Comparison of Four Carnivore Metabarcoding Library-Build Protocols

By

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Abstract

DNA metabarcoding is a relatively new molecular tool for dietary analysis of wildlife populations for monitoring and conservation. Metabarcoding involves use of universal primers to amplify a region of, for example, mitochondrial DNA (mtDNA) that is highly conserved within species and variable among species (a "barcode") extracted from an environmental sample. By utilizing massively parallel sequencing of DNA extracted from predator feces, different prey mtDNA barcodes can be sequenced simultaneously to reveal dietary patterns. Because predator DNA also is abundant in their scats, sequences known as "blockers" are sometimes employed to block the amplification of host mtDNA while allowing prey mtDNA to be amplified preferentially. Before sequencing, a library must be constructed for each sample. Library construction involves amplifying the mtDNA barcode region using universal primers and attaching unique identifiers or indexes to those amplification products. In this study, we designed and tested four library-build protocols on red fox (*Vulpes vulpes*) fecal samples. Two protocols used universal primers attached to sample-specific inline tags, one treated with a blocker and the other without a blocker. The other two protocols used the universal primer without inline tags, relying on a second step for index attachment; one was treated with a blocker and the other without a blocker. We tested our protocols with 3 mock fecal samples (mixtures of prey DNA of known content) and 38 red fox fecal samples. We evaluated the performance of the four protocols based on total read numbers, prey read numbers, and the number of species identified. All four protocols successfully identified some of the prey species from the fox scats. Amplification without inline tag and with blocker yielded the highest average number of prey reads and species identified. Thus, we conclude that this protocol was the most efficient of the four.

Introduction

A method known as DNA metabarcoding is becoming a popular tool for dietary analysis of wildlife used in biodiversity monitoring and conservation (Zizka et al. 2019, Bohmann et al. 2014, Deagle et al., 2018). Compared to traditional techniques used in the dietary analysis (such as visual observation of scat content), metabarcoding is superior in its high resolution, high sensitivity, and high throughput utilizing massively parallel sequencing that can identify food items to the species level (Carew et al., 2013; Nielsen et al., 2017). The principle of metabarcoding involves amplifying and sequencing DNA from ingested organisms corresponding to a specific "barcode" region of, for example, mitochondrial DNA (mtDNA). A barcode is a DNA sequence that is highly variable among taxa, but which is flanked with priming regions that are highly conserved among taxa. Thus, it can be amplified using a pair of universal primers and is capable of distinguishing taxa. The length of the barcode sequence is generally in a range of 100 to 300 base-pairs (Pompanon et al., 2012, Ando et al., 2020). For vertebrate predators, the 12SV5 region of mtDNA is often used, due to the availability of primers conserved sufficiently to amplify in most vertebrate species, but with intervening sequence that is variable enough for species-level identification (Riaz et al., 2011). A recent advance on metabarcoding is the use of blocking oligonucleotides, which block the amplification of the predator's DNA in fecal samples, thereby allowing the universal primers to preferentially amplify mtDNA from food items (Boessenkool et al., 2011; Shehzad et al., 2012). Blocking oligonucleotides are designed to anneal specifically to the target predator's DNA overlapping the universal priming site. Blockers contain a 3-carbon spacer on the 3' end that prevents extension during PCR (Shehzad et al., 2012). The resulting DNA sequences therefore represent proportionally more prey DNA, enabling prey species present in fecal samples to be identified through the "barcode" sequences. The standard strategy of carnivore dietary analysis

through DNA metabarcoding (Ando et al., 2020) is (1) collecting a sample, (2) extracting DNA from the fecal sample, (3) PCR-amplifying a barcoding locus with vertebrate universal primers and with or without inclusion of a carnivore blocker, (4) adding unique identification indexes to each amplicon, (5) pooling individually tagged or indexed PCR products, (6) sequencing, for example, through an Illumina platform, and (7) analyzing sequencing results, including comparison with a reference database to assign taxa through DNA barcodes.

One of the crucial steps in metabarcoding studies is preparing the uniquely labeled DNA sample libraries or so-called library-build, which allows multiplexing, sequencing of multiple samples simultaneously in a single flow cell run (Schnell et al., 2015, Meyer et al. 2010). The use of fusion primers attached through PCR has proven to be an inexpensive, yet efficient, alternative to adapter-ligation-based library preparation methods (Schnell et al., 2015; Zizka et al., 2019; Elbrecht et al., 2017). The fusion primer is one primer sequence that contains Illumina P5/P7 adaptor, sample identification inline tag (eg. unique 8 bp sequence), and the universal primer, so it can amplify the target sequence to make the sequencing-ready library in one PCR run. The general strategy of library-build depends on the sequencing platform. For this study, we used the Illumina MiSeq platform, and our library-build protocol involved two basic steps (Fig. 1). The first step (amplicon PCR) involved PCR-amplification of fecal (or mock fecal) DNA with our fusion primer. The second step was to attach unique indexes (index PCR) to each amplicon. Although the index PCR in our approach introduced unique indexes, an alternative or additional method of uniquely marking samples is to introduce a unique inline tag (unique 8 bp sequence) during the first (amplicon PCR) step (Schnell et al., 2015). Including an inline tag has the advantage of enabling pooling of PCR projects prior to the second PCR step, reducing the overall number of reactions

required. However, the added length of the primer by the length of the tag (e.g., 8 bp) risks reducing the efficiency of the amplicon PCR step (Schnell et al., 2015; Elbrecht et al., 2017).

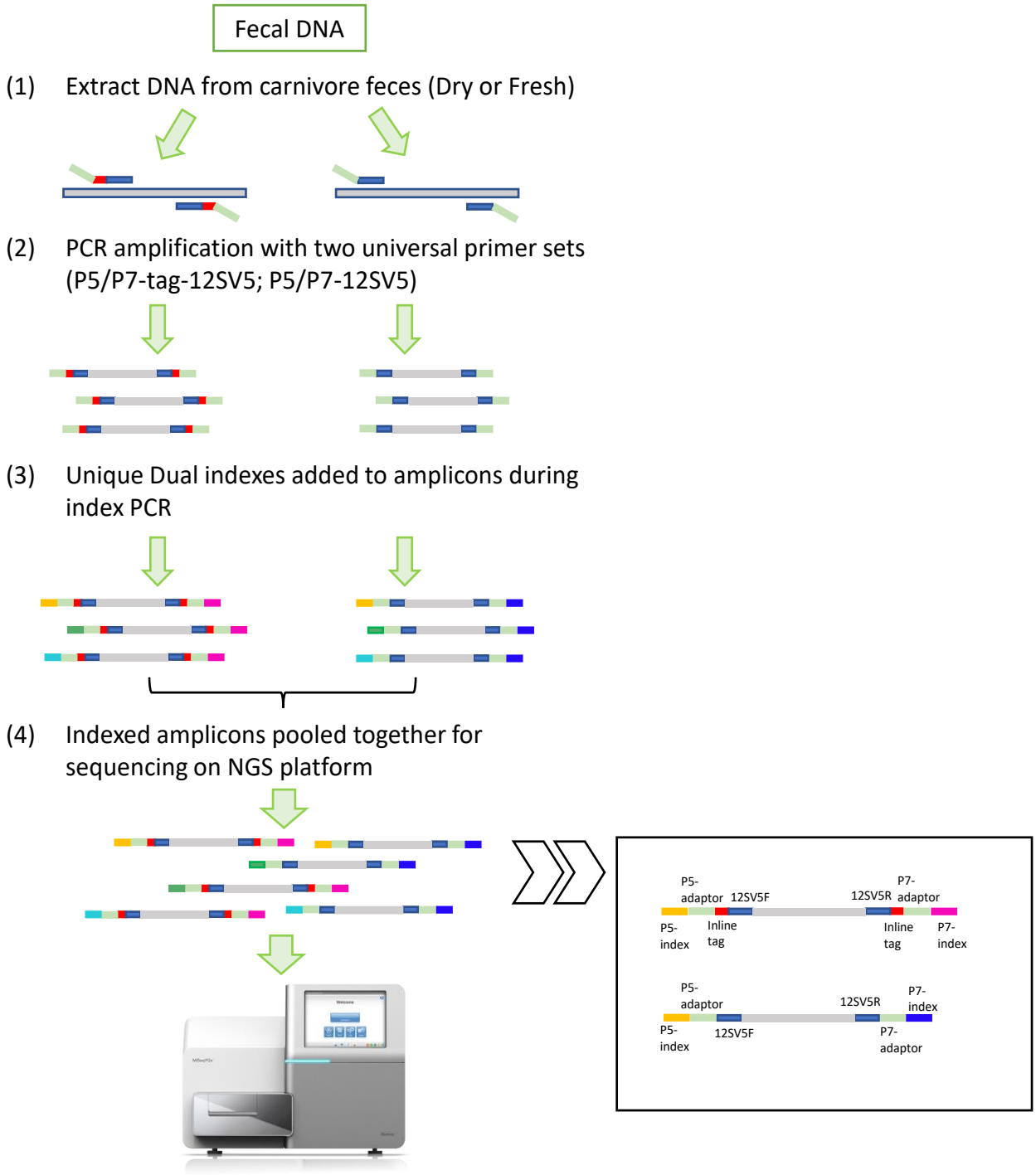


Fig. 1 Four library-build protocols. Two primer designs used in this experiment are shown above. Each primer was treated with and without blocker oligonucleotides, which resulted in four different combinations in total. Amplicons on the left illustrate P5/P7-tag-12SV5 library-builds with inline tag (red); those on the right represent P5/P7-12SV5 library-builds without inline tags; both builds include 12SV5 priming sequence (blue) and P7/P5 index-PCR-compatible overhangs (light green). Uniquely indexed P7 Illumina adapters (yellow, green, light blue) and plate-wide indexed P5 Illumina adapters (blue) are added during the index PCR step.

In this study, we focused on carnivore dietary analysis utilizing DNA metabarcoding to analyze diets of red fox (*Vulpes vulpes*) fecal samples and evaluated four different metabarcoding library construction protocols as well as differing PCR-cleanup protocols. Two library protocols used fusion primers, including P5/P7 overhangs needed for the next step and sample-specific inline tags treated with or without a red fox-specific blocker (hereafter *Vulpes vulpes* blocker); the other two protocols used fusion primers with only P5/P7 overhangs treated with or without *Vulpes vulpes* blocker, but no inline tags. The problems we investigated in this research were (1) assessing the most efficient primer construction for amplicon PCR (a) adding P5/P7 overhangs; (b) adding tags and P5/P7 overhangs; (2) assessing efficacy of blockers, and (3) evaluating workflow(s) performance at identifying prey items in real and synthesized fecal samples.

Methods

1. DNA Extraction and Sample Preparation

In this study, we used fecal DNA extracted from previously confirmed red fox fecal samples using the EURx stool DNA Purification kit (EURx Molecular Biology Products, Gdansk, Poland) and, for one sample, also using the QIAamp DNA Stool Mini Kit (Qiagen, Inc, Valencia, CA, USA) in both cases following the manufacturer's protocols, except that DNA was eluted in 50 µl volumes instead of 200 µl. The fecal samples were collected previously from the Sacramento Valley area (California). We also prepared three positive control samples (mock fecal samples): "Prey-Cocktail," "Prey-Fox," "Fox-Tissue." Prey-Cocktail was prepared by mixing muscle tissue-derived DNA from each of four prey species in equal concentrations of 0.005 ng/µl for a total prey DNA concentration of 0.02 ng/µl. The prey tissue DNA included mule deer (*Odocoileus hemionus*), Laysan albatross (*Phoebastria immutabilis*), Botta's pocket gopher (*Thomomys*

bottae), and deer mouse (*Peromyscus maniculatus*). Fox-Tissue contained only red fox DNA extracted from a red fox's nose sample using the DNEasy blood and tissue kit following manufacturer protocols (Qiagen, Inc.). Prey-Fox was prepared by mixing the prey cocktail (0.02 ng/µl) and fox tissue DNA (0.2 ng/µl). We selected the ratio of 1 part prey DNA to 10 parts fox DNA to simulate actual scats, for which intact host DNA tends to be far more abundant than intact prey DNA (Shezad et al, 2012).

2. PCR Conditions and Preliminary Testing of Primers and Blocking Oligonucleotide

As an initial step, we tested 3 sets of universal vertebrate primers targeting the 12S region of mitochondrial genome with and without both inline tags and red fox blocking oligonucleotides (Table 1). Briefly, the amplicon primer sets differed in length, with the shortest composed (1) solely of bases annealing to flanking region of the target (modified from Riaz et al. 2011 to include degenerate positions, 12SV5degF, 12SV5degR), followed by (2) a set that additionally included P7 and P5 overhangs compatible with Illumina adapters enabling completion of the library through a single additional PCR step (see below, P7-12sv5F, P7-12sv5R), and (3) another including the same bases as (2) but with an 8 bp unique inline tag inserted between annealing bases and P7/P5 overhangs (P7-n-12sv5F, P7-n-12sv5R) (Fig.1). The blocking oligonucleotide (*Vulpes vulpes* Blocker) was designed specifically for the red fox as modified from Vestheim and Jarmann (2008). PCR conditions were adapted from Shehzad et. al. (2012). When no blockers were included, the 25 µl PCR mixture contained 1 U AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA, USA), 10 mM Tris-HCl, 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.1 µM of each universal primer (Table 1), and 5 µg of bovine serum albumin (BSA). For reactions that included a blocker, the same conditions were used except 2.0 µM of blocker oligonucleotide were added (i.e., 20 times the concentration of the universal primers). The PCR thermal profile was

95°C for 10 minutes to denature, followed by 45 cycles of 30s at 95°C, and 30s at 60°C; because our target sequence was shorter than 120 bp, the elongation step was removed to reduce the +A artifact (Riaz et al. 2011). We then electrophoresed products on a 3% agarose gel stained with GelStar Nucleic Acid Gel Stain (Lonza Rockland, Inc., Rockland, ME, USA) to preliminary evaluate amplification success. After determining that the two primer sets with overhangs (with vs without inline tags) amplified at least as well as the short primers, we retained only the overhang primers for subsequent testing.

Table 1. Primers and blocking oligonucleotides used in this study. 12SV5deg primer sets were based on the original universal primer for vertebrates by Riaz et al. (2012), but modified with degenerate bases to accommodate a broader range of species. Other primers set were modified by adding 8-base-pair inline-tags and/or P7/P5 overhangs enabling index PCRs to add Illumina adapter and index sequences. Tag sequences were as follows, respectively, for tags 1–5: ACACACAC; ACAGCACA; GTGTACAT; TATGTCAG; TAGTCGCA.

Primer	Type	Sequence
12sv5degF	Short Forward	YRGAACAGGCTCCTCTAG
12sv5degR	Short Reverse	TTAGATACCCCACTATGY
P5-12SV5	Overhang Forward	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCY RGAACAGGCTCCTCTAG
P7-12SV5	Overhang Reverse	TCTTTCCCTACACGACGCTCTTCCGATCTTAGAT ACCCCACTATGY
P5-[tag]-12sv5	Overhang-tag1 Forward	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC[x xxxxxxx]TAGAACAGGCTCCTCTAG
P7-[tag]-12sv5gopher	Overhang-tag1 Reverse for Gopher DNA	TCTTTCCCTACACGACGCTCTTCCGATC[xxxxxxxx]TTAGATACCCCACTATGT
P7-[tag]-12sv5	Overhang-tag1 Reverse	CTTTCCCTACACGACGCTCTTCCGATC[xxxxxxxx] TTAGATACCCCACTATGC
Vulpes vulpes Blocker	Fox Blocking Oligonucleotide	CCACTATGCTTAGCCCTAAACATAAATAGTTCTA TAAC/3SpC3/

3. Experimental Design

In total, we prepared 5 library plates (96 reactions each). Within each of the 5 library plates, we used each scat sample (n = 20 per plate; Table 2) and each of 4 controls (no-template negative,

prey-cocktail, fox-prey, and fox tissue) 4 times each. Each of the 4 replicates on each plate corresponded to a different combination of blocker (yes/no) vs inline tag (yes/no). Each library plate was prepared using a different combination of PCR product cleanup and pooling methods with the goal of obtaining at least one pair of plates identical with respect to all but one treatment variable (Table 3): magnetic bead versus ExoSAP-IT cleanups for the amplicon PCR (library plates 3, 5), magnetic bead versus spin-column cleanup for index PCR (library plate 1, 2), and pooling index PCR product according to equal DNA mass versus equal PCR product volume (Library plates 1, 3 and 4, 5). However, we used up sample DNA after plate 3 and used a different set of samples for plates 4 and 5 (Table 3), preventing a sample-controlled comparison of amplicon PCR cleanup methods.

Table 2. Four treatments used to amplify target DNA. Fecal DNA was amplified with four treatments. *Vulpes vulpes* blockers were oligonucleotides added with 12SV5 primers to reduce red fox DNA amplification. In-line tags were unique 8-bp sequences (allow sample identification) added to 12SV5 primers so that the amplicons will have the tag sequence between Illumina adaptors and the 12SV5 site.

