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Genetic Structure of Yonahlossee Salamander Populations.

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Genetic Structure of Yonahlossee Salamander Populations

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by

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ABSTRACT

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Plethodon yonahlossee is the largest eastern Plethodontid salamander. It has been classified as a species of greatest conservation need by the Tennessee Wildlife Resources Agency (TWRA). Found only in mountainous areas along the borders of Tennessee, North Carolina, and Virginia, populations of the yonahlossee are considered to be rare and local throughout their range. Genetic differentiation among populations of any species is usually attributable to long-standing, extrinsic barriers to gene flow. Because of their disjunct population structure and some observed morphological variation, genetic differentiation among yonahlossee populations is expected. A genetic structure study of yonahlossee was conducted to identify any genetically differentiated populations as conservation units. One mitochondrial DNA marker as well one nuclear DNA marker were amplified using polymerase chain reaction. After analysis, both markers show genetic differentiation suggesting geographic isolation. This information can be used by management agencies for the protection and conservation of the species.

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CONTENTS

LIST OF TABLES

CHAPTER 1

INTRODUCTION

Purpose

The purpose of this project was to examine the potential for genetic differentiation among populations of the salamander species *Plethodon yonahlossee*. Results of this study were used to evaluate the likelihood of isolated and potentially locally adapted genotypes in this species based on mitochondrial and nuclear DNA variation. This information will then be used by management agencies to identify significant conservation units.

Background

P. yonahlossee is the largest eastern Plethodon and is typically found in deciduous forest habits between 1000-1737 meters and occasionally as low as 436 meters (Petranka 1998). The distribution is rather small, with populations in southwest Virginia, eastern Tennessee, and western North Carolina (AmphibiaWeb 2007). The yonahlossee is characterized by direct development, which means that it has no larval stage outside of the egg. After hatching, the yonahlossee will grow to a length of 11-22cm. During development, individuals form a characteristic, deeply pigmented patch of red on the back. While the general appearance of the yonahlossee is consistent throughout its range, there is notable morphological differentiation among some populations (Highton 1995; Petranka 1998). One such population in the Bat Cave area of North Carolina was considered a completely separate species (*P. longicrus*)

(Alder and Dennis 1962) until a biochemical and morphological analysis was conducted comparing that population with *P. yonahlossee* (Guttman et al. 1978). That study used 21 allozyme loci and conclude that *P. longicrus* is actually synonymous with *P. yonahlossee* based on Roger's genetic similarity and Nei's genetic distance. Coloration was the only morphologically distinguishing characteristic between populations. This difference suggests the possibility of interpopulation genetic isolation within species. Another population with the potential for genetic isolation was noted by some workers of the Tennessee Wildlife Resources Agency who reported morphological variation in yonahlossee specimens in the Rocky Fork Wildlife Management Area in Unicoi County, Tennessee (Wyatt 2006). This potential differentiation could be the result of the isolation of the Rocky Fork population by the Nolichucky River from all more northern populations.

The greatest threat currently facing the yonahlossee is timber harvesting and urbanization (Petranka et al. 1992). TWRA has classified *P. yonahlossee* as a Species of Special Concern and a Species of Greatest Conservation Need (TWRA 2007). To properly define and implement any future conservation efforts, conservation units should be defined. Conservation units can be characterized as populations having a high degree of differentiation at neutral genetic markers. Neutral markers are useful in revealing genetic differentiation among populations by genetic drift (Avise 1994). Genetic differentiation can also be caused by natural selection occurring within a population. For these forces to be effective, a population must have reduced immigration. A theoretical estimate of the magnitude of migration sufficient to allow

genetic differentiation among populations is that the number of migrants be less than 1 per generation (Mills et al. 2002).

A cost effective way to define distinct conservation units would be to conduct a phylogeographic survey among yonahlossee populations using mitochondrial and nuclear DNA sequences. Avise (1994) developed three hypotheses that should be considered when conducting a phylogeographic study. First, most species are subject to phylogeographic differentiation. Second, species that are not subject to phylogeographic differentiation either have life histories conducive to dispersal or are free from long-standing barriers to gene flow. Third, populations of the same species characterized by large phylogenetic gaps usually arise from some long-term, extrinsic barrier to gene flow. Therefore, it is the hypothesis of this study that *P. yonahlossee* is a metapopulation composed of genetically distinguishable subpopulations.

Intraspecific Genetic Differentiation

Two common causes of genetic differentiation between populations of the same species are genetic drift and natural selection (Avise 1994). Genetic drift is the change in allele frequencies within a population over generations caused by random events. These changes can compound over generations eventually leading to the fixation or loss of an allele and any phenotypic traits associated with it. One facet of natural selection is the fixation or loss of an allele caused by its effect on fitness. If an individual of a population has an advantageous biological trait in a unique habitat, it is more likely to survive and pass the allele which codes for that trait on to its offspring.

When considering local adaptation and genetic drift it is important to note that they both occur more rapidly in populations with reduced immigration (Hartl and Clark 1989).

Observed Intraspecific Differentiation

For a species with a widespread and/or disjunct distribution it is not uncommon to find intraspecific genetic differentiation that can approach the interspecific level despite morphological similarity (Zamudio and Wesley 2003). For example, *Ambystoma maculatum*, the spotted salamander, is a widespread species occupying both historically glaciated and unglaciated areas in eastern North America. An analysis of mitochondrial DNA revealed extremely high levels of intraspecific genetic differentiation between populations of *A. maculatum* (Zamudio and Wesley 2003). Results of that research indicated that glaciation created general long-term topographical factors that lead to genetic differentiation. This is evident through the correlation between suture zones and zones of secondary contact. Suture zones are geographic regions described as clumped hybridization zones where genetically differentiated yet closely related species are sympatric. Secondary contact zones are areas where genetically differentiated populations of the same species are sympatric. Zones of secondary contact between spotted salamander populations correlated with three of the four previously identified suture zones in eastern North America (Zamudio and Wesley 2003). The most notable characteristic of these suture zones is that they include species from different communities and lineages (i.e. mammals) (Lessa et al. 2003). With their observed correlation with secondary contact, it would imply that the same topological features that affect the spatial arrangement of species also influences the spatial arrangement and

differentiation within a species. Therefore glaciation and climate change influenced the occurrence of these suture zones that affected differentiation at multiple hierarchical phylogenetic levels. Regardless of mobility, refuge location, and population size, similar signs of genetic differentiation were seen between and among many wide-spread species (Zamudio and Wesley 2003).

Conservation Units

A conservation unit is a population or a group of populations of a species generally defined by at least one of three criteria (Moritz 1994): 1) geographic isolation from other populations, 2) differentiation at neutral genetic markers, and/or 3) the presence of a unique phenotypic trait. This study emphasized the second criterion as the primary definition of a conservation unit. By detecting genetic differentiation between populations, the extent of geographic isolation can be estimated. Criterion 3 is of least concern for this project because it is primarily applicable only to the populations in the Bat Cave area of North Carolina. Based on some reports of possible morphological variation in other populations, future morphometric research on the yonahlossee could focus on this third criterion.

Delimiting Conservation Units. For this project, an analysis of genetic differentiation was performed using one mitochondrial and one nuclear gene. There are strengths and weakness in using both mitochondrial and nuclear loci in delineating conservation units. Mitochondrial DNA has a high mutation rate and is only transmitted maternally. Because females of other salamander species may be less likely to migrate between populations (Jockusch and Wake 2002), one would expect to see a relatively

lower degree of variation within and a higher degree of variation among populations when comparisons are based on mitochondrial loci . Nuclear DNA coding sequences (exons) are likely to be highly conserved between populations even after populations have been isolated for extended periods of time because of selection and repair mechanisms present in nuclear DNA. The non-coding sequences (introns) have no known effect on primary amino acid sequence and are less likely to be influenced by selection. Thus, introns are likely to differentiate at higher rates than exons (Futuyma 1998). Because introns are subject to repair mechanisms, they are expected to differentiate at slower rates than mitochondrial DNA. The genes chosen for this project were the mitochondrial cytochrome b (CYTb) gene and an intron of the nuclear triose-phosphate isomerase gene. These genes have been used in other research to determine genetic differentiation in other salamander species (Baird et al. 2006; Pauly et al. 2006; Wiens et al. 2006).

CHAPTER 2

METHODS

Field Methods

Twelve *P. yonahlossee* populations were chosen for this study (Table 1). These populations cover the extent of the known range and were chosen for potential geographic isolation and/or observed morphological differentiation. Additional information for each specimen is available in APPENDIX A.

Table 1 *P. yonahlossee* populations and number of specimens used for analysis

		Number of Specimens	
Population	ID	CYTb	TPI
Mount Rogers, VA	MR	3	7
Dry Run, VA	DR	3	4
Holston Mountain, TN	HM	9	12
Roan Mountain, TN	RM	3	6
Rocky Fork, TN	RF	4	11
Limestone Cove, TN	LC	2	3
Unaka Mountain, TN	UM	3	2
Iron Mountain, TN	IMT	3	3
Rock Creek Park, TN	RC	3	5
Grandfather Mountain, NC	GM	3	6
Mount Mitchell, NC	MM	3	6
Bat Cave Area, NC	BC	5	7

Capture Methods

Individual salamanders were collected by hand using different search methods. Commonly, yonahlossee salamanders were found under rocks, rotting logs, and pieces of pine bark lying on the ground and at the openings of small mammals burrows along embankments bordering foot trails and forest roads. Independence of specimens at each population was assumed because no two salamanders were collected from under a single rock or log or other single spot. While I had some success capturing individuals during daylight hours, the first several hours after sunset yielded the greatest success. Weather also played a role in capture success. More individuals were captured on mild and wet days than on hot and dry days. Considering all these factors, the most success for capturing yonahlossee salamanders during this research was along an embankment in moist conditions after sunset.

Processing Methods

Upon capture, the salamander was both measured and weighed. Measurements were made using dial calipers and consisted of both total length and snout-vent length. Body length was measure from the tip of the snout to the tip of the tail whereas snout-vent length was measured from the tip of the snout to the beginning of the ventral opening between the hind legs. After measurements were taken, a portable digital scale was used to measure the animal's weight. The salamander was then photographed resting on a data sheet with a grid composed of 1 centimeter squares (APPENDIX C, Figure 1) using a Fujifilm FinePix S8000fd camera with an 8 megapixel

resolution. These photographs will be useful for future morphometric analysis of pigmentation between populations.

Tissue Collection Methods

Tissue samples in the form of tail tips were collected from individuals of each population using a minimally invasive technique (Dinsmore 1977). An individual salamander was first placed into a shallow plastic sandwich container. This allowed the salamander to move freely without easily escaping. Metal forceps were then used to firmly pinch the tail at a 90° angle roughly 10 to 15 mm from the tip. Simply rotating the forceps would then induce tail autotomy, a natural defensive mechanism, just above the forceps. Tail tips were then stored separately in 1.5 ml freestanding screwcap tubes filled with 100% ethanol. Due to the nature of this method, there was no need for follow-up care of the salamander. The live specimen was immediately released.

