
477 other methods of DNA capture, exome capture requires additional considerations when working
478 with noninvansive samples. First, a multitude of factors impact the quality of host genomic
479 material in a natural environment, including time elapsed since excretion (DeMay et al., 2013),
480 field/laboratory storage conditions (Nsubuga et al., 2004; Panek et al., 2018), and enzymatic
481 activity (Deagle et al., 2006). Second, depending on sample quality, it may be necessary to
482 undergo repeated extractions for the same sample, along with multiple double stranded DNA
483 library builds and multiple indexing amplifications. Third, a single capture of the indexed DNA
484 library may lead to a higher duplication rate, which has been cited in several studies as being a
485 barrier to inexpensive and accurate host genome capture (Bansal & Pinney, 2017; Ebbert et al.,
486 2016; García-García et al., 2016).
487 Noninvasive samples have been used across a variety of disciplines for addressing many
488 evolutionary and ecological questions (Beja-Pereira, Oliveira, Alves, Schwartz, & Luikart, 2009)

including investigations into dietary niches, social structures, and diversity of endangered animals (Carroll et al., 2018). Chimpanzees, currently listed as endangered on the IUCN red list, are considered to be flagship species and indicators of environmental stressors in the surrounding area (Wrangham, 2008). Thus, noninvasive genomic methods are critical for monitoring the health of wild populations as well as aspects of local adaptation and population history important for conservation management. This is especially important for small, isolated populations such as that of Gombe National Park, for which there is an effort to maintain genetic diversity (Pusey, Pintea, Wilson, Kamenya, & Goodall, 2007). The results of our study highlight urine as a promising and untapped source of DNA for this and other genomic work in not only chimpanzees, but wild populations of other protected species as well.

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Conflict of Interest Statement

The authors declare no competing interests.

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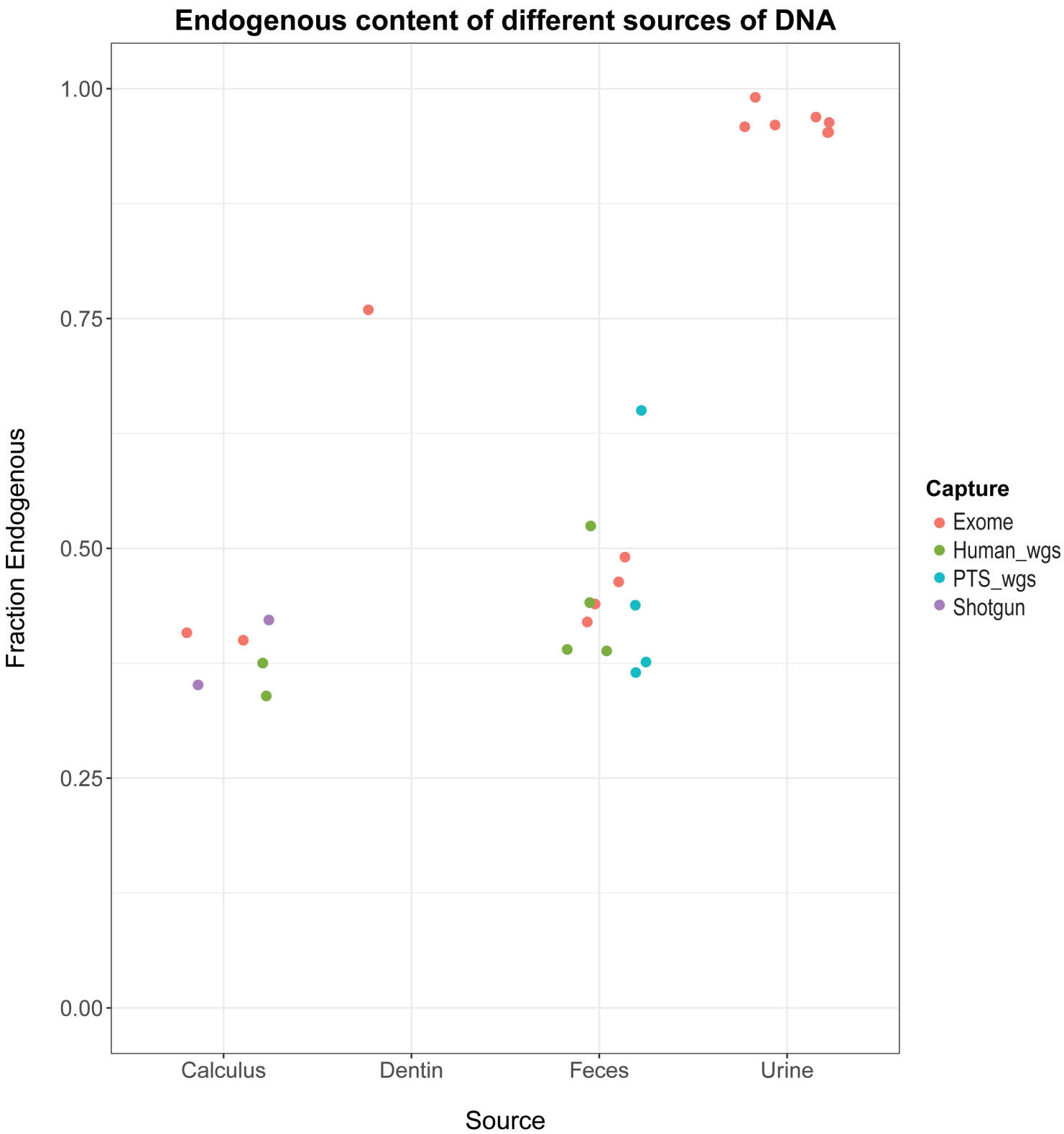
Data Accessibility