Name of Treatments	*Vulpes vulpes* blocker added?	In-line Tags added?
Blocker-NoTag	Yes	No
Blocker-Tag	Yes	Yes
NoBlocker-NoTag	No	No
NoBlocker-Tag	No	Yes

Table 3. Cleanup and normalization methods used in 5 library plates. For libraries 1 to 3, we used the same set of samples (S15-0827 to S15-0845) and for library 4 and 5 we used a different set of samples (S15-0846 to S15-0886). S19-8526Q and S19-8526E were obtained from the same fecal sample extracted with Qiagen (Q) or EURx (E) and were used in all the libraries.

	Cleanup Method Amplicon PCR	**Cleanup Method (index PCR)**	**Normalization by Qubit**	**concentration**
Lib 1	Ampure Beads	Spin column	Yes	**9.45 ng/ul**
Lib 2	Ampure Beads	Ampure Beads	Yes	**2.81 ng/ul**
Lib 3	Ampure Beads	Spin column	No (pooled 2ul from every sample)	**63.7 ng/ul**
Lib 4	ExoSAP-IT	Spin column	Yes	**31.2 ng/ul**
Lib 5	ExoSAP-IT	Spin column	No (pooled 2ul from every sample)	**140 ng/ul**

4. Library Construction and Sequencing

We used a two-stage approach, followed by pooling of samples, which involved (1) amplification of the target DNA through use of universal vertebrate primers (with and without inline tags and blockers) in amplicon PCR and (2) a second index PCR step, which introduced both the necessary P7 and P5 adapter sequences required for Illumina sequencing and unique indexes for each sample, enabling pooling prior to sequencing (Fig. 1). The amplicon PCR procedure was the same as described above for the preliminary PCR tests. The two different PCR cleanup procedures we tested were as follows: AMPure XP magnetic beads (Beckman Coulter Life Science, IN, USA) and ExoSAP-IT (Applied Biosystems, CA, USA) (Table 3). For the index PCRs, 96 different i7 indexes (compatible with P7 overhangs) were used for each library plate and 5 different i5 indexes (compatible with P5 overhangs), each corresponding to a different library plate, were added to amplicon PCR products. The PCR mixture in this step contained 12.5 µl of Q5 Master Mix (Applied Bio), 2.5 µl of i7 index primer, 2.5 µl of i5 index primer, 5 µl nuclease-free water, and 3 µl of cleanup amplicon; the thermocycler profile was 98°C for 30s, followed by 8 cycles of 10s at 98°C for 10 sec and 65°C for 75 sec, and 5 minutes at 65°C, then stored at 4°C. Each library plate shared the same 96 i7 indexes and had a unique (plate-specific) i5 index so every sample could be demultiplexed when sequencing in a single sequencing lane. For the index PCR cleanup, we also tested two methods. Library plates 1, 3, 4, and 5 were purified by QIAquick PCR purification spin columns (Qiagen, Inc) following the manufacturer's instructions; library 2 was cleaned by AMPure XP magnetic beads (Beckman-coulter) following the manufacturer's instructions.

We pooled the cleaned index PCR products of each library plate into a single tube. Samples in library plates 1, 2, and 4 had equal concentrations, which were normalized after quantitation by Qubit fluorometer (Invitrogen, CA, USA); samples in library plates 3 and 5 were not quantitated

prior to pooling equal volumes of PCR product, specifically 2 µl of each. We then quantitated each of the 5 pools and diluted them to 25 ng/µl. Sequences were then pooled in equal volumes and submitted to the Genome Center at the University of California, Davis for paired-end 250 bp sequencing on a single lane of the Illumina MiSeq platform.

5. Bioinformatics

i. Raw Reads Preprocessing and Bioinformatics

We received demultiplexed reads from UC Davis Genome Center in fastq.gz format. The sequences that were not assigned to any samples were removed. Otherwise, the raw sequences were trimmed using cutadapt script (Martin, 2011), which removed adapter and overhang sequences for subsequent analysis. We used MultiQC (Ewels et. al., 2016) to evaluate the overall performance of the sequencing run. Then, we ran the DADA2 (Callahan et al, 2016) pipeline (package version 1.16.0) in R (version 4.0.2) to filter and assemble the forward and reverse reads. Chimera reads were removed using removeBimeraDenovo function from DADA2 package. DADA2 generated an Amplicon Sequence Variant (ASV) table. The ASV table contained every unique sequence assembled in fasta format and their read numbers for each sample.

ii. Species Assignment

The assembled sequences (i.e., ASVs) generated from DADA2 were compared against the nucleotide collection (nr/nt) database with the BLASTn program (version 2.10.1) in NCBI. We manually reviewed and assigned the BLAST results to each unique sequence based on their query coverage, E-value, and percent identity. Results with high query coverage (>98%), and a high percent identity values >95%) were considered accurate species assignments. For BLAST results that identified multiple species with similar query coverage, we determined the species based on the geographic location of their range and selected the species inhabiting in Sacramento Valley,

California, USA because most of the red fox scats samples in this study were collected from this area (the one exception was S19-8526, which was collected from the Lassen National Forest). For all the *Canis lupus* and *Canis lupus familiaris* reads, we converted them to *Vulpes vulpes* reads because many of them appeared in our fox-tissue controls, so they were more likely to be *Vulpes vulpes* reads. Often, multiple ASVs corresponded to the same species and were therefore merged such that for subsequent analyses, species (or higher-level taxon, when species could not be resolved) was the unit of detection.

6. Data Analysis

i. Threshold Determination

Detection thresholds (proportion to the total read number of each sample) can be applied to raw reads to filter out contamination and erroneous reads before subsequent analysis, but if set too high also can reduce detection of valid taxa that were in lower quantities or poorly amplified (De Barba et al. 2014, Zizka et al.2019, Deagle et al. 2019). To assess optimal thresholds for the entire sequencing data, we tested six detection thresholds ranging from 0 to 0.1 (i.e., proportion of reads) with control samples. First, we applied the six thresholds to the species assignment to evaluate the performance. We did this in terms of the four amplicon treatments (Table 2). For a given threshold, any species for which read numbers were below it, was considered undetected, whereas all species with number of reads exceeding the threshold were considered detected. Because the species composition of our controls was known, we could classify the species identified in control samples binarily into four categories: true positive (TP), true negative (TN), false positive (FP), and false negative (FN). We evaluated the sensitivity and specificity of 4 treatments under different detection thresholds using the following equations (Escobar-Zepeda et al. 2018):

Sensitivity = TP/(TP+FN)

Specificity = TN/(TN+FP)

We selected the thresholds that maximized specificity with the least impact on sensitivity based on the control samples.

ii. Blocker Efficacy

For each library plate, we compared data from the Blocker and NoBlocker treatments, in both cases using only samples without inline tags under two detection thresholds, 0.001 and 0.01, selected based on comparisons with the controls. We compared the average total read number, average red fox reads, average prey reads, and average number of species identified between libraries treated with and without blocker. The differences were assessed by pairwise t-tests.

iii. The Performance of Four Library-Build Protocols

To investigate the performance of the libraries when using blockers and inline tags, we tested all four possible combinations of these two variables for each fecal sample (Table 2). We used the same detection thresholds, 0.001 and 0.01. First, we evaluated the performance of four combinations on three aspects: (1) average number of reads per fecal sample; (2) average number of total prey reads per fecal sample; (3) average number of prey species identified per fecal sample. One-way analyses of variance (ANOVA) were used to evaluate the effect of our four library-build treatments on these three aspects.

iv. Comparison of PCR Cleanup and Pooling Procedures

The amplicon cleanup and library pooling procedures for the five library plates are shown in Table 3. First, we evaluated the overall performance of each library plate in terms of average number of

total preys reads and average number of prey species identified under 0.01 and 0.001 detection thresholds. One-way analyses of variance (ANOVAs) were used to analyze the differences among the five libraries in terms of each of these metrics. Second, we evaluated the effects of different PCR cleanup and pooling strategies by comparing relative read abundance (RRA) of the species detected within the libraries under 0.01 detection threshold since it is the more stringent threshold. Relative Read Abundance was calculated as follows:

$$RRA_i = \frac{1}{S}\sum_{k=1}^{S}\frac{n_{i,k}}{\sum_{i=1}^{T} n_{i,k}} \times 100\%$$

In this equation, *T* is the number of taxa, *S* is the number of samples, and $n_{i,k}$ is the number of sequences of food item *i* in sample *k* (Deagle et al., 2018). Except for samples S19-8526Q and S19-8526E, libraries 1,2, and 3 were prepared with a different set of scat samples (S15-0827 to S15-0845) than libraries 4 and 5 (S15-0846 to S15-0886) due to limited availability of DNA. To control for all but a single treatment variable, comparisons were conducted only between libraries using the same scat samples and the same treatments except for the that under comparison.

Results

1. Sequencing Data

We obtained 5,388,092 reads in total from the MiSeq run (Appendixes 1, 2). After demultiplexing and preprocessing, we input the sequencing data into DADA2 for assembly and sample assignment. After this step, we retained 4,902,051 reads (91% of raw reads); 9% of the reads were unpaired or could not be assigned to specific samples. Data from 4 out of 460 samples failed entirely and were not considered further in analysis. DADA2 generated an ASV table containing 360 unique sequences (Appendix 3) with read numbers assigned to 456 uniquely indexed samples.

We reviewed the 360 unique sequences based on their BLAST results and 214 of them met our criteria (i.e.., >98% query coverage and >95% identity to matching sequence in GenBank).

2. Performance of Four Metabarcoding Library Protocols in 2 Control Samples under Different Thresholds

First, we estimated the specificity and sensitivity of the four library-build strategies in the two control samples that included prey species (Table 4, Fig.2). Applying the six detection thresholds ranging from 0 to 10% of prey reads (i.e., excluding red fox reads), maximum specificity was achieved at thresholds ≥0.001 in the prey-cocktail and prey-fox for all but one treatment, the NoBlocker-NoTag group in Prey-Fox, for which the highest specificity was at thresholds ≥0.005. The sensitivities generally decreased with increasing thresholds in both control samples. Because the best performing treatment in Prey-Fox control (NoBlocker-NoTag) had perfect specificity and sensitivity at the 0.01 threshold, we selected this as our more stringent threshold, but also retained a 0.001 threshold which optimized specificity and sensitivity for the other treatments. For analyses of scats, results for both thresholds are presented.

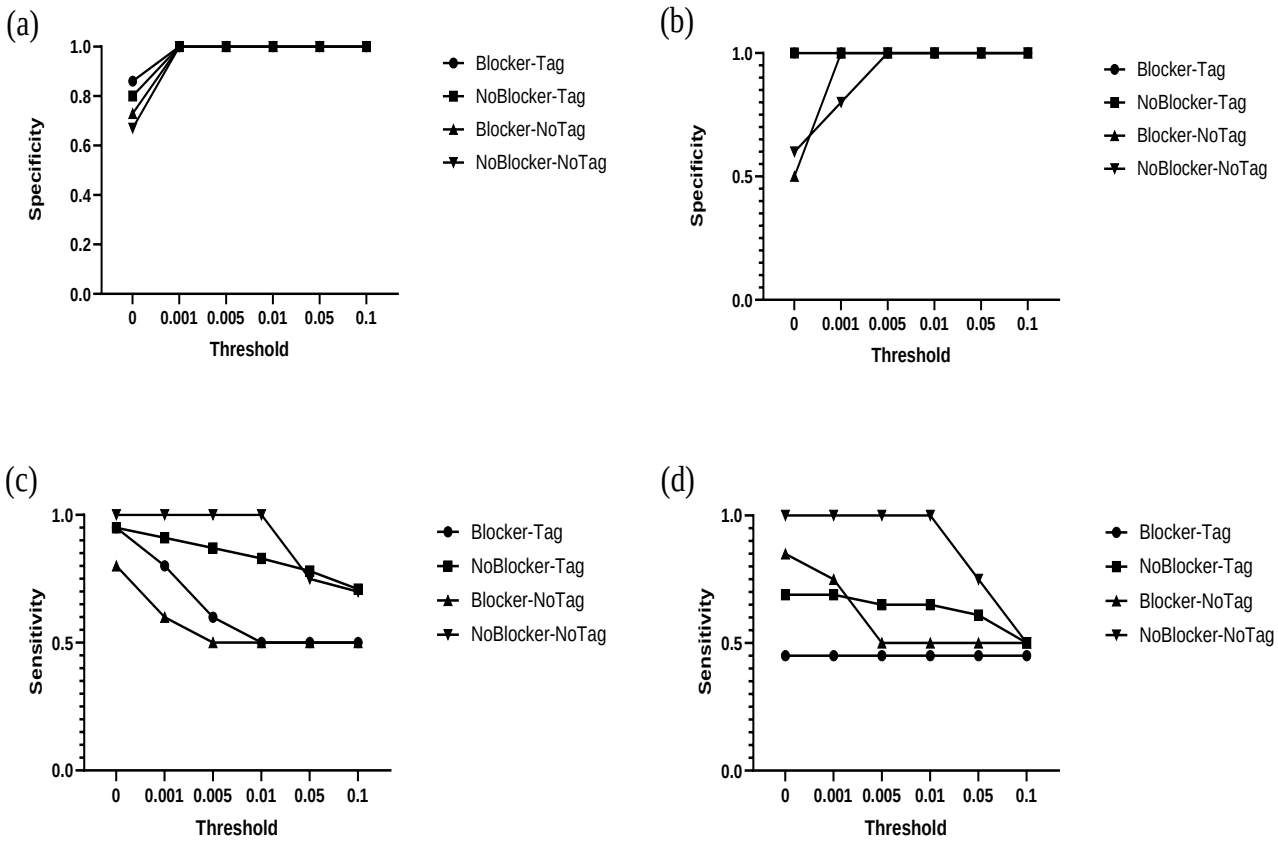


Fig. 2 Specificity (a, b) and sensitivity (c, d) of control samples using 4 NGS library construction treatments over a range of detection thresholds, including (a) specificity of 4-prey mixture, (b) specificity of 4-prey+fox mixture, (c) sensitivity of 4-prey mixture, and (d) sensitivity of 4-prey+fox mixture. Concentrations of all prey DNA were 0.005 ng/µl and red fox DNA (b, d only) was 0. 20 ng/µl in starting template. (b, d) red fox reads were excluded from calculation of specificity, sensitivity, and detection thresholds, which reflect the proportion of all prey reads attributed to a particular prey species (*Odocoileus hemionus*, *Peromyscus maniculatus*, *Thomomys bottae*, and *Phoebastria albatrus*).

Table 4. Specificity and sensitivity of control samples using four metabarcoding library construction treatments with different detection thresholds (proportion of total reads) applied. Prey-cocktail and prey-fox were two control samples we used to determine the filtering threshold. Prey-cocktail was a mock community we made containing black-tailed deer, albatross, deer mouse, gopher tissue DNA in same concentration. Prey-fox was a mock community we made containing the prey-cocktail and fox tissue DNA.

Control sample	Treatment	Detection threshold	Specificity	Sensitivity
Prey-Cocktail	**Blocker-Tag**	0	0.86	0.95
		0.001	1.00	0.80
		0.005	1.00	0.60
		0.01	1.00	0.50
		0.05	1.00	0.50
		0.1	1.00	0.50
	NoBlocker-Tag	0	0.80	0.95
		0.001	1.00	0.91
		0.005	1.00	0.87
		0.01	1.00	0.83
		0.05	1.00	0.78
		0.1	1.00	0.71
	Blocker-NoTag	0	0.73	0.80
		0.001	1.00	0.60
		0.005	1.00	0.50
		0.01	1.00	0.50
		0.05	1.00	0.50
		0.1	1.00	0.50
	NoBlocker-NoTag	0	0.67	1.00
		0.001	1.00	1.00
		0.005	1.00	1.00
		0.01	1.00	1.00
		0.05	1.00	0.75
		0.1	1.00	0.70
Prey-Fox	**Blocker-Tag**	0	1.00	0.45
		0.001	1.00	0.45
		0.005	1.00	0.45
		0.01	1.00	0.45
		0.05	1.00	0.45
		0.1	1.00	0.45
	NoBlocker-Tag	0	1.00	0.69
		0.001	1.00	0.69
		0.005	1.00	0.65
		0.01	1.00	0.65
		0.05	1.00	0.61
		0.1	1.00	0.50
	Blocker-NoTag	0	0.50	0.85
		0.001	1.00	0.75
		0.005	1.00	0.50
		0.01	1.00	0.50
		0.05	1.00	0.50
		0.1	1.00	0.50
	NoBlocker-NoTag	0	0.60	1.00
		0.001	0.80	1.00
		0.005	1.00	1.00
		0.01	1.00	1.00
		0.05	1.00	0.75
		0.1	1.00	0.50

3. Blocker Efficacy

The number of total reads from red fox fecal samples treated with and without blocker oligonucleotides were compared (Fig. 3). The NoBlocker-NoTag treatment had a higher number of total reads at both thresholds and the differences were significant ($P < 0.0001$) based on paired t-test. At the 0.001 detection threshold, the average number of reads for NoTag-Blocker treatment was 27,602 (SD = 13,222) for 100 samples (20 sample per plate), and the average number of reads for NoBlocker-NoTag treatment was 35,656 (SD = 11,350) for 100 samples. At the 0.01 detection threshold, the average number of reads for NoTag-Blocker treatment was 13,480 (SD = 6,472) for 100 samples, and the average number of reads for NoBlocker-NoTag treatment was 17,459 (SD = 5,573) for 100 samples.