Laboratory Methods

All tissue samples were brought to the laboratory promptly after collection and kept frozen at -20°C until use.

Genomic DNA Extraction

Genomic DNA was extracted from tissue samples using a Quiagen DNeasy Tissue Kit. Approximately 5 mm of tail tip was used for each separate preparation. Tissue was first macerated into small pieces on a glass microscope slide with a razor blade. New slides and razors were used for each prep. Macerated tissue was then placed in a clean 1.5 ml screwcap tube along with 180 μl of Buffer ATL, 20 μl of

proteinase K, and a ceramic grinding bead. The sample was then ground and mixed by vortexing in a MP Biomedical FastPrep-24 at 4 m/s for 20 seconds. The sample was then allowed to incubate at 55°C using an Eppendorf ThermoStat Plus until the tissue had completely lysed (roughly 1-3 hours). After lysis, the sample was vortexed for 15 seconds using a Fisher Vortex Genie 2. Two hundred μl of Buffer AL were then added to the sample, mixed by vortexing, and allowed to incubate at 70°C for 10 minutes. Two hundred μl of 100% ethanol were then added to the sample and mixed by vortexing. Using a pipet, the sample mixture was transferred to a DNeasy Mini spin column placed in a 2 ml collection tube and centrifuged at 8,000 rpm for 1 minute. The flow-through and collection tube were then discarded and the DNeasy Mini spin column was placed in a new collection tube. Five hundred μl of Buffer AW1 was added to the spin column and centrifuged at 8,000 rpm for 1 minute. The flow-through and collection tube were discarded and the spin column was placed in another clean collection tube. Five hundred μl of Buffer AW2 was added to the spin column and centrifuged at 14,000 rpm for 3 minutes. The flow-through and collection tube were discarded and the spin column was placed into a clean 1.5 ml flat-top microcentrifuge tube. One hundred μl of elution Buffer AE was added to the spin column and allowed to incubate at room temperature for 1 minute. The sample was then centrifuged for 1 minute at 8,000 rpm. This step was repeated preserving the flow-through each time. This procedure yielded 200 μl of genomic DNA from each sample. Each microcentrifuge tube was labeled according to the individual and frozen at -20°C until use.

Genomic DNA Qualification. To determine the quality of the genomic DNA, each sample was examined by agarose gel electrophoresis. A 1% agarose gel was prepared

by melting 0.6 grams of agarose into 60 ml of 1x TAE buffer. The hot liquid mixture was poured into a form with a 15 well comb. Genomic DNA samples were then mixed with the tracking dye bromophenol blue (BPB) in the 1:6 ratio of 2 μl BPB and 10 μl genomic DNA. Once the gel cooled, the samples were loaded into individual wells of the gel. Lambda DNA was loaded into a separate well to serve as a quality standard. The gel was then submerged in 1x TAE buffer in an electrophoresis chamber. The gel was electrophoressed at 70 volts. When the tracking dye moved to the midway point of the gel, the gel was removed and stained in a 0.05 mg/ml ethidium bromide (EtBr) solution. EtBr binds to DNA and is fluorescent under ultra-violet light. After 1 hour of staining, the gel was viewed and photographed using a UVP EpiChemi II Darkroom.

Genomic DNA Quantification. Genomic DNA was quantified using a NanoDrop ND-1000 and the ND-1000 software. Nucleic acids were selected on the start menu of the software and one μl of water was loaded onto the nanodrop pedestal to initialize the machine. After initialization, the water was wiped off the pedestal using a kimwipe and the machine was zeroed out by loading one μl of Buffer AE onto the pedestal and clicking the zero button in the software. The Buffer AE was then wiped off the pedestal and each sample was measured separately wiping the pedestal between each pair of measurements. The desired DNA concentration for each sample was above 50 ng/ml with a 260/280 ratio of 1.70 or greater which indicated a satisfactory sample quality.

Gene Isolation and Amplification

The polymerase chain reaction (PCR) was used to isolate and amplify the genes to be analyzed for genetic differentiation. Primers for both mitochondrial and nuclear genes were developed through multiple sequence alignments using the program

Geneious Pro 4.6.1 (Table 2). Intron/exon structure was important to note when designing primers for the nuclear marker. For this project, introns were the target of amplification so primers that would bind to the exons surrounding the desired intron were designed. By doing this, a consistent PCR yield was obtained between the populations while capturing any genetic differentiation in the intron. Minimum requirements for primers were that they be at least 20 base pairs long, have a GC ratio of 50%, and have at least a 2 to 3 base pair GC clamp on the 3' end.

Table 2 Primers used for polymerase chain reactions

Gene	Primer	Product Length
CYTb	F: 5'-CCCACATCATACGAAAAGCACACCC-3' R: 5'-GGGTTTGAGTTAAGTCCTGTTGGG-3'	595 bp
TPI Intron 1	F: 5'-AGCCCAGCCATGATTAAGGACTGC-3' R: 5'-TTCTGACCGATGAGCTGTGGAGGG-3'	360 bp

PCR Preparation and Execution. PCR for both genes used Promega's PCR MASTER MIX reagent. This mixture contained the *Taq* DNA polymerase, deoxynucleoside triphosphates (dATP, dGTP, dCTP, dTTP), and Mg^{2+} necessary for the reaction. Reactions were mixed according to the protocol outlined in the usage information sheet provided with the master mix (Table 3). Reactions for the CYTb gene were prepared in PCR dome-capped strip tubes. TPI reactions were prepared in 96-well PCR plates with domed strip-caps. Every reaction set included one positive control reaction (beta-tubulin 1).

Table 3 PCR mixtures. Reactions were based on the 50 μl reaction mixture protocol provided with the Master Mix

H_2O	16 μl
Primers	5 μl
Master Mix	25 μl
Template	4 μl

PCR was performed using an Eppendorf *Mastercycler epgradient* thermal cycler equipped with a heated lid. For reaction cycles and temperatures refer to Table 4.

Table 4 PCR cycles and temperatures. Temperatures are in °C

	Cytochrome b	TPI Intron 1	
Initialization	94	94	
Denaturation	94	94	
Annealing	55	59.5	x30
Elongation	70	70	
Final Elongation	70	70	
Final Hold	4	4	

PCR Product Purification. Cytochrome b product was purified using a QUIAquick PCR Purification Kit. Buffer PBI and the PCR sample were added to a QIAquick spin column inserted into a 2 ml collection tube in a ratio of 5 to 1. The mixture was then centrifuged for 60 seconds at 13,000 rpm to bind the DNA to the spin column filter. All subsequent centrifugations were carried out in this manner. The flow-through was discarded and the spin column was placed back inside the same collection tube. The DNA was then washed by adding 0.75 ml Buffer PE to the spin column and centrifuging.

Again, the flow-through was discarded and the collection tube re-used. The spin column was centrifuged once more to remove any excess ethanol. The collection tube was discarded and the spin column was placed in a clean 1.5 ml microcentrifuge tube. For an increased concentration, the DNA was eluted using 30 μl Buffer EB. The sample was allowed to sit at room temperature for 1 minute and then centrifuged. This procedure yielded roughly 28 μl of purified PCR product. TPI product was sent to the DNA Analysis Facility on Science Hill at Yale University (Yale University 2009) for purification using EXO AP.

Sequencing. The purified cytochrome b samples were sequenced at the Molecular Biology Resource Facility at the University of Tennessee (UT), Knoxville (MBRF). Ten ngs of DNA per every 100 base pairs were require for sequencing. CYTb was 595 base pairs long and samples generally had a concentration of approximately 20 ng/μl. Therefore a typical sequencing reaction required approximately 3 μl of PCR template DNA. One μl of 5 μM primer was also required for each reaction. The PCR samples and primer were shipped to the UT MBRF where they were mixed adding Applied Biosystems' (ABI) Big Dye Terminator v3.1 Cycle Sequencing Mix in a 5x buffer. H_20 was then used to bring the reaction volume up to 12 μl. The sequencing reaction was performed using ABI's 9800 Fast Thermal Cycler. Removal of unused dideoxy-labeled nucleotides was done using Sephedex G50 fine size exclusion columns (University of Tennessee Knoxville 2009).

TPI PCR products were sent to the DNA Analysis Facility on Science Hill at Yale University for purification and sequencing. Five μl of PCR sample and 1 μl of 5 μM primer were requested for each sequencing reaction. Samples and primers were

shipped to Yale University where they were combined and Big Dye Terminator v3.1 was added. Mixed samples were then placed in a thermal cycler. Samples were then cleaned using Edge Sephedex DTR plates. Samples were then sequenced using the ABI 3730xl Genetic Analyzer. Sequence data were then post-processed with Peak trace Software by Nucleics (Yale University 2009).

Sequence Data Analysis

Sequence data were returned electronically from both sequencing facilities. Data were available in both sequence data and chromatogram file formats. The same analysis was applied to both genetic markers separately. Sequence data is available in APPENDIX C.

Sequence Alignment and Haplotype File

Chromatogram files were loaded into the program Geneious Pro v4.6.1 for multiple sequence alignment. Alignments were performed for each population separately. The alignments within populations were then scanned for ambiguities and mismatches between sequences. Ambiguities were evaluated by visually inspecting the chromatogram peak profile. Mismatches between sequences within populations were verified by visually inspecting the chromatogram peak profile. After alignments for each population had been performed, all sequences were aligned together using Clustal W alignment (Larkin et al. 2007). The ends of the sequences in this alignment were trimmed to the shortest sequence and then the alignment was exported to DNASP v4.90 as a fasta file. In DNASP, every sequence was assigned to a population and given a haplotype number. A haplotype is a unique sequence different from other

sequences by one or more nucleotides. If two sequences shared the same nucleotide sequence, they would have the same haplotype number regardless of the population of origin.

Sequence Data Analysis

Sequences were analyzed using the program Arlequin (Excoffier 2005). Arlequin was first used to identify which, and at what frequencies, haplotypes were found in each population by conducting a simple count. Arlequin was also used to calculate a fixation index (F_{ST}) (Wright 1984) analogue using analysis of molecular variance (AMOVA). Pairwise F_{ST} values were calculated in Arlequin by computing different indices of dissimilarities between pairs of populations (Excoffier 2005).