We deposited raw reads in NCBI's Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) under Bioproject PRJNA508503 (Ozga et al., 2020). We implemented the full assembly and analysis pipeline in Snakemake (Köster & Rahmann, 2012), and managed software using Bioconda (Grüning et al., 2018). All code, scripts, and software environments are available on Github (https://github.com/thw17/Gombe_noninvasive_genomic_methods).

Author Contributions

ATO, THW, and ACS designed the study. RSN, ICG, AP, YL, and BH provided samples. ATO completed the lab work. THW analyzed the data. MAW and ACS provided laboratory space and funding. ATO and THW wrote the manuscript. All authors revised and approved the manuscript.


Endogenous content of different sources of DNA
Fraction Endogenous
1.00
0.75
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Calculus
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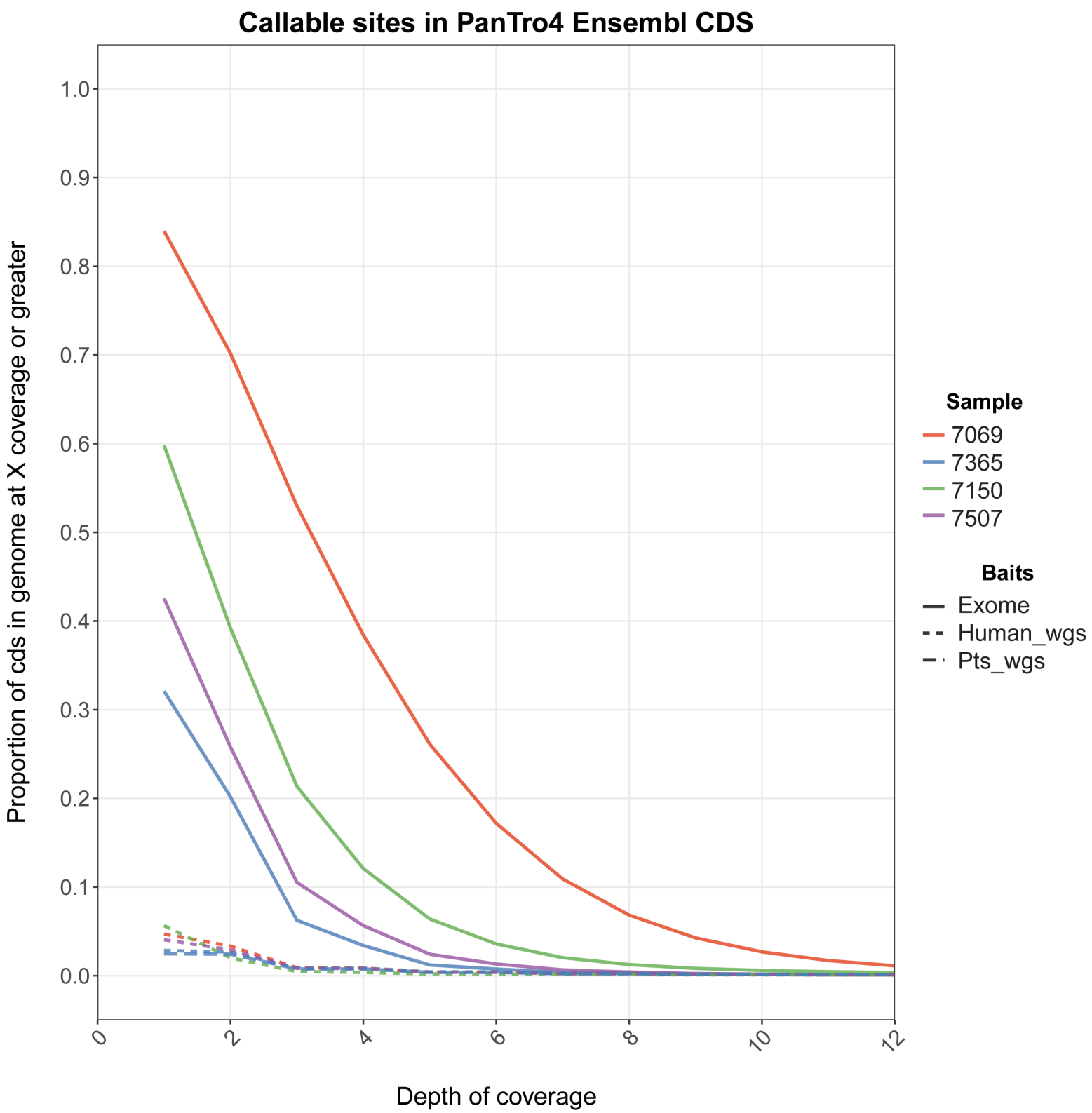
PTS_wgs
Shotgun