Next, we compared the proportions of total reads attributed to red fox from fecal samples treated with and without *Vulpes vulpes* blocker nucleotides (Fig.4a,b). The blocker significantly reduced the proportion of fox reads at both detection thresholds based on a paired t-test at both thresholds ($P < 0.0001$). The average proportion of total reads that were of red fox in 100 samples each treated with versus without blocker was 0.35 and 0.98, respectively, at the 0.001 detection threshold, and similarly 0.35 and 0.99, respectively, at the 0.01 detection threshold.

The average number of prey reads in the blocker group was significantly higher than that of the NoBlocker group among 5 libraries based on a paired t-test ($P < 0.0001$) at both 0.001 and 0.01 detection thresholds (Fig. 4c). The average number of prey reads in the 100 samples each treated with versus without blocker were 8,840 (SD = 2,227) versus 299 (SD = 89), respectively, at the 0.001 detection threshold, and 8,776 (SD = 2,232) versus 240 (SD = 81), respectively, at 0.01 threshold (Fig. 4c).

Next, we compared the number of species identified from red fox fecal samples treated with and without blockers (Fig. 5). The blocker significantly increased the number of species identified in the fecal samples under both detection thresholds based on a paired t-tests ($P < 0.0001$). The average number of species identified from each of the 100 red fox fecal samples treated with versus without blocker was 3.69 (SD = 0.446) and 2.46 (SD = 0.108), respectively, at the 0.001 detection threshold, and 2.26 (SD = 0.380) and 1.44 (SD = 0.129), respectively, at the 0.01 detection threshold.

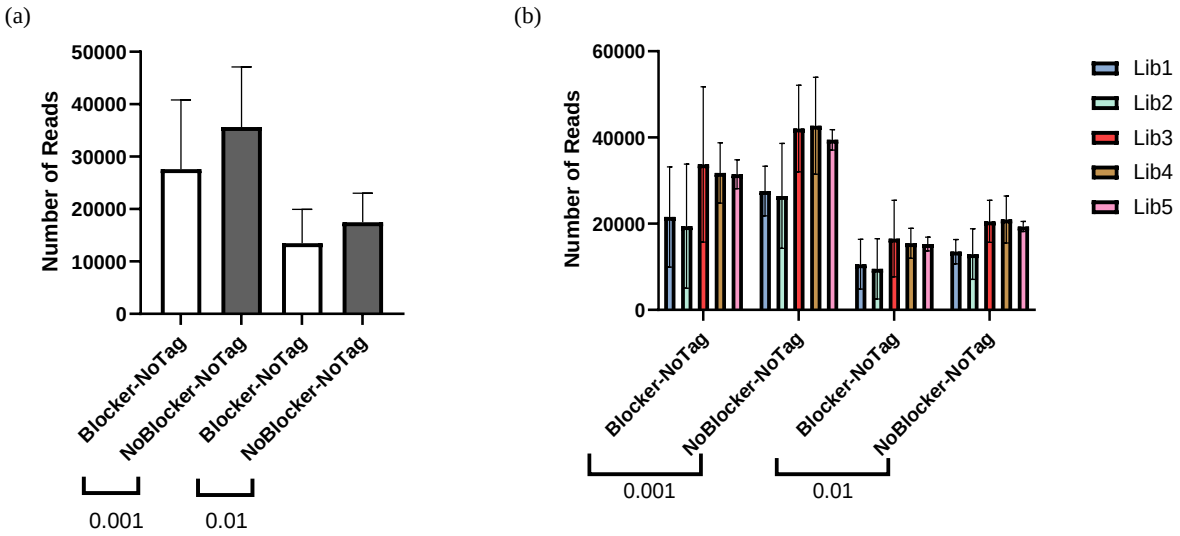


Fig. 3 Bar-plot comparisons of average (+SD) numbers reads of red fox fecal samples with and without *Vulpes vulpes* blocker oligonucleotides at two detection thresholds (0.001, 0.01) for (a) all 5 library plates combined and (b) each library plate shown separately. (a) Paired t-tests indicated that samples treated without blocker had significantly more reads than the samples treated with blocker under both detection thresholds ($P < 0.0001$). Five libraries were constructed with different procedures. Library 3, 4, and 5 had more reads than library 1 and library 2.

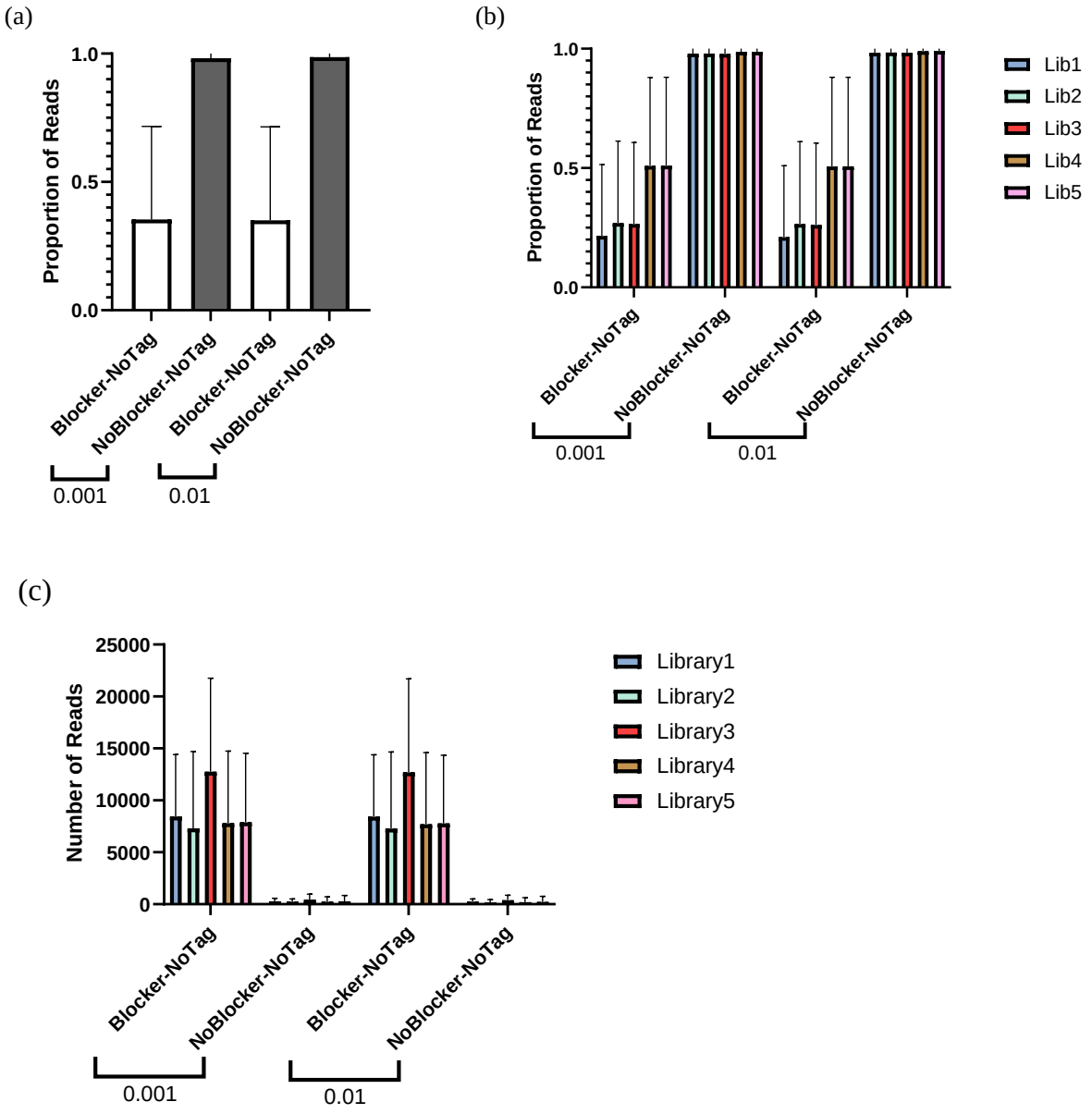


Fig. 4 Bar-plot comparison of sequencing read composition, including proportions of total reads that were red fox in all five libraries combined (a), in each of the five libraries (b), and the total number of prey reads in each of the five libraries (c) in samples treated with and without Vulpes vulpes blocker under 0.001 and 0.01 detection thresholds in 20 red fox scats. Each group included the same red fox fecal samples. (a, b) The blocker reduced the red fox read proportion under both detection thresholds, and the difference was significant ($P < 0.0001$) based on a paired t-test. (c) Samples treated with blockers had significantly more prey reads compared to those treated without blockers for both detection thresholds ($P < 0.0001$).

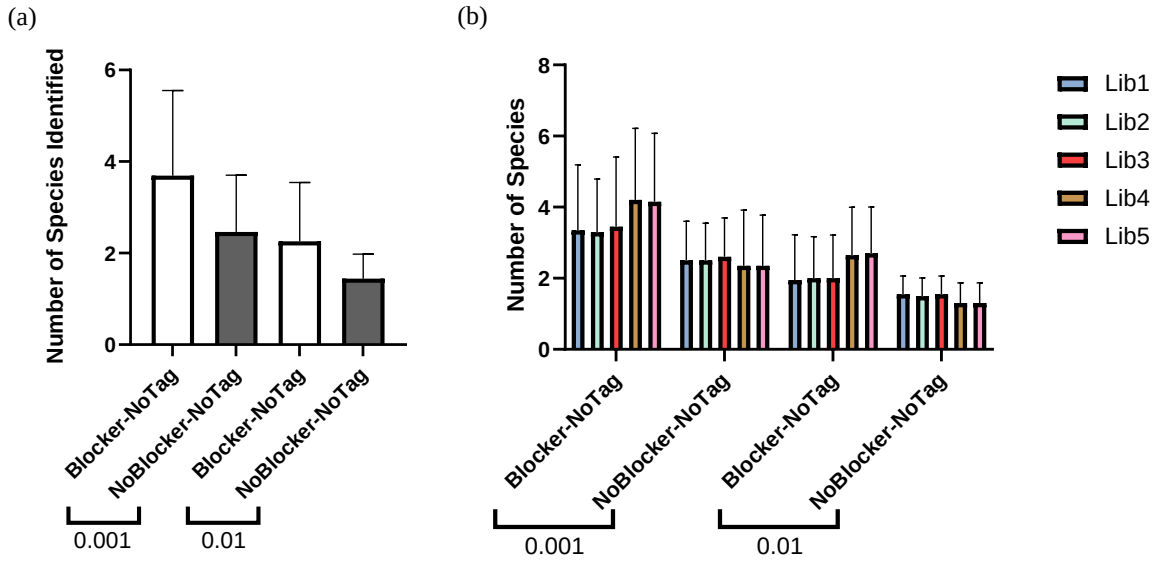


Fig. 5 Bar-plot comparison of the number of species identified from the red fox fecal samples with and without blocker under two detections thresholds (0.001, 0.01), shown for (a) all five libraries combined and (b) for each library shown separately in 20 red fox scats. Each group had the same red fox fecal samples. The blocker increased the number of species identified under both detection thresholds, and the difference was significant ($P < 0.0001$) based on a paired t-test. Numbers of species were higher with a 0.001 detection threshold than with a 0.01 detection threshold.

4. Performance of Four Library-Build Protocols

First, we compared the average total read number of our four library-build protocols (Table 2) under two thresholds (Fig. 6a, b). Under both detection thresholds, the NoBlocker-NoTag group had the highest average total read number and slightly higher than the results of the Blocker-NoTag group. In contrast, the Blocker-Tag group had the lowest average total-read number, which was slightly lower than the results of the NoBlocker-Tag group. At 0.001 detection threshold, the average total read numbers of four groups were significantly different based on the one-way ANOVA test ($F(3, 392)=58.19$, $P < 0.0001$). The total read number for the Blocker-NoTag and the NoBlocker-NoTag group were reported in the blocker efficacy section above. For Blocker-Tag group, the average total read number was 4,376 (SD = 8,380); for the NoBlocker-Tag group, the

average total read number was 5,332 (SD = 9,855). At 0.01 detection thresholds, the one-way ANOVA results indicated that the average total read numbers of four library-build protocols were significantly different at 0.01 detection threshold ($F(3,392)=58.28$, $P<0.0001$). For Blocker-Tag group, the average total read number was 4,376 (SD=8,380); for the NoBlocker-Tag group, the average total read number was 5,286 (SD=9,788).

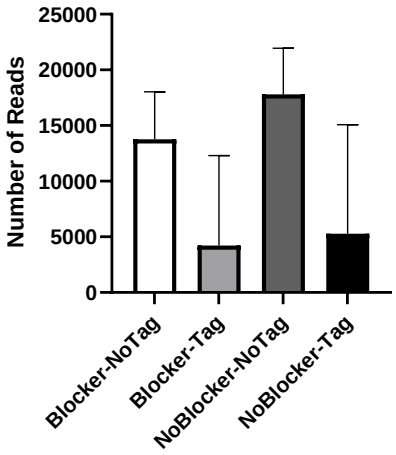
Next, we compared the average prey-read number of our four library-build protocols under two detection thresholds (Fig. 6c, d). Under both detection thresholds, the Blocker-NoTag group had the highest average prey read number among four treatments. At 0.001 detection threshold, the average prey-read numbers of four groups were significantly different based on the one-way ANOVA test ($F(3, 392) = 67.03$, $P < 0.0001$). The prey-read numbers for Blocker-NoTag and NoBlocker-NoTag group were presented in the blocker efficacy section above. For the Blocker-Tag group, the average prey read number was 2,241 (SD=5,859); for the NoBlocker-Tag group, the average prey read number was 61.35 (SD=308.8). At 0.01 detection threshold, the one-way ANOVA results indicated that the average prey read numbers of four library-build protocols were significantly different ($F(3,392)=67.93$, $P<0.0001$). For the Blocker-Tag group, the average prey read number was 2,189 (SD=5,753); for the NoBlocker-Tag group, the average prey read number was 50.85 (SD=287.1).

Last, we compared the average number of prey species identified from our four library-build protocols under two thresholds (Fig. 6e, f). Under both detection thresholds, the Blocker-NoTag group had the highest average number of prey species identified. At 0.001 detection threshold, the average number of prey species identified of four groups were significantly different based on the one-way ANOVA test ($F(3, 392) = 40.15$, $P < 0.0001$). The average number of species identified and the standard deviation for the Blocker-NoTag and the NoBlocker-NoTag group were reported

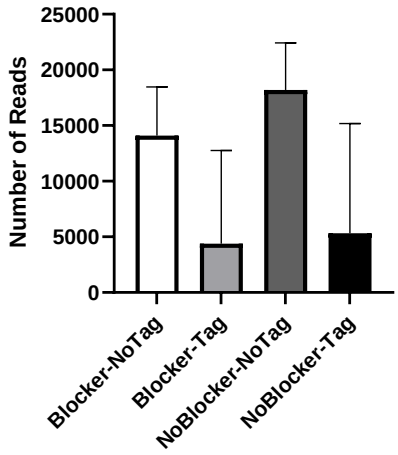
in the blocker efficacy section above. For the Blocker-Tag group, the average number of prey species identified was 1.253 (SD = 1.51); for the NoBlocker-Tag group, the average number of prey species identified was 0.299 (SD = 0.802). At 0.01 detection thresholds, the one-way ANOVA results indicated that the average number of prey species identified of four library-build protocols were significantly different at 0.01 detection threshold ($F(3,392)=36.16$, $P<0.0001$). For the Blocker-Tag group, the average number of prey species identified was 0.943 (SD = 9.962); for the NoBlocker-Tag group, the average number of prey species identified was 0.203 (SD = 0.609).

According to the results above, the Blocker-NoTag group performed the best to generate more prey reads and identifying more prey species under both thresholds. Two tagged groups performed poorly and noticeably inconsistently with different tags (Appendix 1, Appendix 2). The samples containing tag 1, 2, and 3 did not generate many prey reads (~2000 reads with blocker; <100 reads without blocker).