Analysis of Molecular Variance. F_{ST} is a measure of proportional reduction in expected heterozygosity found within subpopulations as compared to a hypothetical panmictic total population and has a magnitude ranging from 0 and 1. This number is used to indicate the level of population differentiation, with 1 being highly differentiated and 0 being no differentiation. AMOVA is an analysis developed around an analysis of variance (ANOVA) framework for the purpose of detecting differentiation at the intraspecific level (Excoffier 1992). It does this by analyzing nucleotide and haplotype diversities within, between, and among groups of subpopulations relating them in the form of squared Euclidean distances. The result of this analysis is an analogue to F_{ST} and is denoted as Φ_{ST}, which indicates the overall level of population differentiation at each marker. Because they are analogous, F_{ST} is used to identify the fixation indices. Migrants per generation is represented by the variable N_m and is directly related to F_{ST} through the equation $N_m = [(1/F_{ST})-1]/4$ (Wright 1984).

CHAPTER 3

RESULTS

Haplotype Frequencies

Four hundred eighty-three bases were resolved from 44 sequences of the Cytb mitochondrial gene. Haplotypes differed by only 1-2 bases resulting in sequence divergences between 0.21%-0.41%. The TPI nuclear intron consisted of 72 sequences each with 324 bases. Haplotypes differed by 2-11 bases resulting in sequence divergences between 0.62%-3.40% There were 6 haplotypes among subpopulations at the CYTb marker and 10 haplotypes at the TPI marker (Table 5). At the CYTb marker, one subpopulation, Limestone Cove (LC), had multiple haplotypes (5,6) while haplotypes 1 and 3 were shared across multiple subpopulations. The TPI marker

Table 5 Haplotype frequencies among populations of *P. yonahlossee*

CYTb - mitochondrial

Haplotype	BC	RM	MM	GM	IMT	MR	UM	RC	DR	RF	HM	LC
1	1.0	1.0			1.0		1.0				1.0	
2			1.0									
3				1.0		1.0		1.0		1.0		
4									1.0			
5												0.5
6												0.5

TPI - nuclear intron

Haplotype	BC	RM	MM	GM	IMT	MR	UM	RC	DR	RF	HM	LC
1				0.167						0.273		
2							0.5					
3				0.167								
4				0.167								
5						1.0						
6		1.0	1.0	0.5	1.0			1.0	1.0	0.273	1.0	1.0
7							0.5					
8										0.455		
9	0.857											
10	0.143											

revealed four subpopulations with multiple haplotypes (BC, GM, UM, RF), while haplotypes 1 and 6 were shared across multiple subpopulations. TPI haplotype six was by far the most widely shared haplotype among subpopulations.

Analysis of Molecular Variance

AMOVA results indicated an overall F_{ST} index of 0.955 for the CYTb marker and 0.633 for the TPI marker. Both F_{ST} indices were statistically significant with p-values of less than 0.05. Among subpopulations there was 95.52% variation at the CYTb marker and 63.38% variation at the TPI marker. Within subpopulation variation was 4.48% at CYTb and 36.62% at TPI. The population specific F_{ST} indices were also very high for all populations at both markers giving them all an estimated N_M of much less than 1 (Table 8). Estimates of migrants per generation were 0.001 for CYTb and 0.15 for TPI (Tables 6,7).

Table 6 Results of AMOVA for CYTb

	d.f.	Sum of Squares	Variance Components	Percentage of Variation
Among Populations	11	13.341	0.333	95.52
Within Populations	32	0.500	0.016	4.48
Total	43	13.841	0.349	
Fixation Index (F_{ST}) : 0.955		Estimated Migrants per Generation (Nm) : 0.001		

Table 7 Results of AMOVA for TPI

	d.f.	Sum of Squares	Variance Components	Percentage of Variation
Among Populations	11	14.125	0.199	63.38
Within Populations	60	6.903	0.115	36.62
Total	71	21.028	0.314	
Fixation Index (F_{ST}) : 0.634		Estimated Migrants per Generation (Nm) : 0.15		

Table 8 Population Specific F_{ST} indices and N_M

Population:	CYTb		TPI	
	F_{ST}	N_M	F_{ST}	N_M
Grandfather Mountain (GM)	0.968	0.001	0.479	0.272
Rocky Fork (RF)	0.968	0.001	0.486	0.264
Unaka Mountain (UM)	0.968	0.001	0.696	0.109
Mount Rogers (MR)	0.968	0.001	0.696	0.109
Limestone Cover (LC)	0.693	0.111	0.696	0.109
Iron Mountain TN (IMT)	0.968	0.001	0.696	0.109
Holston Mountain (HM)	0.968	0.001	0.696	0.109
Roan Mountain (RM)	0.968	0.001	0.696	0.109
Mount Mitchell (MM)	0.968	0.001	0.696	0.109
Dry Run (DR)	0.968	0.001	0.696	0.109
Rock Creek Park (RC)	0.968	0.001	0.696	0.109
Bat Cave (BC)	0.968	0.001	0.617	0.155

Pairwise F_{ST} Comparisons

Pairwise F_{ST} comparisons at the CYTb mitochondrial maker (Table 9) revealed several significantly differentiated populations. Bat Cave (BC) and Rocky Fork (RF) had seven statistically significant F_{ST} pairwise comparisons to other populations. Holston Mountain (HM) had six and the rest had four or fewer statistically significant F_{ST} comparisons.

At the TPI nuclear marker, pairwise F_{ST} comparisons (Table 10) again revealed several significantly differentiated populations. Mount Rogers (MR) had F_{ST} values that were statistically significant when compared to each of the other populations. Bat Cave had 10 and Rocky Fork had 9 statistically significant F_{ST} comparisons. Unaka Mountain (UM) and Holston Mountain had 6 and 5 statistically significant F_{ST} comparisons respectively. The other populations had 4 or fewer statistically significant comparisons.

When the results for both markers are combined into one table (Table 11), the consistencies between both markers are revealed. Using both markers combined, the Bat Cave and Rocky Fork populations have the most statistically significant F_{ST} comparisons. This supports the hypothesis that these populations are the most geographically isolated populations and is reflected in the observation of morphological variation in these populations.

Table 9 Pairwise F_{ST} comparisons between populations of *P. yonahlossee* for CYTb

	BC	RM	MM	GM	IMT	MR	UM	RC	DR	RF	HM	LC
BC	-	0.99	**0.01**	**0.05**	0.99	**0.04**	0.99	**0.02**	**0.00**	**0.02**	0.99	**0.04**
RM	0.00	-	0.08	0.10	0.99	**0.05**	0.99	0.81	0.10	**0.00**	0.99	0.12
MM	1.00	1.00	-	0.16	0.09	0.10	0.06	0.07	0.11	**0.03**	**0.00**	0.07
GM	1.00	1.00	1.00	-	0.11	0.99	0.12	0.99	0.16	0.99	**0.01**	0.06
IMT	0.00	0.00	1.00	1.00	-	0.09	0.99	0.10	0.07	**0.04**	0.99	0.16
MR	1.00	1.00	1.00	0.00	1.00	-	0.08	0.99	0.63	0.99	**0.00**	0.07
UM	0.00	0.00	1.00	1.00	0.00	1.00	-	0.11	**0.05**	**0.01**	0.99	0.11
RC	1.00	1.00	1.00	0.00	1.00	0.00	1.00	-	0.12	0.99	**0.00**	0.11
DR	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-	**0.04**	**0.01**	0.19
RF	1.00	1.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	-	**0.00**	0.12
HM	0.00	0.00	1.00	1.00	0.00	1.00	0.00	1.00	1.00	1.00	-	0.08
LC	0.77	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.72	0.87	-

CYTb pairwise F_{ST} below diagonal, significance values above diagonal ($P < 0.05$)

Table 10 Pairwise F_{ST} comparisons between populations of *P. yonahlossee* for TPI

	BC	RM	MM	GM	IMT	MR	UM	RC	DR	RF	HM	LC
BC	-	**0.00**	**0.00**	**0.00**	**0.01**	**0.00**	0.09	**0.00**	**0.00**	**0.00**	**0.00**	**0.01**
RM	0.85	-	0.99	0.23	0.99	**0.00**	**0.02**	0.99	0.99	**0.01**	0.99	0.99
MM	0.85	0.00	-	0.23	0.99	**0.00**	**0.03**	0.99	0.99	**0.00**	0.99	0.99
GM	0.47	0.20	0.20	-	0.47	**0.00**	0.59	0.18	0.23	0.17	**0.03**	0.49
IMT	0.79	0.00	0.00	0.40	-	**0.00**	0.08	0.99	0.99	**0.05**	0.99	0.99
MR	0.86	1.00	1.00	0.63	1.00	-	**0.03**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
UM	0.56	0.81	0.81	0.14	0.65	0.83	-	0.06	**0.05**	**0.05**	**0.00**	0.15
RC	0.83	0.00	0.00	0.16	0.00	1.00	0.77	-	0.99	**0.02**	0.99	0.99
DR	0.82	0.00	0.00	0.11	0.00	1.00	0.72	0.00	-	**0.05**	0.99	0.99
RF	0.47	0.42	0.42	0.80	0.32	0.59	0.22	0.40	0.36	-	**0.00**	0.16
HM	0.90	0.00	0.00	0.35	0.00	1.00	0.90	0.00	0.00	0.53	-	0.99
LC	0.79	0.00	0.00	0.40	0.00	1.00	0.65	0.00	0.00	0.32	0.00	-

TPI pairwise F_{ST} below diagonal, significance values above diagonal ($P < 0.05$)

Table 11 Statistically significant pairwise F_{ST} values for both markers

	BC	RM	MM	GM	IMT	MR	UM	RC	DR	RF	HM	LC
BC	-			+		+		+	+	+		+
RM		-				+				+		
MM			-							+		
GM	+			-							+	
IMT					-					+		
MR	+	+				-					+	
UM							-		+	+		
RC	+							-				
DR	+						+		-	+		
RF	+	+	+		+		+		+	-	+	
HM				+		+				+	-	
LC	+											-

Graph expanded above and below diagonal for easier viewing

CHAPTER 4

DISCUSSION

As with similar studies of this nature, such as the work done by Drew et al. in 2003 with the fisher *Martes pennanti,* the low level of sequence divergence among *P. yonahlossee* populations (CYTb = 0.21%-0.41%; TPI = 0.62%-3.40%) did not allow for phylogenetic analysis. Genetic distance matrices created using the Tajima-Nei model in Mega (Kumar et al. 2008) did not reveal any substantial hierarchal genetic structure (APPENDIX B). Hierarchal genetic structure was, however, detectable at the population level using AMOVA.

At the mitochondrial cytb marker, more variation is observed between populations when compared to the variation within populations. At the nuclear tpi marker, there was less variation between populations and more within populations than at the mitochondrial locus. These patterns of variation are reflected in the distribution of the haplotypes..

The overall and population specific F_{ST} values indicate that the yonahlossee salamander consists of many geographically isolated subpopulations. By equating the overall F_{ST} to the estimated number of migrants per generation (N_m), both the mitochondrial and nuclear markers estimate an N_m of much less than 1.0 (0.001 and 0.15 respectively). This suggests that overall gene flow, even male-mediated, has been reduced to a level that may allow for genetic drift or local adaptation leading to genetic differentiation among subpopulations. Also, the low estimated migration rates indicate that there is likely to be demographic independence among subpopulations.