Callable sites in PanTro4 Ensembl CDS
Proportion of cds in genome at X coverage or greater
1.0
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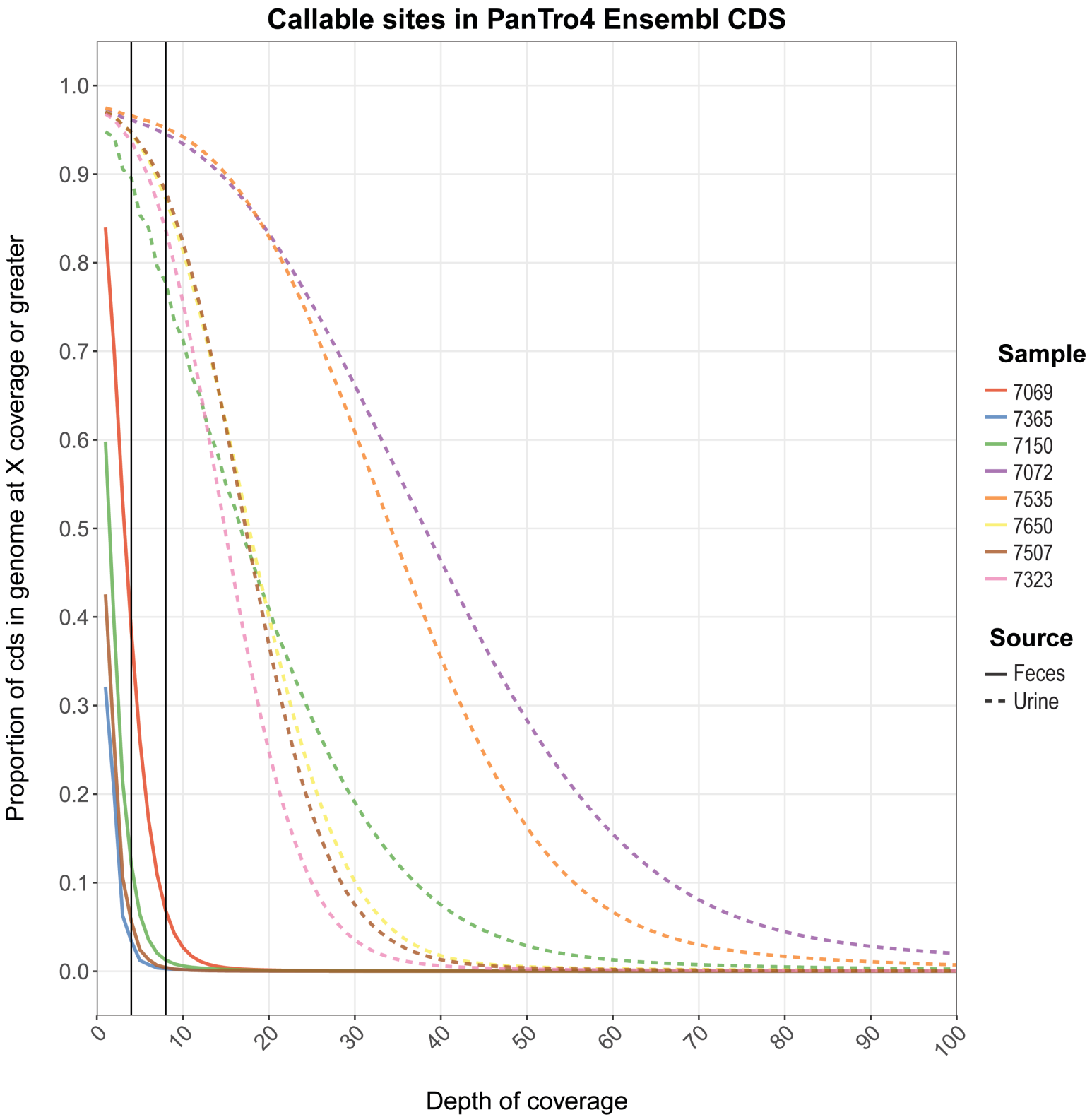
12
Depth of coverage
Sample
7069
7365
7150
7507
Baits
Exome
Human_wgs
Pts_wgs


