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# World Wide Diversity of Phytophthora capsici 

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To the Graduate Council:
I am submitting herewith a dissertation written by Dylan B. Storey entitled "World Wide Diversity of Phytophthora capsici." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Life Sciences.

Kurt H. Lamour, Major Professor
We have read this dissertation and recommend its acceptance:
Albrecht Von Arnim, Todd Reynolds, Juan Luis Jurat Fuentes
Accepted for the Council:
Carolyn R. Hodges
Vice Provost and Dean of the Graduate School
(Original signatures are on file with official student records.)

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A Dissertation Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Dylan B. Storey
August 2014
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To the shoulder I have stood on.

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"There is a single light of science, and to brighten it anywhere is to brighten it everywhere." - Isaac Asimov

## Abstract

The plant pathogen Phytophthora capsici is a genetically diverse organism that is a global problem. It effects many crops across the Solanacea, Cucurbitacea, and Leguminosea. As a result of the large diversity between individuals (and by extension populations) it has been previously near impossible to make meaningful comparisons between individuals of geographically distinct locations. Here we present the results of applying Next Generation Sequencing (NGS) technologies to a representative panel of isolates. This information and data is further used to demonstrate how this diversity has a functionally relevent effect on a class of proteins responsible for the infectious process. We also demonstrate the application of these technologies and techniques to even lesser studied plant pathogen systems and how they can be used to make intelligent decisions about isolate selection for future studies.

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## Chapter 1

## Literature Review

### 1.1 The Plant Pathogen Phytophthora Capsici

### 1.1.1 Basic Biology

Phytophthora capsici is an aggressive filamentous plant pathogen belonging to the class Oomycota, in Clade 2b of the Phytophthora genus [1]. It is the cause of significant crop loss in the United States where it threatens an estimated one-billion dollars in crops annually [2]. First described on a New Mexico agricultural research station in 1922 [3]; it is the causal agent of root, crown, and fruit rot on a number of economically important crops [4]. While it was initially thought to only infect chili peppers; it has been since described on: tomato, eggplant, cucurbits, lima, and snap beans [5-11].

Phytophthora capsici is a heterothallic species where each isolate has one mating type (A1 or A2). When both mating types are in close proximity, sexual reproduction can occur [12]. Sexual reproduction results in the production of thick walled oospores that can survive harsh environmental conditions for greater than 5 years [13-15]. While this cycle is important for the pathogen to survive fallow periods and produce recombinant offspring, when under favorable conditions the pathogen produces asexual sporangia that release motile zoospores into standing water [15, 16].


Figure 1.1: The life cycle of Phytophthora capsici. The pathogen grows as mycelia in the field. If it encounters mycelia of another mating type the two individuals will undergo meiosis and produce thick walled oospores. These structures allow the pathogen to survive fallow periods. Upon germination, an oospore can create both sporangia and mycelia. Both of these structures are capable of infecting new plant tissue. In the case of sporangia, the structure contains and release motile bi-flagellated zoo-spores that will encyst onto plant tissue prior to forming mycellial tissue.

This allows the pathogen to produce a poly-cyclic infection cycle and can cause entire crops to be lost in a few days [17].

### 1.1.2 Infection

The infectious process can start in one of two ways : the germination of an oospore, or adherence of a zoospore. In the first method, an oospore germinates resulting in the production of mycellia and sporangia. These sporangia then go on to produce and release 20-40 flagellated zoospores. These zoospores demonstrate chemotactic responses toward plants and a negative geotropism. Once these zoospores encounter
plant tissue they adhere and produce a germ-tube. This germ tube penetrates the host cuticle using secreted enzymes and colonizes the host tissue $[18,19]$. Infection then goes through two distinct stages : biotrophy and necrotrophy. Early on in infection host cells do not appear to be largely affected indicating there is a local suppression of host defense responses. As the pathogen continues to develop and mature the pathogen kills infected cells and causes significant tissue damage. Upon tissue collapse, new sporangia begin emerge allowing the pathogen to disperse and begin a poly-cyclic infection cycle. In the presence of warm weather (25-30) and high relative humidity the time period between initial infection by a germ tube and sporulation is 2-3 days [17]. While the necrotrophic phase of infection causes obvious crop loss by damaging the host plant, the biotrophic phase is responsible for post harvest losses. This loss occurs because biotrophic infection may not be readily apparent during harvest and necrotrophic symptoms will not become obvious until fruit reaches a packing plant [15].

### 1.1.3 Pathogen Control

Genetic Resistance: Currently only a few varieties of C. annuum have been found to have natural host resistance to Phytophthora capsici. Specifically in pepper a number of studies on the genetics and inheritence of resistance have led to the identification of numerous Quantitative Trait Loci (QTLs) for resistance. Unfortunately only one land race of Capsicum annuum, var. Criollo de Morelos, has demonstrated near absolute resistance to all isolates of Phytopthora capsici. This variety of $C$. annum is not a commercial variety and the broad pathogen resistance trait appears to be complex; this means that there are currently no crops with any appreciable durable resistance to this pathogen at this time and there are not likely to be any soon as pyramiding multiple resistance QTLs requires long periods for breeding and verification.

Crop Rotation: Crop rotation strategies ,while still advisable [20], are likely nonfunction for control as oospores are able to go dormant for long periods of time [21] and the pathogen has been associated with non canonical species both in the field $[22,23]$ and lab [24]. This long term oospore survival coupled with continual reports [5-11,24] of host expansion and association with weed species $[22,23]$ indicate that this pathogen may be much better at surviving crop rotation than previously expected.

Exclusion: Exclusion practices when executed properly and maintained are capable of controlling the introduction of $P$. capsici to crop land. Irrigation water [4, 25, 26] and field run off [ $15,27,28$ ] have been demonstrated as common routes by which spores may enter a field, providing several obvious targets on which exclusionary practices may be employed.

Cultural Methods: With the infectious cycle, dispersal, and biology of P.capsici being so tightly linked with standing water it should come as no surprise that cultural methods that limit this resource are highly effective at control. General recommendations for controlling the pathogen include: using raised planting beds [20], bedding in soil that allows for good drainage, using irrigation drip tape [29], wide row and plant spacing, and grading fields to ensure water isn't allowed to stand [30]. Unsurprisingly, these methods work especially well in arid areas with little rainfall (Central California and New Mexico), but are often harder to implement in areas that are generally wetter and may receive significant rainfall (Michigan, Tennessee, North Carolina).

Chemical: There are a limited number of chemicals registered for use in controlling P. capsici: dimethomorph (Acrobat), metalaxyl(MetaStar), mefanoxam (Ridomil), cynofamid (Ranman), propamocarb (Previcur Flex) [31,32]. Usage of these chemicals along with good cultural practices and crop rotation has been successful at controlling serious outbreaks. As a result of $P$. capsici's high diversity, mitotic recombination, and the high selection pressure of these chemicals, resistance often develops quickly and absolutely if care is not taken $[?, 33]$. While in the past methylbromide fumigation was an efficacious method for protecting a field it has been in the
process of being phased-out of usage and is now only available through critical use exemptions.

### 1.1.4 Population Structures

The structure of individual populations of $P$. capsici has been studied globally using a number of marker systems (SSR,AFLP,SNP,SSR, physiological race systems etc.) and study designs (spatio-temporal, survey, etc). These studies demonstrate that the population structure and genetic compostition varies greatly by geographic location and potentially on the availability of hosts. It is important to note that while physiological race systems have been used to describe and classify populations of $P$. capsici these systems are reliant on plant host interactions and environmental conditions making calculating a proper distance metric impossible [34-36].

In the United States and South Africa, populations are diverse, short lived, narrowly dispersed, and out-crossing. Furthermore spatio-temporal studies of U.S. populations have demonstrated that during initial stages of an epidemic a population is genetically very diverse and as time progresses the population becomes genetically pure. These populations are generally confined to individual growers or fields and do not survive fallow periods as the following year a new group of competitive genotypes germinates from infectious materials in the soil [21,37-41].

In Peru and Argentina populations are characterized as being clonal, long lived, and widely dispersed [42-44]. It is thought that this population structure primarily arises from the wet and humid climate combined with a perennial cropping system.

In China it appears that there may be four populations that are long lived and clonal along with a considerable reservoir of highly unique long lived genotypes present $[45,46]$. This situation may be similar to that described in South America masquerading as something more complex due to geographic scale.

### 1.2 Oomycete Effectors

### 1.2.1 Biology and Function

Effectors are secreted by plant pathogens and are broadly defined as: a protein or small molecule that alters the host cell structure or function [47-51]. These molecules can : facilitate infection through the modulation of resistance pathways or cell function, trigger host defense responses by being recognized, or potentially do both [52-55]. These effectors provide an extended phenotype for the pathogen as their physical manifestation of the origin of the phenotype [51,56]. This broad definition of effectors while very convienient means that a number of small molecules, toxins, enzymes, and pathogen associated molecular patterns (PAMPs) are all subterms that may fit into this class. This means that with the acceleration of next generation sequencing technologies the identification of putative effectors is increasing, actual identification of function can be difficult as it requires isolating and measuring phenotypes in a second host plant $[50,51]$.

Effectors are important for the pathogens ability to subvert and suppress the host cell function [52-54, 57-59]. Many effectors have been identified as being avirulence factors ; being identified by resistance genes (R-genes) inside of plants and triggering hypersensitive responses to the pathogen. This duality of effectors as being required for infection and responsible for host detection of the pathogen has kicked off a molecular arms race between host and pathogen [47] resulting in the expansion of most effector families and plant resistance genes.

This detection of effectors by plant cells have also provided a number of very specific targets for breeders to use in the development of genetically resistant strains of crops $[60,61]$ and the identification of important $R$ genes in resistant germ-plasm.


Figure 1.2: The basic structure of an RxLR effector. An RxLR effector is defined by a signal peptide within 30 amino acids of a start codon, followed by a linear RxLR motif between 30 and 60 amino acids from the signal peptide, the dEER motif is usually within 20-40 amino acids of the RxLR and is within the effector domain.

### 1.2.2 RxLR Effectors

This class of effectors has been historically very important in the field mainly as several members from this group were the first described avirulence genes in the Phytophthora genus $[59,62,63]$ and has recently been studied and reviewed in a number of publications [51,64,65]. Of all described Oomycete effectors, this class is the largest.

The oomycete RxLR effectors are a class of cytoplasmic effectors that are defined by an signal peptide followed by: an immutable RxLR motif (Arginine-Anything-Leucine-Arginine), a high frequency of acidic residues( $\mathrm{D} / \mathrm{E}$ ), and a diverse effector domain that is responsible for function (Figure 1.2). This conserved RxLR motif has allowed for the use of bio-informatic pipelines that screen potential proteins for the preceeding criteria and allow the $a b$ initio prediction of these proteins [66,67]. In brief these workflows take the six frame translation of a genome and search for sequences greater than 70 amino acids that have: a signal peptide within 30 amino acids of the start codon and an RxLR sequence between 30 and 60 amino acids of the start and behind the signal peptide.

RxLR effectors display signatures of strong positive (diversifying) selection, especially on the C-terminal end $[61,66]$. This is believed to be primarily due to the fact that this family is under pressure to evolve away from plant resistance genes in order to survive. This information paired with a crystal structure for several effectors [68-70] has provided new insight into how these mutational events may allow an effector to evade detection while maintaining function. Based on the highly
conserved helical bundle structure of many of these proteins it is believed that these proteins mutate away from R genes by extending N and C terminal lengths, inserting loops between conserved helical bundles, and limiting mutations to exposed amino acids.

While initial reports indicated that the RxLR domain was solely responsible as a host cell targeting motif $[67,71]$ and responsible for the entrance of the protein into cells, later reports have demonstrated that other higher order structures in the N terminal domain are likely to be playing a more important role $[68,72]$ in directly binding specific lipids presented at the cytoplasmic surface. A recent thorough review of this problem has come to the conclusion that the delivery of these effectors is a complex process that involves multiple steps and may go through multiple different pathways in order to arrive at their destination [73].

### 1.2.3 CRN Effectors



Figure 1.3: The modular structure of the CRN Family. A CRN has a signal peptide, and LFLAK domain, a potential DI domain, a DWL domain, and a trailing effector domain [74].

The Crinkler(CRN) effector family is another highly redundant group of cytoplasmic effectors highly conserved across the Phytopthora genus. This family of effectors was named based on a crinkling leaf phenotype observed during ectopic expression of these proteins in plant [75].

The CRNs also share the RxLR effectors modularity but are generally larger and are sequentially much more complex (Figure 1.3). A typical CRN protein is
compromised of a signal peptide, followed by an LFLAK domain, possibly a DI domain, a DWL domain, and an effector domain.

Currently the best method of annotating CRN effectors from genomic sequences is to : generate a library of open reading frames greater than 300 amino acids in length, detect secreted proteins, search for an LFLAK domain that is behind the signal peptide and within 66 amino acids of the start, search for a DWL domain down stream of the LFLAK domain followed by an effector domain. This pipeline has shown to be both sensitive and specific within $P$. capsici [74].

### 1.3 Next Generation Sequencing - The Illumina Platform

Sequencing starts with the preparation of raw material and generation of a sequencing library. In the case of genomic samples this means the extraction of high quality molecular weight DNA, followed by shearing and size selection of fragments between 200-500 base pairs in length, and the ligation of Illumina specific adapters. In RNASeq experiments samples go through either poly-A enrichment or rRNA depletion to enrich transcripts. After this enrichment : a cDNA library is generated and then the following processing steps are the exact same as the steps required for genomic re-sequencing.

Libraries are then loaded onto a glass flow cell and attached to its surface. Each of these single molecules then goes through an amplification step that creates a cluster of several million identical sequences directly surrounding the originally bound DNA molecule. The free end is then bound to the flow cell as well creating a bridged molecule.

A sequencing by synthesis approach is then used to determine the sequence of each cluster. In such an approach, a DNA polymerase and all four nucleotides are added to a flow channel at every step. These nucleotides include fluorescent labels.

After incorporation of nucleotides onto a cluster, an image of the flow cell is taken. After imaging a blocking group is removed from all molecules and the next round of nucleotides is applied and another image is taken for a defined number of times. If a single end sequencing experiment is all that was required, the sequencing process ends at the completion of this step. In the event that a paired end approach is required, the clusters are regenerated so that the opposite side of the original molecule is now being visualized and the sequencing reaction is carried out again.

### 1.4 Processing NGS Data

### 1.4.1 Common File Formats

FASTA: The fasta format is a format for the description of nucleotide and protein sequences. In the format each sequences consists of a header denoted with a " $>$ " symbol followed by a descriptor of the following sequence. While early versions of this format were strict about a 72 character per line format because of computational needs, this standard is rarely if ever forced in modern formats.

FASTQ: The fastq format is a format that describes sequencing data using four lines per sequence. Line one begins with an "@" symbol and is followed by a sequence identifier and/or description. Line two contains the raw sequence called by whatever technology generated the read. Line 3 begins with a " + " symbol and may have the same sequence identifier as line 1 . Line 4 contains the phred quality values that correspond to each nucleotide of line 2. The phred score of a base is determined by converting the ascii character of a symbol into its corresponding integer and subtracting the vendor specific offset(Figure 1.4).

GFF: The Generic Feature Format are used to annotate features within a genome. It consists of 9 columns of data that are tab delimited: Sequence ID, Source, Type, Start, End, Score, Strand, Phase, and Attributes. Any feature in a sequence can

```
SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS
```





```
!"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJKLMNOPQRSTUVWXYZ[\]^_`abcdefghijklmnopqrstuvwxyz{|}~
\begin{tabular}{rrr|r}
\(\mid\) & \(\mid\) & \(\mid\) & \(\mid\) \\
33 & 59 & 64 & 73
\end{tabular}
```



```
    -5...0........9.................... . . . . . . . . . . . . . 40
0.........9..... . . . . . . . . . . . . . . . . . . . . . . . . 40
0........................... . . 26... . 31......... . 41
S - Sanger Phred+33, raw reads typically (0, 40)
X - Solexa Solexa+64, raw reads typically (-5, 40)
I - Illumina 1.3+ Phred+64, raw reads typically (0, 40)
J - Illumina 1.5+ Phred+64, raw reads typically (3, 40)
    with 0=unused, 1=unused, 2=Read Segment Quality Control Indicator (bold)
    (Note: See discussion above)
L - Illumina 1.8+ Phred+33, raw reads typically (0, 41)
```

Figure 1.4: The phred quality score for different sequencing vendors. The integer value of valid ascii characters used to encode this information, and the resulting quality score after offset.
be described using this format and it is generally very flexible to individual users applying internal lab ontologies to gene features.

GTF: The Gene Transfer Format is a based partially on the GFF format but has additional structure and requirements. While the individual fields in a line are the same as that of a GFF, the GTF has increased requirement for descriptions that must be present for each annotated feature. In this case a start codon, stop codon, and CDS feature type must be attributed to each sequence identifier provided.

SAM/BAM Format: The SAM format is specified in the samtools [76] package and has been widely adopted as being the format to describe how a read aligns to a reference genome. The minimal record identifies on a single line: the query name, a bitwise flag, reference sequence name, 1 base left most mapping position, mapping quality, a CIGAR string, Reference name of the mate if paired, observed template length, the segment sequence, and a 33 offset phred score for the sequence. The BAM format is simply a binary version of the SAM format that is compressed and can be indexed for quick sequence lookup and retrieval.

### 1.4.2 Short Read Aligners

The alignment of DNA and protein sequences is an old and well described problem in bio-informatics. In this problem, two strings are presented for comparison. By applying a user defined set of rewards for matches and penalties for mismatches and gaps alignment algorithms compare the possible ways that any two sequences could be related two each other and alignment with the best score is selected as being correct.

In bio-informatics there are two basic alignment methods: the NeedlemanWunsch [77] and Smith-Waterman [78] algorithms for alignment. Both are considered classic examples of dynamic programming algorithms and are used to report an optimal alignment between two sequences. The major difference between these two algorithms is that the Needleman-Wunsch is a globally optimized algorithm while Smith-Waterman is a locally optimizing algorithm.

Global alignment methods attempt to align the two sequences over the entire length of the sequence and only work well when two sequences are highly related to each other with few mismatches. Local alignment methods find regions of high similarity and minimize scores over those regions allowing them to be used on sequences that are potentially divergent or only have small regions of similarity.