Pairwise F_{ST} comparisons revealed concurrent statistically significant F_{ST} values at both markers. The Rocky Fork and Bat Cave populations were statistically significant at both markers when compared to seven and six other populations respectively. The Dry Run, Mount Rogers, and Holston Mountain populations were each statistically significant at both markers for three populations comparisons. The same was true for the Grandfather Mountain, Roan Mountain, and Unaka Mountain populations when compared to two populations. The Mount Mitchell, Iron Mountain, Rock Creek, and Limestone Cove populations had only one concurrent statistically significant pairwise F_{ST} comparison. These significant F_{ST} comparisons at both markers suggest that the Bat Cave and Rocky Fork populations are the most genetically differentiated subpopulations in this species.

Based on sequence divergence of the mitochondrial cytb gene (0.21%-0.41%) an estimated divergence time falls sometime between 21,000 to 41,000 years ago, assuming 10% sequence divergence per million years (Aquadro and Greenberg 1982). These dates correspond to the end of the last Pleistocene glacial maximum. This same time frame has been shown to have greatly affected differentiation within other species (Zamudio and Savage 2003). The distribution of the yonahlossee populations post-glaciation may be “islands” separated from each other by lower elevation habitat. During glaciation, the climate may have cooled enough to allow populations of the yonahlossee to achieve a much lower minimum habitat elevation. This would potentially have allowed their distribution to be much more continuous with relatively little intraspecific genetic differentiation by the end of glaciation and could explain the apparent lack of a phylogeographic pattern among present yonahlossee populations.

As glaciation receded with climate warming, an increase in minimum habitat elevation produced the mountain-top “island” population structure seen today.

The current distribution appears to be a metapopulation. A metapopulation, consists of a “population of populations” (Levins 1969). Each population is generally independent of the other and is at risk of extinction as a result of stochastic events. When migration is facilitated, there is the potential for a “rescue effect” to occur when individuals from source populations immigrate to potential sink populations. With the disjunct nature of yonahlossee populations and the threat of habitat loss in the southeastern U.S., the migration levels detected in this study indicate that individual yonahlossee populations are demographically isolated and at potential risk of extinction because a “rescue effect” is not likely to occur. This could potentially result in the loss of locally adapted and evolutionarily significant subpopulations.

In conclusion, it appears the yonahlossee salamander has a metapopulation structure consisting of relict subpopulations that may have been more continuously distributed during much of the pleistocene. It is proposed that all subpopulations of the yonahlossee salamander should be considered as significant conservation units for management purposes.

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APPENDICES

APPENDIX A: Specimens

Table 12 Detailed list of individual specimens

ID#	Date	Total Length (mm)	Snout/ Vent Length (mm)	Weight (g)	Location	State	ng/ul
BC-1	8.28.2008	123.7	62.8	6	Bat Cave Area	NC	171.6
BC-2	8.28.2008	137.8	65.9	6.9	Bat Cave Area	NC	148.8
BC-3	8.28.2008	137.8	58.3	5.5	Bat Cave Area	NC	120.3
BC-4	8.28.2008	127.2	69.6	7.1	Bat Cave Area	NC	153.5
BC-5	8.28.2008	163.5	70	9.7	Bat Cave Area	NC	146.7
BC-6	8.28.2008	144.4	69.8	8.3	Bat Cave Area	NC	190.8
BC-7	8.28.2008	139.7	70	7.8	Bat Cave Area	NC	156.2
BC-8	8.28.2008	90.5	69.3	6.3	Bat Cave Area	NC	177.6
BC-9	8.28.2008	150.1	64.9	7.8	Bat Cave Area	NC	113.5
BC-10	8.28.2008	154.3	67	6.6	Bat Cave Area	NC	117.7
BC-11	8.28.2008	87	56.8	4.5	Bat Cave Area	NC	234.0
BC-12	8.28.2008	102	55.1	3.3	Bat Cave Area	NC	124.2
BC-13	8.28.2008	77.8	58.6	4.2	Bat Cave Area	NC	171.5
BC-14	8.28.2008	135.8	59.9	5	Bat Cave Area	NC	
CCB-M1	7.16.2008	-	-	-	Camp Creek Bald	TN	87.2
CCB-M2	7.16.2008	-	-	-	Camp Creek Bald	TN	97.9
CCB-M3	7.16.2008	-	-	-	Camp Creek Bald	TN	184.5
DR-1	7.14.2008	141.9	50.1	8.2	Dry Run - Iron Mountains	VA	73.4
DR-2	7.14.2008	133.1	55.3	5.1	Dry Run - Iron Mountains	VA	110.7
DR-3	7.14.2008	51.5	28.9	0.7	Dry Run - Iron Mountains	VA	128.1
DR-4	7.14.2008	103.5	47.3	2.7	Dry Run - Iron Mountains	VA	76.8
DR-5	7.14.2008	37.6	19.2	0.3	Dry Run - Iron Mountains	VA	135.6
GM-1	7.5.2008	146	67	-	Grandfather Mountain	NC	114.8
GM-2	7.5.2008	135	65	-	Grandfather Mountain	NC	112.9
GM-3	7.5.2008	175	75	-	Grandfather Mountain	NC	101.2
GM-4	9.1.2008	109.1	54.9	4.2	Grandfather Mountain	NC	154.9
GM-5	9.1.2008	141.8	57.8	5.4	Grandfather Mountain	NC	137.8
GM-6	9.1.2008	112.8	56.9	4.2	Grandfather Mountain	NC	103.7
HOM-1	5.20.2008	82.7	35	1.6	Holston Mountain	TN	63.0
HOM-2	5.20.2008	143.9	64.1	6.2	Holston Mountain	TN	42.0
HOM-3	5.20.2008	130.5	57	3.7	Holston Mountain	TN	78.2
HOM-4	5.20.2008	88.1	52.7	2	Holston Mountain	TN	72.8
HOM-5	5.20.2008	137.4	71.9	5.7	Holston Mountain	TN	69.9
HOM-6	5.20.2008	85	46.7	1.9	Holston Mountain	TN	121.8
HOM-7	5.20.2008	140.8	57.4	5	Holston Mountain	TN	96.7

Table 12 Detailed list of individual specimens

ID#	Date	Total Length (mm)	Snout/ Vent Length (mm)	Weight (g)	Location	State	ng/ul
HOM-8	5.20.2008	98.9	57.8	3.4	Holston Mountain	TN	114.5
HOM-9	5.20.2008	108	45.7	2.5	Holston Mountain	TN	62.0
HOM-10	5.20.2008	92.7	43.3	1.8	Holston Mountain	TN	116.6
HOM-11	5.20.2008	76.7	42.3	1.3	Holston Mountain	TN	88.5
HOM-12	5.20.2008	62	40.7	1	Holston Mountain	TN	91.3
IMT-1	8.27.2008	123.9	55.9	4.5	Iron Mountain	TN	75.8
IMT-2	8.27.2008	71.7	37.9	1.2	Iron Mountain	TN	84.7
IMT-3	8.27.2008	131.3	57.9	5.4	Iron Mountain	TN	154.7
IMT-G1	8.27.2008	-	-	-	Iron Mountain	TN	67.4
IMT-G2	8.27.2008	-	-	-	Iron Mountain	TN	91.7
IMT-G3	8.27.2008	-	-	-	Iron Mountain	TN	89.1
LC-Glut	6.24.2008	-	-	-	Limestone Cove	TN	77.4
LC-1	6.24.2008	129.5	63.7	-	Limestone Cove	TN	133.0
LC-2	6.25.2008	155.5	75.9	7.8	Limestone Cove	TN	127.0
LC-3	6.30.2008	148.5	58.5	5.9	Limestone Cove	TN	117.2
MM-1	7.7.2008	166.8	70.3	12	Mount Mitchell	NC	72.7
MM-2	7.7.2008	153.6	67	6.7	Mount Mitchell	NC	80.4
MM-3	7.7.2008	160.3	67.5	7.7	Mount Mitchell	NC	64.0
MM-4	7.7.2008	141.7	68.9	8.7	Mount Mitchell	NC	77.8
MM-5	7.7.2008	173.8	77.5	10.9	Mount Mitchell	NC	58.0
MM-6	7.7.2008	112.5	52.7	3.5	Mount Mitchell	NC	53.8
MR-1	5.17.2008	147.9	61.6	5.4	Mt. Rogers	VA	50.0
MR-2	5.17.2008	80.2	40.5	1.4	Mt. Rogers	VA	69.0
MR-3	5.17.2008	111.4	50.5	2	Mt. Rogers	VA	93.0
MR-4	5.17.2008	102	48.6	1.8	Mt. Rogers	VA	90.0
MR-5	6.10.2008	153.4	78.8	-	Mt. Rogers	VA	83.0
MR-6	6.10.2008	143.7	69.1	8.4	Mt. Rogers	VA	49.0
MR-7	6.10.2008	153.2	64.2	8.5	Mt. Rogers	VA	81.0
RM-1	5.23.2008	153.4	65.7	5.9	Roan Mountain	TN	125.3
RM-2	5.23.2008	141.2	72.7	8.1	Roan Mountain	TN	140.6
RM-3	5.23.2008	120.3	48.8	3.1	Roan Mountain	TN	162.1
RM-4	5.23.2008	126	59.4	5	Roan Mountain	TN	172.5
RM-5	5.23.2008	144	73.3	7.2	Roan Mountain	TN	152.9
RM-6	9.6.2008	144.1	64.1	5.6	Roan Mountain	TN	118.0
RC-1	6.28.2008	159.9	67.8	9.7	Rock Creek Park	TN	154.9
RC-2	6.28.2008	53.8	24.7	0.6	Rock Creek Park	TN	75.2
RC-3	6.28.2008	51	25.7	0.4	Rock Creek Park	TN	89.8
RC-4	7.3.2008	157	66.9	8.3	Rock Creek Park	TN	95.9
RC-5	7.3.2008	137.4	64.5	9.4	Rock Creek Park	TN	146.4
RF-G1	6.11.2008	-	-	-	Rock Fork	TN	236.5
RF-1	6.11.2008	136.8	70.6	9.6	Rock Fork	TN	146.8