Callable sites in PanTro4 Ensembl CDS
Proportion of cds in genome at X coverage or greater
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Depth of coverage
Sample
7069
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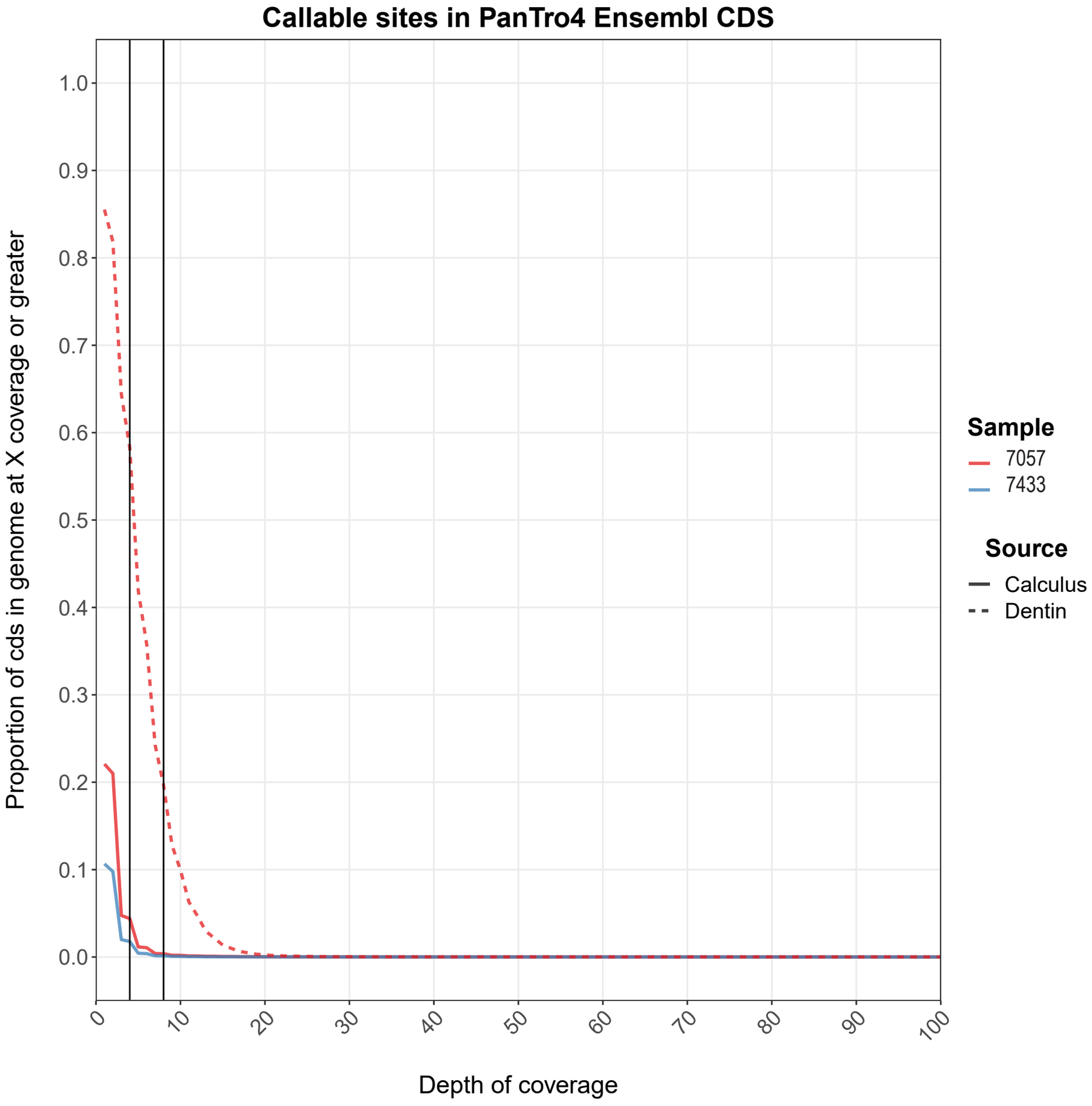
7323
Source
Feces
Urine