The alignment of short reads from high throughput sequencing technologies to a genome provide a similar if different sort of problem in aligning two sequences together. Because the two previously described algorithms are quadratic in nature more advanced methods to limit search spaces or modify search parameters are required to align millions of reads to genomes in a useful time. As a result of this need more than 30 alignment algorithms have been described to solve this informatics problem. These aligners broadly fall into two categories and attempt to increase search speeds by limiting the amount of space required to search for best matches. A summary of popular open source search tools is presented in Table 1.1. Please keep in mind that this is a brief list of only the most commonly used software and is by no means a complete or comprehensive list.

Table 1.1: Summary of popular short read aligners, the underlying technique used for computing matches, and available features.

|  | Algorithm | Allows Gaps | Paired End | Junction Splicing | Reference |
| :--- | :--- | :--- | :--- | :--- | :--- |
| Bfast | Hashing ref | Yes | Yes | No | $[79]$ |
| Bowtie2 | Trie | Yes | Yes | No | $[80]$ |
| Blat | Hashing ref | Yes | No | No | $[81]$ |
| BWA | Trie | Yes | Yes | No | $[82]$ |
| MAQ | Hashing reads | Yes | Yes | No | $[83]$ |
| SOAP2 | Trie | No | Yes | No | $[84]$ |
| Tophat2 | Trie | Yes | Yes | Yes | $[85]$ |

Hash table aligners: The hash aligner has its roots in the BLAST algorithm [86]. In the BLAST algorithm a series of sub words of length k are generated from a query, the algorithm then uses the words from the query and words from each potential match to find subjects with many word pairs in common and define where alignments may exist between two strings, from each of these seeds alignments are locally extended until their alignment score decreases. This process is completed for all sequences in a database of subject sequences, alignments are refined using a Smith-Waterman alignment and significant outputs are reported. This allows the algorithm to first limit its search space to likely candidates before executing computationally expensive exact alignments. Hash table aligners follow a similar pattern of seeding many small alignments from a short read and then extending them in order to match a short read against the genome. In this scheme a hash table is generated for the genome and held in memory and local alignments are only done when a query sequence has many words that hit near each other within the hash table of the genome. As a class these aligners are highly sensitive but, suffer from severe performance considerations when the detection of indels and mismatches is required.

Trie tree aligners: Aligners in this group identify exact matches within a genome and then build inexact alignments that are supported by many exact matches. In order to do this, these algorithms first build a suffix trie for the genome. A suffix tree is a representation of all of the possible suffixes of a word, this tree is usually compressed
into a structure to make indexed searching feasible. A search is then started by taking a single letter from the end of a query and eliminating any indexed positions that it does not match to. The next letter from the query is added to the search and the search is repeated to find eliminate any sequences that do not match. In this way an exact string match can be found in any database, in order to do mismatches searches must be repetitively seeded with mismatches allowed at individual and/or multiple sites and final alignment scores calculated upon completion of a search. From this list the best score is reported.

As a group these aligners tend to use less memory as a result of compression on the suffix trie and are quick to run but suffer from issues of sensitivity as they require the reseeding of queries against the tree in order to ensure that all possible mismatches and indels are taken into consideration before reporting a best alignment.

### 1.4.3 Intron Junction Mappers

The mapping of reads originating from transcripts is a particularly difficult problem as it combines all of the normal problems associated with the above mappers with the added wrinkle that proper alignments may have large gaps of several thousand letters in them as the result of intron splicing. One method of dealing with this problem is to simply extract and generate all of the known (or predicted) transcripts from a genome and then searching your reads against this database. This method is great because it is computationally trivial and intuitive for most people to understand but requires that researchers have great confidence in their prior knowledge of the gene structure and content of an organism. Another method is to make a two pass approach to mapping. In this approach reads are mapped against the genome as previously described and any unmapped reads are then used in the second round of mapping. This second round of mapping splits each read into two reads in order to simulate a splice event. Both of these reads are then mapped to the genome and any
alignments in which the left read and the right read map in a linear fashion within a set number of base pairs is kept.

### 1.4.4 Genotype and SNP Calling from NGS Sequencing Data

NGS data can suffer from high error rates due to errors in the actual sequencing of data and down stream errors in mis-alignment during mapping. Furthermore, low coverage regions ( $<5 \mathrm{x}$ coverage) have a high probability of having only one chromosome in a diploid individual being sequenced [87]. These conditions make the accurate calling of genotypes a difficult problem that imparts high levels of uncertainty in results.

A common method of reducing these compounding errors is to only consider sites that have "deep coverage" ( $>19 \mathrm{x}$ ). Unfortunately for many studies, this criteria makes the usage of next generation sequencing a prohibitively costly venture. It is much more cost effective to reduce the uncertainty of resulting genotpe and SNP calls in lower coverage regions than to over sequence samples.

## Criteria Based

Early methods of base calling were heuristic models in which researchers would filter sites based on a number of criteria. Commonly pipelines would: filter out all "low quality sites", check for a minimum depth of coverage, and use the ratios of bases called to assign a genotype. Common practice is to call a site homozygous if any allele is more than $80 \%$ of all alleles seen and heterozygous if any two alleles are both between $20 \%$ and $80 \%$ of all alleles seen. An example of this process is presented in Figure 1.5.

These methods are simple to understand, calculate, and work well in high coverage areas. Unfortunately, in low coverage areas these methods result in either: no call being made or a high proportion of heterozygous sites being mis identified as homozygotes. These methods can be improved using empirically estimated cut-offs but still suffer in low coverage experiments [88].


Figure 1.5: An example of a criteria based genotype method. A positions bases and associated qualities are read. Positions that have a low quality (score of $<20$ ) are removed. The read depth $(17 x)$ is calculated as are the proportions of all sequenced bases. The position is called A because proportion of $\mathrm{A}>.8$ and $\mathrm{T}<.2$.

## Bayesian Methods

For low sequencing depth samples, using criteria based methods results in: a large number of heterozygous sites mis-identified as homozygous and the loss of information from read qualities. Criteria based methods also do not allow for any measurement of uncertainty in a called genotype.

Bayesian methods, apply Bayes Theorem to this problem by calculating the likelihood of an observed allele and utilizing prior probability of a variant to calculate the posterior probability of the genotype given the data. The general model [89, 90] for genotype $G$ given the reads $D$ (where $D=d_{1}, d_{2}, \ldots d_{n}$ ) in individual $i$ is :

$$
\begin{gathered}
P(G \mid D)=\frac{P(G) P(D \mid G)}{\sum_{i=1}^{n} P\left(G_{i}\right) P\left(D \mid G_{i}\right)} \\
P(D \mid G)=\prod_{j}\left(\frac{P\left(D_{j} \mid H_{1}\right)}{2}+\frac{P\left(D_{j} \mid H_{2}\right)}{2}\right) \\
P\left(D_{j} \mid H\right)=\operatorname{Pr} D_{j} \mid b \\
P\left(D_{j} \mid b\right)=\begin{array}{c}
1-e_{j} \text { if } D_{j}=b \\
e_{j} \text { else }
\end{array}
\end{gathered}
$$

In the case that an insertion or deletion is to be called at a site: haplotypes $H_{i}$ are discovered from reads at a position, the diploid genotypes $G$ of all possible haplotype
combinations $H_{i} H_{j}$ is created, for each haplotype the likelihood of each read $D_{j}$ over all possible $\pi$ alignments.

$$
P\left(D_{j} \mid H\right)=\sum_{\pi} P\left(D_{j}, \pi\right)
$$

Genotype likelihoods $(P(D \mid G))$ can be improved by: re-calibrating per base quality scores [89, 91], filtering used reads based on base and mapping quality, [90, 92]. Prior probabilities $(P(G))$ can be assigned as being completely naive (all genotypes having an equal weight), assigned based from allele frequencies of previous studies [84, 89], or incorporating calls from other individuals in the same study [76] assuming Hardy-Weinberg equilibrium.

## Other Statistical Methods

The assignment of genotype from sequencing data can also be completed through the application of Fischer's Exact Test or Pearson's chi-squared test [93, 94]. These two tests are both for a $2 \times 2$ contingency table in which the probability of the observed table is calculated compared to any that are more extreme. Fischer's Exact Test is particularly useful in these situations as sequencing data is often sparse enough that a chi-squared test is inappropriate.

Given the table:

$$
\left(\begin{array}{ccc}
a & b & r_{1}=a+b \\
c & d & r_{2}=c+d \\
c_{1}=a+c & c_{2}=b+c & n=(a+b+c+d)
\end{array}\right)
$$

The probability of obtaining a set of values can be calculated as:

$$
p=\frac{r_{1}!r_{2}!c_{1}!c_{2}!}{a!b!c!d!n!}
$$

This equation gives the exact probability of observing this arrangement of marginal totals against the null hypothesis that columns are equally distributed. In the case of genotype calling we are also interested in the probabilities associated with more extreme conditions. This is done by calculating all potential p-values for tables that have the same marginal values and calculating the sum of all $p$-values that are less than or equal to the first test. This is clearly a non trivial matter in the case that there are large numbers of observations. The final significance level can be calculated in a simpler matter by organizing the table such that $c 1$ is the smallest of the four marginal totals and a can be increased in the direction specified by the alternative hypothesis. In this case the generalized equation:

$$
\alpha=\sum_{x=a}^{x=\min \left(r_{1}, c_{1}\right)} \frac{\binom{r_{1}}{x}\binom{r_{2}}{c_{1}-x}}{\binom{n}{c_{1}}}
$$

This computation is still non trivial for a large number of observations and is the cause of computational bottle necks when using these methods to make genotype calls.

### 1.5 Phytophthora tropicalis a brief history and overview

It has always been clear that Phytopthora capsici is a diverse species and carries large levels of variation between individuals [95-97].

Prior to the extensive use of molecular tools in species identification, P. capsici was described using isozymes and morphological data. These descriptions classified $P$. capsci into three groups: CAP1,CAP2, and CAP3 [95]. The CAP1 group contained individuals that were primarily from vegetable plants with some from black pepper
and cacao, the CAP2 were primarily from tropical woody plants, and CAP3 was exclusively populated by individuals isolated from Brazilian cacao. This classification was later revised to merge CAP2 and CAP3 into a single group, CAPB, and CAP1 was re-designated CAPA [96]. As more data and markers have been applied to this problem the description of Phytophthora tropicalis emerged [98]. This reclassification redefined isolates of from CAPA (CAP1) and Phytopthora palmivora MF4 into the species [98].

This species description relies heavily on previous studies morphological and isozyme descriptions and adds to it the addition of host range and avirulence measurements. Phytophthora tropicalis being isolated from perennial woody species such as: Piper nigrum, Macademia itergrifolia, and Theobroma cacao.

The generation of molecular phylogenies within the genus Phytopthora using the ITS region of the genome, cytochrome oxidase genes from the mitochondria, and other genomic loci has further muddied the waters by providing evidence that isolates from tropical and temperate regions have a high continuity of relatedness based on the observed morphological and molecular markers. [27, 99-101]. Furthermore, it has been demonstrated that in some rare cases $P$. capsici and $P$. tropicalis are capable of producing viable offspring [102]. Taken in total, it is clear that while P. tropicalis is a distinct, albeit related, species from P.capsici but current molecular markers have a difficult time distinguishing both inter and intra specific variation.

## Chapter 2

## Population Structures of Phytophthora capsici: A Global Context

### 2.1 Introduction

Phytophthora capsici, first described on chili peppers in 1922 New Mexico [3], has since been reported in most cucurbits, tomato, eggplant, snap, and lima beans. $P$. capsici is a worldwide problem. P.capsici is a heterothallic organism that has two mating types (A1 and A2); when these mating types encounter each other it results in the creation of a thick walled, genetically recombinant, oospore. This pathogen also produces large numbers of deciduous sporangia on the surface of infected tissue; capable of directly infecting tissue or releasing many motile zoospores into standing water. In warm wet conditions the pathogen is capable of destroying a field within days of introduction.

The epidemiology and genetic structure of P.capsici has been investigated at locations around the globe using a number of genetic markers and it is clear that populations vary greatly. In the US and South Africa both mating types are
commonly found in the same field and infectious material does not appear to survive fallow periods. This results in populations that are short lived, genetically diverse, and are spatially restricted. In contrast in Argentina, Peru, and China populations are spatially large, long lived, and clonal.

With the publication of a high quality genome in 2012 [103], it has become possible to begin leveraging high throughput sequencing technologies in order to asses and measure genome scale diversity in this pathogen. This publication was also the first to document mitotic recombination; while the mechanism for such a process is unclear signatures of it are easily detected as the switching of heterozygous regions to homozygous. This loss of heterozygosity has been linked with physical growth characteristics, change of mating type, and virulence in lab populations. This process has been suggested as a major driver for the pathogens clonal profile in a number of studies and causes issues in biological replication of sequencing results.

This makes the application of high throughput sequencing technologies especially exciting as it gives an approach that allows for the comparison of all sites in the genome, a method to measure events of mitotic recombination, and the development of informative markers between geographically distinct isolates. These resources are especially needed as there is obvious need to develop prior knowledge of allele frequencies so that researchers do not need to rely on heuristic criteria based methods or frequentest approaches that require high depth of sequencing.

In this study we present a comparison in the genetic diversity, distance, and structure of a global panel of Phytophthora capsici isolates. Because of the inclusion of transcriptomic samples taken while isolates were infecting plant material, it is especially suited for dissecting the population structure using SNP markers that are functionally linked to the survival of the pathogen. By considering a greater number of markers than previously used in these types of studies we also avoid extreme calculations of distance that arise from only a few differences in between individuals. This approach demonstrates that isolates from Chinese regions are genomically distinct from their western counterparts both in absolute distance and
based on regional patterns of variation. The presence of fine grained population structures within western populations is also discussed.

### 2.2 Materials and Methods

### 2.2.1 Isolate Growth and Genomic DNA Extraction:

Tufts of mycelia were placed into 50 mL Falcon tubes of liquid V8-PARP media (160 mL of $\mathrm{V} 8,3 \mathrm{~g}$ CaCo3, 960 mL of ddH2O, 25ppm pimaricin, 25ppm rifampacin, 25ppm PCNB, 100ppm ampacillin). Tubes were grown with agitation at 28 degrees Celsius for 3 days. Tissue was harvested and lyopholized; then powdered using liquid nitrogen, mortar, and pestle. Genomic DNA was extracted using standard phenol/chloroform extraction methods. All sample concentrations were checked via 260/280 absorption readings using a NanoDrop ND-1000 and high molecular weight DNA verified using $1 \%$ agarose gel.

### 2.2.2 Infection Assays and Total RNA Extraction:

Tufts of mycelia were placed onto V8-PARP agar and allowed to grow for 3 days at room temperature under fluorescent light. A 6 mm punch was then used to take multiple plugs from fully covered portions of agar. 4 plugs were placed mycelia side down on the underside of 6 week old tomato plants (var. Moneymaker). At 24 and 72 hours post inoculation infected tissue was taken from the area directly under the agar plug from 4 separate sites ( 2 separate leaves), frozen in liquid nitrogen, and stored for later extraction. Total RNA was then extracted using RNA-Easy (Qiagen) extraction kit using the manufacturer's instructions. All samples were treated with DNAse (Ambion) on the column prior to final elution. Sample quality was assessed using a NanoDrop ND-100 and 1.5\% agarose gel.

### 2.2.3 Library Construction and Sequencing (Genomic) :

Genomic sequencing was carried out on an Illumina HiSeq 2000 with 100bp single end reads being output. All sample and library preparation was carried out by staff at Children's Mercy Hospital (Kansas City, MO, USA) as per manufacturers instructions.

### 2.2.4 Library Construction and Sequencing (Transcript) :

RNA sequencing was carried out on an Illumina HiSeq 2000 with 100bp paired end reads being output. All sample and library preperation was carried out by staff at GeneWiz Inc (South Plainfield, NJ, USA) as per manufacturers instructions.

### 2.2.5 Read Mapping (Genomic):

Reads were mapped to the Phytophthora capsici genome [103] using Bowtie2 [80]. Bowtie was employed using the end-to-end high sensitivity mode and the following modifications: -D $40-\mathrm{R} 10-\mathrm{L} 10-\mathrm{N} 1$. Only uniquely mapping reads were used for consensus sequence determination and SNP calling. Potential PCR duplicates were then removed from these mapped reads using the samtools utility [76].

### 2.2.6 Read Mapping (Transcript):

Reads originating from transcript sequences were mapped to a concatenation of the Phytophothora capsici and Solanum lycopersicum genomes [103, 104] using the program TopHat2 [?]. TopHat2 was employed with the following settings : read mismatches 8 , read gap length 8 , microexon search True, splice mismatches 2, mate inner distance 100, mate standard deviation 100, read edit distance 8, read realign edit distance 0 , max insertion length 8 , max deletion length 8 , min anchor length 15 , GFF guided mapping True, max multi hits 40. Bowtie 2 settings were : N 1, L 10,

I S,1,1.5, D 20 , R 10. Only uniquely mapping ,conchordant, reads were used for consensus sequence determination and SNP calling.

### 2.2.7 Consensus Sequence Determination (Transcriptomic and Genomic) and SNP calling:

SNPs were called using the VarScan2 (ver 2.3.4) program [93] with the following settings: 8 x coverage, 3 x alternate allele minimum coverage, minimum quality score of 20 , minimum variant frequency of 0.1 , and a P -value threshold of 0.01 .

### 2.2.8 Between Isolate Distance and Clustering:

All sites in which mapping data was available for all samples were considered for calculating distance. The distance between two individuals being defined as :

$$
D_{i j}=\frac{1}{L} \sum_{l=1}^{L} d_{i j}^{l}
$$

Where $L$ is the number of sites observed for comparison and was set to 1 base calls with no overlap, 0 for matching consensus base calls, 0.5 for heterozygous/homozygous overlap (i.e. : A to $[\mathrm{W}, \mathrm{M}]$, T to $[\mathrm{Y}, \mathrm{K}]$, G to $[\mathrm{S}, \mathrm{K}]$, C to $[\mathrm{Y}, \mathrm{S}]$ ), or 0.83 for heterozygous/heterozygous overlap ( i.e. : R to $[\mathrm{Y}, \mathrm{S}, \mathrm{W}, \mathrm{K}, \mathrm{M}]$, Y to $[\mathrm{S}, \mathrm{W}, \mathrm{K}, \mathrm{M}]$, $S$ to $[R, Y, K, M], W$ to $[R, Y, K, M], M$ to $[R, Y, S, W, M])$.