Table 12 Detailed list of individual specimens

ID#	Date	Total Length (mm)	Snout/ Vent Length (mm)	Weight (g)	Location	State	ng/ul
RF-2	6.11.2008	153.2	70.8	9.9	Rock Fork	TN	165.8
RF-3	6.11.2008	153.4	71.8	8.1	Rock Fork	TN	155.8
RF-4	6.11.2008	92.1	39.9	2	Rock Fork	TN	115.9
RF-5	6.11.2008	138.9	58.5	7.1	Rock Fork	TN	161.6
RF-6	6.11.2008	117.3	52.6	4.6	Rock Fork	TN	67.6
RF-7	6.11.2008	123.8	53.5	3.8	Rock Fork	TN	86.4
RF-8	6.11.2008	147.9	67.7	8.1	Rock Fork	TN	97.1
RF-9	6.11.2008	153.8	76.7	10.3	Rock Fork	TN	137.0
RF-10	6.11.2008	140.5	67.2	6.2	Rock Fork	TN	152.5
RF-11	6.11.2008	144.4	62.5	5.8	Rock Fork	TN	192.2
UM-1	6.25.2008	57.7	27.2	0.5	Unaka Mountain	TN	119.7
UM-2	6.25.2008	63.8	31.8	0.9	Unaka Mountain	TN	138.6
UM-3	6.25.2008	87.3	44.1	2.5	Unaka Mountain	TN	193.2

APPENDIX B: Genetic Distances

Table 13 Genetic distances for CYTB

	GM	RF	UM	MR	LC	IMT	HM	RM	MM	DR	RC	BC
GM		0.003	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.01
RF	0.006		0.004	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.009
UM	0.008	0.01		0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.01
MR	0.003	0.005	0.007		0	0	0	0	0	0	0	0.01
LC	0.003	0.005	0.007	0		0	0	0	0	0	0	0.01
IMT	0.003	0.005	0.007	0	0		0	0	0	0	0	0.01
HM	0.003	0.005	0.007	0	0	0		0	0	0	0	0.01
RM	0.003	0.005	0.007	0	0	0	0		0	0	0	0.01
MM	0.003	0.005	0.007	0	0	0	0	0		0	0	0.01
DR	0.003	0.005	0.007	0	0	0	0	0	0		0	0.01
RC	0.003	0.005	0.007	0	0	0	0	0	0	0		0.01
BC	0.032	0.029	0.035	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	

Genetic distances were calculated using the Tajima-Nei method.
Genetic distances are below the diagonal, standard error is above the diagonal.

Table 14 Genetic distances for CYTB

	BC	RM	MM	GM	IMT	MR	UM	RC	DR	RF	HM	LC
BC		0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.00
RM	0.000		0.002	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.002
MM	0.002	0.00		0.002	0.002	0.002	0.002	0.002	0.003	0.002	0.002	0.00
GM	0.000	0.000	0.002		0	0	0	0	0	0	0	0.00
IMT	0.000	0.000	0.002	0		0	0	0	0	0	0	0.00
MR	0.000	0.000	0.002	0	0		0	0	0	0	0	0.00
UM	0.000	0.000	0.002	0	0	0		0	0	0	0	0.00
RC	0.000	0.000	0.002	0	0	0	0		0	0	0	0.00
DR	0.002	0.002	0.004	0	0	0	0	0		0	0	0.00
RF	0.000	0.000	0.002	0	0	0	0	0	0		0	0.00
HM	0.000	0.000	0.002	0	0	0	0	0	0	0		0.00
LC	0.003	0.003	0.005	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	

Genetic distances were calculated using the Tajima-Nei method.
Genetic distances are below the diagonal, standard error is above the diagonal.

APPENDIX C: Collection Form and Nucleotide Sequences

SPECIES:_____ **DATE:**_____ **ID#:**_____

TL:_____ **SVL:**_____ **WT:**_____ **SEX:**_____

LOCATION:_____ **OBS:**_____

GPS:_____ **ELV:**_____

JPEG#:_____

NOTES:_____

_____

_____

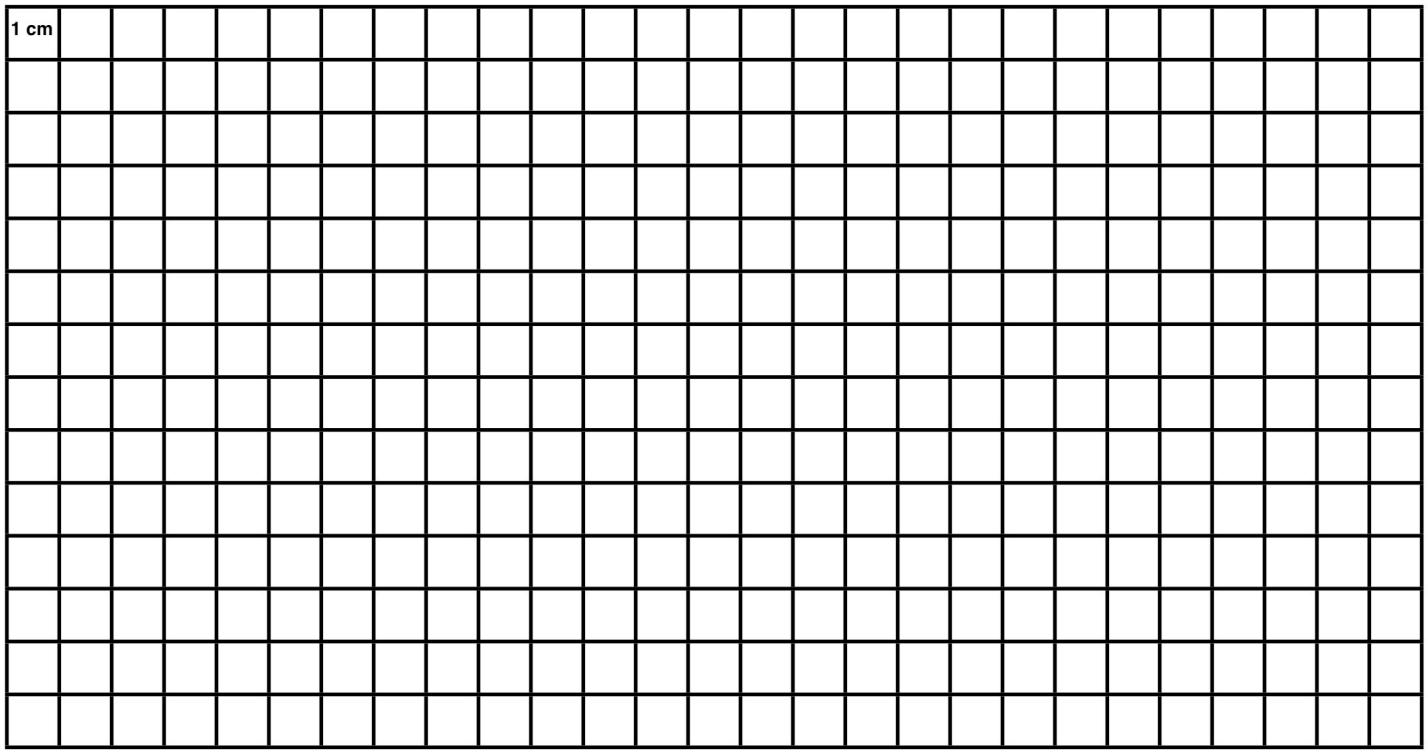


Figure 1 Collection Data Sheet - Sample of collection data sheet and photography grid. Not to scale.

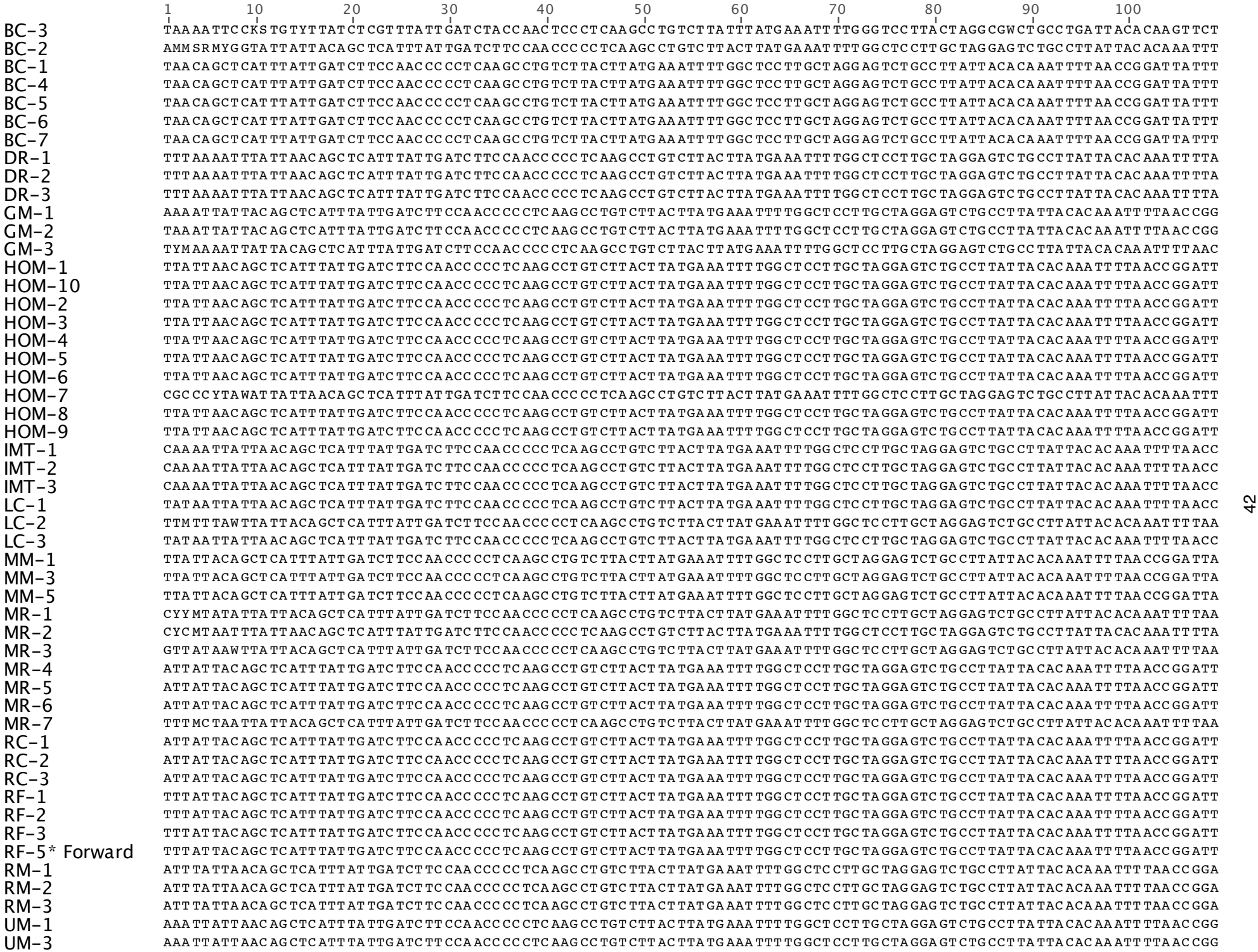
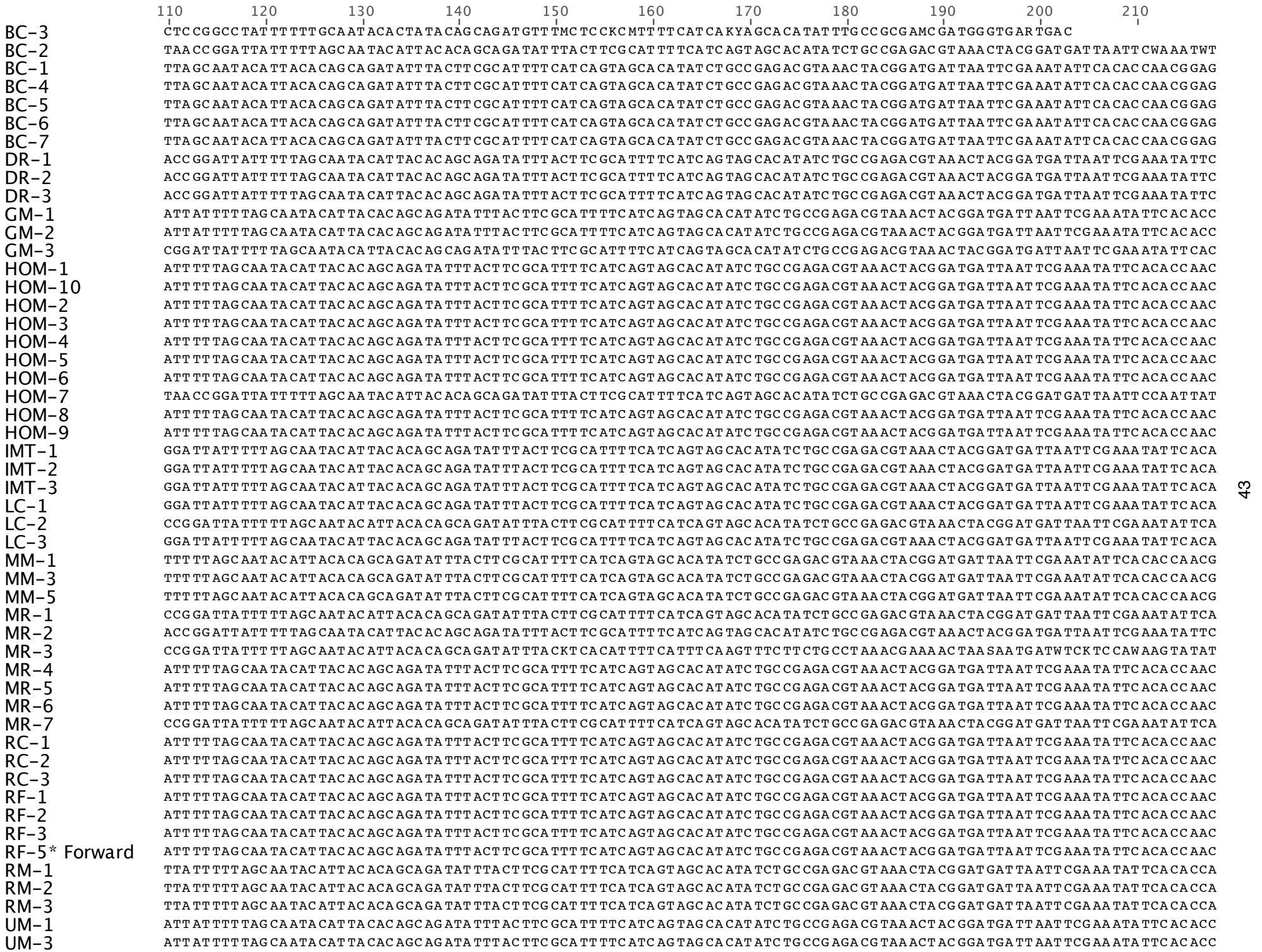