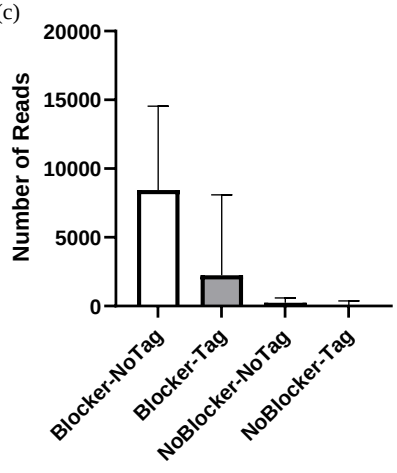
(a)


Number of Reads
25000
20000
15000
10000
5000
0
Blocker-NoTag
Blocker-Tag
NoBlocker-NoTag
NoBlocker-Tag

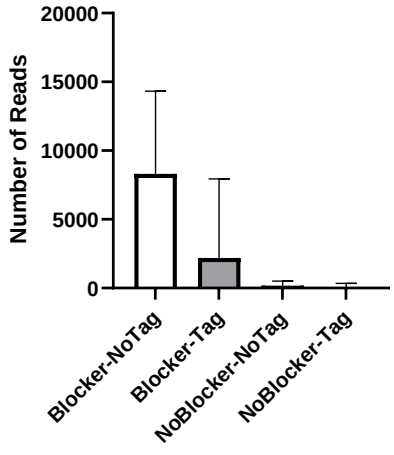
(b)


Number of Reads
25000
20000
15000
10000
5000
0
Blocker-NoTag
Blocker-Tag
NoBlocker-NoTag
NoBlocker-Tag

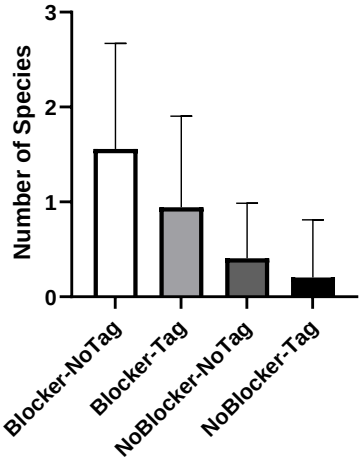
(c)


Number of Reads
20000
15000
10000
5000
0
Blocker-NoTag
Blocker-Tag
NoBlocker-NoTag
NoBlocker-Tag

(d)


Number of Reads
20000
15000
10000
5000
0
Blocker-NoTag
Blocker-Tag
NoBlocker-NoTag
NoBlocker-Tag

(e)


Number of Species
3
2
1
0
Blocker-NoTag
Blocker-Tag
NoBlocker-NoTag
NoBlocker-Tag

(f)

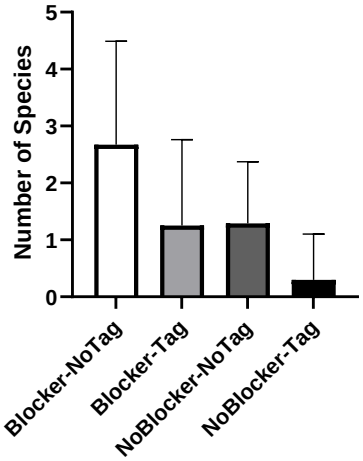

Number of Species
5
4
3
2
1
0
Blocker-NoTag
Blocker-Tag
NoBlocker-NoTag
NoBlocker-Tag

Fig. 6 Bar-plot comparison of four library-build protocols with respect to average total read number (a, b), prey read number (c, d), and numbers of prey species identified (e, f) under 0.001 (a, c, e) and 0.01 (b, d, f) detection thresholds in 20 red fox scats. The libraries built without tags had significantly higher total numbers of reads (a, b), prey reads (c, d), and prey species (e, f) than that of tagged libraries. The libraries using the blocker had slightly lower numbers of total reads (a, b) but higher numbers of prey reads (c, d) and prey species identified (e, f) than libraries built without blocker. The library built with the combination of blocker and non-tagged primer had the highest average prey species number.

5. Comparison of Library Plates, PCR Cleanup, and Pooling Procedures.

Library plates each contained the same series of blocker and tag combinations but differed according to their PCR cleanup and pooling procedures. Each plate contained usable data from 80 samples, except for library plates 1 (S15-0836-T3A, S15-0845-T3A, and S15-0846NB-T1A failed completely) and 2 (S15-0846-T1B failed completely). To assess whether results were sensitive to PCR cleanup and pooling procedures, we compared several performance measures among the 5 library plates. First, we evaluated the average number of prey reads under the two thresholds (Fig. 7a, b) and found no significant differences among the five library plates under either threshold based on one-way ANOVA tests ($F(4,391) = 0.979$, $P = 0.419$ under 0.001 threshold; $F(4,391) = 0.979$, $P = 0.419$ under 0.01 threshold). Next, we evaluated the average number of prey species identified from the five library plates under the two thresholds (Fig. 7c,d). Again, there were no significant differences under either threshold based on one-way ANOVA test ($F(4,391) = 0.0426$, $P = 0.9966$ under 0.001 threshold; $F(4,391) = 0.410$, $P = 0.801$ under 0.01 threshold). Finally, we compared the relative read abundances of prey species identified from each library plate at the 0.01 threshold (Table 5, Fig. 8). Using four paired t-tests ($P>0.99$) we found no significant differences between plates 1 and 2 (spin column vs AMPure beads for index PCR cleanup), plates 1 vs 3 or 4 vs 5 (normalization vs non- normalization), and between plates 2 vs 4 (AMPure Beads vs ExoSAP-IT for amplicon PCR cleanup).

Table 5. Relative Read Abundance (RRA) of prey species identified in each library at the 0.01 threshold. We compared library 1 with library 3 and library 4 with library 5 to determine the effects of pooling strategies on RRA. The differences were not significant according to a pairwise t-test.

Prey taxon	Library 1 (%)	Library 2 (%)	Library 3 (%)	Library 4 (%)	Library 5 (%)
Thomomys bottae	47.49	52.27	45.37	48.74	49.89
Martes americana	20.09	7.67	19.53	29.47	27.13
Terricola subterraneus	8.33	8.53	10.4	0.56	0.35
Sayornis phoebe	3.82	15.88	4.04	1.14	1.02
Gallus gallus	7.62	4.06	9.03	0	0
Microtus savii	4.39	6.14	3.41	2.25	3.01
Sturnus vulgaris	4.74	2.88	4.11	0	0
Zenaida macroura	0	0	0	5.23	4.82
Lepus californicus	0	0	0	4.56	5.44
Corvus corax	1.96	1.81	2.73	0.51	0.53
Mus musculus	0	0	0	2.13	2.06
Morone saxatilis	0	0	0	1.92	2.16
Martes americana caurina	0.52	0.25	0.45	0.45	0.49
Callospermophilus lateralis	0.23	0.03	0.25	1.81	1.84
Campylorhynchus brunneicapillus	0.26	0.09	0.27	0.27	0.21
Sciurus griseus	0	0	0	0.42	0.52
Peromyscus	0.14	0.05	0.13	0.3	0.26
Callipepla californica	0.22	0.18	0.19	0	0
Sylvilagus floridanu	0	0	0	0.18	0.2
Pipilo maculatus	0.12	0.15	0.09	0	0
Sylvilagus floridanus	0	0	0	0.07	0.08
Rattus rattus	0.05	0	0	0	0

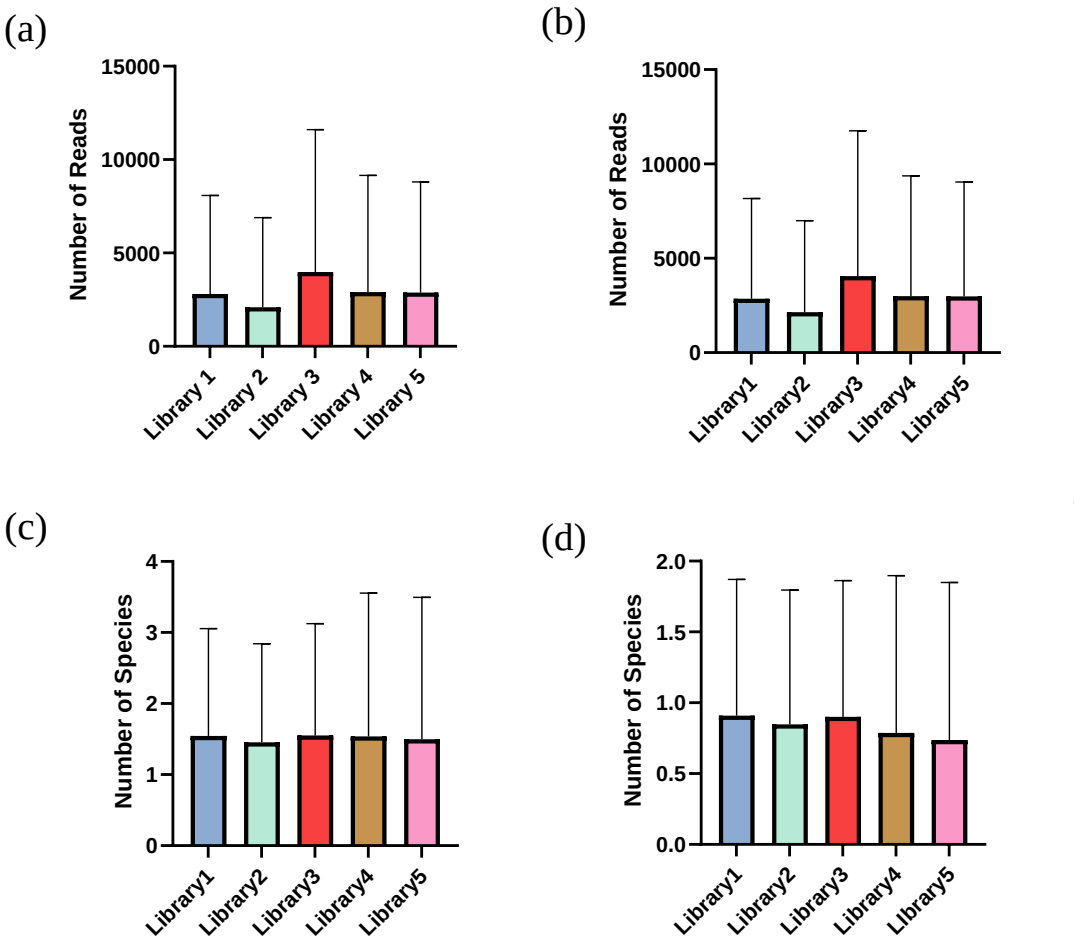


Fig. 7 Bar-plot comparisons of five libraries, each with a distinct amplicon cleanup procedure, showing numbers of prey reads (+SD) (a, b) and prey species identified (c, d) at 0.001 (a, c) and 0.01 (b, d) detection thresholds in 20 red fox scats. Five libraries differed by the amplicon cleanup procedures and pooling strategies (shown in Table 3). (a, b) Differences among libraries were not significant based on the ANOVA test (F = 0.979, 0.979; P = 0.419, 0.419) for 0.001(a) and 0.01(b) detection thresholds, respectively. (c, d) Differences among libraries were not significant based on the ANOVA tests (F = 0.0426, 0.410; P = 0.997, 0.801) for 0.001 (c) and 0.01 (d) detection thresholds, respectively.

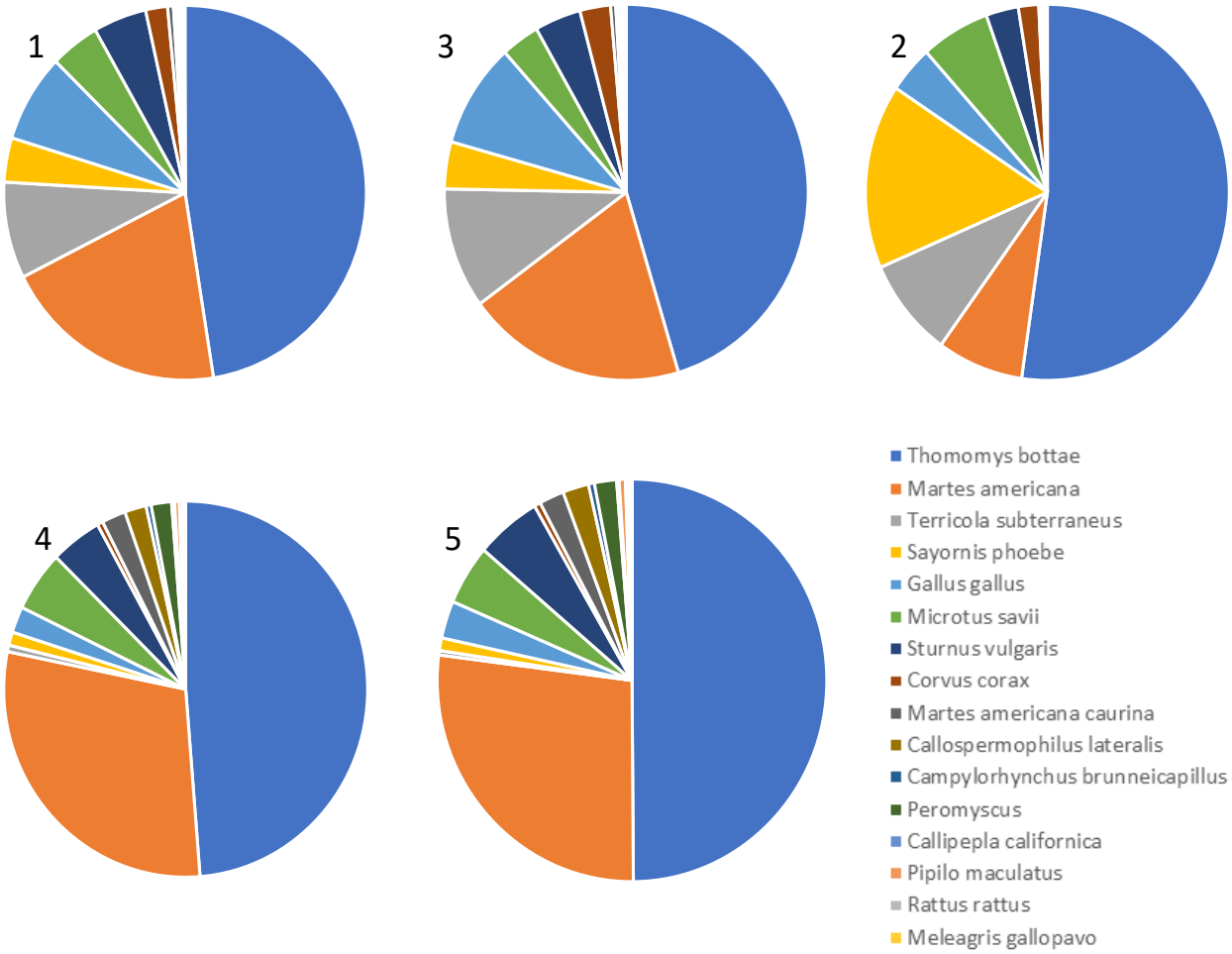


Fig. 8 Relative Read Abundance of Prey Species from Five Library Plates with a Threshold of 0.01. Five library preparation procedures are shown in Table 3. To evaluate the effect of normalization before pooling indexed amplicons, we compared library 1 with library 3 and library 4 with library 5, so the only variable was limited to pooling strategy. To evaluate the effect of using a magnetic bead versus spin column protocol for index PCR cleanup, we compared plates 1 and 2. To evaluate the effect of using a magnetic bead versus ExoSAP-IT protocols for amplicon PCR cleanup, we compared plates 3 and 5. There was no significant difference observed in relative read abundances across the five plates ($P > 0.99$), indicating that the effects of normalization before pooling indexed amplicons and of using magnetic bead versus spin-column or ExoSAP-IT for PCR clean-up were not significant.

Discussion

We evaluated the capability of several workflows in identifying the food items from synthetic mock communities and actual fecal samples. In terms of the performance of the tagged versus untagged primer sets, those without inline tags produced more reads than the primer set with inline tags. The differences suggested that the primer sets without inline tags had a higher amplification efficiency. For instance, the average prey read number for the NoBlocker-Tag group was less than 100, which is not enough to generate reliable dietary data for further analysis. This result was not surprising because the two primer sets we used in this study were different in length (with 8 bp inline tags). Thus, in agreement with previous studies, our findings suggest the longer fusion primer sets can substantially reduce the PCR amplification efficiency (Schnell et al.,2015; Elbrecht et al., 2017).