Hierarchical clustering was performed on the calculated distance matrix using euclidean distances and complete linkage in R .

### 2.2.9 SNP Matrix Construction and PCA analysis:

SNP data was transformed into a numerical matrix and a PCA analysis was performed by the EIGENSOFT (ver 5.1) suite of programs [105,106]. SNP data was transformed by comparing each site against the re-sequenced LT1534 genomic sequence and assigning a score between $0-2$ where: 0 is a match , 1 is a heterozygous match , 2 is
a mismatch. The significance of eigenvectors was determined by the Tracey-Widom test, also implemented in the EIGENSOFT package.

### 2.3 Results and Discussion

### 2.3.1 Sequencing

Individual files were concatenated into single files for each library and summary statistics were calculated using internally developed scripts and programs. Cumulative quality scores (Figure 2.1, Figure 2.2, Figure 2.3) for genomic and transcriptomic samples showed a large number of good to high quality bases in each sample and for each run overall. Nucleotide proportions at each position (Figure 2.4, Figure 2.5, Figure 2.6) showed a near even distribution at most reported positions. Quality scores (Figure 2.7, Figure 2.8, Figure 2.9) at demonstrated that $>95 \%$ of position qualities were over 30 in the transcriptomic samples and $>80 \%$ of position quality scores were over 30 in the genomic samples.


Figure 2.1: Cumulative proportion of sequencing quality scores for the genomic re-sequencing run.


Figure 2.2: Cumulative proportion of sequencing quality scores for the biotrophic nucleotide sample.


Figure 2.3: Cumulative proportion of sequencing quality scores for the necrotrophic nucleotide sample.


Figure 2.4: Proportion of nucleotides reported at each position in the genomic sequencing run.


Figure 2.5: Proportion of nucleotides reported at each position in the biotrophic nucleotide sample.


Figure 2.6: Proportion of nucleotides reported at each position in the necrotrophic nucleotide sample.


Figure 2.7: Distribution of quality scores at each position in the genomic sequencing run.


Figure 2.8: Distribution of quality scores at each position in the biotrophic nucleotide sample.


Figure 2.9: Distribution of quality scores at each position in the necrotrophic nucleotide sample.

### 2.3.2 Mapping and SNP Calling

Mapping was completed using either the Bowtie2 [80] or TopHat2 [?] programs using the NewtonHPC cluster at the University of Tennessee, Knoxville. Initial mapping rates for genomic samples were curiously low (64-86\%). As isolates were grown in a rich medium to promote growth, it was decided to take the initially unmapped reads and map them against the mitochondrial genome of Phytophthora capsici. Final mapping rates after this step were much higher (2.10). Transcriptomic samples showed a generally high mapping rate $(69-91 \%, 2.11)$. In order to ascertain how many of these reads were mapped properly to P.capsici counts of primary concordant reads against each genome were gathered. While it was clear that there were different proportions depending on what stage in the life cycle the transcript was taken (data not presented here), overall a large proportion of high quality reads mapped to the Phytophthora capsici genome(53-99\%, 2.12).


Figure 2.10: Mapping rate of genomic samples as reported by Bowtie2.

BAM files for the biotrophic and necrotrophic life stages of each isolate were merged into a single file. This file was then filtered to only include primary concordant reads that mapped to the P.capsici genome. Reads that sat across exon junction were then removed to ensure that ambiguous bases were not included in the pileup file. A pileup file was produced using samtools [76] for all genomic samples, all transcriptomic samples, and all samples combined.

SNP calling was carried out on each pileup file using the VarScan2 [93] program's mpileup2snp command. Table 2.1 contains a summary of the number of sites considered for SNP calling, number of SNPs called in each pool, and the number of sites shared across all isolates. In order to asses the effects of using transcriptomic samples for SNP calling comparisons between identical isolates used in both data collection sets was completed (Table 2.2). These comparisons showed that while pairwise samples had a calculated distance between 0.05 and 0.081 from each other, absolute differences(homozygous mismatch, heterozygous mismatch) in SNP calls did


Figure 2.11: Average overall mapping rate of transcriptomic samples. Reported mapping statistics as reported by TopHat2 were arithmetically averaged between each isolate.
not make up many of the disagreements. A number of the disagreements generated came from calls switching from heterozygous to homozygous sites. This may be a statistical artifact of low coverage, the conversion of heterozygous sites has been described as a normally occurring event in P. capsici [103] and may also be driving a portion of these disagreements. While these type of errors may be problematic, it is more important for the community that the allele frequency at each site for a large number of individuals is available in order to allow for the usage of Bayesian methods of SNP calling in low coverage samples. This will help future projects by allowing more individuals to be sampled and the usage of low coverage sites in a statistically sound manner.


Figure 2.12: Average overall mapping rate of transcriptomic samples for each genome. The proportion of primary, concordant was calculated for each sample. These proportions were then arithmetically averaged for each isolate.

Table 2.1: SNP calling statistics for each pool of called individuals.

|  | Sites Considered | SNPs Called | Completely Shared |
| :--- | :--- | :--- | :--- |
| Transcriptomic Individuals | $42,143,345$ | $1,505,241$ | 377,832 |
| Genomic Individuals | $56,178,139$ | $2,740,759$ | 287,301 |
| All Individuals | $56,178,139$ | $3,522,934$ | 12,507 |

Table 2.2: Comparison of Transcriptomic and Genomic SNP calls from re-sequenced isolates. Isolate - Isolate Identifier, Distance - Distance between Transcriptomic and Genomic samples, Total Mismatch - Total number of loci not in agreement, Hom-Hom - Number of Alleles that showed two different homozygous calls, Het-Het - Number of alleles that showed two different heterozygous alleles, Het-Hom - Number of loci that switched from heterozygous-homozygous. T(het)-G(hom) The number of loci that were heterozygous in the transcriptomic sample and homozygous in the genomic sample, $G$ (het)$T$ (hom) - The number of loci that went from heterozygous in the genome-homozygous in the transcriptome, Ratio - The ratio of events between T (het)-G(hom) and G (het)-T(hom).

| Isolate | Distance | Total Mis-Match | Hom-Hom | Het-Het | Het-Hom | T(het)-G(hom) | G (het)-T(hom) | Ratio |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| 7395 | .050 | 1170 | 3 | 14 | 1155 | 153 | 1002 | $13.25 \%: 86.75 \%$ |
| 6745 | .050 | 1144 | 10 | 8 | 1129 | 451 | 678 | $39.95 \%: 60.05 \%$ |
| 6503 | .062 | 1471 | 10 | 10 | 1456 | 875 | 581 | $60.10 \%: 39.90 \%$ |
| 51 | .061 | 1485 | 8 | 12 | 1470 | 475 | 995 | $32.31 \%: 67.69 \%$ |
| 263 | .061 | 1460 | 13 | 5 | 1445 | 1183 | 262 | $81.87 \%: 18.13 \%$ |
| 1534 | .040 | 961 | 8 | 9 | 947 | 374 | 573 | $39.49 \%: 60.51 \%$ |
| 9397 | .081 | 1962 | 25 | 11 | 1931 | 1504 | 426 | $77.89 \%: 22.06 \%$ |

### 2.3.3 Hierarchical Clustering and Principle Component Analysis on Populations



Figure 2.13: Dendogram of all samples distance matrix based on euclidean distance and complete linkage. Colored dots indicate which group an individual was assigned to based on this dendogram. Cyan - China A, Pink - China B, Blue - Western A, Red - Western B, Green - Lab

VarScan2 native output was transformed into a distance matrix using an internally developed script (Appendix A). It was decided to only use sites where all individuals had a base called for this operation.

Hierarchical clustering was performed on the calculated distance matrices for each pooled set using R, between row distances were calculated using a euclidean method and complete linkage was used for merging groups (Figure 2.13). The resulting dendogram shows that: all of the isolates originating from western countries are closer to each other than to those from China, the Chinese group most likely has at least two major groups, the western group has at least three major groups. When a heatmap (Figure 2.14) is produced by applying the dendogram to order the initial distance matrix some of the drivers of the clustering become more apparent.

While no re sampled isolates were identical, there was only one instance where they were merged with another isolates before their counterpart (LT_1534-T and LT_263-T). While this was unexpected it is not terribly surprising as LT263 is


Figure 2.14: Clustered heat map of all individuals. The dendogram has been colored to match the dendogram colors in Figure 2.13.
the primary parent in the back-cross that created LT1534 and in the event that transcriptional bias is driving genotype calls one would expect them to have a small calculated distance from each other. It should be noted that a group of isolates (LT1021,LT1422,LT263,LT1534) formed a very distinct group far away from the other western samples. These isolates are all lab strains that were created for the building of the draft genome of P. capsici [107] or LT1534 with TD-TOM fluorescent label (LT9378). These isolates are derivatives of LT263. This brings up some concerns about the usefulness of these lab strains for translative based studies as they are
genetically distinct and have a life history that is wholly unique and artificial to the species.

If you look at the largest western population (Western B), it is clear that all of these individuals are largely distant from all other isolates and show little similarity to any other individuals. This group comprises a broad geographic range and while geographically linked isolates do appear to be more closely related to eachother (LT5473,LT5474,LT5475 ; LT10296,10297), distance calculations and clustering alone are incapable of resolving higher order differentiations.

Western A shows a higher level of internal similarity. This group contains individuals who: are the other parent of the back cross scheme (LT51,Michigan), geographically close (Wisconsin/Michigan), or from a research field in North Carolina. Whether this decrease in distance is due to inbreeding or similar selection pressures; it is clear that there is a difference from Western B .

The Chinese isolates are very distant from western and Lab groups and split into two distinct sub-groups: China A and China B. This dichotomy is due to the over representation of a genotype in China A; LT9401,LT9402,and LT9419 are all sectors of LT9400 that demonstrated major shifts while growing under lab conditions on V8-agar. These types of extreme phenotype shifts while under vegetative growth are indicitive of loss of heterozygosity. These samples give us a measurement for how much distance between individuals could be ascribed to this event. The China B clade is comprised of several related individuals that show very little similarity to any western or Lab Strains. These measurements parallel observations from previous studies $[45,46]$ but are not replicated enough to support the idea that a few highly clonal lineages exist in China.

Calculating distances and then clustering based on the resulting patterns of relationships means that differences are aggregated before any sort of group assignment occurs. In order to gain more fine scaled groupings based on the genomic location and pattern of SNPs principle component analysis was applied to this dataset using the EIGENSOFT 5.1 software package. Native Varscan2 outputs were transformed
into inputs for the EIGENSOFT package [105] using an internally developed script (Appendix B). The EIGENSOFT analysis was run with individuals binned into a number of groups: groups as defined by hierarchical clustering, country of origin, and state of origin(For US Samples).

Table 2.3: Statistical significance of eigenvectors based on Tracy-Widdom test statistic. Calculated using twstats from the EIGENSOFT software package.

| Number | Eigenvalue | TW-pvalue |
| :--- | :--- | :--- |
| 1 | 4.984158 | $4.83 \mathrm{E}-018$ |
| 2 | 3.223916 | $6.69 \mathrm{E}-008$ |
| 3 | 2.46997 | 0.0231457 |
| 4 | 2.276269 | 0.0748581 |
| 5 | 2.072959 | 0.330732 |
| 6 | 1.883087 | 0.819575 |
| 7 | 1.804541 | 0.863314 |
| 8 | 1.63595 | 0.99796 |
| 9 | 1.557456 | 0.999759 |
| 10 | 1.552837 | 0.997547 |



Figure 2.15: A screeplot of the eigenvalues calculated from principle component analysis.

The Tracy-Widom statistic was calculated and a scree plot generated on the resulting eigenvalues from the PCA analysis (Table 2.3, Figure 2.15). While the output from calculating Tracy-Widom test statistics indicates that only the first three factors are significant, the scree plot indicates that more factors may still be informative. As a result of this it was decided to continue all resulting analyses in EIGENSOFT while keeping the top ten principle components. The ten SNPs with the highest singular value decomposition for each eigenvector are presented in Appendix C.


Figure 2.16: Plot of the first two Eigenvectors of PCA analysis; populations based on hierarchical clustering coloring corresponds to 2.13.

Table 2.4: Statistical significance of differences between populations as defined on groups found during hierarchical clustering are shown on the upper triangle. The statistically significant (pvalue $<1 \mathrm{e}-3$ ) eigenvectors between each group is on the lower triangle.

|  | Lab | Western_A | Western_B | China_A | China_B |
| :--- | :--- | :--- | :--- | :--- | :--- |
| Lab | x | $5.5441 \mathrm{e}-19$ | $1.02768 \mathrm{e}-21$ | $2.86361 \mathrm{e}-49$ | $6.79034 \mathrm{e}-30$ |
| Western_A | $1,2,4,5$ | x | $1.40091 \mathrm{e}-12$ | $3.12257 \mathrm{e}-22$ | $1.23574 \mathrm{e}-24$ |
| Western_B | 1,2 | $1,2,5$ | x | $1.51584 \mathrm{e}-22$ | $1.34019 \mathrm{e}-19$ |
| China_A | $1,2,3,4,7,8,9,10$ | $1,2,8$ | 1,2 | x | $2.99121 \mathrm{e}-30$ |
| China_B | $1,2,5,8$ | $1,2,4$ | 1,2 | $2,3,4,7,8,9,10$ | x |

When labeling samples by the large groups defined during hierarchical clustering, it is apparent that the populations are readily identifiable along the first two
eigenvectors of analysis and all groups are statistically significant from eachother along a number of components and in overall space (Table 3.4,Fig 2.16).

These results are interesting but hardly surprising based on previously measured genetic distances. The Chinese isolates show low within group variation. This may be indicative of long term inbreeding and/or mitotic recombination combined with a lack of introduced allelic diversity from outside sources. What is unclear is whether this has resulted in previously reported clonal populations ( $[45,46]$ ) or simply reduced allelic complexity, the variance along eigen vectors within the Chinese population would seem to indicate the latter. the Chinese population would seem to indicate the latter.

Lab strains of the pathogen continue to show that they are infact highly distinct from all other individuals in the analysis (including their parents) and very unique when compared to Chinese isolates. This should help serve as a strong reminder that the strain sequenced for the published genome has a unique life history resulting in what is a comparatively abnormal genomic landscape.

WesternA continues to show that while similar to Western B , it is statistically unique. Western B also showed the highest within group variance in eigenvalues along each vector indicating that it may be comprised of many smaller groups.

In order to determine if there are differences at different levels of geographic granularity, individuals were relabeled on first a country (Table 2.5,Fig 2.17) then for US isolates a state basis(Table 2.6,Fig 2.18). Based on these results is evident that there is a distinct difference between individuals from different countries and even between states in the US. Because this partitioning of data results in a lower sample number for subsequent statistical tests, only samples with at least $\mathrm{n}=3$ have been reported. These results demonstrate that the WesternB group is made up of a large number of smaller groups that contain local similarities but between group variability.


Figure 2.17: Plot of the first two Eigenvectors of PCA analysis; populations defined by country.

Table 2.5: Statistical significance of differences between countries. Significant ChiSquared tests are on the upper triangle. The statistically significant (p-value $<1 \mathrm{e}-3$ ) eigenvectors between each group is on the lower triangle.

|  | US | Lab | Mexico | France | China |
| :--- | :--- | :--- | :--- | :--- | :--- |
| US | x | $1.57 \mathrm{E}-14$ | 0.00024 | $1.68 \mathrm{E}-10$ | $8.01 \mathrm{E}-21$ |
| Lab | $1,2,4$ | x | $1.29 \mathrm{E}-16$ | $6.75 \mathrm{E}-19$ | $2.16 \mathrm{E}-26$ |
| Mexico | 4 | $1,2,5$ | x | $2.59 \mathrm{E}-8$ | $1.45 \mathrm{E}-15$ |
| France | 3,4 | $1,2,4$ | 4,5 | x | $9.17 \mathrm{E}-28$ |
| China | 1,2 | $1,2,4,5$ | 1,2 | $1,4,5$ | x |



Figure 2.18: Plot of the first two Eigenvectors of PCA analysis; populations defined by country and state.

Table 2.6: Statistical significance of differences between states. States without enough samples to be considered are not included in this table. Significance as a result of a Chi-Squared test is on the upper triangle, significant eigenvectore (p-value $<1 \mathrm{e}-3$ ) are on the lower triangle.

|  | TN | NY | MI | CA | NM | CT | NC |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| TN | X | 0.00102285 | $2.17 \mathrm{E}-10$ | $7.20 \mathrm{E}-6$ | $1.27 \mathrm{E}-11$ | $2.71 \mathrm{E}-9$ | 0.000821334 |
| NY |  | X | $1.06 \mathrm{E}-16$ | $1.02 \mathrm{E}-10$ | $9.75 \mathrm{E}-11$ | 0.000112663 | $1.87 \mathrm{E}-8$ |
| MI | 1 | $1,7,9$ | X | $2.15 \mathrm{E}-17$ | $3.87 \mathrm{E}-22$ | $2.60 \mathrm{E}-19$ | $5.43 \mathrm{E}-11$ |
| CA |  | 5 | $1,2,5$ | X | $2.97 \mathrm{E}-10$ | $6.84 \mathrm{E}-9$ | $1.20 \mathrm{E}-9$ |
| NM | 1 |  | $1,2,4,5$ |  | X | $3.09 \mathrm{E}-11$ | $8.19 \mathrm{E}-15$ |
| CT | 8 |  | $1,7,8$ |  |  | X | $2.19 \mathrm{E}-15$ |
| NC |  |  | 10 |  | 2 |  | X |

This first ever comparasion of isolates across distinct geographic locations provides a number of insights into the life history of the pathogen Phytophthora capsici. While the within population variation of this pathogen has been studied numerous times [21,37-46], markers used in these studies were not conserved across studies making it impossible to do a global comparison. With the application of next generation sequencing it becomes a relatively trivial matter make these comparasions using a unified marker set.