Table 15 CYTb sequence data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DR-2 ACCGGATTATTTTTAGCAATACATTACACAGCAGATATTTACTTCGCATTTTCATCAGTAGCACATATCTGCCGAGACGTAAACTACGGATGATTAATTCGAAATATTC
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GM-3 CGGATTATTTTTAGCAATACATTACACAGCAGATATTTACTTCGCATTTTCATCAGTAGCACATATCTGCCGAGACGTAAACTACGGATGATTAATTCGAAATATTCAC
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RF-5* Forward ATTTTTAGCAATACATTACACAGCAGATATTTACTTCGCATTTTCATCAGTAGCACATATCTGCCGAGACGTAAACTACGGATGATTAATTCGAAATATTCACACCAAC
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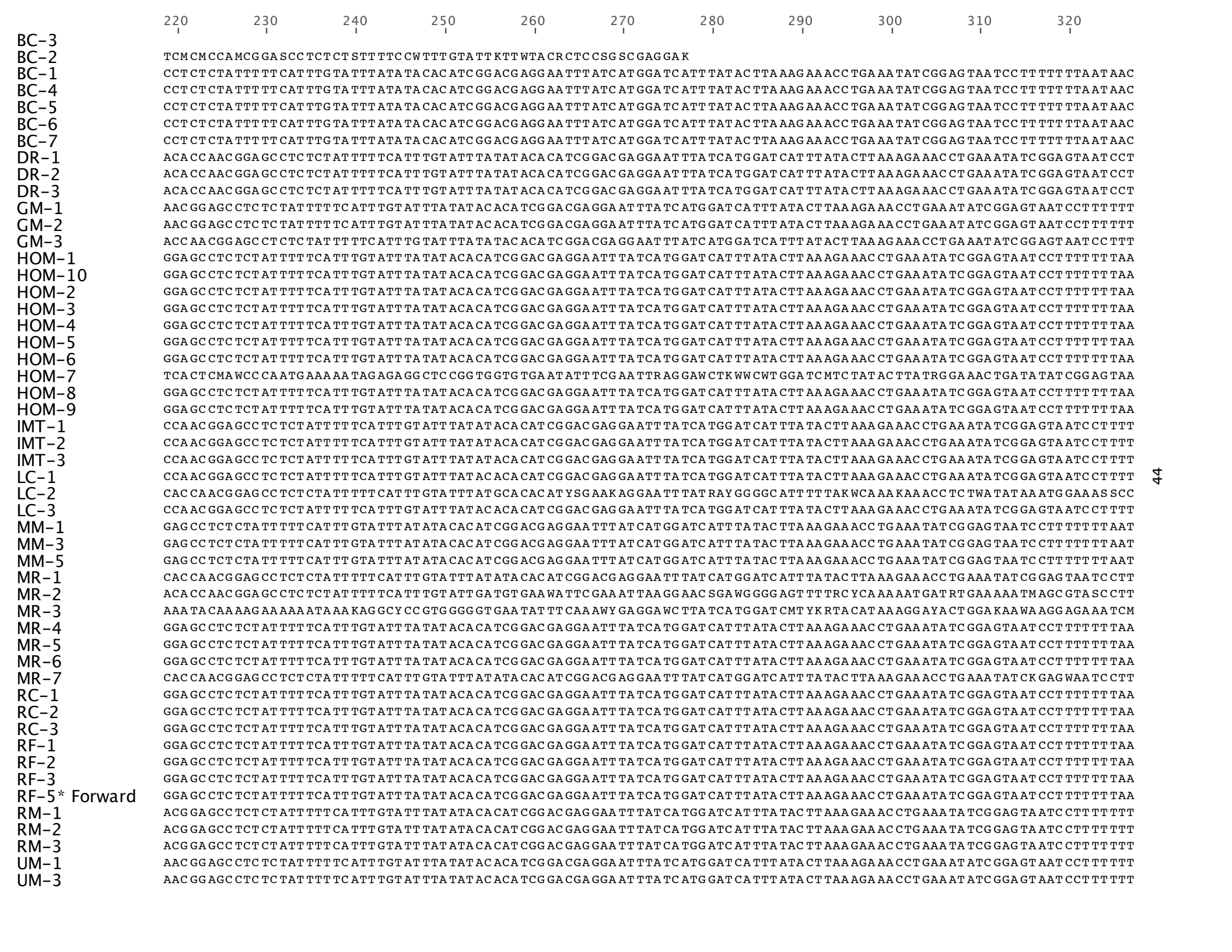
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RF-5* Forward GGAGCCTCTCTATTTTTCATTTGTATTTATATACACATCGGACGAGGAATTTATCATGGATCATTTATACTTAAAGAAACCTGAAATATCGGAGTAATCCTTTTTTTAA
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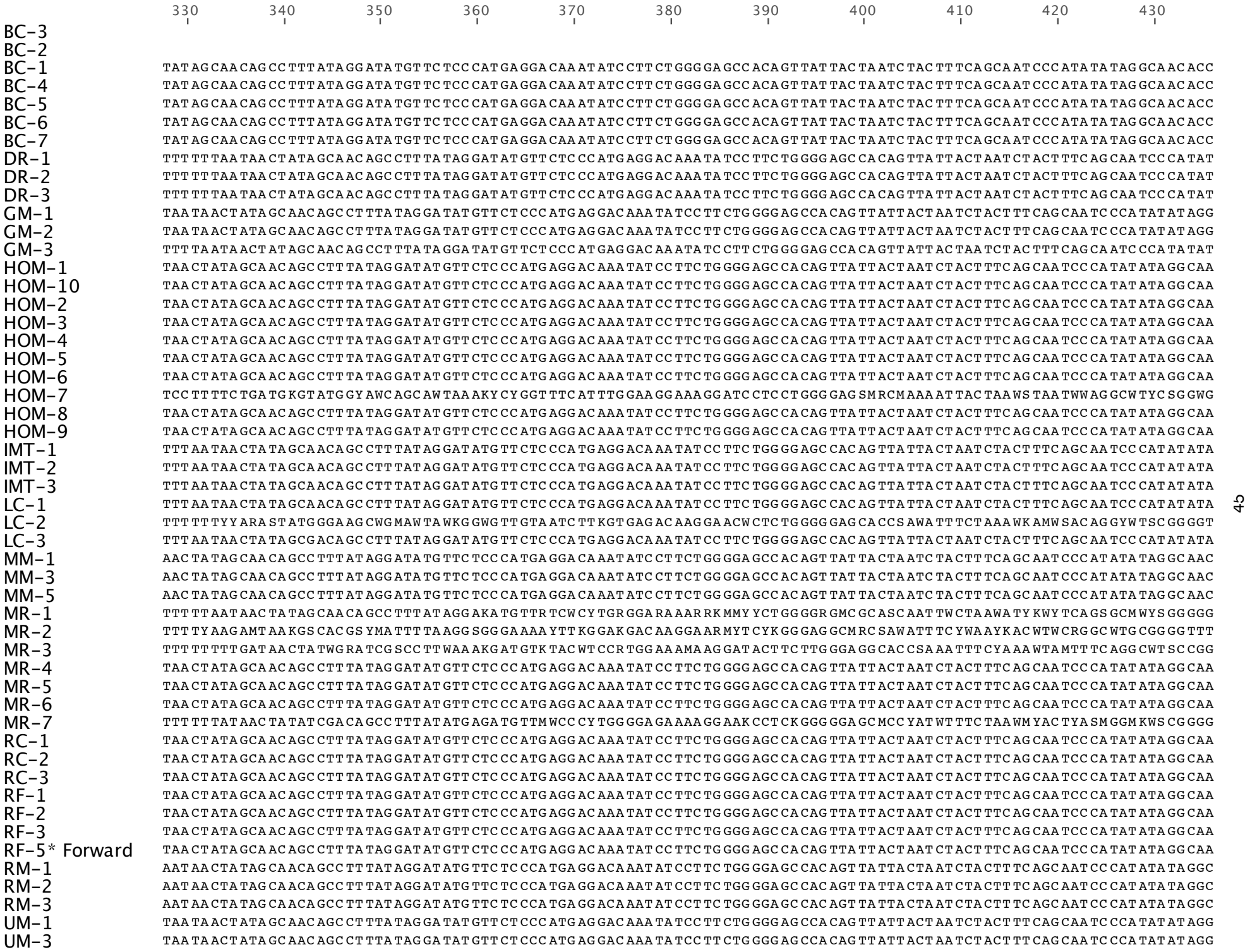
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BC-3
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MR-4 TAACTATAGCAACAGCCTTTATAGGATATGTTCTCCCATGAGGACAAATATCCTTCTGGGGAGCCACAGTTATTACTAATCTACTTTCAGCAATCCCATATATAGGCAA
MR-5 TAACTATAGCAACAGCCTTTATAGGATATGTTCTCCCATGAGGACAAATATCCTTCTGGGGAGCCACAGTTATTACTAATCTACTTTCAGCAATCCCATATATAGGCAA
MR-6 TAACTATAGCAACAGCCTTTATAGGATATGTTCTCCCATGAGGACAAATATCCTTCTGGGGAGCCACAGTTATTACTAATCTACTTTCAGCAATCCCATATATAGGCAA
MR-7 TTTTTTATAACTATATCGACAGCCTTTATATGAGATGTTMWCCCYTGGGGAGAAAAGGAAKCCTCKGGGGGAGCMCCYATWTTTCTAAWMYACTYASMGGMKWSCGGGG
RC-1 TAACTATAGCAACAGCCTTTATAGGATATGTTCTCCCATGAGGACAAATATCCTTCTGGGGAGCCACAGTTATTACTAATCTACTTTCAGCAATCCCATATATAGGCAA
RC-2 TAACTATAGCAACAGCCTTTATAGGATATGTTCTCCCATGAGGACAAATATCCTTCTGGGGAGCCACAGTTATTACTAATCTACTTTCAGCAATCCCATATATAGGCAA
RC-3 TAACTATAGCAACAGCCTTTATAGGATATGTTCTCCCATGAGGACAAATATCCTTCTGGGGAGCCACAGTTATTACTAATCTACTTTCAGCAATCCCATATATAGGCAA
RF-1 TAACTATAGCAACAGCCTTTATAGGATATGTTCTCCCATGAGGACAAATATCCTTCTGGGGAGCCACAGTTATTACTAATCTACTTTCAGCAATCCCATATATAGGCAA
RF-2 TAACTATAGCAACAGCCTTTATAGGATATGTTCTCCCATGAGGACAAATATCCTTCTGGGGAGCCACAGTTATTACTAATCTACTTTCAGCAATCCCATATATAGGCAA
RF-3 TAACTATAGCAACAGCCTTTATAGGATATGTTCTCCCATGAGGACAAATATCCTTCTGGGGAGCCACAGTTATTACTAATCTACTTTCAGCAATCCCATATATAGGCAA
RF-5* Forward TAACTATAGCAACAGCCTTTATAGGATATGTTCTCCCATGAGGACAAATATCCTTCTGGGGAGCCACAGTTATTACTAATCTACTTTCAGCAATCCCATATATAGGCAA
RM-1 AATAACTATAGCAACAGCCTTTATAGGATATGTTCTCCCATGAGGACAAATATCCTTCTGGGGAGCCACAGTTATTACTAATCTACTTTCAGCAATCCCATATATAGGC
RM-2 AATAACTATAGCAACAGCCTTTATAGGATATGTTCTCCCATGAGGACAAATATCCTTCTGGGGAGCCACAGTTATTACTAATCTACTTTCAGCAATCCCATATATAGGC
RM-3 AATAACTATAGCAACAGCCTTTATAGGATATGTTCTCCCATGAGGACAAATATCCTTCTGGGGAGCCACAGTTATTACTAATCTACTTTCAGCAATCCCATATATAGGC
UM-1 TAATAACTATAGCAACAGCCTTTATAGGATATGTTCTCCCATGAGGACAAATATCCTTCTGGGGAGCCACAGTTATTACTAATCTACTTTCAGCAATCCCATATATAGG
UM-3 TAATAACTATAGCAACAGCCTTTATAGGATATGTTCTCCCATGAGGACAAATATCCTTCTGGGGAGCCACAGTTATTACTAATCTACTTTCAGCAATCCCATATATAGG
4b