However, results also suggested that the efficiency of inline tags could be partly dependent on the tag sequence, which also is not ideal for studies aimed at sampling all scats equitably. Among the five tags we used, primers containing tag 1, tag 2, and tag 3 failed almost completely to generate reads, while primers containing tag 4 and tag 5 performed much better. The possible explanation could involve our two-staged library-build protocol. According to the preliminary gel image from the amplicon PCR, the difference between the two primer sets was visually insignificant. This observation suggests that amplification failure could have happened during the index PCR stage, although the reasons for this failure are unclear. Failure at the indexing PCR step might also explain a large number (~9%) of undetermined reads in our data. Usually, the rate of tag-jump or mislabeling during sequencing is only 1% to 3% (Zizka et al., 2019; Schnell et al., 2015; Elbrecht et al., 2017), so unlikely to explain this result. Another potential drawback of using tagged primers is that the fusion primer can lead to PCR bias towards particular DNA templates (certain species)

even with tags less than 10bp, so it can substantially change the community composition and each prey's relative read abundance (Zizka et al., 2019; Elbrecht et al., 2015, 2017a, 2017b).

A second question we investigated was the efficacy of blockers. Theoretically, we expect the blocker oligonucleotide to block most of the host DNA so we can have more data on prey DNA. In our study, the application of blockers decreased the overall number of reads. Nevertheless, blockers increased the composition prey reads from around 2% of the total reads to about 70% of the total reads of each sample, resulting in the average number of species identified being about two times that of the group that did not use the blocker. Such a vast improvement in prey reads was also reported in previous studies (Shezad et al., 2012). Therefore, we have more prey reads for the subsequent analysis, which is cost-effective.

However, these results were at odds with those of our control sample (4-prey-cocktail and prey-fox) data, which indicated that the NoBlocker-NoTag treatment group performed the best in terms of sensitivity and specificity. In contrast to the control samples, the results of our fecal samples indicated that the Blocker-NoTag group performed the best in terms of prey read count and number of species identified. A possible explanation could be that the samples amplified without blockers did not generate enough prey reads. The technique we used to extract fecal DNA before this study was intended to enrich the host by selectively collecting and homogenizing the fox scats' outer layer. This technique was optimized for STR genotyping to confirm the predator species and the sex. Another potential issue of the blocker is that the blocker also inhibited some prey species while blocking the host DNA in our control samples. Mule deer (*Odocoileus hemionus*) and Deer mouse (*Peromyscus maniculatus*) yielded lower read numbers than the other two species in our control samples. The inhibition of certain prey species by blockers was also reported in previous studies (Pinol et al.2014, Robeson et al. 2017, Shi et al., 2021). A possible explanation could be

suboptimal design of the blocker. For example, if the blocker was not long enough to bind with high specificity to the predator DNA, it will also bind with some, but not all, prey mtDNA, which the sequence is most similar to the host mtDNA sequence. Overall, our study suggests that the blocker can be a powerful tool. However, before using the blocker in the study of specific prey species, we recommend testing the blocker first and adjusting the design if it also appears to block the target prey DNA. Without prior knowledge, it is impossible to determine the co-blocking from a fecal sample. It is also essential to decide on the concentration of the blocker oligonucleotides empirically. The range of the blocker concentration is 5 to 20 times higher than the universal primer (De Barba et al., 2014, Shehzad et al., 2012, Shi et al., 2021). In our study, we used the maximum ratio in this range (i.e., 20x). Our findings of suppression of some prey in the controls suggests that the blockers concentration should be kept at a minimum.

The AMPure XP magnetic beads cleanup was the most labor-intensive protocol for post-PCR cleanup. Therefore, we also assessed the less time-consuming protocols for better efficiency, in this case, ExoSAP-IT for amplicon PCR cleanup and QIAquick Spin columns for post-index PCR cleanup. According to our results, the influence of different cleanup protocols in terms of average read numbers and average prey species identified was insignificant. However, based on the bioanalyzer results before sequencing, our five library plates still contained a small amount of primer dimers after the cleanup, which might require an additional cleanup procedure. When pooling the samples, according to our results, the effects of quantitation and normalization of each sample before pooling were not clear. We did not observe significant differences when comparing the relative read abundance of each prey item between library plates (e.g., Normalization vs Non-normalization, library 1 vs library 3; Normalization vs Non-normalization, library 4 vs library 5). In general, we still recommend pooling after the normalization of each sample. It can prevent the

more concentrated samples from disproportionally dominating the flow cell lane during sequencing, which can lead to underestimating the diversity.

Overall, our carnivore metabarcoding workflow without inline tag and with blocker proved to be a low-labor and cost-effective approach that can be applied to carnivore dietary studies. As a non-invasive approach, metabarcoding is a powerful tool for dietary analysis. It provides qualitative data such as the list of food items and their occurrence, and the resolution can be taxa level. Moreover, the quantitative data generated by metabarcoding such as the relative proportion of food items in dietary allows more precise estimation and modeling of the complex interaction between different populations and species, which is the goal for dietary analysis. Application of the technology requires awareness of potential problems, such as primer bias, low abundance of rare food items, taxa that are absent from the reference database, tag switch, overestimates of the diversity due to contamination (although, applying more stringent filtering thresholds can avoid inflated diversity but it will sacrifice the detection sensitivity), and large specimens may dominate the dataset.

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Appendix 1: Raw Data of Control Samples

Sample	Blocker	Tag	Library plate	red fox	albatr oss	gopher	deer	mouse	non-target
4-preycocktail-A	Y	N	1	0	5330	3527	0	7	0
4-prey-cocktail-B	Y	N	2	0	2255	1673	0	0	0
4-prey-cocktail-C	Y	N	3	0	9275	6306	0	10	0
4-prey-cocktail-D	Y	N	4	0	8982	8033	6	37	12
4-prey-cocktail-U	Y	N	5	8	10300	9111	0	33	8
4-preycocktailNB-A	N	N	1	0	7606	5631	1647	563	5
4-prey-cocktailNB-B	N	N	2	0	6069	4259	1190	415	4
4-prey-cocktailNB-C	N	N	3	0	11770	7598	2276	720	14
4-prey-cocktailNB-D	N	N	4	0	7805	5439	1892	605	8
4-prey-cocktailNB-U	N	N	5	0	9708	7212	2495	793	13
4-preycocktailNB-T5A	N	5	1	0	15645	12305	4017	1893	13
4-prey-cocktailNB-T5B	N	5	2	0	1488	1170	387	192	0
4-prey-cocktailNB-T5C	N	5	3	0	16186	12275	4106	1919	13
4-prey-cocktailNB-T5D	N	5	4	0	12211	2673	1877	862	6
4-prey-cocktailNB-T5U	N	5	5	0	15102	3356	2386	983	9
4-preycocktail-T5A	Y	5	1	0	7491	7025	8	96	3
4-prey-cocktail-T5B	Y	5	2	0	4037	3891	0	33	0
4-prey-cocktail-T5C	Y	5	3	0	11091	9827	14	122	14
4-prey-cocktail-T5D	Y	5	4	0	15468	13840	16	120	24
4-prey-cocktail-T5U	Y	5	5	0	12635	11035	13	81	16
preyxfox-A	Y	N	1	19	7773	6568	0	22	3
preyxfoxNB-A	N	N	1	5753	4557	2940	939	263	0
preyxfoxNB-T2A	N	2	1	34	7	6	0	4	0
preyxfox-T2A	Y	2	1	0	49	26	0	0	0
preyxfox-B	Y	N	2	11	9031	7803	0	35	4
preyxfoxNB-B	N	N	2	4102	2980	2050	615	183	3
preyxfoxNB-T2B	N	2	2	82	41	18	10	0	0
preyxfox-T2B	Y	2	2	0	12	12	0	0	0
preyxfox-C	Y	N	3	51	12982	10496	0	67	4
preyxfoxNB-C	N	N	3	8050	6181	4123	1254	392	6
preyxfoxNB-T2C	N	2	3	43	17	12	0	0	0
preyxfox-T2C	Y	2	3	0	46	35	0	0	0
preyxfox-D	Y	N	4	32	13376	11540	10	71	17
preyxfoxNB-D	N	N	4	5530	5537	4058	1149	499	27
preyxfoxNB-T2D	N	2	4	8	15	0	0	0	0
preyxfox-T2D	Y	2	4	3	3	0	0	0	0
preyxfox-U	Y	N	5	31	12020	10388	11	42	20
preyxfoxNB-U	N	N	5	6334	6241	4686	1349	618	29
preyxfoxNB-T2U	N	2	5	12	12	2	0	0	0
preyxfox-T2U	Y	2	5	0	8	13	0	0	0
foxtissue-A	Y	N	1	9295	0	0	0	0	0

foxtissueNB-A	N	N	1	16425	0	0	0	0	0
foxtissueNB-T1A	N	1	1	30	0	0	0	0	0
foxtissue-T1A	Y	1	1	71	0	0	0	0	0
foxtissue-B	Y	N	2	15558	0	0	0	0	0
foxtissueNB-B	N	N	2	25596	0	0	0	0	0
foxtissueNB-T1B	N	1	2	70	0	0	0	0	0
foxtissue-T1B	Y	1	2	51	0	0	0	0	0
foxtissue-C	Y	N	3	15505	0	4	0	0	0
foxtissueNB-C	N	N	3	22428	0	0	0	0	0
foxtissueNB-T1C	N	1	3	110	0	0	0	0	0
foxtissue-T1C	Y	1	3	94	0	0	0	0	0
foxtissue-D	Y	N	4	14354	0	0	0	0	0
foxtissueNB-D	N	N	4	18274	0	0	0	0	0
foxtissueNB-T1D	N	1	4	31	0	0	0	0	0
foxtissue-T1D	Y	1	4	14	0	0	0	0	0
foxtissue-U	Y	N	5	16007	0	0	0	0	0
foxtissueNB-U	N	N	5	20335	0	0	0	0	0
foxtissueNB-T1U	N	1	5	25	0	0	0	0	0
foxtissue-T1U	Y	1	5	26	0	0	0	0	0

Appendix 2: Data of Each Library under Two Detection Threshold (0.01 and 0.001)

			0.01			
Sample Name	Blocker	Tag	NumberPrey Species	Number Read	NumberPrey Read	*Vulpes vulpes*
S15-0827-A	Y	N	1	16547	16012	535
S15-0827-B	Y	N	1	6631	6379	252
S15-0827-C	Y	N	1	29292	28282	1010
S15-0827NB-A	N	N	1	10041	116	9925
S15-0827NB-B	N	N	1	10727	108	10619
S15-0827NB-C	N	N	1	19410	261	19149
S15-0827NB-T1A	N	1	0	66	0	66
S15-0827NB-T1B	N	1	0	115	0	115
S15-0827NB-T1C	N	1	0	59	0	59
S15-0827-T1A	Y	1	1	179	175	4
S15-0827-T1B	Y	1	1	154	146	8
S15-0827-T1C	Y	1	1	74	68	6
S15-0829-A	Y	N	2	12952	12952	0
S15-0829-B	Y	N	2	6830	6830	0
S15-0829-C	Y	N	2	21650	21650	0
S15-0829NB-A	N	N	2	10570	568	10002
S15-0829NB-B	N	N	2	10560	634	9926
S15-0829NB-C	N	N	2	20379	1191	19188
S15-0829NB-T2A	N	2	0	59	0	59
S15-0829NB-T2B	N	2	0	63	0	63
S15-0829NB-T2C	N	2	1	101	3	98
S15-0829-T2A	Y	2	2	113	103	10
S15-0829-T2B	Y	2	2	67	67	0
S15-0829-T2C	Y	2	2	78	73	5
S15-0830-A	Y	N	1	12478	12478	0
S15-0830-B	Y	N	1	6020	6020	0
S15-0830-C	Y	N	1	24560	24560	0
S15-0830NB-A	N	N	1	10846	863	9983
S15-0830NB-B	N	N	1	9017	721	8296
S15-0830NB-C	N	N	1	19013	1528	17485
S15-0830NB-T3A	N	3	0	1	0	1
S15-0830NB-T3B	N	3	0	1	0	1
S15-0830NB-T3C	N	3	0	5	0	5
S15-0830-T3A	Y	3	0	0	0	0
S15-0830-T3B	Y	3	0	0	0	0

S15-0830-T3C	Y	3	0	0	0	0
S15-0831-A	Y	N	2	22170	22170	0
S15-0831-B	Y	N	2	5718	5718	0
S15-0831-C	Y	N	2	24668	24668	0
S15-0831NB-A	N	N	1	11097	409	10688
S15-0831NB-B	N	N	1	9776	353	9423
S15-0831NB-C	N	N	1	15989	622	15367
S15-0831NB-T4A	N	4	0	17773	0	17773
S15-0831NB-T4B	N	4	0	22669	0	22669
S15-0831NB-T4C	N	4	1	21883	236	21647
S15-0831-T4A	Y	4	1	13698	13548	150
S15-0831-T4B	Y	4	1	5993	5923	70
S15-0831-T4C	Y	4	1	12703	12565	138
S15-0832-A	Y	N	1	4516	1197	3319
S15-0832-B	Y	N	1	10430	2751	7679
S15-0832-C	Y	N	1	9528	2504	7024
S15-0832NB-A	N	N	0	11158	0	11158
S15-0832NB-B	N	N	0	16075	0	16075
S15-0832NB-C	N	N	0	22255	0	22255
S15-0832NB-T5A	N	5	0	12223	0	12223
S15-0832NB-T5B	N	5	0	11928	0	11928
S15-0832NB-T5C	N	5	0	14563	0	14563
S15-0832-T5A	Y	5	2	13112	2934	10178
S15-0832-T5B	Y	5	2	7473	1598	5875
S15-0832-T5C	Y	5	2	13626	3031	10595
S15-0833-A	Y	N	0	9390	0	9390
S15-0833-B	Y	N	0	13290	0	13290
S15-0833-C	Y	N	0	20493	0	20493
S15-0833NB-A	N	N	0	11092	0	11092
S15-0833NB-B	N	N	0	8317	0	8317
S15-0833NB-C	N	N	0	19698	0	19698
S15-0833NB-T1A	N	1	0	7	0	7
S15-0833NB-T1B	N	1	0	48	0	48
S15-0833NB-T1C	N	1	0	97	0	97
S15-0833-T1A	Y	1	1	112	11	101
S15-0833-T1B	Y	1	1	77	7	70
S15-0833-T1C	Y	1	1	89	9	80
S15-0835-A	Y	N	1	18404	10196	8208
S15-0835-B	Y	N	1	9045	4726	4319
S15-0835-C	Y	N	1	23595	13038	10557
S15-0835NB-A	N	N	0	17072	0	17072

S15-0835NB-B	N	N	0	22986	0	22986
S15-0835NB-C	N	N	0	28010	0	28010
S15-0835NB-T2A	N	2	0	21	0	21
S15-0835NB-T2B	N	2	0	161	0	161
S15-0835NB-T2C	N	2	0	102	0	102
S15-0835-T2A	Y	2	1	147	43	104
S15-0835-T2B	Y	2	1	107	35	72
S15-0835-T2C	Y	2	1	65	22	43
S15-0836-A	Y	N	0	0	0	0
S15-0836-B	Y	N	0	6	0	6
S15-0836-C	Y	N	0	10	0	10
S15-0836NB-A	N	N	1	10103	108	9995
S15-0836NB-B	N	N	0	7771	0	7771
S15-0836NB-C	N	N	0	14040	0	14040
S15-0836NB-T3A	N	3	1	1	1	0
S15-0836NB-T3B	N	3	0	0	0	0
S15-0836NB-T3C	N	3	0	8	0	8
S15-0836-T3B	Y	3	0	0	0	0
S15-0836-T3C	Y	3	1	2	2	0
S15-0838-A	Y	N	2	1095	1079	16
S15-0838-B	Y	N	2	682	665	17
S15-0838-C	Y	N	2	455	443	12
S15-0838NB-A	N	N	0	11315	0	11315
S15-0838NB-B	N	N	1	8866	97	8769
S15-0838NB-C	N	N	1	6458	99	6359
S15-0838NB-T1A	N	1	0	57	0	57
S15-0838NB-T1B	N	1	0	254	0	254
S15-0838NB-T1C	N	1	0	134	0	134
S15-0838-T1A	Y	1	3	69	69	0
S15-0838-T1B	Y	1	3	76	66	10
S15-0838-T1C	Y	1	3	92	79	13
S15-0839-A	Y	N	1	6007	6007	0
S15-0839-B	Y	N	1	25798	25798	0
S15-0839-C	Y	N	1	8022	8022	0
S15-0839NB-A	N	N	0	17253	0	17253
S15-0839NB-B	N	N	0	21474	0	21474
S15-0839NB-C	N	N	0	18621	0	18621
S15-0839NB-T2A	N	2	0	49	0	49
S15-0839NB-T2B	N	2	0	29	0	29
S15-0839NB-T2C	N	2	0	116	0	116
S15-0839-T2A	Y	2	1	63	57	6