This is the first time it has been possible to make direct comparisons between isolates of different countries. These comparisons make it quite obvious that there are definte large scale differences between countries (Table 2.5); the most considerable difference being between that of China and Western countries. This difference is not just the result of relative distance (Figure 2.14 ) but also genetic content (Figure 2.17) and may be the result of a founder effect coupled with inbreeding loss of heterozygosity and the lack of gene flow coming into the Chinese region. This type of situation means that the use of genetic resistance in crops to control this pathogen is tenable but that breeders need to ensure that they are developing these crops using isolates from the area. This lack of genetic diversity, signatures of inbreeding, and lack of gene flow also mean that genetic resistance is more likely to be robust and long lasting.

On the other hand, the Western isolates continue to show that they are genetically distant when compared to each other even when coming from the same region(Figure 2.14). This variability is patterned enough to allow regions to be statistically separated from each other (Table 2.6) indicating that there is some underlying structure to what appears to be a homogeneous group of individuals. This may create a more difficult situation when it comes to using genetic control in the pathogen in these areas and if there is any sort of drift between these populations make generating plant germ-lines with robust resistance an intractable problem.

This study demonstrates the relative and absolute differences between individuals drawn from a global panel of $P$. capsici isolates. These similarities and differences are especially important when thought of from the context of effector based breading
strategies that are becoming increasingly popular in an effort to exploit genetic resistance for control. While not directly addressed in this study , it is highly probable and likely that a number of these differences fall within avirulence factors that are important for infection and are detected by host R genes. These levels of absolute difference between isolates and conservation at a local regional level indicate that great care needs to be made in these breeding schemes to either : identify and target highly conserved breeding targets in the pathogen or develop multiple strains of resistant plants that can be targeted to the local genetic structure of the pathogen.

## Chapter 3

## Molecular description of Phytopthora tropicalis

### 3.1 Introduction

Phytophthora tropicalis is relatively recently described species in the Phytophthora genus [98]. The original description included certain isolates that were originally classified as Phytophthora capsici and Phytophthora palmivora MF4 [4]. Initial characteristics that were used to differentiate P.tropicalis from P. capsici were morphological in nature and included: the production of chlamydospores, poor to no growth at 35 degrees Celsius, a lack of pathogenicity on Capsicum seedlings, and narrow sporangia. Most isolates of $P$. tropicalis were also isolated from woody perennial plants like : Piper nigrum , Macademia itergrifolia, and Theobroma cacao [98].

Before $P$. tropicalis was formally described there was evidence that there were extreme levels of variation within the $P$. capsici species, and through the usage of morphological data and isozymes the species was initially described as having three and later two subgroups [95-97].

The development of multiple gene molecular phylogenies in the genus Phytophthora using the internal transcribed spacer (ITS) sequence [99], mitochondrial cytochrome oxidase genes [100], and other molecular [27,101,108] markers has created a situation where while it is clear that $P$. tropicalis is a distinct species its classification is difficult due to the continuum of variation that spans between both species $[102,109,110]$.

One of the objectives of our laboratory is to develop and describe molecular tools for the identification and classification of Phytophthora capsici. As a result of historically fluid description of this species it has also become important for us to be able to quickly and succinctly identify if an isolate is instead Phytophthora tropicalis so that we may properly include or exclude it in relevant studies. Here we present evidence for the continued description of $P$. tropicalis as at least one species and molecular data that can be used to distinguish isolates.

### 3.2 Materials and Methods

### 3.2.1 Isolate Selection:

Isolates were selected based on geographic diversity and host range. Please note that $P$. subnubulus has not been described and was named such due to its host of collection but lack of $P$. capscici characteristics. Table 3.1 contains a description of isolates used in this study.

| Isolate | Species | Location | Host | Citation |
| :--- | :--- | :--- | :--- | :--- |
| LT_5230 | P. subnubulus | Oxapampa, Peru | C. Pubescens | $[43,44]$ |
| LT_5287 | P. subnubulus | Oxapampa, Peru | C. Pubescens | $[43,44]$ |
| LT_5395 | P. subnubulus | Oxapampa, Peru | C. Pubescens | $[43,44]$ |
| LT_5761 | P. tropicalis | Oahu | C. papaya | $[98]$ |
| LT_35 | P. tropicalis | Brazil | T. cacao | $[96]$ |


| LT_232 | P. tropicalis | Murfreesboro, TN | Rhododendron | [102] |
| :---: | :---: | :---: | :---: | :---: |
| LT_29 | P. tropicalis | Brazil | T. cacao | [96] |
| LT_5762 | P. tropicalis | Hawaii | Anthurium | [98] |
| LT_5763 | P. tropicalis | Oahu | D. caryophylus | [98] |
| LT_1021 | P. capsici | Lab |  | [107] |
| LT_1422 | P. capsici | Lab |  | [107] |
| LT_1534 | P. capsici | Lab |  | [103] |
| LT_2135 | P. capsici | Lab |  | [107] |
| LT_263 | P. capsici | Tennessee | Cucurbita |  |
| LT_51 | P. capsici | Michigan | Cucurbita | [38] |
| LT_6149 | P. capsici | NY | Cucurbita |  |
| LT_62 | P. capsici | Michigan | Cucurbita | [38] |
| LT_6503 | P. capsici | Conneticut | P. vulgaris |  |
| LT_6535 | P. capsici | Argentina | C. Annuum |  |
| LT_6745 | P. capsici | Mexico | C. Annuum |  |
| LT_72 | P. capsici | Michigan | Cucurbita | [38] |
| LT_7395 | P. capsici | France | C. Annuum |  |
| LT_7701 | P. capsici | France | C. Annuum |  |
| LT_7704 | P. capsici | France | C. Annuum |  |
| LT_9288 | P. capsici | China | C. Annuum | [45, 46] |
| LT_9378 | P. capsici | Lab |  | [103] |
| LT_9397 | P. capsici | China | C. Annuum | [45, 46] |
| LT_9398 | P. capsici | China | C. Annuum | [45,46] |
| LT_9400 | P. capsici | China | C. Annuum | [45, 46] |
| LT_9401 | P. capsici | China | C. Annuum | [45, 46] |
| LT_9402 | P. capsici | China | C. Annuum | [45,46] |
| LT_9415 | P. capsici | Wisconsin | Cucurbita |  |


| LT_9417 | P. capsici | North Carolina | C. lanatus |  |
| :--- | :--- | :--- | :--- | :--- |
| LT_9418 | P. capsici | North Carolina | C. lanatus |  |
| LT_9419 | P. capsici | China | C. Annuum | $[45,46]$ |

Table 3.1: A brief description of isolates used in this study, their initial species description, collection location, and host collected from.

### 3.2.2 Isolate Growth and Genomic DNA extraction:

Tufts of mycellia were placed into 50mL Falcon tubes of liquid V8-PARP media (160 mL of $\mathrm{V} 8,3 \mathrm{~g}$ CaCo3, 960 mL of ddH2O, 25ppm pimaricin, 25ppm rifampacin, 25 ppm PCNB, 100ppm ampacillin). Tubes were grown with agitation at 28 degrees Celsius for 3 days. Tissue was harvested and lyopholized; then powdered using liquid nitrogen, mortar, and pestle. Genomic DNA was extracted using standard phenol/chloroform extraction methods. All sample concentrations were checked via 260/280 absorption readings using a NanoDrop ND-1000 and high molecular weight DNA verified using $1 \%$ agarose gel.

### 3.2.3 Library Construction and Sequencing:

Genomic sequencing was carried out on an Illumina HiSeq 2000 with 100bp single end reads being output. All sample and library preperation was carried out by staff at Children's Mercy Hospital (Kansas City, MO, USA) as per manufacturers instructions.

### 3.2.4 Read Mapping:

Reads were mapped to the Phytophthora capsici genome [103] using Bowtie2 [80]. Bowtie was employed using the end-to-end high sensitivity mode and the following modifications: -D 40 -R 10 -L10 -N1. Only uniquely mapping reads were used for
consensus sequence determination and SNP calling. Potential PCR duplicates were then removed from these mapped reads using the samtools utility [76].

### 3.2.5 Consensus Sequence Determination (Transcriptomic and Genomic) and SNP calling:

SNPs were called using the VarScan2 (ver 2.3.4) program [93] with the following settings: 8 x coverage, 3 x alternate allele minimum coverage, minimum quality score of 20 , minimum variant frequency of 0.1 , and a P -value threshold of 0.01 .

### 3.2.6 Between Isolate Distance and Clustering:

All sites in which mapping data was available for all samples were considered for calculating distance. The distance between two individuals being defined as :

$$
D_{i j}=\frac{1}{L} \sum_{l=1}^{L} d_{i j}^{l}
$$

Where $L$ is the number of sites observed for comparison and was set to 1 base calls with no overlap, 0 for matching consensus base calls, 0.5 for heterozygous/homozygous overlap (i.e. : A to $[\mathrm{W}, \mathrm{M}]$, T to $[\mathrm{Y}, \mathrm{K}]$, G to $[\mathrm{S}, \mathrm{K}]$, C to $[\mathrm{Y}, \mathrm{S}]$ ), or 0.83 for heterozygous/heterozygous overlap ( i.e. : R to $[\mathrm{Y}, \mathrm{S}, \mathrm{W}, \mathrm{K}, \mathrm{M}]$, Y to $[\mathrm{S}, \mathrm{W}, \mathrm{K}, \mathrm{M}]$, $S$ to $[R, Y, K, M], W$ to $[R, Y, K, M], M$ to $[R, Y, S, W, M])$.

Hierarchical clustering was performed on the calculated distance matrix using euclidean distances and complete linkage in R .

### 3.2.7 SNP Matrix Construction and PCA analysis:

SNP data was transformed into a numerical matrix and a PCA analysis was performed by the EIGENSOFT (ver 5.1) suite of programs $[105,106]$. SNP data was transformed by comparing each site against the re-sequenced LT1534 genomic sequence and assigning a score between $0-2$ where: 0 is a match, 1 is a heterozygous match , 2 is
a mismatch. The significance of eigenvectors was determined by the Tracey-Widom test, also implemented in the EIGENSOFT package.

### 3.3 Results and Discussion

### 3.3.1 Raw Sequencing, Mapping of Data, and SNP calling

Individual files were combined into single files for each library library and summary statistics were calculated using internally developed scripts and programs. Cumulative quality scores (Figure 3.1) for all samples showed a large number of good to high quality bases in each sample and for each run overall. Nucleotide proportions at each position (Figure 3.2) showed a near even distribution at most reported positions. Quality scores (Figure 3.3) showed $>80 \%$ of position quality scores were over 30 in all samples.

Mapping rates were expectedly low for P.tropicalis and $P$. subnubulu isolates, between $15 \%$ and $20 \%$, while P.capsci rates were at levels from $70-90 \%$ (data not shown). All mapping files had potential PCR duplicates removed using the samtools package [76].

Variant calling was carried out on pileup files of all samples using the VarScan2 utility. Of $56,174,635$ considiered bases in pileup file $2,766,390$ SNP positions were called and 2,740,759 positions were maintained after filtering for strand bias.


Figure 3.1: Cumulative proportion of sequencing quality scores for the run.


Figure 3.2: Cumulative proportion of sequencing quality scores for the run.


Figure 3.3: Distribution of quality scores at each position in the run.

### 3.3.2 Hierarchical Clustering and Principle Component Analysis

VarScan2 native output was transformed into a distance matrix using internally developed scripts (Appendix A). It was decided to only use sites where all individuals had a base called for this operation. After this filtering step 35,163 sites were used for all further analysis.

Hierarchical clustering was performed on the calculated distance matrix in R , between row distances were calculated using a euclidean method and complete linkage was used for merging groups (Figure 3.4). The resulting dendrogram shows that: all $P$. capsici isolates predictably cluster together, these isolates are predictably distant from other isolates, the $P$. tropicalis isolates break into two distinct clades, and the P. subnublus sample form a grouping next to but relatively distant from P.tropicalis samples. When a heatmap (Figure 3.5) is produced with this dendrogram it is
apparent that there are at least three sub-groups of individuals in the $P$. tropicalis / P. subnubulus line and the group comprised of LT5762, LT5761, LT5763, LT232 may be comprised of even more sub groups.

dist(data_matrix)
hclust ( ${ }^{*}$, "complete")
Figure 3.4: Dendrogram of all individuals calculated from the SNP distance matrix. Generated using complete language and the euclidean distance between all samples.


Figure 3.5: Heatmap of all individuals.

SNP data was transformed into a matrix suitable for PCA analysis using internally developed scripts (B). PCA was carried out using the EIGENSOFT software package with population labels applied derived from both presumed species name and hierarchical clustering groups. Tracy-Widom statistics were calculated on the resultant eigenvectors of analysis and show that the first four eigenvectors were of statistical significance, a screeplot on the same data shows the same (Table 3.2 and Figure 3.6).

PCA plots of the first three eigenvectors were plotted using labels either garnered from hierarchical clustering or assigned species name (Figures 3.7, 3.8 and Figures 3.9, 3.10 respectively). These plots show that while it is clear on a genomic level that P.capsci and P.tropicalis are distinct from eachother, there may be at least 2 sub groups of $P$. tropicalis and the tenatively named $P$. subnubulus is also its own group. Interestingly, the isolates identified as $P$. subnubulus demonstrate a number of morphological and genomic similarites to $P$. tropicalis but were collected from pepper plants in Peruvian farms near the jungle. This may indicate that like $P$. capsici this species may be capable of jumping to new hosts, or more likely simply was in an environment provided it with an environment that was conducive to growth. $P$. tropicalis group B also warrants further attention as this grouping shows a high level of within group variation and may harbor multiple sub-populations.

Calculations of significance between both plots was also performed and is reported on Tables 3.4 and 3.3 for both sets of labels. It is clear both from the PCA and these calculations that while $P$. tropicalis is clearly its own species compared to P. capcisi, there may be further sub groups or species left to describe.


Figure 3.6

Table 3.2: Statistical significance of eigenvectors generated during PCA, calculated using the twstats package from EIGENSOFT.

| $\# \mathrm{~N}$ | eigenvalue | p-value |
| :--- | :--- | :--- |
| 1 | 12.158865 | 0.01587 |
| 2 | 6.476862 | 0.00778839 |
| 3 | 4.580823 | $8.61 \mathrm{E}-006$ |
| 4 | 1.437583 | $5.26 \mathrm{E}-007$ |
| 5 | 0.786518 | 0.154235 |



Figure 3.7: Plot of the first two Eigenvectors of PCA analysis,groups assigned by herarchical clustering.


Figure 3.8: Plot of the Second two Eigenvectors of PCA analysis, groups assigned by hierarchical clustering.


Figure 3.9: Plot of the first two Eigenvectors of PCA analysis, groups assigned by species.


Figure 3.10: Plot of the Second two Eigenvectors of PCA analysis, groups assigned by species.

Table 3.3: Statistical significance of differences between species. Significance as a result of a Chi-Squared test is on the upper triangle, significant eigenvectors ( p -value $<1 \mathrm{e}-3$ ) are on the lower triangle.

|  | P. tropicalis | P. subnubulus | P. capsici |
| :--- | :--- | :--- | :--- |
| P. tropicalis | x | 0.00259772 | $8.37168 \mathrm{e}-19$ |
| P. subnubulus |  | X | $5.24046 \mathrm{e}-137$ |
| P. capsici | $1,2,3,4$ | 1,2 | X |

These results taken as a whole strongly support the continued separation of P.tropicalis from P. capsici and continue to support previous findings that suggest that there is reason to re-structure the $P$. tropicalis species into more sub groups [102]. They further point to a need to further study this species as it is clear that while there

Table 3.4: Statistical significance of differences between groups assigned by hierarchical clustering. Significance as a result of a Chi-Squared test is on the upper triangle, significant eigenvectors ( p -value $<1 \mathrm{e}-3$ ) are on the lower triangle.

|  | P. Tropicalis-A | P. Tropicalis-B | P. Subnubulus | P. Capsici |  |
| :--- | :--- | :--- | :--- | :--- | :--- |
|  | $7.48 \mathrm{e}-18$ | $4.75 \mathrm{e}-08$ | $3.48 \mathrm{E}-122$ |  |  |
| P. Tropicalis-A | X | X | $1.14 \mathrm{e}-23$ | $7.82 \mathrm{E}-073$ |  |
| P. Tropicalis-B | $1,2,3$ | $1,2,3$ | X | $5.24 \mathrm{E}-137$ | ( 1,3 |
| P. Subnubulus | 1,3 | $1,2,3$ | $1,2,3$ | X |  |
| P. Capsici | $1,2,3$ |  |  |  |  |

are potentially highly divergent groups within $P$. tropicalis there was not enough data in this group to adequately describe it.

It has been clear for some time that the usage of standard highly conserved molecular markers for phylogeny reconstruction while powerful offers little in situations where highly divergent species show overlapping variations at the same loci [27,95-102, 108-110]. In order to properly measure and determine the structure of groups from each other it is important that data collection be done in a more agnostic matter, allowing for the sampling of sites that are not classically used (such as ITS and COX)and instead using sites that maximize chances of observing variation.

We further demonstrate the power of applying NGS technologies and population level analysis to non model organisms that have historically been overlooked in these fields. As the costs associated with these techniques continues to drop it is crucial that researchers of these less studied (but still highly important) species begin adopting and utilizing these tools. As we have demonstrated here it is possible to apply these techniques and technologies even without classically needed resources such as a reference genome. These technologies have already allowed for us to provide a series of SNP markers that allow researchers to assign isolates to these provisional groups D.

## Chapter 4

## Effector Diversity in Phytophthora capsici

### 4.1 Introduction

Plant pathogens secrete proteins that target host proteins within the host cytoplasm and directly modify host cell processes in order to promote infection [47, 49, 51, 111]. Within the oomycetes there are two large and widely studied classes of effectors: the RxLR so named because of a linear Arginine-Anything-Leucine-Arginine motif [66, 67], and the Crinkler (CRN) intially named after their demonstrated phenotype of leaf crinkling $[112,113]$. These effector classes have been widely studied in the Phytophthora infestans and Phytophthora sojae patho systems and have been demonstrated to play a large role in the pathogens ability to infect a host. They have also been demonstrated as being responsible for host recognition by host R genes $[53,54,114]$.