440
450
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470
480
490
500
505
BC-3
BC-2
BC-1 CTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTTATCCCGAG
BC-4 CTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTTATCCCGAG
BC-5 CTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTTATCCCGAG
BC-6 CTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTTATCCCGAG
BC-7 CTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTTATCCCGAG
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DR-2 ATAGGCAACACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGGGGACCAAGCCACCTTATTCCCGAG
DR-3 ATAGGCAACACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGGGGACCAAGCCACCTTATTCCCGAG
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GM-2 CAACACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTTATCCCG
GM-3 AGGCAACACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTTATCCCGA
HOM-1 CACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTT
HOM-10 CACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTT
HOM-2 CACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTT
HOM-3 CACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTT
HOM-4 CACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTT
HOM-5 CACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTT
HOM-6 CACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTT
HOM-7 TTGGGARAMMACCTAATKMKYTGRAAYTWRAGGGWAKTMYAGGGKGKGCTTTYCGYCTGATGCGGG
HOM-8 CACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTT
HOM-9 CACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTT
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IMT-2 GGCAACACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTTTATCCCGA
IMT-3 GGCAACACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTTTATCCCGA
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LC-3 GGCAACACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTTAT
MM-1 ACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTTATTCCCGA
MM-3 ACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTTATTCCCGA
MM-5 ACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTTATTCCCGA
MR-1 TKTGAGRCACCCYMKWKSTMTGAGWATRAGGGGGRGAYTCAGGGGGGRCCAYCCCCYCWGAYCGSGR
MR-2 GGRMGACCMCCTAATKCYWTGRAAYTGRAGGGGAATCYAMGGTGAGCAWWCCGWCTGATGCSGG
MR-3 GTTTGGGSAACMMCCTAATTCTATGAAWCTGAAGGGGATTCTWGGGGGASCYARCCACCTGWTSCCGG
MR-4 CACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTTATCCCG
MR-5 CACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTTATCCCG
MR-6 CACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTTATCCCG
MR-7 TKGAGGRSACMMWCAARTGMTRTGAATMTGRGGGAGASTCSGGGGGGGASCWAMCCMWCMGATSCSGGA
RC-1 CACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTTATCCCGAG
RC-2 CACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTTATCCCGAG
RC-3 CACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTTATCCCGAG
RF-1 CACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTTA
RF-2 CACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTTA
RF-3 CACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTTA
RF-5* Forward CACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTTA
RM-1 AACACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTTATATCCCGAG
RM-2 AACACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTTATATCCCGAG
RM-3 AACACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTTATATCCCGAG
UM-1 CAACACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTTATCCCGAG
UM-3 CAACACCCTAGTTCTATGAATCTGAGGGGGATTCTCAGTGGACCAAGCCACCTTATCCCGAG