S15-0839-T2B	Y	2	2	142	118	24
S15-0839-T2C	Y	2	1	99	90	9
S15-0840-A	Y	N	1	8054	8054	0
S15-0840-B	Y	N	1	26032	26032	0
S15-0840-C	Y	N	1	12522	12522	0
S15-0840NB-A	N	N	1	12001	191	11810
S15-0840NB-B	N	N	1	13333	190	13143
S15-0840NB-C	N	N	1	20799	289	20510
S15-0840NB-T3A	N	3	0	2	0	2
S15-0840NB-T3B	N	3	0	0	0	0
S15-0840NB-T3C	N	3	1	3	3	0
S15-0840-T3A	Y	3	0	3	0	3
S15-0840-T3C	Y	3	0	4	0	4
S15-0841-A	Y	N	1	11081	4229	6852
S15-0841-B	Y	N	1	8372	2991	5381
S15-0841-C	Y	N	1	22120	8665	13455
S15-0841NB-A	N	N	0	15475	0	15475
S15-0841NB-B	N	N	0	9158	0	9158
S15-0841NB-C	N	N	0	22360	0	22360
S15-0841NB-T4A	N	4	0	21363	0	21363
S15-0841NB-T4B	N	4	0	10460	0	10460
S15-0841NB-T4C	N	4	0	28490	0	28490
S15-0841-T4A	Y	4	0	12075	0	12075
S15-0841-T4B	Y	4	0	5515	0	5515
S15-0841-T4C	Y	4	0	12899	0	12899
S15-0842-A	Y	N	3	10012	6565	3447
S15-0842-B	Y	N	2	7649	4864	2785
S15-0842-C	Y	N	3	22724	14714	8010
S15-0842NB-A	N	N	0	12420	0	12420
S15-0842NB-B	N	N	0	9023	0	9023
S15-0842NB-C	N	N	0	17645	0	17645
S15-0842NB-T5A	N	5	0	26157	0	26157
S15-0842NB-T5B	N	5	0	4649	0	4649
S15-0842NB-T5C	N	5	0	38509	0	38509
S15-0842-T5A	Y	5	2	12879	4938	7941
S15-0842-T5B	Y	5	2	8047	3028	5019
S15-0842-T5C	Y	5	2	24805	9612	15193
S15-0843-A	Y	N	3	6488	4776	1712
S15-0843-B	Y	N	3	6838	5047	1791
S15-0843-C	Y	N	3	7786	5680	2106
S15-0843NB-A	N	N	1	13284	163	13121

S15-0843NB-B	N	N	0	8229	0	8229
S15-0843NB-C	N	N	1	20600	233	20367
S15-0843NB-T1A	N	1	0	25	0	25
S15-0843NB-T1B	N	1	0	22	0	22
S15-0843NB-T1C	N	1	0	59	0	59
S15-0843-T1A	Y	1	3	88	56	32
S15-0843-T1B	Y	1	3	85	47	38
S15-0843-T1C	Y	1	3	123	69	54
S15-0844-A	Y	N	2	16334	16334	0
S15-0844-B	Y	N	2	14423	14423	0
S15-0844-C	Y	N	2	26353	26353	0
S15-0844NB-A	N	N	1	14407	473	13934
S15-0844NB-B	N	N	1	5021	153	4868
S15-0844NB-C	N	N	1	22599	783	21816
S15-0844NB-T2A	N	2	0	33	0	33
S15-0844NB-T2B	N	2	0	27	0	27
S15-0844NB-T2C	N	2	0	89	0	89
S15-0844-T2A	Y	2	1	67	67	0
S15-0844-T2B	Y	2	1	94	91	3
S15-0844-T2C	Y	2	1	67	67	0
S15-0845-A	Y	N	2	17575	8835	8740
S15-0845-B	Y	N	2	15928	7984	7944
S15-0845-C	Y	N	2	26466	13061	13405
S15-0845NB-A	N	N	0	16044	0	16044
S15-0845NB-B	N	N	0	27205	0	27205
S15-0845NB-C	N	N	0	24443	0	24443
S15-0845NB-T3A	N	3	1	2	2	0
S15-0845NB-T3B	N	3	0	0	0	0
S15-0845NB-T3C	N	3	0	1	0	1
S15-0845-T3B	Y	3	0	0	0	0
S15-0845-T3C	Y	3	0	5	0	5
S15-0846-A	Y	N	1	7835	6967	868
S15-0846-B	Y	N	1	11319	10057	1262
S15-0846-C	Y	N	1	10388	9255	1133
S15-0846NB-A	N	N	0	14164	0	14164
S15-0846NB-B	N	N	0	13217	0	13217
S15-0846NB-C	N	N	0	21867	0	21867
S15-0846NB-T1B	N	1	0	2	0	2
S15-0846NB-T1C	N	1	0	4	0	4
S15-0846-T1A	Y	1	1	58	37	21
S15-0846-T1B	Y	1	1	134	85	49

S15-0846-T1C	Y	1	1	137	88	49
S15-0847-A	Y	N	4	8883	7639	1244
S15-0847-B	Y	N	4	9768	8398	1370
S15-0847-C	Y	N	4	9180	7932	1248
S15-0847NB-A	N	N	1	15148	221	14927
S15-0847NB-B	N	N	1	13359	186	13173
S15-0847NB-C	N	N	1	25143	438	24705
S15-0847NB-T2A	N	2	2	59	34	25
S15-0847NB-T2B	N	2	3	138	101	37
S15-0847NB-T2C	N	2	3	214	98	116
S15-0847-T2A	Y	2	1	36	24	12
S15-0847-T2B	Y	2	1	63	42	21
S15-0847-T2C	Y	2	1	91	63	28
S15-0848-D	Y	N	4	12550	7061	5489
S15-0848NB-D	N	N	1	15543	176	15367
S15-0848NB-T3D	N	3	0	2	0	2
S15-0848NB-T3U	N	3	0	0	0	0
S15-0848NB-U	N	N	1	18032	245	17787
S15-0848-T3D	Y	3	0	4	0	4
S15-0848-T3U	Y	3	0	9	0	9
S15-0848-U	Y	N	4	16962	9460	7502
S15-0849-D	Y	N	2	15045	2826	12219
S15-0849NB-D	N	N	0	14910	0	14910
S15-0849NB-T1D	N	1	0	3	0	3
S15-0849NB-T1U	N	1	0	0	0	0
S15-0849NB-U	N	N	0	18197	0	18197
S15-0849-T1D	Y	1	0	40	0	40
S15-0849-T1U	Y	1	0	40	0	40
S15-0849-U	Y	N	2	12390	2475	9915
S15-0850-D	Y	N	3	13800	3811	9989
S15-0850NB-D	N	N	0	22943	0	22943
S15-0850NB-T2D	N	2	0	186	0	186
S15-0850NB-T2U	N	2	0	181	0	181
S15-0850NB-U	N	N	0	19404	0	19404
S15-0850-T2D	Y	2	0	10	0	10
S15-0850-T2U	Y	2	0	26	0	26
S15-0850-U	Y	N	3	13602	3876	9726
S15-0854-D	Y	N	0	16310	0	16310
S15-0854NB-D	N	N	0	19949	0	19949
S15-0854NB-T1D	N	1	0	35	0	35
S15-0854NB-T1U	N	1	0	25	0	25

S15-0854NB-U	N	N	0	19533	0	19533
S15-0854-T1D	Y	1	0	12	0	12
S15-0854-T1U	Y	1	0	13	0	13
S15-0854-U	Y	N	0	16701	0	16701
S15-0855-D	Y	N	0	12035	0	12035
S15-0855NB-D	N	N	0	17417	0	17417
S15-0855NB-T2D	N	2	0	20	0	20
S15-0855NB-T2U	N	2	0	23	0	23
S15-0855NB-U	N	N	0	19109	0	19109
S15-0855-T2D	Y	2	1	31	5	26
S15-0855-T2U	Y	2	0	29	0	29
S15-0855-U	Y	N	0	13686	0	13686
S15-0856-D	Y	N	3	16211	9995	6216
S15-0856NB-D	N	N	0	19974	0	19974
S15-0856NB-T3D	N	3	0	0	0	0
S15-0856NB-T3U	N	3	0	0	0	0
S15-0856NB-U	N	N	0	19027	0	19027
S15-0856-T3D	Y	3	1	4	4	0
S15-0856-T3U	Y	3	0	2	0	2
S15-0856-U	Y	N	3	14910	9266	5644
S15-0857-D	Y	N	2	14830	13029	1801
S15-0857NB-D	N	N	0	28244	0	28244
S15-0857NB-T4D	N	4	0	38311	0	38311
S15-0857NB-T4U	N	4	0	21484	0	21484
S15-0857NB-U	N	N	0	21004	0	21004
S15-0857-T4D	Y	4	3	18943	16287	2656
S15-0857-T4U	Y	4	3	22079	18804	3275
S15-0857-U	Y	N	2	15677	13811	1866
S15-0858-D	Y	N	0	16038	0	16038
S15-0858NB-D	N	N	0	19042	0	19042
S15-0858NB-T5D	N	5	0	23947	0	23947
S15-0858NB-T5U	N	5	0	21854	0	21854
S15-0858NB-U	N	N	0	19000	0	19000
S15-0858-T5D	Y	5	3	24779	2256	22523
S15-0858-T5U	Y	5	3	21475	1969	19506
S15-0858-U	Y	N	0	14594	0	14594
S15-0859-D	Y	N	4	13126	8389	4737
S15-0859NB-D	N	N	0	19607	0	19607
S15-0859NB-T1D	N	1	0	9	0	9
S15-0859NB-T1U	N	1	0	21	0	21
S15-0859NB-U	N	N	0	18173	0	18173

S15-0859-T1D	Y	1	1	26	6	20
S15-0859-T1U	Y	1	0	28	0	28
S15-0859-U	Y	N	4	15897	10096	5801
S15-0869-D	Y	N	2	13033	7448	5585
S15-0869NB-D	N	N	0	24756	0	24756
S15-0869NB-T2D	N	2	0	18	0	18
S15-0869NB-T2U	N	2	0	25	0	25
S15-0869NB-U	N	N	0	19991	0	19991
S15-0869-T2D	Y	2	1	17	4	13
S15-0869-T2U	Y	2	0	24	0	24
S15-0869-U	Y	N	2	16612	9598	7014
S15-0870-D	Y	N	2	23968	6461	17507
S15-0870NB-D	N	N	0	19946	0	19946
S15-0870NB-T3D	N	3	1	4	4	0
S15-0870NB-T3U	N	3	0	16	0	16
S15-0870NB-U	N	N	0	16707	0	16707
S15-0870-T3D	Y	3	0	6	0	6
S15-0870-T3U	Y	3	1	18	3	15
S15-0870-U	Y	N	2	13269	3532	9737
S15-0871-D	Y	N	2	13880	4119	9761
S15-0871NB-D	N	N	0	30647	0	30647
S15-0871NB-T1D	N	1	0	52	0	52
S15-0871NB-T1U	N	1	0	29	0	29
S15-0871NB-U	N	N	0	18383	0	18383
S15-0871-T1D	Y	1	0	31	0	31
S15-0871-T1U	Y	1	0	27	0	27
S15-0871-U	Y	N	2	14352	4259	10093
S15-0880-D	Y	N	4	13542	10443	3099
S15-0880NB-D	N	N	0	36171	0	36171
S15-0880NB-T2D	N	2	0	18	0	18
S15-0880NB-T2U	N	2	0	24	0	24
S15-0880NB-U	N	N	0	20405	0	20405
S15-0880-T2D	Y	2	0	5	0	5
S15-0880-T2U	Y	2	1	29	9	20
S15-0880-U	Y	N	4	15160	11624	3536
S15-0881-D	Y	N	1	12325	9231	3094
S15-0881NB-D	N	N	1	24041	611	23430
S15-0881NB-T3D	N	3	0	2	0	2
S15-0881NB-T3U	N	3	0	0	0	0
S15-0881NB-U	N	N	1	18652	491	18161
S15-0881-T3D	Y	3	0	4	0	4

S15-0881-T3U	Y	3	1	7	7	0
S15-0881-U	Y	N	1	15069	11127	3942
S15-0882-D	Y	N	1	16608	490	16118
S15-0882NB-D	N	N	0	19134	0	19134
S15-0882NB-T4D	N	4	0	19798	0	19798
S15-0882NB-T4U	N	4	0	26659	0	26659
S15-0882NB-U	N	N	0	19872	0	19872
S15-0882-T4D	Y	4	2	16711	370	16341
S15-0882-T4U	Y	4	2	23998	616	23382
S15-0882-U	Y	N	1	15048	314	14734
S15-0884-D	Y	N	2	23865	22799	1066
S15-0884NB-D	N	N	0	16235	0	16235
S15-0884NB-T5D	N	5	0	17485	0	17485
S15-0884NB-T5U	N	5	0	23534	0	23534
S15-0884NB-U	N	N	0	18960	0	18960
S15-0884-T5D	Y	5	3	25585	21523	4062
S15-0884-T5U	Y	5	3	23767	19837	3930
S15-0884-U	Y	N	1	16268	15513	755
S15-0885-D	Y	N	2	13394	13394	0
S15-0885NB-D	N	N	1	18651	268	18383
S15-0885NB-T1D	N	1	0	25	0	25
S15-0885NB-T1U	N	1	0	37	0	37
S15-0885NB-U	N	N	1	19474	282	19192
S15-0885-T1D	Y	1	1	12	12	0
S15-0885-T1U	Y	1	0	0	0	0
S15-0885-U	Y	N	3	18002	17811	191
S15-0886-D	Y	N	1	12452	540	11912
S15-0886NB-D	N	N	0	17252	0	17252
S15-0886NB-T2D	N	2	0	32	0	32
S15-0886NB-T2U	N	2	0	24	0	24
S15-0886NB-U	N	N	0	20535	0	20535
S15-0886-T2D	Y	2	0	13	0	13
S15-0886-T2U	Y	2	0	15	0	15
S15-0886-U	Y	N	1	12903	507	12396
S19-8526Q-A	Y	N	2	8491	8491	0
S19-8526Q-B	Y	N	2	2393	2365	28
S19-8526Q-C	Y	N	2	13890	13890	0
S19-8526Q-D	Y	N	1	19607	19365	242
S19-8526QNB-A	N	N	2	18610	444	18166
S19-8526QNB-B	N	N	1	19679	286	19393
S19-8526QNB-C	N	N	1	23018	341	22677

S19-8526QNB-D	N	N	2	17754	728	17026
S19-8526QNB-T3A	N	3	0	2	0	2
S19-8526QNB-T3B	N	3	1	2	2	0
S19-8526QNB-T3C	N	3	0	5	0	5
S19-8526QNB-T3D	N	3	0	0	0	0
S19-8526QNB-T3U	N	3	0	10	0	10
S19-8526QNB-U	N	N	1	20518	618	19900
S19-8526Q-T3A	Y	3	0	2	0	2
S19-8526Q-T3B	Y	3	0	0	0	0
S19-8526Q-T3C	Y	3	0	0	0	0
S19-8526Q-T3D	Y	3	0	2	0	2
S19-8526Q-T3U	Y	3	0	0	0	0
S19-8526Q-U	Y	N	1	16591	16374	217
S19-8626E-A	Y	N	1	13543	13543	0
S19-8626E-B	Y	N	1	3333	3333	0
S19-8626E-C	Y	N	1	17047	17047	0
S19-8626E-D	Y	N	1	16697	16697	0
S19-8626ENB-A	N	N	2	18080	885	17195
S19-8626ENB-B	N	N	2	15130	758	14372
S19-8626ENB-C	N	N	2	28526	1403	27123
S19-8626ENB-D	N	N	3	16909	1834	15075
S19-8626ENB-T4A	N	4	3	30619	1740	28879
S19-8626ENB-T4B	N	4	3	4420	258	4162
S19-8626ENB-T4C	N	4	3	47153	2808	44345
S19-8626ENB-T4D	N	4	3	22003	1787	20216
S19-8626ENB-T4U	N	4	3	27328	2260	25068
S19-8626ENB-U	N	N	3	21862	2361	19501
S19-8626E-T4A	Y	4	3	20919	20644	275
S19-8626E-T4B	Y	4	3	6511	6382	129
S19-8626E-T4C	Y	4	3	30208	29813	395
S19-8626E-T4D	Y	4	3	31425	30654	771
S19-8626E-T4U	Y	4	3	26301	25603	698
S19-8626E-U	Y	N	1	17856	17856	0