As potential agents for a hosts ability to recognize a pathogen, effectors have held the promise of providing strong breeding targets in the screening and development of pathogen resistant germplasm $[115,116]$. This method has proven to be a successful strategy in creating crop lines resistant to $P$. infestans [60,61] and is in varying levels
of development in other Phytophthora patho-systems. The physical assaying of protein in plant/pathogen pathosystems continues to be a major bottle neck in the description and functional classification of effector proteins [117] and as our ability to describe potential effectors in a genome is ever increasing this problem has been exacerbated.

Phytophthora capsici is a globally important plant pathogen known to infect a broad range of hosts in the field [36]. It has also been demonstrated that it has high levels of between isolate variation and likely uses a number of mechanisms to quickly both quickly fix alleles and maintain population diversity [46, 103]. The diversifying selection pressure placed on these families of effectors coupled with the natural diversity of $P$. capsici potentially compounds the problem of too many effectors and not enough time by providing more unique targets than a lab could reasonably expect to assay.

Here we describe a set of effectors based on the reference guided assembly of a panel of $P$. capsici isolates and a systematic classification system by which effectors can be assigned to a larger paralog family. These paralog families can then serve as proxies of core conserved effectors. This approach allows researchers to bypass the lack of physical syntenic conservation absent between most effectors in $P$. capsici and instead utilize sequence identities and patterns of conservation to assign proteins to groups. This approach allows for increased power in the detection of sites that are under varying levels of selection and guide future interrogations of effector functionality. We use the Avr3a effector protein as a case study in how this classification may be used to determine the relative importance of a molecule.

### 4.2 Methods and Materials

### 4.2.1 Isolate Growth and Genomic DNA extraction:

Tufts of mycellia were placed into 50 mL Falcon tubes of liquid V8-PARP media (160 mL of $\mathrm{V} 8,3 \mathrm{~g}$ CaCo3, 960 mL of ddH2O, 25ppm pimaricin, 25ppm rifampacin, 25 ppm

PCNB, 100ppm ampacillin). Tubes were grown with agitation at 28 degrees Celsius for 3 days. Tissue was harvested and lyophilized; then powdered using liquid nitrogen, mortar, and pestle. Genomic DNA was extracted using standard phenol/chloroform extraction methods. All sample concentrations were checked via 260/280 absorption readings using a NanoDrop ND-1000 and high molecular weight DNA verified using $1 \%$ agarose gel.

### 4.2.2 Library Construction and Sequencing:

Genomic sequencing was carried out on an Illumina HiSeq 2000 with 100bp single end reads being output. All sample and library preperation was carried out by staff at Children's Mercy Hospital (Kansas City, MO, USA) as per manufacturers instructions.

### 4.2.3 Read Mapping:

Reads were mapped to the Phytophthora capsici genome [103] using Bowtie2 [80]. Bowtie was employed using the end-to-end high sensitivity mode and the following modifications: -D $40-\mathrm{R} 10$-L10 -N1. Only uniquely mapping reads were used for consensus sequence determination and SNP calling. Potential PCR duplicates were then removed from these mapped reads using the samtools utility [76].

### 4.2.4 Consensus Sequence Determination sequence construction:

SNPs were called using the VarScan2 (ver 2.3.4) program [93] with the following settings: 8x coverage, 3x alternate allele minimum coverage, minimum quality score of 20 , minimum variant frequency of 0.1 , and a P -value threshold of 0.01 . Resulting VCF files were then used to modify the reported P.capsici genome using the vcftools command vcf-consensus.

### 4.2.5 ORF Detection, Local Phasing, and Translation:

Six frame translation was carried out on resulting genomes for each sample using an internally developed program (Appendix E). Open reading frames were defined as any set of sequences that had an in frame start and stop codon and were at least 213 nucleotides in length. In the event that an open reading frame had multiple start codons, the longest open reading frame was retained. We also allowed for a maximum of six missing amino acids within an open reading frame before an ORF was excluded from consideration.

### 4.2.6 RxLR Effector Detection:

The RxLR effector pipeline described by Win et al [66] was implemented and used to detect RxLR like effectors. In brief, translated open reading frames from a genome were scanned for a signal peptide within 30 amino acids of a start site and RxLR motif within the first 60 amino acids of a start site and behind the signal peptide cleavage point.

### 4.2.7 CRN Effector Detection:

An RxLR effector pipeline described by Stam et al was [74] implemented and used to dect CRN effectors. In brief: translated open reading frames were filtered for records longer than 300 amino acids in length and then scanned for a secretion signal. This sub set of records is then subjected to a search of the LFLAK and DWL domains using previously developed hidden markov models. This sub-set is further subjected to searches for effector domains using another set of previously described hidden markov models for effector domains [118].

### 4.3 Results and Discussion

### 4.3.1 Sequencing and Mapping Results:

Mapping was completed using the Bowtie2 program [80] against the Phytophthora capsci genome as a reference [103]. Mapping rates were between $60 \%$ and $80 \%$ (data not shown)for all samples and after subsequent filtering of PCR duplicates using the samtools [76] package estimates of total genome coverage showed generally favorable results (Table 4.1). Mapped reads were then compiled into locally phased genomes and entered effector detection pipelines.

Table 4.1: Total number of uniquely aligning reads for each sample and estimated genome coverage based on a 64 Mb genome of Phytophthora capsici for each isolate in this study.

| Isolate | Reads | Estimated Coverage (64Mb) |
| :--- | :--- | :--- |
| LT 263 | 11448613 | 17.9 |
| LT 7395 | 40535291 | 63.3 |
| LT 62 | 13007162 | 20.3 |
| LT 2135 | 18141183 | 28.3 |
| LT 6535 | 14696770 | 23 |
| LT 51 | 22039048 | 34.4 |
| LT 7704 | 18311991 | 28.6 |
| LT 7701 | 19914895 | 31.1 |
| LT 9397 | 8004572 | 12.5 |
| LT 6503 | 12563504 | 19.6 |
| LT 9417 | 10729665 | 16.8 |
| LT 9418 | 14258492 | 22.3 |
| LT 9415 | 18268665 | 28.5 |
| LT 6745 | 14686079 | 22.9 |
| LT 9288 | 23995631 | 37.5 |

### 4.3.2 Effector Detection:

The RxLR effector pipeline detected a total number of 31,175 mature sequences across all sampled isolates; 15,083 were completely unique at an amino acid level. The
detected RxLR effectors had a median length of 145 amino acids and a mean length of 296 amino acids.

The CRN effector pipeline detected a total number of 3575 mature sequences across all isolates; 2,629 of which were complete unique at the amino acid level. The detected mature CRN effetors had a median length of 285 amino acids and mean length of 322 amino acids.

This raw number of unique sequences demonstrates the need for a classification system that can assign effectors to larger groups with potentially similar function for future study.

### 4.3.3 Effector Relatedness and Clustering:

In order to assess the relatedness of all sequences within a family, iterative clustering of records was performed using the USEARCH [119] program and for each inclusion identity measuring the resulting values: number of clusters, the number of records in the largest cluster, number of records in the smallest cluster, the average number of records per cluster, the number of individual sequences that formed their own cluster. These measures provide estimates of the relatedness of sequences within each of these families.

In the case of the RxLR effectors, it is clear that clusters form at low similarities and remain stable until high ( $>95 \%$ ) measures of similarity are reached. This is indicative that there are a large number of groups that have high internal similarity and low between group similarities(Fig 4.1). In contrast to the RxLR effectors, CRN effectors steadily break into groups as percent identities for group inclusion are increased. This is indicative of CRN effectors groups having high internal similarity and moderate levels of between group similarity (Fig ??).


Figure 4.1: Distribution of quality scores at each position in the run. The X axis the simmilarity measures used to determine if an individual belonged in a group, and the y -axis is the measure of the corresponding value.


Figure 4.2: Distribution of quality scores at each position in the run. The X axis the the simmilarity measures used to determine if an individual belonged in a group, and the $y$-axis is the measure of the corresponding value.

While this high level overview is useful in describing the relatedness of individual sequences to each other, it was decided to perform a less naive approach to clustering related sequences. Effectors were merged into "nodes" by combining any described
effector that demonstrated an overlap in physical location within the genome. In order to be considered valid a node had to have at least three effectors associated with it. These nodes are described in Appendix F. The RxLR effectors mapped to a total of 1,936 nodes ; 1,051 of which had at least 3 sequences. The CRN effectors mapped to a total of 140 loci; 139 of which had at least 3 sequences. No nodes in either family were found in all resequenced isolates.

### 4.3.4 Determining Paralagous Effector Groups:

As no nodes were completely conserved across all sampled isolates it was impossible to determine a core set of conserved effectors for either family. As these proteins are known to be highly modular [74, 118], exist within transposable elements [113, 120], and have undergone recent duplication events [120-122], the definition of broader paralagous group that contain many nodes may provide a better description of core effector groups.

The average sequence similarity between each node was calculated for each family. In order to do this the similarities of all sequences in one node was calculated by comparison to all of the sequences in a second node. These similarities were calculated by global alignment using a BLOSUM62 matrix [123]. Each query was required to be half the length of the target and the final alignment must cover $60 \%$ of the query. This was carried out for all pair-wise combinations of nodes. The average similarity was then calculated by the arithmetic mean of distances between each node. This resulting matrix of average similarity measures was then filtered to include only values of great than or equal to $60 \%$ so that only potentially homologous values would be included in down stream analysis.

The minimum spanning tree of the matrix was calculated using Chu-Liu-Edmods algorithm for computing shortest routes of a directed graph [124, 125]. Because only connections with a weight of greater than $60 \%$ were considered, each family resulted in a forest of minimum spanning trees. Each of these trees can be considered to be
a paralogous group of sequences.The RxLR family has 121 total paralagous groups containing between 2 and 12 nodes each. The CRN family has 24 total paralagous groups containing between 2 and 14 nodes in each graph. These results show good agreement of the clustering patterns previously described based on just sequence data. These trees are presented in Appendix G and Appendix H for the RxLR and CRN families respectively.

By using both the physical location of described effectors in combination with average sequence identity as a proxy for evolutionary distance some conclusions about the evolutionary history of each of these families can be made.

In the case of RxLR effectors, most ( 68 groups , $57 \%$ of total described), of the paralagous groups contain only 2 nodes. Many of the paralagous groups extracted from the RxLR effector group also demonstrated a contiguous relation ship existing on the same scaffold in a linear fashion. This observation combined with high similarity measures may indicate evolutionarily recent duplication events. It should be noted as well that the paralagous groups with 5 or more nodes all are made up of sequences from each of the sampled isolates which may indicate sequence within these groups are important for virulence.

In contrast the CRN family of effectors paralagous groups contain on average many more nodes and a nodes most similar neighbor is most likely to be on a different scaffold than on the same scaffold. These observations combined with the much more consistent discovery of sequences from each isolate belonging to individual nodes would indicate that this family of effectors is necessary for the pathogen to successfully infect a host.

### 4.3.5 Selection Pressure Calculations:

In order to more effectively determine the types of selection pressures on each paralagous group (and loci under selection pressure) all mature sequences from paralagous groups were aligned into a multiple sequence alignment in an automated
fashion using MUSCLE [126]. Corresponding nucleotide sequences were fetched and aligned based on MUSCLE alignments using pal2nal [127]. These alignements were then tested for codon level selection pressures using the HyPhy package FUBAR [128, 129].

Graphs of codon level selection pressures and amino acids present at loci are included as part of Appendix I and Appendix J for RxLR and CRN families respectively. Measures of overall selection pressure in the RxLR family indicate that few residues in any paralagous group are under strong purifying pressure.
P. capsici is a pathogen that shows great diversity between individuals and the RxLR family of effectors has been described as being highly diverse within genomes of the Phytophthora genus $[66,67]$. It should therefore come as no surprise that there is an extreme level of diversity in this class of proteins when comparisons are made between individuals of $P$. capsci. What is surprising is that if this if this class of proteins is also crucial for the pathogen's ability to infect host plants, no RxLR effectors were conserved based on physical location. This is highly problematic if breeders and researchers wish to use these proteins as breeding targets to generate resistant lines as there does not appear to be a core conserved set of effectors that is shared across all isolates. This may indicate that while $P$. capsici still carries a relatively large number of RxLR effectors in it's genome they may not be as functionally important to infection. While the CRN family shows much higher levels of conservation based both on selection levels and physical conservation between isolates; it still does not provide any candidates that demonstrate absolute physical conservation.

By further systematically defining paralagous families of effectors we have provided a system by which effectors can be assigned to a paralagous group through sequence identity and access this information to determine potentially significant residues. This classification system provides a frame work through which a core set of effectors can be described and assigned.

Based on previous observations that $P$. capsici has 4 major sub groups the possibility exists that each major group would have its own set of core effectors.

This may be especially true in China where it is clear that there is much less diversity between isolates. The inclusion of more isolates into this classification system coupled with division of data based on population may provide a more reasonable and powerful classification system for effectors. While the research presented here paints a fairly bleak picture in the application of effector based screening programs in $P$. capsci, it should be noted that the limited number of isolates included in this sample coupled with the extreme diversity of this organism is likely the cause.

### 4.3.6 The Avr3a Effector Group:

The Avr3a effector has been widely studied in a number of Phytophthora species as it is a locus that has been long associated with virulence in Phytopthora pathosystems. With the publication of a crystal structure of this effector [69] and subsequent controversy surrounding which portion of the protein is responsible for binding external cell lipids [68,72,130-132] it was decided to investigate this specific effector in depth.

A hidden markov model based on previously described Avr3a sequences was used to identify which paralagous group was comprised of the most Avr3a like sequences(Figure 4.3). This sequence fell into a paralagous group containing at least one sequence from each individual. As a representative sequence from each isolate sequenced is contained within this group, it is likely a core effector and important for infection.

The previously calculated selection pressure for group 51 was then mapped onto the three dimensional structure of Avr3a (Figure 4.4 and Figure 4.4). These explorations demonstrate that residues predicted and demonstrated to have importance in the entry of Avr3a to host cells are under high (red) to absolute (yellow) levels of purifying selection [68]. There are a number of residues on the back side that have also been predicted to of importance for the structural integrity of the protein that are under strong levels of purifying selection [70]. Interestingly regions of the
protein that are disordered loops and that if mutated could break certain turns are under diversifying selection. It has been hypothesized that this protein may undergo conformational shifts to escape cell detection and the placement of these diversifying sites would indicate that many potenially structurally important sites are under such pressures.

## Selection Pressure on Cluster 51



Figure 4.3: The calculated codon level selection levels on RxLR paralagous group 51.


Figure 4.4: The positive charged face of the Avr3a effector with amino acids colored as red or yellow for negative selection pressures and green for positive selection pressures.


Figure 4.5: The face opposite of the positively charged face on the Avr3a effector with amino acids colored as red for negative selection pressures and green for positive selection pressures.

## Chapter 5

## Conclusions

This dissertation demonstrates: the variation present within P. capsici, the genetic structure of the organism based on geographic location, the effects that diversity has on expanding gene families, and how next generation sequencing technologies can be applied to clarify the classification cryptic non-model organisms.

While it has been demonstrated by multiple studies that this pathogen shows high levels of diversity within locally sampled populations, this is the first time that individuals between geographic locations have been compared. The application of next generation sequencing technologies to this problem provides the ability to select a high number of sites that are both conserved and variable across the entire genome of organism. This increased power allows for very granular measurements of variation based on calculations of raw distance and fine scaled measurements based on patterns of inheritance within the genome. These measures have resulted in the observation that there may be multiple distinct sub groups of individuals across the globe and that even within the United States there are signatures of local selection pressures and allele fixation. We now also have a number of markers that have a quantified ability to distinguish these groups from each other allowing for the quick screening and identification of samples with unknown origin. While this study benefited from large sample size, it did demonstrate the problems associated with low coverage samples
and the use of criteria based SNP callers and the issues of bad prior assumption in bayesian classifiers. Future research creating, curating and maintaining a SNP data base based on these initial observations and the re-calibration of priors for higher heterozygous rates should prove to be a valuable resource for the community as a whole.

We applied the same technologies and analyses to a closely related but separate sister species $P$. tropicalis and potentially un-described species $P$. subnubulus. By doing this we help clarify that $P$. tropicalis is in fact its own distinct species and that it may be comprised of multiple subgroups. We were also able to demonstrate that while $P$. subnubulus may not be its own species it is at least a distinct sub group of $P$. tropicalis. Because these two species are morphologically very similar to $P$. capsici and commonly used sequences such as ITS and COX often are unable to differentiate between them the addition of loci that can be easily assayed is of great value to the community. While initial sampling of $P$. tropicalis should have provided enough samples for stronger statistical analyses, the realization that there may be multiple sub populations within this described species created a dearth of individuals in each group. Never the less this provides a strong indication that further investigation into genome wide variation within isolates that have been classified to this species will provide be valuable and that the species classification within this group needs to be revisited.

Finally, by using reference guided genome construction techniques we have been able to generate a number of resequenced genomes of reasonable quality. Because of the high rate of heterozygous sites within the organism we have also been able to apply read backed phasing to short areas of interest a practice that has been developed for some time but because of biological limitations is rarely feasible. By applying this method to the description of an biologically important class of effectors we were able to measure and demonstrate the lack of a conserved set of proteins within this species and have put forward a different classification system that can be used to assign proteins to larger groups. This last study provides a good look at how low sample size,
high variability, and diverse between group population structures within P. capsici stymies common tasks in comparative genomics.

Taken in toto these findings illustrate the need for future research to refine these observations with increased sampling of isolates, to identify and measure population stratification based on whole genome measures, and to take into account the population strucutre of samples when conducting both comparative and translational.