Callable sites in PanTro4 Ensembl CDS
Proportion of cds in genome at X coverage or greater
1.0
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30
40
50
60
70
80
90
100
Depth of coverage
Sample
7057
7433
Source
Calculus
Dentin

Individual ID	Depth[a]	Source	Passing Variant Sites[b]	Passing in Both[c]	Shared Sites[d]	Dropout Sites[e]	Ambiguous Sites[f]	Dropout Rate[g]
7365	4	Feces	2,484	453	441	3	5	0.66%
7365	4	Urine	17,127	453	441	4	5	0.88%
7365	6	Feces	711	147	139	3	4	2.04%
7365	6	Urine	11,522	147	139	1	4	0.68%
7365	8	Feces	315	79	74	3	1	3.80%
7365	8	Urine	7,486	79	74	1	1	1.27%
7365	10	Feces	227	59	54	3	1	5.08%
7365	10	Urine	4,952	59	54	1	1	1.69%
7150	4	Feces	9,933	4,317	3,918	38	26	0.88%
7150	4	Urine	107,629	4,317	3,918	335	26	7.76%
7150	6	Feces	5,039	2,580	2,391	38	19	1.47%
7150	6	Urine	103,639	2,580	2,391	132	19	5.12%
7150	8	Feces	2,947	1,778	1,650	36	16	2.02%
7150	8	Urine	98,441	1,778	1,650	76	16	4.27%
7150	10	Feces	2,014	1,309	1,212	36	15	2.75%
7150	10	Urine	93,244	1,309	1,212	46	15	3.51%
7507	4	Feces	3,153	1,497	1,436	19	14	1.27%
7507	4	Urine	144,600	1,497	1,436	28	14	1.87%
7507	6	Feces	1,284	666	629	19	9	2.85%
7507	6	Urine	135,002	666	629	9	9	1.35%
7507	8	Feces	611	362	332	18	9	4.97%
7507	8	Urine	125,793	362	332	3	9	0.83%
7507	10	Feces	368	229	203	18	6	7.86%
7507	10	Urine	115,955	229	203	2	6	0.87%
7057	4	Calculus	3,555	322	291	10	8	3.11%
7057	4	Dentine	74,516	322	291	13	8	4.04%
7057	6	Calculus	1,291	132	113	10	3	7.58%
7057	6	Dentine	58,088	132	113	6	3	4.55%
7057	8	Calculus	349	56	40	10	2	17.86%
7057	8	Dentine	40,438	56	40	4	2	7.14%

7057	10	Calculus	115	38	27	7	1	18.42%
7057	10	Dentine	27,190	38	27	3	1	7.89%