			0.001			
Sample Name	Blocker	Tag	NumberPrey Species	Number Read	NumberPrey Read	*Vulpes vulpes*
S15-0827-A	Y	N	1	16547	16012	535
S15-0827-B	Y	N	1	6631	6379	252
S15-0827-C	Y	N	1	29292	28282	1010
S15-0827NB-A	N	N	1	10041	116	9925
S15-0827NB-B	N	N	1	10727	108	10619
S15-0827NB-C	N	N	1	19410	261	19149
S15-0827NB-T1A	N	1	0	66	0	66
S15-0827NB-T1B	N	1	0	115	0	115
S15-0827NB-T1C	N	1	0	59	0	59
S15-0827-T1A	Y	1	1	179	175	4
S15-0827-T1B	Y	1	1	154	146	8
S15-0827-T1C	Y	1	1	74	68	6
S15-0829-A	Y	N	3	13158	13158	0
S15-0829-B	Y	N	3	6868	6856	12
S15-0829-C	Y	N	3	21950	21950	0
S15-0829NB-A	N	N	3	10648	631	10017
S15-0829NB-B	N	N	3	10633	692	9941
S15-0829NB-C	N	N	3	20520	1285	19235
S15-0829NB-T2A	N	2	0	59	0	59
S15-0829NB-T2B	N	2	0	63	0	63
S15-0829NB-T2C	N	2	1	101	3	98
S15-0829-T2A	Y	2	2	113	103	10
S15-0829-T2B	Y	2	2	67	67	0
S15-0829-T2C	Y	2	2	78	73	5
S15-0830-A	Y	N	2	12665	12574	91
S15-0830-B	Y	N	2	6086	6036	50
S15-0830-C	Y	N	2	24915	24713	202
S15-0830NB-A	N	N	1	11150	863	10287
S15-0830NB-B	N	N	1	9274	721	8553
S15-0830NB-C	N	N	1	19595	1528	18067
S15-0830NB-T3A	N	3	0	1	0	1
S15-0830NB-T3B	N	3	0	1	0	1
S15-0830NB-T3C	N	3	0	5	0	5
S15-0830-T3A	Y	3	0	0	0	0
S15-0830-T3B	Y	3	0	0	0	0
S15-0830-T3C	Y	3	0	0	0	0
S15-0831-A	Y	N	2	22364	22338	26
S15-0831-B	Y	N	2	5744	5735	9

S15-0831-C	Y	N	2	24930	24888	42
S15-0831NB-A	N	N	2	11126	438	10688
S15-0831NB-B	N	N	2	9803	380	9423
S15-0831NB-C	N	N	2	16042	675	15367
S15-0831NB-T4A	N	4	2	17999	226	17773
S15-0831NB-T4B	N	4	2	22962	293	22669
S15-0831NB-T4C	N	4	2	21976	299	21677
S15-0831-T4A	Y	4	2	13745	13576	169
S15-0831-T4B	Y	4	2	6016	5938	78
S15-0831-T4C	Y	4	2	12751	12588	163
S15-0832-A	Y	N	2	4565	1221	3344
S15-0832-B	Y	N	3	10575	2830	7745
S15-0832-C	Y	N	3	9663	2588	7075
S15-0832NB-A	N	N	0	11759	0	11759
S15-0832NB-B	N	N	0	16904	0	16904
S15-0832NB-C	N	N	0	23823	0	23823
S15-0832NB-T5A	N	5	0	12656	0	12656
S15-0832NB-T5B	N	5	0	12474	0	12474
S15-0832NB-T5C	N	5	0	15299	0	15299
S15-0832-T5A	Y	5	3	13681	3102	10579
S15-0832-T5B	Y	5	3	7702	1706	5996
S15-0832-T5C	Y	5	3	14068	3080	10988
S15-0833-A	Y	N	1	9660	11	9649
S15-0833-B	Y	N	1	13745	25	13720
S15-0833-C	Y	N	1	21357	27	21330
S15-0833NB-A	N	N	0	11533	0	11533
S15-0833NB-B	N	N	1	8580	12	8568
S15-0833NB-C	N	N	1	20462	26	20436
S15-0833NB-T1A	N	1	0	7	0	7
S15-0833NB-T1B	N	1	0	48	0	48
S15-0833NB-T1C	N	1	0	97	0	97
S15-0833-T1A	Y	1	1	112	11	101
S15-0833-T1B	Y	1	1	77	7	70
S15-0833-T1C	Y	1	1	89	9	80
S15-0835-A	Y	N	2	18709	10267	8442
S15-0835-B	Y	N	2	9181	4760	4421
S15-0835-C	Y	N	2	24135	13161	10974
S15-0835NB-A	N	N	0	17072	0	17072
S15-0835NB-B	N	N	0	22986	0	22986
S15-0835NB-C	N	N	1	28039	29	28010
S15-0835NB-T2A	N	2	0	21	0	21

S15-0835NB-T2B	N	2	0	161	0	161
S15-0835NB-T2C	N	2	0	102	0	102
S15-0835-T2A	Y	2	1	147	43	104
S15-0835-T2B	Y	2	1	107	35	72
S15-0835-T2C	Y	2	1	65	22	43
S15-0836-A	Y	N	0	0	0	0
S15-0836-B	Y	N	0	6	0	6
S15-0836-C	Y	N	0	10	0	10
S15-0836NB-A	N	N	2	10133	124	10009
S15-0836NB-B	N	N	2	7859	66	7793
S15-0836NB-C	N	N	2	14281	155	14126
S15-0836NB-T3A	N	3	1	1	1	0
S15-0836NB-T3B	N	3	0	0	0	0
S15-0836NB-T3C	N	3	0	8	0	8
S15-0836-T3B	Y	3	0	0	0	0
S15-0836-T3C	Y	3	1	2	2	0
S15-0838-A	Y	N	3	1098	1082	16
S15-0838-B	Y	N	2	682	665	17
S15-0838-C	Y	N	2	455	443	12
S15-0838NB-A	N	N	3	11674	245	11429
S15-0838NB-B	N	N	3	9047	210	8837
S15-0838NB-C	N	N	3	6598	194	6404
S15-0838NB-T1A	N	1	0	57	0	57
S15-0838NB-T1B	N	1	0	254	0	254
S15-0838NB-T1C	N	1	0	134	0	134
S15-0838-T1A	Y	1	3	69	69	0
S15-0838-T1B	Y	1	3	76	66	10
S15-0838-T1C	Y	1	3	92	79	13
S15-0839-A	Y	N	6	6202	6131	71
S15-0839-B	Y	N	4	26586	26387	199
S15-0839-C	Y	N	5	8265	8193	72
S15-0839NB-A	N	N	2	17485	167	17318
S15-0839NB-B	N	N	2	21785	184	21601
S15-0839NB-C	N	N	1	18952	206	18746
S15-0839NB-T2A	N	2	0	49	0	49
S15-0839NB-T2B	N	2	0	29	0	29
S15-0839NB-T2C	N	2	0	116	0	116
S15-0839-T2A	Y	2	1	63	57	6
S15-0839-T2B	Y	2	2	142	118	24
S15-0839-T2C	Y	2	1	99	90	9
S15-0840-A	Y	N	2	8157	8071	86

S15-0840-B	Y	N	2	26603	26344	259
S15-0840-C	Y	N	2	12816	12697	119
S15-0840NB-A	N	N	2	12024	214	11810
S15-0840NB-B	N	N	2	13356	213	13143
S15-0840NB-C	N	N	2	20847	337	20510
S15-0840NB-T3A	N	3	0	2	0	2
S15-0840NB-T3B	N	3	0	0	0	0
S15-0840NB-T3C	N	3	1	3	3	0
S15-0840-T3A	Y	3	0	3	0	3
S15-0840-T3C	Y	3	0	4	0	4
S15-0841-A	Y	N	3	11510	4348	7162
S15-0841-B	Y	N	3	8663	3040	5623
S15-0841-C	Y	N	3	23166	8921	14245
S15-0841NB-A	N	N	0	15733	0	15733
S15-0841NB-B	N	N	0	9250	0	9250
S15-0841NB-C	N	N	0	22780	0	22780
S15-0841NB-T4A	N	4	0	21740	0	21740
S15-0841NB-T4B	N	4	0	10674	0	10674
S15-0841NB-T4C	N	4	0	29008	0	29008
S15-0841-T4A	Y	4	1	12404	33	12371
S15-0841-T4B	Y	4	1	5707	9	5698
S15-0841-T4C	Y	4	1	13246	41	13205
S15-0842-A	Y	N	3	10163	6636	3527
S15-0842-B	Y	N	3	7833	4984	2849
S15-0842-C	Y	N	3	23088	14893	8195
S15-0842NB-A	N	N	2	12689	50	12639
S15-0842NB-B	N	N	1	9155	28	9127
S15-0842NB-C	N	N	1	18007	60	17947
S15-0842NB-T5A	N	5	1	26688	82	26606
S15-0842NB-T5B	N	5	1	4709	21	4688
S15-0842NB-T5C	N	5	1	39241	143	39098
S15-0842-T5A	Y	5	3	13358	4957	8401
S15-0842-T5B	Y	5	2	8333	3028	5305
S15-0842-T5C	Y	5	3	26246	9764	16482
S15-0843-A	Y	N	4	6727	4790	1937
S15-0843-B	Y	N	5	7066	5071	1995
S15-0843-C	Y	N	5	7911	5708	2203
S15-0843NB-A	N	N	1	14017	163	13854
S15-0843NB-B	N	N	1	8640	81	8559
S15-0843NB-C	N	N	1	21858	233	21625
S15-0843NB-T1A	N	1	0	25	0	25

S15-0843NB-T1B	N	1	0	22	0	22
S15-0843NB-T1C	N	1	0	59	0	59
S15-0843-T1A	Y	1	3	88	56	32
S15-0843-T1B	Y	1	3	85	47	38
S15-0843-T1C	Y	1	3	123	69	54
S15-0844-A	Y	N	2	16553	16496	57
S15-0844-B	Y	N	2	14614	14581	33
S15-0844-C	Y	N	2	26823	26766	57
S15-0844NB-A	N	N	1	14407	473	13934
S15-0844NB-B	N	N	1	5029	153	4876
S15-0844NB-C	N	N	1	22671	783	21888
S15-0844NB-T2A	N	2	0	33	0	33
S15-0844NB-T2B	N	2	0	27	0	27
S15-0844NB-T2C	N	2	0	89	0	89
S15-0844-T2A	Y	2	1	67	67	0
S15-0844-T2B	Y	2	1	94	91	3
S15-0844-T2C	Y	2	1	67	67	0
S15-0845-A	Y	N	2	18006	8835	9171
S15-0845-B	Y	N	2	16195	7984	8211
S15-0845-C	Y	N	2	27134	13061	14073
S15-0845NB-A	N	N	1	16980	151	16829
S15-0845NB-B	N	N	2	28785	277	28508
S15-0845NB-C	N	N	2	26033	275	25758
S15-0845NB-T3A	N	3	1	2	2	0
S15-0845NB-T3B	N	3	0	0	0	0
S15-0845NB-T3C	N	3	0	1	0	1
S15-0845-T3B	Y	3	0	0	0	0
S15-0845-T3C	Y	3	0	5	0	5
S15-0846-A	Y	N	2	8037	7079	958
S15-0846-B	Y	N	2	11632	10228	1404
S15-0846-C	Y	N	2	10733	9485	1248
S15-0846NB-A	N	N	2	14797	125	14672
S15-0846NB-B	N	N	2	13847	138	13709
S15-0846NB-C	N	N	3	22855	214	22641
S15-0846NB-T1B	N	1	0	2	0	2
S15-0846NB-T1C	N	1	0	4	0	4
S15-0846-T1A	Y	1	1	58	37	21
S15-0846-T1B	Y	1	1	134	85	49
S15-0846-T1C	Y	1	1	137	88	49
S15-0847-A	Y	N	4	9003	7668	1335
S15-0847-B	Y	N	4	9871	8410	1461

S15-0847-C	Y	N	4	9293	7960	1333
S15-0847NB-A	N	N	1	15527	221	15306
S15-0847NB-B	N	N	1	13540	186	13354
S15-0847NB-C	N	N	1	25842	438	25404
S15-0847NB-T2A	N	2	2	59	34	25
S15-0847NB-T2B	N	2	3	138	101	37
S15-0847NB-T2C	N	2	3	214	98	116
S15-0847-T2A	Y	2	1	36	24	12
S15-0847-T2B	Y	2	1	63	42	21
S15-0847-T2C	Y	2	1	91	63	28
S15-0848-D	Y	N	7	12934	7220	5714
S15-0848NB-D	N	N	1	16023	176	15847
S15-0848NB-T3D	N	3	0	2	0	2
S15-0848NB-T3U	N	3	0	0	0	0
S15-0848NB-U	N	N	2	18722	265	18457
S15-0848-T3D	Y	3	0	4	0	4
S15-0848-T3U	Y	3	0	9	0	9
S15-0848-U	Y	N	6	17502	9638	7864
S15-0849-D	Y	N	2	15219	2826	12393
S15-0849NB-D	N	N	0	15637	0	15637
S15-0849NB-T1D	N	1	0	3	0	3
S15-0849NB-T1U	N	1	0	0	0	0
S15-0849NB-U	N	N	0	19254	0	19254
S15-0849-T1D	Y	1	0	40	0	40
S15-0849-T1U	Y	1	0	40	0	40
S15-0849-U	Y	N	2	12499	2475	10024
S15-0850-D	Y	N	3	14259	3890	10369
S15-0850NB-D	N	N	0	24770	0	24770
S15-0850NB-T2D	N	2	0	186	0	186
S15-0850NB-T2U	N	2	0	181	0	181
S15-0850NB-U	N	N	0	20869	0	20869
S15-0850-T2D	Y	2	0	10	0	10
S15-0850-T2U	Y	2	0	26	0	26
S15-0850-U	Y	N	4	14252	3991	10261
S15-0854-D	Y	N	1	17130	96	17034
S15-0854NB-D	N	N	0	21025	0	21025
S15-0854NB-T1D	N	1	0	35	0	35
S15-0854NB-T1U	N	1	0	25	0	25
S15-0854NB-U	N	N	0	20659	0	20659
S15-0854-T1D	Y	1	0	12	0	12
S15-0854-T1U	Y	1	0	13	0	13

S15-0854-U	Y	N	1	17350	100	17250
S15-0855-D	Y	N	1	12199	106	12093
S15-0855NB-D	N	N	1	17481	64	17417
S15-0855NB-T2D	N	2	0	20	0	20
S15-0855NB-T2U	N	2	0	23	0	23
S15-0855NB-U	N	N	1	19213	82	19131
S15-0855-T2D	Y	2	1	31	5	26
S15-0855-T2U	Y	2	0	29	0	29
S15-0855-U	Y	N	1	13872	123	13749
S15-0856-D	Y	N	5	16475	10133	6342
S15-0856NB-D	N	N	2	20575	54	20521
S15-0856NB-T3D	N	3	0	0	0	0
S15-0856NB-T3U	N	3	0	0	0	0
S15-0856NB-U	N	N	2	19539	43	19496
S15-0856-T3D	Y	3	1	4	4	0
S15-0856-T3U	Y	3	0	2	0	2
S15-0856-U	Y	N	5	15315	9384	5931
S15-0857-D	Y	N	2	15198	13355	1843
S15-0857NB-D	N	N	1	28463	219	28244
S15-0857NB-T4D	N	4	0	38311	0	38311
S15-0857NB-T4U	N	4	0	21484	0	21484
S15-0857NB-U	N	N	1	21234	203	21031
S15-0857-T4D	Y	4	3	19638	16599	3039
S15-0857-T4U	Y	4	3	23093	19336	3757
S15-0857-U	Y	N	2	16224	14299	1925
S15-0858-D	Y	N	0	16601	0	16601
S15-0858NB-D	N	N	1	19096	24	19072
S15-0858NB-T5D	N	5	0	23947	0	23947
S15-0858NB-T5U	N	5	0	21854	0	21854
S15-0858NB-U	N	N	1	19063	27	19036
S15-0858-T5D	Y	5	5	25960	2552	23408
S15-0858-T5U	Y	5	5	22439	2205	20234
S15-0858-U	Y	N	0	15102	0	15102
S15-0859-D	Y	N	7	13439	8552	4887
S15-0859NB-D	N	N	1	19688	81	19607
S15-0859NB-T1D	N	1	0	9	0	9
S15-0859NB-T1U	N	1	0	21	0	21
S15-0859NB-U	N	N	1	18268	73	18195
S15-0859-T1D	Y	1	1	26	6	20
S15-0859-T1U	Y	1	0	28	0	28
S15-0859-U	Y	N	7	16131	10301	5830