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## Appendix

## Appendix A

## Varscan2 to Distance Utilility

```
#!/ usr/bin / perl
use warnings;
use strict;
#indel comparisons using Levenshtein distance;
use Text:: Levenshtein qw(fastdistance);
use Getopt::Long;
#Global_Variables
my @file_names;
my %distance_matrix ;
my %number_comparisons_matrix;
my $allowed_missing = 0;
my $varscan_file;
my $inventory_file;
my $snps_only = 0;
my $file_prefix;
my $help = 0;
```

```
GetOptions("missing=i" => \$allowed_missing,
            "varscan=s" }\quad>\$\mathrm{ varscan_file,
            "inventory=s" => \$inventory_file,
            "snps_only+" => \$snps_only,
            "prefix=s" }\quad>\$\mathrm{ file_prefix,
            "help+" }\quad>\$\mathrm{ help
        ) or die usage();
usage() if $help = 1;
open (INVENTORY , "<", $inventory_file) || die $!;
open(VARSCAN ,"<",$varscan_file) || die $!;
while (my $line = <INVENTORY>){
    chomp $line;
    if ($line eq ','){
        next;
        }
    else{
        my ($prefix , $suffix) = split (/\./ , $line);
        push @file_names , $prefix;
        }
    }
close INVENTORY;
print "loading file \n";
my $line_counter = 0;
my @lines =<VARSCAN>;
print " . . . in\n";
```

```
open (KEPT , ">", 'kept_lines.txt') || die $!;
foreach my $line (@lines){
    chomp $line;
    $line_counter ++;
    if ($line_counter % 1000000 ==0){
        print STDOUT $line_counter . " completed \n";
        }
    if ($line eq "Chrom Position Ref Var Cons:Cov:Reads1:Reads2:
    Freq:P-value StrandFilter:R1+:R1-:R2+:R2-:pval SamplesRef
    SamplesHet SamplesHom SamplesNC Cons:Cov:Reads1:Reads2:Freq:
    P-value"){
        next;
    }
```

my @tmp $=$ split $(/ \backslash \mathrm{s}+/, \$ \operatorname{line})$;
my @individual_info $=@ \operatorname{tmp}[10 . . \$ \# \mathrm{tmp}] ; \#$ taking an array slice so its
less maths to adjust indices
my \$number_of_Ns = count_missing ( $\backslash$ @individual_info);
if $\left((\$ \operatorname{tmp}[9]<=\$\right.$ allowed_missing $) \& \&\left(\$ n u m b e r \_o f \_N s<=\right.$
\$allowed_missing) ) \{
print KEPT \$line . " n ";
for (my $\$ \mathrm{x}=0 ; \$ \mathrm{x}<=\$ \#$ individual_info $; ~ \$ \mathrm{x}++$ ) $\{$
for $(m y \$ y=\$ x ; \$ y=\$ \#$ individual_info $; \$ y++)\{$
my $\$$ info_string_1 $=$ \$individual_info $[\$ \mathrm{x}]$;
my $\$$ info_string_ $2=\$ i n d i v i d u a l \_i n f o[\$ y] ;$


\{ \#non informative comparisons are dumped.
\}

```
elsif(($info_string_1 =~ /\+|\ [ATGC]+/) &&(
    $info_string_2 = ~}\\+|\[ATGC]+/)){ #two indel typ
    positions
        if ($snps_only=0){
            $distance_matrix { $file_names [$x]}{ {file_names [$y
            ]} +=($info_string_1 eq $info_string_2) ? 0
                : compare_indel_indel($info_string_1,
                $info_string_2);
        $number_comparisons_matrix {$file_names[$x]}{
            $file_names [$y]} +=($info_string_1 =~/\*/
            || $info_string_2=~}/\*/) ? 2 : 1; ##if ther
                is a * in any of the string it technically
            represents two positions for comparison)
        }
    }
elsif(($info_string_1 =~}/\+|\-[ATGC]+/) || (
    $info_string_2 =~ /\+| \\[ATGC]+/)){ #indel to non
    indel
        if ($snps_only=0){
            $distance_matrix { $file_names [$x]}{$file_names [$y
            ]} += compare_indel_base($info_string_1,
            $info_string_2);
            $number_comparisons_matrix { $file_names[$x]}{
            $file_names [$y]} +=($info_string_1 =~ /\*/
            || $info_string_2 =~/\*/) ? 2 : 1; ##if there
                is a * in any of the string it technically
            represents two positions)
            }
    }
else{
    (my $position_call1 ,my @trash) =split (/\:/,
            $info_string_1);
    (my $position_call2, @trash)=split (/\:/ ,
            $info_string_2);
```

```
                        $distance_matrix {$file_names[$x]}{$file_names[$y]}
                                    +=($position_call1 eq $position_call2) ? 0 :
                                    compare_bases($position_call1,$position_call2);
                    $number_comparisons_matrix {$file_names[$x]}{
                                    $file_names[$y]} += 1;
                                    }
                }
            }
    }
}
```

my \%weighted_distance;
foreach my $\$ \mathrm{x}\left(@ f i l e \_n a m e s\right)\{$
foreach my \$y(@file_names) \{
if ((exists \$distance_matrix $\{\$ x\}\{\$ y\}) \& \&(e x i s t s$
\$number_comparisons_matrix $\{\$ x\}\{\$ y\})$ ) $\{$
my $\$$ avg $=\$$ distance_matrix $\{\$ \mathrm{x}\}\{\$ \mathrm{y}\} /$
\$number_comparisons_matrix $\{\$ x\}\{\$ y\} ;$
$\$$ weighted_distance $\{\$ \mathrm{x}\}\{\$ \mathrm{y}\}=\$ \mathrm{avg} ;$
\}
\}
\}

```
my $dout = $file_prefix . '_distance.matrix';
my $wout = $file_prefix . ', weighted.matrix';
my $cout = $file_prefix . '_comparisons.matrix';
pretty_printer ($dout, \%distance_matrix , \@file_names);
```

```
pretty_printer ($wout , \%weighted_distance,\@file_names);
pretty_printer ($cout, \%number_comparisons_matrix,\@file_names);
pretty_printer_complete ("$wout\.complete",\%weighted_distance,\
    @file_names);
print "Run complete \n";
exit;
sub compare_bases{
    my ($base1 , $base2)= @_;
    if ($base1 eq $base2) {
        return 0;
        }
    ## Both Items are homozygous ##
    if ((($base1 eq 'A')|($base1 eq 'T')|($base1 eq 'G')|($base1 eq '
        C')) &&(($base2 eq 'A')|($base2 eq 'T')|($base2 eq 'G')||(
        $base2 eq '(C'))){
            return 1;
            }
    ## base1 is homozygous base2 is heterozygous
    if ((($base1 eq 'A')|($base1 eq 'T')|($base1 eq 'G')|($base1 eq '
        C')) && (($base2 eq 'Y')|($base2 eq 'R')|($base2 eq 'W')|(
        $base2 eq 'S')|($base2 eq 'K')|($base2 eq 'M'))){
        #Make our comparisons#
        if($base2 eq 'Y' &&($base1 eq 'C'| |base1 eq 'T') ) {
            return 0.5;
            }
        if($base2 eq 'R'&&($base1 eq 'A'|| |base1 eq 'G') ) {
```

```
        return 0.5;
        }
    if($base2 eq 'W' && ($base1 eq 'A'||base1 eq 'T') ) {
        return 0.5;
        }
    if($base2 eq 'S' &&($base1 eq 'G'||base1 eq 'C') ){
        return 0.5;
        }
    if($base2 eq 'K' && ($base1 eq 'T'||base1 eq 'G') ) {
        return 0.5;
        }
    if($base2 eq 'M' && ($base1 eq 'C'||base1 eq 'A') ) {
        return 0.5;
        }
        return 1;
    }
```

```
## base2 is homozygous base1 is heterozygous
```


## base2 is homozygous base1 is heterozygous

if ((($base2 eq 'A')||($base2 eq 'T')||($base2 eq 'G')||($base2 eq '
if ((($base2 eq 'A')||($base2 eq 'T')||($base2 eq 'G')||($base2 eq '
C'))\&\& (($base1 eq 'Y')|($base1 eq 'R')||($base1 eq 'W')||
    C'))&& (($base1 eq 'Y')|($base1 eq 'R')||($base1 eq 'W')||
$base1 eq 'S')||($base1 eq 'K')|(\$base1 eq 'M'))){
$base1 eq 'S')||($base1 eq 'K')|($base1 eq 'M'))){
    return compare_bases($base2 , $base1); #swap our variable and
    return compare_bases($base2 , \$base1); \#swap our variable and
call the subroutine again;
call the subroutine again;
}
}

## both bases are heterozygotes

## both bases are heterozygotes

if (($base1 eq 'Y') && ($base2 eq 'R')){
if (($base1 eq 'Y') && ($base2 eq 'R')){
return 1;
return 1;
}
}
if (($base1 eq 'W') && ($base2 eq 'S')){
if (($base1 eq 'W') && ($base2 eq 'S')){
return 1;
return 1;
}
}
if (($base1 eq 'M') && ($base2 eq 'K')){

```
if (($base1 eq 'M') && ($base2 eq 'K')){
```

```
            return 1;
        }
    if (($base1 eq 'R') && ($base2 eq 'Y')){
        return 1;
        }
    if (($base1 eq 'S') && ($base2 eq 'W')){
        return 1;
        }
    if (($base1 eq 'K') && ($base2 eq 'M')){
        return 1;
        }
    return 0.83;
}
sub compare_indel_indel{
    (my $insert_1 , my @trash)= split (/\:/ , $_ [0]); #grab the sequence
    (my $insert_2, @trash)= split (/\:/ , $_[1]); #grab the sequence
    if ($insert_1 eq $insert_2){
        return 0; #perfect match = zero distance;
        }
    if ($insert_1 =~ /\// && $insert_2 =~
        comparisons
        ## average levenstein distance between all everything
        my ($string_1_a, $string_1_b )= split (/\//,$insert_1);
        my ($string_2_a, $string_2_b)= split (/\//,$insert_2);
    my $distance = 0;
    my $comparisons = 0;
    foreach my $x($string_1_a,$string_1_b){
        foreach my $y ($string_2_a, $string_2_b){
            $distance += fastdistance ($x , $y);
```

```
                    $comparisons++;
                    }
        }
        return ($distance / $comparisons);
        }
    }
sub compare_indel_base{
    (my $insert_1 , my @trash)= split (/\:/ ,$_[0]); #grab the sequence
    (my $insert_2 , @trash)= split (/\:/ ,$_[1]); #grab the sequence
    ($insert_1,$insert_2) =($insert_1 =~/\+|-/)? ($insert_1, $insert_2) :
        ($insert_2, $insert_1); # Check our string 1 has the indel, if
        it doesnt flip them;
    my ($string_1_a , $string_1_b)=split (/\//,$insert_1);
    $string_1_a =~ s/\+|-//;
    $string_1_b =~ s/\+|-//;
    if ($string_1_a eq $string_1_b){
        return fastdistance($string_1_a,$insert_2);
        }
elsif($string_1_a ne $string_1_b){
    my $distance = 0;
    my $comparisons = 0;
    foreach ($string_1_a , $string_1_b){
        $distance += fastdistance($_ , $insert_2);
        $comparisons ++;
        }
    ($distance==0) ? (return 0) : (return ($distance/$comparisons));
    }
}
sub pretty_printer{
```

```
    my \(\$\) file_out \(=\$\) - \([0]\);
    my \%hash \(=\%\{\$-[1]\}\);
    my @files \(=@\{\$-[2]\} ;\)
    open (OUT , ">" , \$file_out) \| die \$!;
    \#HEADER
    print OUT" \(\backslash \mathrm{t}\) ".scalar (@files) ." \({ }^{\text {n" }}\);
    \#END HEADER
    for (my \(\$ \mathrm{x}=0 ; \$ \mathrm{x}<=\$ \#\) files \(; \$ \mathrm{x}++\) ) \(\{\)
        print OUT \$files [\$x] ." \(\backslash t " ;\)
        for (my \(\$ \mathrm{y}=\$ \mathrm{x} ; \$ \mathrm{y}<=\$ \#\) files \(; \$ \mathrm{y}++)\{\)
        if (exists \$hash\{\$files [\$x]\}\{\$files[\$y]\}) \{
            print OUT \$hash\{\$files [\$x]\}\{\$files[\$y]\}."\t"
            \}
        else \{print OUT \$hash\{ \(\$\) files [\$y]\}\{\$files [\$x]\} ." \(\backslash \mathrm{t}\); \(\}\)
        \}
        print OUT " \(\backslash \mathrm{n} "\);
        \}
    return;
\}
sub pretty_printer_complete\{
my \(\$\) file_out \(=\$\) - \([0]\);
my \(\%\) hash \(=\%\{\$-[1]\}\);
my @files \(=@\{\$[2]\} ;\)
    open (OUT , ">" , \$file_out) || die \$!;
\# HEADER
    print OUT" \(\backslash \mathrm{t}\) ".scalar (@files) ." \({ }^{\text {n" }}\);
\#END HEADER
```

```
for(my $x = 0; $x <= $#files; $x++){
    print OUT $files[$x] ."\t";
    for (my $y = 0; $y<=$#files ; $y++){
    if (exists $hash{$files [$x]}{$files[$y]}) {
        print OUT $hash{$files[$x]}{$files [$y]} ."\t"
        }
    else {print OUT $hash{$files [$y]}{$files [$x]}."\t";}
```

        \}
        print OUT " n n";
    \}
    return;
\}
sub usage\{
print " $\backslash \mathrm{n} \backslash \mathrm{nB}$ Basic Usage: $\backslash \mathrm{n} "$;
print "./Varscan2_to_Distance.pl --varscan <varscan_file> --
inventory $<$ list of isolates $>--$ prefix $<$ string $>\backslash n " ;$
print " $\backslash t \backslash t-v a r s c a n<$ string $>$ mpileup $2 c n s$ file from varscan $2 \backslash n "$;
print " $\backslash t \backslash t-i n v e n t o r y<s t r i n g>~ i n v e n t o r y ~ f i l e ~ f o r ~ t h e ~ p i l e u p s ~(~$
isolate name on each line) $\backslash \mathrm{n}$ ";
print " $\backslash t \backslash t —$ prefix $<$ string $>$ prefix you want for out put files $\backslash n$ ";
print "optional arguments: $\backslash \mathrm{n}$ ";

allowed for a locus $\backslash \mathrm{n}$ ";
print " $\backslash t \backslash t —$ onnssonly compare snp sites, this will skip
over calculations of levenstein distance between sites with
indels $\backslash$ n";
print " $\backslash n "$;
print "This program take two files: \n";
print " $\backslash t \backslash t$ 1) Standard Varscan mpileup2cns output $\backslash n "$;
print " $\backslash t \backslash t 2)$ The inventory list from that mpileup2cns run $\backslash n "$;

```
    print "It will then process the lines to calulate distance between
        each individual using the following rules: \n";
    print " \(\backslash \mathrm{t} \backslash \mathrm{t}\) Homozygous match \(=0 \backslash \mathrm{n} "\);
    print " \(\backslash \mathrm{t} \backslash \mathrm{t}\) Homozygous mismatch \(=1 \backslash \mathrm{n} "\);
    print " \(\backslash \mathrm{t} \backslash \mathrm{t}\) Homozygous/Heterozygous overlap \(=0.5 \backslash \mathrm{n} "\);
    print " \(\backslash \mathrm{t} \backslash \mathrm{t}\) Heterozygous/Heterozygous overlap \(=0.83 \backslash \mathrm{n} "\);
    print " \(\backslash t \backslash t\) Indel/Indel \(=\) Levenshtein distance between the two
        strings \(\backslash \mathrm{n}\) ";
    print "Scores are summed up for all comparisons made then averaged
        out by the number of comparisons made \(\backslash n\) ";
    print "The program then outputs 3 files, the upper right triangle
        of : \n";
    print " \(\backslash t \backslash\) tTotal scores \(\backslash n \backslash t \backslash t\) Total comparisons \(\backslash n \backslash t \backslash t\) Weighted
        distance \(\backslash \mathrm{n} \backslash \mathrm{n} "\);
    print "All files are formatted for immediate use in the Phylip
        program \(\backslash \mathrm{n"}\);
    print "Some notes: \n";
    print " \(\backslash t \backslash t\) Its recommended to only input variant sites from
        varscan2 (--variants_only option) \n";
    exit;
    \}
sub count_missing \{
    my @array \(=@\{\$+[0]\} ;\)
    my \(\$\) missing \(=0\);
    foreach my \$test(@array)\{
    (my \$position_call,my @trash) =split (/\:/, \$test);
    if (\$position_call \(\left.=^{\sim} / \mathrm{N} / \mathrm{g}\right)\{\) \#skip lines with \(N\) positions calls
    \$missing ++ ;
    \}
```


## \}

return \$missing;
\}

## Appendix B

## VarScan2 to EIGENSOFT Utility

```
#!/ usr/bin / perl
use warnings;
use strict;
use Getopt::Long;
#Global_Variables
my @file_names;
my %matrix ;
my $allowed_missing = 0;
my $compare_to = "ref";
my $line_counter = 0;
my $output_prefix;
my $varscan_file;
my $inventory_file;
my $help = 0;
my $index = - 1; #holds the position of the column to compare to.
GetOptions("missing=i" 
    "varscan=s" }\quad>\$\mathrm{ varscan_file,
```

```
        "inventory=s" => \$inventory_file,
        "prefix=s" => \$output_prefix,
        "help+" => \$help,
        "compare_to=s" => \$compare_to
        ) or usage();
usage () if $help = 1;
#output files
my $snp_out = $output_prefix . ".snp";
my $geno_out = $output_prefix . ".geno";
foreach ($inventory_file, $varscan_file, $compare_to, $output_prefix) {
    usage() if $_ eq '';
    }
open(VARSCAN ,"<",$varscan_file) || die $!;
open (GEN , ">", $geno_out) || die $!;
open (SNP , ">" , $snp_out ) || die $!;
```

```
    open(INVENTORY , "<", $inventory_file) || die $!;
```

    open(INVENTORY , "<", $inventory_file) || die $!;
    while (my $line = <INVENTORY>){
    while (my $line = <INVENTORY>){
        chomp $line;
        chomp $line;
        push @file_names , $line;
        push @file_names , $line;
        }
        }
    close INVENTORY;
    \#inventory to list of file names
if (\$compare_to ne "ref"){
\#\# get the index number for the inventory list
for (my \$i = 0; $i< @file_names; ++$i){
if ($file_names[$i] eq \$compare_to){

```
```