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BC-10-TPI_INT1_f.ab1  CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
BC-11-TPI_INT1_f.ab1  CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
BC-12-TPI_INT1_f.ab1  CCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTAAGACTGGAC
BC-13-TPI_INT1_f.ab1  CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
BC-2-TPI_INT1_f.ab1   CCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTAAGACTGGAC
BC-3-TPI_INT1_f.ab1   CCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTAAGACTGGAC
BC-4-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
BC-5-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
BC-6-TPI_INT1_f.ab1   CCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTAAGACTGGAC
BC-7-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
BC-8-TPI_INT1_f.ab1   CCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTAAGACTGGAC
BC-9-TPI_INT1_f.ab1   CCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTAAGACTGGAC
DR-1-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
DR-2-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
DR-3-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
DR-4-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
DR-5-TPI_INT1_f.ab1   GGGTCATATCTGGCTGAGATCCTCTGAGAGGAGGATGTCTTTGGGGAGTCGGACAAGGTAAAAACCAACCTTATTCTTCTTG
GM-1-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
GM-2-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
GM-3-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
GM-4-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
GM-5-TPI_INT1_f.ab1   CTTCTTGGGGCTCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGA
GM-6-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
HM-1-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
HM-10-TPI_INT1_f.ab1  CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
HM-11-TPI_INT1_f.ab1  CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
HM-12-TPI_INT1_f.ab1  CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
HM-2-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
HM-3-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
HM-4-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
HM-5-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
HM-6-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
HM-7-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
HM-8-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
HM-9-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
IMT-1-TPI_INT1_f.ab1  CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
IMT-2-TPI_INT1_f.ab1  CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
IMT-3-TPI_INT1_f.ab1  CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
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LC-2-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
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MM-1-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
MM-2-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
MM-3-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
MM-4-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
MM-5-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
MM-6-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
MR-1-TPI_INT1_f.ab1   CTTCTTGGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTT
MR-2-TPI_INT1_f.ab1   CTTCTTGGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTT
MR-3-TPI_INT1_f.ab1   CTTCTTGGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTT
MR-4-TPI_INT1_f.ab1   CTTCTTGGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTT
MR-5-TPI_INT1_f.ab1   CTTCTTGGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTT
MR-6-TPI_INT1_f.ab1   CTTCTTGGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTT
MR-7-TPI_INT1_f.ab1   CTTCTTGGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTT
RC-1-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
RC-2-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
RC-3-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
RC-4-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
RC-5-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
RF-1-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
RF-10-TPI_INT1_f.ab1  CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
RF-11-TPI_INT1_f.ab1  CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
RF-2-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
RF-3-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
RF-4-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
RF-5-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
RF-6-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
RF-7-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
RF-8-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
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RM-1-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
RM-2-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
RM-3-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
RM-4-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
RM-5-TPI_INT1_f.ab1   CTTCTTGGCCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTA
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UM-1-TPI_INT1_f.ab1   CTTCTTGGCCTCTGAGAGGAGGCATGTCTTTGGGGAGTCGGACGAGGTAAGAACCAACCTATATTCTTCTTGTGGGATTTAA
UM-2-TPI_INT1_f.ab1   GGATCGGGATGTCTCTGCCCCACGGAGGGGGTGGTCTTTGGGAACCGAACAAGGAAAAACCCCCCTATTTTCTTCTTGTGGA
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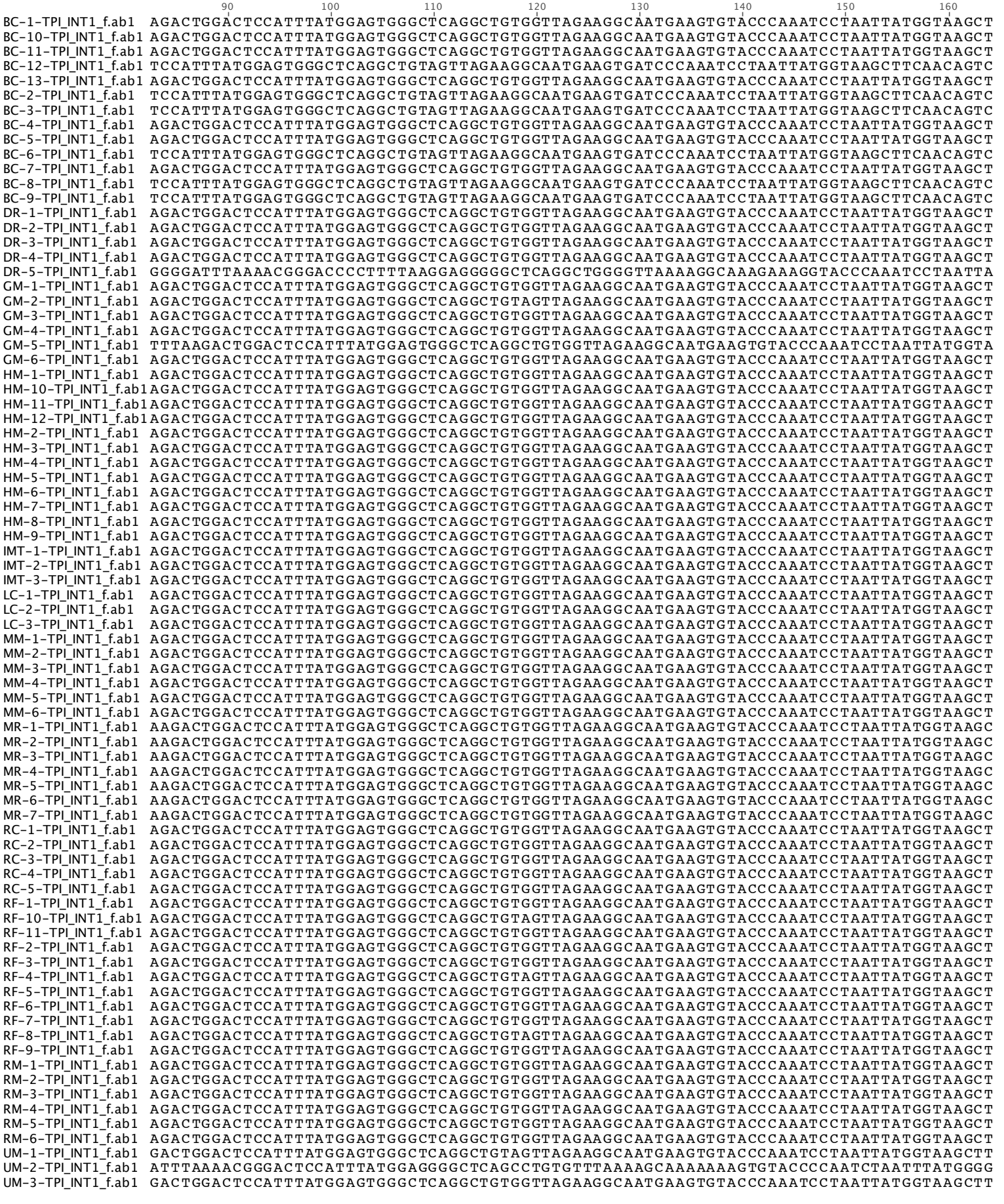
Table 16 TPI sequence data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BC-13-TPI_INT1_f.ab1 AGACTGGACTCCATTTATGGAGTGGGCTCAGGCTGTGGTTAGAAGGCAATGAAGTGTACCCAAATCCTAATTATGGTAAGCT
BC-2-TPI_INT1_f.ab1 TCCATTTATGGAGTGGGCTCAGGCTGTAGTTAGAAGGCAATGAAGTGATCCCAAATCCTAATTATGGTAAGCTTCAACAGTC
BC-3-TPI_INT1_f.ab1 TCCATTTATGGAGTGGGCTCAGGCTGTAGTTAGAAGGCAATGAAGTGATCCCAAATCCTAATTATGGTAAGCTTCAACAGTC
BC-4-TPI_INT1_f.ab1 AGACTGGACTCCATTTATGGAGTGGGCTCAGGCTGTGGTTAGAAGGCAATGAAGTGTACCCAAATCCTAATTATGGTAAGCT
BC-5-TPI_INT1_f.ab1 AGACTGGACTCCATTTATGGAGTGGGCTCAGGCTGTGGTTAGAAGGCAATGAAGTGTACCCAAATCCTAATTATGGTAAGCT
BC-6-TPI_INT1_f.ab1 TCCATTTATGGAGTGGGCTCAGGCTGTAGTTAGAAGGCAATGAAGTGATCCCAAATCCTAATTATGGTAAGCTTCAACAGTC
BC-7-TPI_INT1_f.ab1 AGACTGGACTCCATTTATGGAGTGGGCTCAGGCTGTGGTTAGAAGGCAATGAAGTGTACCCAAATCCTAATTATGGTAAGCT
BC-8-TPI_INT1_f.ab1 TCCATTTATGGAGTGGGCTCAGGCTGTAGTTAGAAGGCAATGAAGTGATCCCAAATCCTAATTATGGTAAGCTTCAACAGTC
BC-9-TPI_INT1_f.ab1 TCCATTTATGGAGTGGGCTCAGGCTGTAGTTAGAAGGCAATGAAGTGATCCCAAATCCTAATTATGGTAAGCTTCAACAGTC
DR-1-TPI_INT1_f.ab1 AGACTGGACTCCATTTATGGAGTGGGCTCAGGCTGTGGTTAGAAGGCAATGAAGTGTACCCAAATCCTAATTATGGTAAGCT
DR-2-TPI_INT1_f.ab1 AGACTGGACTCCATTTATGGAGTGGGCTCAGGCTGTGGTTAGAAGGCAATGAAGTGTACCCAAATCCTAATTATGGTAAGCT
DR-3-TPI_INT1_f.ab1 AGACTGGACTCCATTTATGGAGTGGGCTCAGGCTGTGGTTAGAAGGCAATGAAGTGTACCCAAATCCTAATTATGGTAAGCT
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BC-9-TPI_INT1_f.ab1  TGACTTTGTTCATCAGCCTCTACATTCCCGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
DR-1-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
DR-2-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
DR-3-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
DR-4-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
DR-5-TPI_INT1_f.ab1  CTTTGACTTTGTTCATCCCCCTCTACTTTCAAGATTGCCTTCTGCCCTCCACCGCTCATCGGAAAAAAAAA
GM-1-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCTCGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
GM-2-TPI_INT1_f.ab1  GGAGAGCTTTGACTTTGTTCATCAGCCTCTACATTCCCGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
GM-3-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
GM-4-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
GM-5-TPI_INT1_f.ab1  ACTTTGTTCATCAGCCTCTACATTCACAATTGCCTTCTGCCCTCCACAGCTCATCGGTCAAAA
GM-6-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
HM-1-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
HM-10-TPI_INT1_f.ab1 TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
HM-11-TPI_INT1_f.ab1 TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
HM-12-TPI_INT1_f.ab1 TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
HM-2-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
HM-3-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
HM-4-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
HM-5-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
HM-6-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
HM-7-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
HM-8-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
HM-9-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
IMT-1-TPI_INT1_f.ab1 TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
IMT-2-TPI_INT1_f.ab1 TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
IMT-3-TPI_INT1_f.ab1 TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
LC-1-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
LC-2-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
LC-3-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
MM-1-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
MM-2-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
MM-3-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
MM-4-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
MM-5-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
MM-6-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
MR-1-TPI_INT1_f.ab1  TTGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
MR-2-TPI_INT1_f.ab1  TTGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
MR-3-TPI_INT1_f.ab1  TTGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
MR-4-TPI_INT1_f.ab1  TTGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
MR-5-TPI_INT1_f.ab1  TTGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
MR-6-TPI_INT1_f.ab1  TTGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
MR-7-TPI_INT1_f.ab1  TTGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
RC-1-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
RC-2-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
RC-3-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
RC-4-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
RC-5-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
RF-1-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
RF-10-TPI_INT1_f.ab1 GGAGAGCTTTGACTTTGTTCATCAGCCTCTACATTCCCGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
RF-11-TPI_INT1_f.ab1 TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
RF-2-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCAGAGCTCATCGGTCAAAA
RF-3-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCAGAGCTCATCGGTCAAAA
RF-4-TPI_INT1_f.ab1  GGAGAGCTTTGACTTTGTTCATCAGCCTCTACATTCCCGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
RF-5-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCAGAGCTCATCGGTCAAAA
RF-6-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
RF-7-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCAGAGCTCATCGGTCAAAA
RF-8-TPI_INT1_f.ab1  GGAGAGCTTTGACTTTGTTCATCAGCCTCTACATTCCCGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
RF-9-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCAGAGCTCATCGGTCAAAA
RM-1-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
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RM-4-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
RM-5-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
RM-6-TPI_INT1_f.ab1  TGTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
UM-1-TPI_INT1_f.ab1  GAGAGCTTTGACTTTGTTCATCAGCCTCTACATTCCCGATTGCCTTCTGCCCTCCACAGCTCATCGGTCAGAA
UM-2-TPI_INT1_f.ab1  GATTTGACTTTGTTCATCATCCTCTATATTTTCCCAATGCCTTCTGTCCCCCACAGCTCACGGGCCACAAAAAAAT
UM-3-TPI_INT1_f.ab1  GTTCATCAGCCTCTACATTCACGATTGCCTTCTGCCCTCCACAGCTCACGGGTCAGAA
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Honnors and Awards	Marcia Davis Research Award (East Tennessee State University, Department of Biological Sciences) - To support research toward the conservation of natural resources in Tennessee.