S15-0869-D	Y	N	2	13187	7448	5739
S15-0869NB-D	N	N	0	24756	0	24756
S15-0869NB-T2D	N	2	0	18	0	18
S15-0869NB-T2U	N	2	0	25	0	25
S15-0869NB-U	N	N	0	19991	0	19991
S15-0869-T2D	Y	2	1	17	4	13
S15-0869-T2U	Y	2	0	24	0	24
S15-0869-U	Y	N	2	16834	9598	7236
S15-0870-D	Y	N	3	24462	6584	17878
S15-0870NB-D	N	N	1	20193	32	20161
S15-0870NB-T3D	N	3	1	4	4	0
S15-0870NB-T3U	N	3	0	16	0	16
S15-0870NB-U	N	N	1	16886	27	16859
S15-0870-T3D	Y	3	0	6	0	6
S15-0870-T3U	Y	3	1	18	3	15
S15-0870-U	Y	N	3	13564	3591	9973
S15-0871-D	Y	N	2	14327	4119	10208
S15-0871NB-D	N	N	0	32168	0	32168
S15-0871NB-T1D	N	1	0	52	0	52
S15-0871NB-T1U	N	1	0	29	0	29
S15-0871NB-U	N	N	0	19147	0	19147
S15-0871-T1D	Y	1	0	31	0	31
S15-0871-T1U	Y	1	0	27	0	27
S15-0871-U	Y	N	2	14802	4259	10543
S15-0880-D	Y	N	6	14072	10639	3433
S15-0880NB-D	N	N	2	36660	171	36489
S15-0880NB-T2D	N	2	0	18	0	18
S15-0880NB-T2U	N	2	0	24	0	24
S15-0880NB-U	N	N	2	20701	110	20591
S15-0880-T2D	Y	2	0	5	0	5
S15-0880-T2U	Y	2	1	29	9	20
S15-0880-U	Y	N	6	15781	11881	3900
S15-0881-D	Y	N	4	12686	9487	3199
S15-0881NB-D	N	N	2	24114	684	23430
S15-0881NB-T3D	N	3	0	2	0	2
S15-0881NB-T3U	N	3	0	0	0	0
S15-0881NB-U	N	N	2	18700	539	18161
S15-0881-T3D	Y	3	0	4	0	4
S15-0881-T3U	Y	3	1	7	7	0
S15-0881-U	Y	N	4	15545	11470	4075
S15-0882-D	Y	N	2	17118	558	16560

S15-0882NB-D	N	N	0	19134	0	19134
S15-0882NB-T4D	N	4	0	19798	0	19798
S15-0882NB-T4U	N	4	0	26659	0	26659
S15-0882NB-U	N	N	0	19892	0	19892
S15-0882-T4D	Y	4	3	17409	571	16838
S15-0882-T4U	Y	4	4	25117	894	24223
S15-0882-U	Y	N	2	15628	455	15173
S15-0884-D	Y	N	4	24201	22997	1204
S15-0884NB-D	N	N	2	16439	134	16305
S15-0884NB-T5D	N	5	0	17506	0	17506
S15-0884NB-T5U	N	5	0	23561	0	23561
S15-0884NB-U	N	N	2	19172	153	19019
S15-0884-T5D	Y	5	4	26214	21715	4499
S15-0884-T5U	Y	5	4	24386	20018	4368
S15-0884-U	Y	N	4	16695	15844	851
S15-0885-D	Y	N	6	14054	13885	169
S15-0885NB-D	N	N	3	18896	491	18405
S15-0885NB-T1D	N	1	0	25	0	25
S15-0885NB-T1U	N	1	0	37	0	37
S15-0885NB-U	N	N	3	19752	536	19216
S15-0885-T1D	Y	1	1	12	12	0
S15-0885-T1U	Y	1	0	0	0	0
S15-0885-U	Y	N	6	18617	18348	269
S15-0886-D	Y	N	1	12986	540	12446
S15-0886NB-D	N	N	0	17305	0	17305
S15-0886NB-T2D	N	2	0	32	0	32
S15-0886NB-T2U	N	2	0	24	0	24
S15-0886NB-U	N	N	0	20671	0	20671
S15-0886-T2D	Y	2	0	13	0	13
S15-0886-T2U	Y	2	0	15	0	15
S15-0886-U	Y	N	1	13466	507	12959
S19-8526Q-A	Y	N	5	8616	8576	40
S19-8526Q-B	Y	N	5	2415	2384	31
S19-8526Q-C	Y	N	6	14260	14164	96
S19-8526Q-D	Y	N	5	20090	19757	333
S19-8526QNB-A	N	N	3	18656	490	18166
S19-8526QNB-B	N	N	3	19910	517	19393
S19-8526QNB-C	N	N	4	23316	639	22677
S19-8526QNB-D	N	N	6	17883	857	17026
S19-8526QNB-T3A	N	3	0	2	0	2
S19-8526QNB-T3B	N	3	1	2	2	0

S19-8526QNB-T3C	N	3	0	5	0	5
S19-8526QNB-T3D	N	3	0	0	0	0
S19-8526QNB-T3U	N	3	0	10	0	10
S19-8526QNB-U	N	N	5	20807	907	19900
S19-8526Q-T3A	Y	3	0	2	0	2
S19-8526Q-T3B	Y	3	0	0	0	0
S19-8526Q-T3C	Y	3	0	0	0	0
S19-8526Q-T3D	Y	3	0	2	0	2
S19-8526Q-T3U	Y	3	0	0	0	0
S19-8526Q-U	Y	N	5	17045	16742	303
S19-8626E-A	Y	N	7	13780	13748	32
S19-8626E-B	Y	N	6	3404	3375	29
S19-8626E-C	Y	N	8	17407	17322	85
S19-8626E-D	Y	N	6	17251	17133	118
S19-8626ENB-A	N	N	4	18300	1105	17195
S19-8626ENB-B	N	N	4	15304	932	14372
S19-8626ENB-C	N	N	4	28854	1731	27123
S19-8626ENB-D	N	N	4	17033	1937	15096
S19-8626ENB-T4A	N	4	4	30751	1872	28879
S19-8626ENB-T4B	N	4	4	4450	288	4162
S19-8626ENB-T4C	N	4	4	47403	3058	44345
S19-8626ENB-T4D	N	4	4	22131	1885	20246
S19-8626ENB-T4U	N	4	4	27465	2364	25101
S19-8626ENB-U	N	N	4	21981	2480	19501
S19-8626E-T4A	Y	4	6	21417	21024	393
S19-8626E-T4B	Y	4	5	6627	6498	129
S19-8626E-T4C	Y	4	6	30858	30325	533
S19-8626E-T4D	Y	4	8	32925	31774	1151
S19-8626E-T4U	Y	4	8	27552	26570	982
S19-8626E-U	Y	N	7	18457	18357	100

Appendix 3: ASV Table Generated by DADA2

	Top Blast Result	Number of reads
ASV_1	*Vulpes vulpes*	3079213
ASV_2	*Thomomys bottae*	319812
ASV_3	*Phoebastria albatrus*	291241
ASV_4	*Martes americana*	265326
ASV_5	*Thomomys bottae*	258809
ASV_6	*Thomomys bottae*	215780
ASV_7	*Vulpes vulpes*	120568
ASV_8	*Terricola subterraneus*	74364
ASV_9	*Vulpes vulpes*	69642
ASV_10	*Vulpes vulpes*	67810
ASV_11	*Sayornis phoebe*	56804
ASV_12	*Gallus gallus*	55095
ASV_13	*Microtus savii*	46855
ASV_14	*Vulpes vulpes*	44313
ASV_15	*Thomomys bottae mohavensis*	32933
ASV_16	*Sturnus vulgaris*	30692
ASV_17	*Odocoileus hemionus*	29813
ASV_18	*Vulpes vulpes*	26553
ASV_19	*Lepus californicus*	25425
ASV_20	*Zenaida macroura*	23986
ASV_21	*Vulpes vulpes*	22982
ASV_22	*Corvus corax*	17621
ASV_23	*Vulpes vulpes*	14095
ASV_24	*Vulpes vulpes*	11927
ASV_25	*Vulpes vulpes*	11646
ASV_26	*Vulpes vulpes*	11553
ASV_27	*Peromyscus aztecus*	11220
ASV_28	*Mus musculus*	10424
ASV_29	*Morone saxatilis*	10305
ASV_30	*Vulpes vulpes*	9315
ASV_31	*Vulpes vulpes*	8046
ASV_32	*Martes americana caurina*	7137
ASV_33	*Vulpes vulpes*	6614
ASV_35	*Callospermophilus lateralis*	5918
ASV_34	*Vulpes vulpes*	6025
ASV_36	*Vulpes vulpes*	5282
ASV_38	*Thomomys bottae mohavensis*	4540
ASV_39	*Callospermophilus lateralis*	4424
ASV_41	*Peromyscus polionotus*	3735
ASV_40	*Callospermophilus lateralis*	4076

ASV_43	*Campylorhynchus brunneicapillus*	3286
ASV_42	*Vulpes vulpes*	3387
ASV_44	*Thomomys bottae mohavensis*	3094
ASV_46	*Vulpes vulpes*	2847
ASV_45	*Sciurus griseus*	3062
ASV_47	*Vulpes vulpes*	2657
ASV_48	*Corvus corax*	2178
ASV_49	*Terricola subterraneus*	1880
ASV_37	*Homo sapiens*	4632
ASV_51	*Peromyscus attwateri*	1627
ASV_50	*Homo sapiens*	1671
ASV_52	*Callipepla californica*	1467
ASV_53	*Sylvilagus floridanu*	1407
ASV_54	*Vulpes vulpes*	1172
ASV_55	*Vulpes vulpes*	1152
ASV_59	*Terricola subterraneus*	955
ASV_56	*Pipilo maculatus*	1021
ASV_60	*Thomomys bottae mohavensis*	945
ASV_57	*Myocastor coypus*	993
ASV_62	*Thomomys bottae mohavensis*	904
ASV_58	*Vulpes vulpes*	958
ASV_63	*Thomomys bottae mohavensis*	838
ASV_64	*Homo sapiens*	831
ASV_61	*Vulpes vulpes*	922
ASV_65	*Vulpes vulpes*	691
ASV_66	*Vulpes vulpes*	686
ASV_68	*Gallus gallus*	664
ASV_69	*Vulpes vulpes*	624
ASV_67	*Callospermophilus lateralis*	665
ASV_70	*Sayornis phoebe*	600
ASV_72	*Callospermophilus lateralis*	530
ASV_74	*Vulpes vulpes*	510
ASV_75	*Vulpes vulpes*	508
ASV_76	*Vulpes vulpes*	503
ASV_71	*Rattus rattus*	533
ASV_77	*Vulpes vulpes*	467
ASV_78	*Thomomys bottae mohavensis*	462
ASV_73	*Callospermophilus lateralis*	522
ASV_79	*Vulpes vulpes*	432
ASV_80	*Callospermophilus lateralis*	389
ASV_82	*Vulpes vulpes*	382
ASV_83	*Vulpes vulpes*	359

ASV_84	*Sayornis phoebe*	353
ASV_81	*Sylvilagus floridanus*	383
ASV_86	*Callipepla californica*	335
ASV_85	*Sciurus niger*	353
ASV_87	*Homo sapiens*	321
ASV_88	*Thomomys bottae mohavensis*	307
ASV_89	*Sylvilagus floridanus*	298
ASV_90	*Vulpes vulpes*	295
ASV_91	*Vulpes vulpes*	287
ASV_92	*Thomomys bottae mohavensis*	274
ASV_93	*Thomomys bottae mohavensis*	274
ASV_94	*Thomomys bottae mohavensis*	273
ASV_95	*Thomomys bottae mohavensis*	273
ASV_96	*Thomomys bottae mohavensis*	216
ASV_97	*Terricola subterraneus*	208
ASV_100	*Vulpes vulpes*	187
ASV_99	*Callospermophilus lateralis*	203
ASV_103	*Eonycteris spelaea*	169
ASV_104	*Vulpes vulpes*	165
ASV_105	*Callospermophilus lateralis*	159
ASV_102	*Vulpes vulpes*	173
ASV_107	*Thomomys bottae*	149
ASV_108	*Vulpes vulpes*	147
ASV_106	*Macaca mulatta*	150
ASV_109	*Catharus ustulatus*	142
ASV_111	*Vulpes vulpes*	140
ASV_112	*Vulpes vulpes*	135
ASV_113	*Vulpes vulpes*	134
ASV_114	*Thomomys bottae*	132
ASV_110	*Urocyon cinereoargenteus*	141
ASV_115	*Thomomys bottae*	128
ASV_116	*Tamias ruficaudus simulans*	127
ASV_118	*Vulpes vulpes*	124
ASV_119	*Thunnus orientalis*	120
ASV_120	*Vulpes vulpes*	117
ASV_122	*Thomomys bottae mohavensis*	111
ASV_123	*Odocoileus hemionus*	110
ASV_125	*Thomomys bottae mohavensis*	109
ASV_127	*Vulpes vulpes*	108
ASV_128	*Martes americana caurina*	108
ASV_126	*Vulpes vulpes*	108
ASV_129	*Vulpes vulpes*	106
ASV_130	*Vulpes vulpes*	105

ASV_131	*Vulpes vulpes*	104
ASV_132	*Vulpes vulpes*	101
ASV_134	*Vulpes vulpes*	97
ASV_135	*Sayornis phoebe*	97
ASV_133	*Vulpes vulpes*	97
ASV_136	*Terricola subterraneus*	96
ASV_137	*Thomomys bottae mohavensis*	96
ASV_138	*Sylvilagus floridanus*	92
ASV_139	*Callospermophilus lateralis*	91
ASV_140	*Odocoileus hemionus*	89
ASV_141	*Homo sapiens*	87
ASV_142	*Vulpes vulpes*	86
ASV_124	*Chrysocyon brachyurus*	110
ASV_143	*Vulpes vulpes*	85
ASV_145	*Chrysocyon brachyurus*	79
ASV_144	*Vulpes vulpes*	79
ASV_146	*Martes americana caurina*	75
ASV_147	*Callospermophilus lateralis*	72
ASV_149	*Vulpes vulpes*	66
ASV_152	*Thomomys bottae*	54
ASV_155	*Vulpes vulpes*	52
ASV_154	*Capra hircus*	52
ASV_156	*Vulpes vulpes*	52
ASV_157	*Vulpes vulpes*	52
ASV_158	*Vulpes vulpes*	52
ASV_159	*Meleagris gallopavo*	50
ASV_160	*Thomomys bottae*	48
ASV_161	*Vulpes vulpes*	47
ASV_163	*Procyon lotor*	45
ASV_162	*Vulpes vulpes*	45
ASV_164	*Sus scrofa*	43
ASV_165	*Callospermophilus lateralis*	42
ASV_166	*Vulpes vulpes*	36
ASV_167	*Vulpes vulpes*	36
ASV_168	*Vulpes vulpes*	34
ASV_171	*Cratogeomys estor*	30
ASV_170	*Puma concolor*	30
ASV_172	*Bos taurus*	29
ASV_173	*Thomomys bottae*	29
ASV_176	*Martes americana caurina*	28
ASV_174	*Microtus richardsoni*	28
ASV_175	*Vulpes vulpes*	28
ASV_177	*Phoebastria albatrus*	27

ASV_179	*Vulpes vulpes*	26
ASV_178	*Homo sapiens*	26
ASV_180	*Peromyscus maniculatus*	25
ASV_181	*Vulpes vulpes*	22
ASV_182	*Cratogeomys estor*	22
ASV_183	*Picoides pubescens*	21
ASV_184	*Microtus richardsoni*	20
ASV_185	*Thomomys bottae*	18
ASV_186	*Thomomys bottae*	18
ASV_187	*Peromyscus attwateri*	15
ASV_188	*Odocoileus hemionus*	14
ASV_190	*Martes americana caurina*	13
ASV_189	*Thomomys bottae*	13
ASV_191	*Vulpes vulpes*	12
ASV_193	*Homo sapiens*	12
ASV_192	*Sciurus niger*	12
ASV_196	*Callospermophilus lateralis*	11
ASV_195	*Sylvilagus audubonii*	11
ASV_194	*Thomomys bottae*	11
ASV_215	*Vulpes vulpes*	7
ASV_224	*Callospermophilus lateralis*	6
ASV_233	*Mephitis mephitis*	5
ASV_234	*Tamiasciurus hudsonicus*	5
ASV_251	*Bos taurus*	4
ASV_252	*Vulpes vulpes*	4
ASV_250	*Thomomys bottae mohavensis*	4
ASV_269	*Martes americana caurina*	3
ASV_268	*Phoebastria albatrus*	3
ASV_299	*Vulpes vulpes*	2
ASV_300	*Terricola subterraneus*	2
ASV_295	*Thomomys bottae mohavensis*	2
ASV_296	*Thomomys bottae mohavensis*	2
ASV_298	*Thomomys talpoides*	2
ASV_297	*Vulpes vulpes*	2
ASV_117	*Callipepla californica*	125
ASV_169	*Callospermophilus lateralis*	31
ASV_121	*Campylorhynchus brunneicapillus*	117
ASV_150	*Corvus corax*	62
ASV_151	*Corvus corax*	56
ASV_203	*Eonycteris spelaea*	9
ASV_153	*Homo sapiens*	53
ASV_204	*Oryctolagus cuniculus*	9

ASV_101	*Peromyscus attwateri*	183
ASV_98	*Peromyscus maniculatus*	206
ASV_273	*Picoides pubescens*	3
ASV_148	*Terricola subterraneus*	70
ASV_197	NoResult	10
ASV_198	NoResult	10
ASV_199	NoResult	9
ASV_200	NoResult	9
ASV_201	NoResult	9
ASV_202	NoResult	9
ASV_205	NoResult	9
ASV_206	NoResult	9
ASV_207	NoResult	9
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ASV_213	NoResult	8
ASV_214	NoResult	8
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