                $index = $i;
            }
    }
    die "couldn't find the column you want me to compare to" if (\$index =
-1);
}

```
print "loading varscan file ... n ";
my @lines =<VARSCAN \(>\);
print " \(\backslash \mathrm{t} \backslash \mathrm{t} \backslash \mathrm{t} \ldots\) loaded \(\backslash \mathrm{n} \backslash \mathrm{n}\) ";
foreach my \$line (@lines) \{
    chomp \$line;
    \$line_counter ++ ;
    if \((\$\) line_counter \(\% 1 e 6==0)\{\)
        print STDOUT \$line_counter . " completed \(\backslash n "\);
        \}
    if (\$line eq "Chrom Position Ref Var Cons:Cov:Reads1:Reads2:
        Freq: P-value StrandFilter:R1+:R1-:R2+:R2-:pval SamplesRef
        SamplesHet SamplesHom SamplesNC Cons:Cov:Reads1:Reads2:Freq:
        P-value") \{
        next;
        \}
    my @tmp \(=\) split \((/ \backslash \mathrm{s}+/, \$\) line \() ;\)
    my @individual_info \(=@ \operatorname{tmp}[10 . . \$ \# t m p] ; \# t a k i n g\) an array slice so its
        less maths to adjust indices
    if \((\$ \operatorname{tmp}[9]=0)\{\)
```

if (check_for_indels_or_missing(\@individual_info) == 42){
my \$position_scaff = $1 if ($tmp[0] =~/(\d+)/);
my \$position_name = \$position_scaff . " _" . \$tmp[1];
my \$i = 0;
my %local_hash;
print SNP \$position_name . "\t". \$position_scaff . "\t0.0\t"
. \$tmp [1]."\n";
foreach (@individual_info){
(my \$position_call1,my @trash) =split (/\:/, \$_);
my $position_call2;
        if ($compare_to ne "ref"){
( \$position_call2, @trash)=split (/\:/ ,
$individual_info[$index]);
}
else{
\$position_call2 = \$tmp[2];
}
my $value = compare_bases($position_call1,
$position_call2);
        if ($value > -1){
print GEN \$position_name . "\t" . $file_names[$i].
"\t" . \$value . "\n";
}
else{
die "WAKA WAKA WAKA\n";
}
\$i++;
}
}
}

```
```

exit;

```
sub usage \(\{\)
print "\n\nBasic Usage: \n";
print "./Varscan2_to_Matrix.pl ——varscan <varscan_file> --compare_to \(<\) isolate_to_compare_to \(>--m i s s i n g<m i s s i n g>--i n v e n t o r y<l i s t\) of isolates \(>-\) prefix \(<\) string \(>\backslash \mathrm{n}^{\prime \prime}\);
print " \(\backslash t \backslash t — v a r s c a n<s t r i n g>m p i l e u p 2 c n s\) file from varscan \(2 \backslash n "\);
print " \(\backslash t \backslash t\) —inventory \(<\) string \(>\) inventory file for the pileups ( isolate name on each line) \(\backslash \mathrm{n}\) ";
print " \(\backslash t \backslash t — p r e f i x<s t r i n g>~ p r e f i x ~ y o u ~ w a n t ~ f o r ~ o u t p u t ~ f i l e s ~ \ n " ; ~\)
print " \(\backslash \mathrm{n} "\);
print "This program take two files: \(\backslash \mathrm{n}\) ";
print " \(\backslash t \backslash t\) 1) Standard Varscan mpileup2cns output \(\backslash n "\);
print " \(\backslash t \backslash t 2)\) The inventory list from that mpileup2cns run \(\backslash n\) ";
print "It will then process the lines and encode snps against the chosen individual using the following scheme \(\backslash \mathrm{n}\) ";
print " \(\backslash \mathrm{t} \backslash \mathrm{t}\) Homozygous match \(=0 \backslash \mathrm{n} "\);
print " \(\backslash \mathrm{t} \backslash \mathrm{t}\) Homozygous mismatch \(=2 \backslash \mathrm{n} "\);
print " \(\backslash t \backslash t\) Homozygous/Heterozygous overlap \(=1 \backslash n " ;\)
print " \(\backslash t \backslash t\) Heterozygous/Heterozygous overlap \(=1 \backslash n \backslash n " ;\)
print "The program then outputs 2 files : \n";
print " \(\backslash t \backslash t\) List of all snps scored \(\backslash n "\);

print " \(\backslash t \backslash t A l l\) files are formatted for the EigenSNP software \(\backslash n \backslash n\) ";
print "Some notes: \n";
```

    print "\t\t Its recommended to only input variant sites from
        varscan2 (--variants_only option)\n";
    exit;
    }
    sub compare_bases{
my (\$base1 , $base2) = @_;
if ($base1 eq $base2) {
        return 0;
        }
if ((($base1 eq 'A')|($base1 eq 'T')||($base1 eq 'G')|($base1 eq
    C')) &&(($base2 eq 'A')|($base2 eq 'T')|($base2 eq 'G')||(
\$base2 eq 'C'))){
return 2;
}

## base1 is homozygous base2 is heterozygous all of these cases are

    for when only one base needs to change for them to match
    if ((($base1 eq 'A')||($base1 eq 'T')||($base1 eq 'G')||($base1 eq
C'))\&\& (($base2 eq 'Y')|($base2 eq 'R')|(\$base2 eq 'W')||(
$base2 eq 'S')||($base2 eq 'K')||($base2 eq 'M'))){
    #Make our comparisons#
    if($base2 eq 'Y' \&\& ($base1 eq 'C'||base1 eq 'T') ) {
                return 1;
        }
    if($base2 eq 'R'\&\&($base1 eq 'A'|| |base1 eq 'G') ) {
        return 1;
        }
    if($base2 eq 'W'\&\&(\$base1 eq 'A'||base1 eq 'T') ){
return 1;
}

```
```

if($base2 eq 'S' &&($base1 eq 'G'|| |base1 eq 'C') ){
return 1;
}
if($base2 eq 'K' &&($base1 eq 'T'||base1 eq 'G') ) {
return 1;
}
if($base2 eq 'M' &&($base1 eq 'C'| | $ase1 eq 'A') ) {
    return 1;
    }
if($base2 eq 'R'\&\&(\$base1 eq 'C'| | $base1 eq 'T') ) {
    return 2;
    }
if($base2 eq 'Y' \&\&($base1 eq 'A'| |base1 eq 'G') ){
    return 2;
    }
if($base2 eq 'S'\&\&($base1 eq 'A'|| |base1 eq 'T') ) {
    return 2;
    }
if($base2 eq 'W' \&\&($base1 eq 'G'| |base1 eq 'C') ) {
        return 2;
        }
if($base2 eq 'M' \&\& ($base1 eq 'T'||base1 eq 'G') ) {
    return 2;
    }
if($base2 eq 'K' \&\&(\$base1 eq 'C'|| |base1 eq 'A') ) {
return 2;
}

```
```

            }
    ## base2 is homozygous base1 is heterozygous
    if ((($base2 eq 'A')||($base2 eq 'T')||($base2 eq 'G')||($base2 eq
        C'))&& (($base1 eq 'Y')|($base1 eq 'R')||($base1 eq 'W')||(
        $base1 eq 'S')||($base1 eq 'K')|($base1 eq 'M'))){
        return compare_bases($base2 , $base1); #swap our variable and
            call the subroutine again;
        }
        if ((($base1 eq 'Y')||($base1 eq 'R')||($base1 eq 'W')||($base1 eq
        S')||($base1 eq 'K')|($base1 eq 'M')) && (($base2 eq 'Y')||(
        $base2 eq 'R')||($base2 eq 'W')||($base2 eq 'S')||($base2 eq 'K'
        )|($base2 eq 'M')) ) {
    ## both bases are heterozygotes with no overlap ##
        if (($base1 eq 'R') && ($base2 eq 'Y')) {
            return 2;
            }
        if (($base1 eq 'Y') &&($base2 eq 'R')){
            return 2;
            }
        if (($base1 eq 'S') && ($base2 eq 'W')){
            return 2;
            }
        if (($base1 eq 'W') && ($base2 eq 'S')){
            return 2;
            }
        return 1; # default behavior
        }
    return - 1;
}
sub check_for_indels_or_missing {

```
```

my @array = @{ \$_ [0]};
foreach my \$test(@array){
(my \$position_call,my @trash) =split (/\:/, $test);
    if ($position_call=~}/\+|-|*/g || $position_call eq ','){ #non
        informative comparisons are dumped.
        return -1;
        }
    if ($position_call=~}/N/g){ \#skip lines with N positions call
return -1;
}
}
return 42;
}

```

\section*{Appendix C}

\section*{High Value SNPs within \(P\).}

\section*{capsici.}

The ten SNPs with the highest singular value decomposition for each eigenvector as reported by the EIGENSOFT package.
\begin{tabular}{|l|l|l|l|l|l|}
\hline EigenVector & SNP Name & Scaffold & Position & SVD & Alleles Present \\
\hline 1 & 14_552797 & 14 & 552797 & 4.867 & A,M,C \\
\hline 1 & \(14 \_526491\) & 14 & 526491 & 4.782 & A,R,G \\
\hline 1 & \(17 \_12019\) & 17 & 12019 & 4.209 & T,C,Y \\
\hline 1 & 6_998905 & 6 & 998905 & 4.171 & A,W,T \\
\hline 1 & 9_287111 & 9 & 287111 & 4.168 & T,C,Y \\
\hline 1 & \(20 \_559866\) & 20 & 559866 & 4.093 & T,C,Y \\
\hline 1 & 6_460442 & 6 & 460442 & 4.089 & A,R,G \\
\hline 1 & 2_1616779 & 2 & 1616779 & 3.99 & A,R,G \\
\hline 1 & 2_1616861 & 2 & 1616861 & 3.986 & T,C,Y \\
\hline 1 & \(20 \_572811\) & 20 & 572811 & 3.863 & T,K,G \\
\hline 2 & 15_726217 & 15 & 726217 & 3.791 & A,R,G \\
\hline 2 & \(11 \_182933\) & 11 & 182933 & 3.618 & S,C,G \\
\hline 2 & \(7 \_693612\) & 7 & 693612 & 3.613 & A,R,G \\
\hline
\end{tabular}
\begin{tabular}{|c|c|c|c|c|c|}
\hline 2 & 13_1028113 & 13 & 1028113 & 3.603 & A,R,G \\
\hline 2 & 3_880189 & 3 & 880189 & 3.596 & T, C, Y \\
\hline 2 & 21_227318 & 21 & 227318 & 3.583 & A,R,G \\
\hline 2 & 18_914098 & 18 & 914098 & 3.577 & T,C,Y \\
\hline 2 & 10_1087317 & 10 & 1087317 & 3.557 & T, C, Y \\
\hline 2 & 8_732616 & 8 & 732616 & 3.534 & A,R,G \\
\hline 2 & 15_681752 & 15 & 681752 & 3.509 & T, C, Y \\
\hline 3 & 1_1640307 & 1 & 1640307 & 5.608 & A,G \\
\hline 3 & 2_1003184 & 2 & 1003184 & 5.608 & C,G \\
\hline 3 & 4_1413785 & 4 & 1413785 & 5.608 & A, G \\
\hline 3 & 8_1125200 & 8 & 1125200 & 5.608 & T, C \\
\hline 3 & 2_463967 & 2 & 463967 & 4.576 & A,R,G \\
\hline 3 & 13_596134 & 13 & 596134 & 4.576 & A,R,G \\
\hline 3 & 13_596135 & 13 & 596135 & 4.576 & A,R,G \\
\hline 3 & 2_1325240 & 2 & 1325240 & 4.165 & A,W,T \\
\hline 3 & 2_1379175 & 2 & 1379175 & 3.993 & T, C, Y \\
\hline 3 & 2_782680 & 2 & 782680 & 3.966 & A,T,W \\
\hline 4 & 13_597579 & 13 & 597579 & 4.758 & T, C, Y \\
\hline 4 & 13_597576 & 13 & 597576 & 4.563 & T, C, Y \\
\hline 4 & 1_55802 & 1 & 55802 & 3.986 & A,R,G \\
\hline 4 & 13_595983 & 13 & 595983 & 3.963 & T, C, Y \\
\hline 4 & 2_439383 & 2 & 439383 & 3.789 & T, C, Y \\
\hline 4 & 22_239274 & 22 & 239274 & 3.651 & R,G \\
\hline 4 & 22_240165 & 22 & 240165 & 3.651 & R,G \\
\hline 4 & 5_1163140 & 5 & 1163140 & 3.362 & R,G \\
\hline 4 & 6_551821 & 6 & 551821 & 3.362 & R,G \\
\hline 4 & 16_655190 & 16 & 655190 & 3.357 & T,K,G \\
\hline
\end{tabular}
\begin{tabular}{|c|c|c|c|c|c|}
\hline 5 & 11_765770 & 11 & 765770 & 5.292 & T, C, Y \\
\hline 5 & 17_323455 & 17 & 323455 & 4.934 & T,K,G \\
\hline 5 & 17_440448 & 17 & 440448 & 4.836 & A,R,G \\
\hline 5 & 13_610564 & 13 & 610564 & 4.672 & A,R,G \\
\hline 5 & 7_93053 & 7 & 93053 & 4.59 & A,R,G \\
\hline 5 & 17_309213 & 17 & 309213 & 4.533 & A, G \\
\hline 5 & 10_449580 & 10 & 449580 & 4.431 & A,R,G \\
\hline 5 & 4_166065 & 4 & 166065 & 4.344 & A,R,G \\
\hline 5 & 15_855110 & 15 & 855110 & 4.305 & A,R,G \\
\hline 5 & 17-440452 & 17 & 440452 & 4.302 & A,M,C \\
\hline 6 & 22_782188 & 22 & 782188 & 4.155 & S,C,G \\
\hline 6 & 6_440129 & 6 & 440129 & 3.855 & T, C, Y \\
\hline 6 & 1_606386 & 1 & 606386 & 3.803 & C, Y \\
\hline 6 & 17_170207 & 17 & 170207 & 3.775 & T, C, Y \\
\hline 6 & 17_170216 & 17 & 170216 & 3.775 & A,T,W \\
\hline 6 & 15_595665 & 15 & 595665 & 3.741 & S,C,GY \\
\hline 6 & 4_332284 & 4 & 332284 & 3.65 & T, C, Y \\
\hline 6 & 21_101774 & 21 & 101774 & 3.591 & A,R,G \\
\hline 6 & 10_884017 & 10 & 884017 & 3.493 & A,R,G \\
\hline 6 & 22_789496 & 22 & 789496 & 3.464 & A,R,G \\
\hline 7 & 19_356721 & 19 & 356721 & 4.444 & A,R,G \\
\hline 7 & 9_185259 & 9 & 185259 & 3.718 & A,R,G \\
\hline 7 & 21_245518 & 21 & 245518 & 3.649 & T,K,G \\
\hline 7 & 11_187518 & 11 & 187518 & 3.541 & A,M,C \\
\hline 7 & 19_356894 & 19 & 356894 & 3.432 & S,C,G \\
\hline 7 & 11_318193 & 11 & 318193 & 3.419 & R,G \\
\hline 7 & \(4 \_45737\) & 4 & 45737 & 3.41 & S,C,G \\
\hline
\end{tabular}
\begin{tabular}{|c|c|c|c|c|c|}
\hline 7 & 11_186176 & 11 & 186176 & 3.395 & R,G \\
\hline 7 & 6_770682 & 6 & 770682 & 3.352 & T,C,Y \\
\hline 7 & 18_916283 & 18 & 916283 & 3.342 & T, Y \\
\hline 8 & 5_677190 & 5 & 677190 & 4.54 & A,R,G \\
\hline 8 & 21_429359 & 21 & 429359 & 4.459 & A,R,G \\
\hline 8 & 21_501247 & 21 & 501247 & 4.246 & A,R,G \\
\hline 8 & 14_555854 & 14 & 555854 & 3.973 & R,G \\
\hline 8 & 6_931389 & 6 & 931389 & 3.804 & A,R,G \\
\hline 8 & 13_940310 & 13 & 940310 & 3.581 & T, C, Y \\
\hline 8 & 4_214861 & 4 & 214861 & 3.552 & A,R,G \\
\hline 8 & 9_453826 & 9 & 453826 & 3.532 & S,C,G \\
\hline 8 & 10_353315 & 10 & 353315 & 3.519 & A,M,C \\
\hline 8 & 16_936671 & 16 & 936671 & 3.443 & S,C,G \\
\hline 9 & 17_321960 & 17 & 321960 & 5.015 & T, C, Y \\
\hline 9 & 17_323751 & 17 & 323751 & 4.281 & T, C, Y \\
\hline 9 & 3_1019857 & 3 & 1019857 & 4.259 & A,R,G \\
\hline 9 & 3_1019864 & 3 & 1019864 & 4.259 & A,R,G \\
\hline 9 & 3_1026294 & 3 & 1026294 & 4.259 & T, C, Y \\
\hline 9 & 8_180944 & 8 & 180944 & 4.109 & A,M,C \\
\hline 9 & 1_68897 & 1 & 68897 & 3.96 & A,G \\
\hline 9 & 4_353869 & 4 & 353869 & 3.96 & A,T \\
\hline 9 & 5_1314613 & 5 & 1314613 & 3.96 & A,G \\
\hline 9 & 7_680173 & 7 & 680173 & 3.96 & A, C \\
\hline 10 & 1_68897 & 1 & 68897 & 4.285 & A,G \\
\hline 10 & 4_353869 & 4 & 353869 & 4.285 & A,T \\
\hline 10 & 5_1314613 & 5 & 1314613 & 4.285 & A,G \\
\hline 10 & 7_680173 & 7 & 680173 & 4.285 & A, C \\
\hline
\end{tabular}
\begin{tabular}{|l|l|l|l|l|l|}
\hline 10 & \(7 \_693648\) & 7 & 693648 & 4.285 & T,G \\
\hline 10 & \(5 \_665315\) & 5 & 665315 & 3.959 & T,C,Y \\
\hline 10 & \(4 \_274962\) & 4 & 274962 & 3.953 & A,R,G \\
\hline 10 & \(4 \_169465\) & 4 & 169465 & 3.855 & A,T,W \\
\hline 10 & \(14 \_489467\) & 14 & 489467 & 3.8 & A,R,G \\
\hline 10 & \(7 \_702402\) & 7 & 702402 & 3.796 & T,K,G \\
\hline
\end{tabular}

Table C.1: SNP markers that explain the most variation along each eigenvector, and the alleles present at these loci.

\section*{Appendix D}

\section*{High Value SNPs between \(\boldsymbol{P}\). capsici and P. tropicalis}

The ten SNPs with the highest singular value decomposition for each eigenvector as reported by the EIGENSOFT package.
\begin{tabular}{|l|l|l|l|l|l|l|l|}
\hline EigenVector & SNP Name & Scaffold & Position & SVD & P. Subnubulis & P. Tropicalis & P. Capsici \\
\hline 1 & 1_73805 & 1 & 73805 & 2.169 & T & T,C & G \\
\hline 1 & 1_99952 & 1 & 99952 & 2.169 & C & C & T \\
\hline 1 & 1_140290 & 1 & 140290 & 2.169 & T & T & C \\
\hline 1 & 1_140304 & 1 & 140304 & 2.169 & C & C & G \\
\hline 1 & 1_258552 & 1 & 258552 & 2.169 & T & T & C \\
\hline 1 & 1_258568 & 1 & 258568 & 2.169 & T & T & C \\
\hline 1 & 1_287061 & 1 & 287061 & 2.169 & C & C & T \\
\hline 1 & 1_406498 & 1 & 406498 & 2.169 & C & C & T \\
\hline 1 & 1_409267 & 1 & 409267 & 2.169 & C & C & A \\
\hline 1 & 1_421573 & 1 & 421573 & 2.169 & T & T & C \\
\hline 2 & 1_59737 & 1 & 59737 & 2.93 & A & G,A & A \\
\hline 2 & 1_59741 & 1 & 59741 & 2.93 & G & G,A & G \\
\hline 2 & 1_59743 & 1 & 59743 & 2.93 & G & G,C & G \\
\hline
\end{tabular}
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline 2 & 1_59758 & 1 & 59758 & 2.93 & A & C,A & A \\
\hline 2 & 1_59761 & 1 & 59761 & 2.93 & G & G,A & G \\
\hline 2 & 1_59769 & 1 & 59769 & 2.93 & G & G,A & G \\
\hline 2 & 1_75994 & 1 & 75994 & 2.93 & C & C,T & C \\
\hline 2 & 1_75996 & 1 & 75996 & 2.93 & C & C,G & C \\
\hline 2 & 1_228922 & 1 & 228922 & 2.93 & G & G,T & G \\
\hline 2 & 1_287058 & 1 & 287058 & 2.93 & T & T, C & T \\
\hline 3 & 1_55810 & 1 & 55810 & 2.827 & G & A,G & G \\
\hline 3 & 1_59772 & 1 & 59772 & 2.827 & A & T, A & A \\
\hline 3 & 1_59773 & 1 & 59773 & 2.827 & C & T, C & C \\
\hline 3 & 1_73692 & 1 & 73692 & 2.827 & A & C,A & A \\
\hline 3 & 1_73697 & 1 & 73697 & 2.827 & C & T, C & C \\
\hline 3 & 1_89603 & 1 & 89603 & 2.827 & C & T, C & C \\
\hline 3 & 1_89632 & 1 & 89632 & 2.827 & C & T, C & C \\
\hline 3 & 1_115845 & 1 & 115845 & 2.827 & G & A,G & G \\
\hline 3 & 1_136920 & 1 & 136920 & 2.827 & T & A, G & T \\
\hline 3 & 1_148635 & 1 & 148635 & 2.827 & T & C,T & T \\
\hline 4 & 1_644862 & 1 & 644862 & 4.689 & A & A, G & A \\
\hline 4 & \(1 \_1147588\) & 1 & 1147588 & 4.689 & G & A,G & G \\
\hline 4 & 1_1256579 & 1 & 1256579 & 4.689 & T & T, C & T \\
\hline 4 & 1_1258758 & 1 & 1258758 & 4.689 & C & C,G & C \\
\hline 4 & 1_1841529 & 1 & 1841529 & 4.689 & T & T, C & T \\
\hline 4 & 1_1864629 & 1 & 1864629 & 4.689 & A & A, G & A \\
\hline 4 & 2_581994 & 2 & 581994 & 4.689 & T & T, A & T \\
\hline 4 & 3_1183603 & 3 & 1183603 & 4.689 & A & A, G & A \\
\hline 4 & 6_1104888 & 6 & 1104888 & 4.689 & C & C,T & C \\
\hline 4 & 16_637463 & 16 & 637463 & 4.689 & C & C,T & C \\
\hline
\end{tabular}
\begin{tabular}{|l|l|l|l|l|l|l|l|}
\hline 5 & 2_1374321 & 2 & 1374321 & 7.731 & G & G & G,K,T \\
\hline 5 & \(9 \_277144\) & 9 & 277144 & 7.652 & C & C & C,Y,T \\
\hline 5 & \(7 \_962612\) & 7 & 962612 & 7.562 & C & C & C,Y,T \\
\hline 5 & \(9 \_211073\) & 9 & 211073 & 7.562 & C & C & C,S,G \\
\hline 5 & \(18 \_89948\) & 18 & 89948 & 7.365 & T & T & C,Y,T \\
\hline 5 & 4_87070 & 4 & 87070 & 7.297 & C & C & C,Y,T \\
\hline 5 & \(14 \_597493\) & 14 & 597493 & 7.297 & A & A & A,R,G \\
\hline 5 & 2_1502322 & 2 & 1502322 & 6.976 & C & T,C & C,Y,T \\
\hline 5 & \(21 \_497740\) & 21 & 497740 & 6.96 & G & G & A,R,G \\
\hline 5 & \(22 \_575348\) & 22 & 575348 & 6.918 & G & G & G,R,A \\
\hline
\end{tabular}

Table D.1: SNP markers that explain the most variation along each eigenvector, and the alleles present at these loci.

\section*{Appendix E}

\section*{Six Frame and Translation Utility}
```

\#!/ usr / bin / perl
use warnings;
use strict;
use threads;
use Config;
use Getopt::Long;
\#compiling our regular expressions\#

# Get our settings

my \$threads = 4;
my \$orf_size = 70;
my \$informatic_stops = 0;
my @files =();
my \$missing_max = 6;
GetOptions(
'threads|i' => \$threads,
'orf_size|i' => \$orf_size,
'informatic_stops' }=>\$\mathrm{ \$informatic_stops,
'missing_data' }=>\$missing_max
'}<>'=>\&file_or_somethin

```
```

);
\#load our codon table
my %g = load_codon_table();
\#Check Dependencies\#
\$Config{useithreads} || die "Recompile Perl with threads in order to use
this program";
\#checking for files to work on\#
if (@files < 0){
die "You didn't provide files to work on.\n";
usage();
}
print "Current settings are: \n\tthreads -> \$threads\n\torfsize ->
\$orf_size\n\tinformatic stops -> \$informatic_stops \n\tMissing
Information -> \$missing_max\n\tfiles -> " .join(', , , @files)."\n\
n";

## Main Program

my \$terminator = \$/; \#saving our EOL
foreach my \$file (@files){
(my \$filename = \$file) =~ s /\.[^.] + \$//; \#\# get the prefix
local \$/ = ">"; \#splitting file on > character now Scoped to this
loop and all sub_routines called from here.
open (FASTA , '<', \$file) || die $!;
    open (AAOUT , '>', "$filename\.sfaa" ) || die $!;
    open (NTOUT , '>', "$filename\.sfnt") || die \$!;
my @records = <FASTA > ; \#slurp
shift @records;
my @executing_threads = (); \#list of executing threads

```
```

    while (@records) {
        for(my $i = 0 ; $i < $threads ; $i++){ ## only want to execute
        threads that we can actually work on
            if (@records){ ## assuming we have stuff to work on
            push @executing_threads , (threads ->create(\& 
                six_frame_translate , pop(@records))); ## execute a
                thread to do the work , denote the return variable
                as an array by encasing the create in ()
            }
        }
        while (@executing_threads){ # while I have threads to clean up
            (my $nt_print ,my $aa_print) = (pop(@executing_threads) -> 
                join()); ## clean the threads and get our print returns
                back
            print NTOUT uc $nt_print; ## we're doing this out of the
                thread so that we don't have buffer flushes screwing up
            prints
        print AAOUT uc $aa_print;
        }
            }
    }
    exit;

```
```

sub usage{

```
sub usage{
    return;
    return;
    }
```

    }
    ```
```

sub file_or_something{
if (-f \$_[0]){
push @files , shift @_;
}
else{
print"@_ either doesn't exist or an unknown option !\n";
}
}

```
sub load_codon_table\{
    \#simply returns a hash containing an extended table of codon \(\Rightarrow\) AA
        converstions
    my \(\% \mathrm{~g}=(\)
        'GCA' \(\Rightarrow\) ' A ',
        'GCT' \(\Rightarrow{ }^{\prime} \mathrm{A}^{\prime}\),
        'GCG' \(\Rightarrow{ }^{\prime} A^{\prime}\),
        'GCC' \(\Rightarrow{ }^{\prime} \mathrm{A}^{\prime}\),
        'GCW' \(\Rightarrow\) 'A',
        \({ }^{\prime} \mathrm{GCS}^{\prime} \Rightarrow{ }^{\prime} \mathrm{A}^{\prime}\),
        'GCM' \(\Rightarrow{ }^{\prime} \mathrm{A}^{\prime}\),
        'GCK' \(\Rightarrow\) 'A',
        'GCR' \(\Rightarrow{ }^{\prime} \mathrm{A}^{\prime}\),
        'GCY' \(\Rightarrow\) 'A',
        'GCB' \(\Rightarrow{ }^{\prime} \mathrm{A}^{\prime}\),
        'GCD' \(\Rightarrow\) ' A ',
        'GCH' \(\Rightarrow\) 'A',
        'GCV' \(\Rightarrow\) 'A',
        'GCN' \(\Rightarrow{ }^{\prime} \mathrm{A}^{\prime}\),
        'CGT' \(\Rightarrow\) 'R',
        'CGC' \(\Rightarrow{ }^{\prime} \mathrm{R}^{\prime}\),
        'CGA' \(\Rightarrow\) 'R',
        'CGG' \(\Rightarrow\) 'R',
\[
\begin{aligned}
& \text { 'AGA' } \Rightarrow{ }^{\prime} \mathrm{R}^{\prime} \text {, } \\
& \text { 'AGG' } \Rightarrow \text { 'R', } \\
& \text { 'CGN' }{ }^{\prime} \Rightarrow{ }^{\prime} \mathrm{R} \text { ', } \\
& \text { 'CGW' } \Rightarrow \text { 'R', } \\
& \text { 'CGS' } \Rightarrow{ }^{\prime} \mathrm{R}^{\prime} \text {, } \\
& \text { 'OGM' } \Rightarrow \text { 'R', } \\
& \text { 'CGK' } \Rightarrow \text { 'R', } \\
& \text { 'CGR' } \Rightarrow \text { ' }{ }^{\prime} \text { ', } \\
& \text { 'CGY' } \Rightarrow \text { 'R', } \\
& \text { 'CGB' } \Rightarrow \text { 'R', } \\
& \text { 'CGD' } \Rightarrow{ }^{\prime} \text { ' } \text { ', } \\
& \text { 'CGH' } \Rightarrow \text { 'R', } \\
& \text { 'CGV' } \Rightarrow{ }^{\prime} \mathrm{R}^{\prime} \text {, } \\
& \text { 'MGR' } \Rightarrow{ }^{\prime} \mathrm{R}^{\prime} \text {, } \\
& { }^{\prime} A A T{ }^{\prime} \Rightarrow{ }^{\prime} \mathrm{N}^{\prime}, \\
& \text { ' } \mathrm{AAC}{ }^{\prime} \Rightarrow \mathrm{N}^{\prime} \text {, } \\
& { }^{\prime} A A Y ' \Rightarrow{ }^{\prime} N^{\prime} \text {, } \\
& \text { 'GAT' } \Rightarrow \text { 'D', } \\
& \text { 'GAC' } \Rightarrow \text { 'D', } \\
& \text { 'GAY' } \Rightarrow{ }^{\prime} \mathrm{D}^{\prime} \text {, } \\
& \text { 'TGT' } \Rightarrow{ }^{\prime} \mathrm{C} \text { ', } \\
& \text { 'TGC' } \Rightarrow{ }^{\prime} \mathrm{C}^{\prime} \text {, } \\
& \text { 'TGY' } \Rightarrow{ }^{\prime}{ }^{\prime}{ }^{\prime} \text { ', } \\
& \text { 'CAA' } \Rightarrow{ }^{\prime} \mathrm{Q}^{\prime} \text {, } \\
& \text { 'CAG' } \Rightarrow{ }^{\prime} \mathrm{Q}^{\prime} \text {, } \\
& \text { 'CAR' } \Rightarrow{ }^{\prime} \mathrm{Q}^{\prime} \text {, } \\
& \text { 'GAA' } \Rightarrow{ }^{\prime} E \text { ', } \\
& \text { 'GAG' } \Rightarrow{ }^{\prime} \mathrm{E}^{\prime} \text {, } \\
& \text { 'GAR' } \Rightarrow{ }^{\prime} \mathrm{E}^{\prime} \text {, }
\end{aligned}
\]
\[
\begin{aligned}
& \text { 'GGT' } \Rightarrow \text { ' } \mathrm{G}^{\prime} \text {, } \\
& \text { 'GGC' } \Rightarrow{ }^{\prime} \mathrm{G}^{\prime} \text {, } \\
& \text { 'GGA' } \Rightarrow \text { ' } \mathrm{G}^{\prime} \text {, } \\
& \text { 'GGG' } \Rightarrow \text { ' }{ }^{\prime} \text { ', } \\
& \text { 'GGN' } \Rightarrow{ }^{\prime} \mathrm{G}^{\prime} \text {, } \\
& \text { 'GGW' } \Rightarrow \text { ' } \mathrm{G}^{\prime} \text {, } \\
& \text { 'GGS' } \Rightarrow \text { ' }{ }^{\prime} \text { ', } \\
& \text { 'GGM' } \Rightarrow \text { 'G', } \\
& \text { 'GGK' } \Rightarrow{ }^{\prime} \mathrm{G}^{\prime} \text {, } \\
& \text { 'GGR' } \Rightarrow{ }^{\prime} \mathrm{G}^{\prime} \text {, } \\
& { }^{\prime} \mathrm{GGY}^{\prime} \Rightarrow{ }^{\prime} \mathrm{G}^{\prime} \text {, } \\
& \text { 'GGB' } \Rightarrow \text { ' } \mathrm{G}^{\prime} \text {, } \\
& \text { 'GGD' } \Rightarrow{ }^{\prime} \mathrm{G}^{\prime} \text {, } \\
& \text { 'GGH' } \Rightarrow \text { ' }{ }^{\prime} \text { ', } \\
& \text { 'GGV' } \Rightarrow \text { ' } \mathrm{G}^{\prime} \text {, } \\
& \text { 'CAT' } \Rightarrow \text { 'H', } \\
& \text { 'CAC' } \Rightarrow{ }^{\prime} \mathrm{H}^{\prime} \text {, } \\
& \text { 'CAY' } \Rightarrow{ }^{\prime} \mathrm{H}^{\prime} \text {, } \\
& \text { 'ATT' } \Rightarrow{ }^{\prime} \text { I', } \\
& \text { 'ATC' } \Rightarrow{ }^{\prime} \text { I', } \\
& \text { 'ATA' } \Rightarrow{ }^{\prime} I^{\prime} \text {, } \\
& \text { 'ATH' } \Rightarrow \text { ' I', } \\
& \text { 'ATM' } \Rightarrow{ }^{\prime} \text { I', } \\
& \text { 'ATW' } \Rightarrow{ }^{\prime} \text { I', } \\
& { }^{\prime} \text { ATY' }^{\prime} \Rightarrow{ }^{\prime} I^{\prime}, \\
& { }^{\prime} \text { ATG' } \Rightarrow{ }^{\prime} \mathrm{M}^{\prime}, \\
& \text { 'TTA' } \Rightarrow \text { 'L', } \\
& \text { 'TTG' } \Rightarrow \text { 'L', } \\
& \text { 'CTT' } \Rightarrow{ }^{\prime} \mathrm{L}^{\prime} \text {, }
\end{aligned}
\]
\[
\begin{aligned}
& \text { 'CTC' } \Rightarrow{ }^{\prime} \mathrm{L}^{\prime} \text {, } \\
& \text { 'CTA' } \Rightarrow{ }^{\prime} \mathrm{L}^{\prime} \text {, } \\
& \text { 'CTG' } \Rightarrow{ }^{\prime} \mathrm{L}^{\prime} \text {, } \\
& \text { 'YTR' } \Rightarrow{ }^{\prime} L^{\prime} \text {, } \\
& \text { 'CTN' } \Rightarrow{ }^{\prime} \mathrm{L}^{\prime} \text {, } \\
& \text { 'CTW' } \Rightarrow{ }^{\prime} \mathrm{L}^{\prime} \text {, } \\
& \text { 'CTS' } \Rightarrow{ }^{\prime} \mathrm{L}^{\prime} \text {, } \\
& \text { 'CTM' } \Rightarrow{ }^{\prime} \text { L', } \\
& \text { 'CTK' } \Rightarrow{ }^{\prime} \mathrm{L}^{\prime} \text {, } \\
& \text { 'CTR' } \Rightarrow{ }^{\prime} \mathrm{L}^{\prime} \text {, } \\
& \text { 'CTY' } \Rightarrow{ }^{\prime} \mathrm{L}^{\prime} \text {, } \\
& { }^{\prime} \text { CTB' } \Rightarrow{ }^{\prime} \mathrm{L}^{\prime} \text {, } \\
& \text { 'CTD' } \Rightarrow{ }^{\prime} \mathrm{L}^{\prime} \text {, } \\
& { }^{\prime} \text { CTH' } \Rightarrow{ }^{\prime} \mathrm{L}^{\prime} \text {, } \\
& \text { 'CTV' } \Rightarrow{ }^{\prime} \mathrm{L}^{\prime} \text {, } \\
& \text { 'AAA' } \Rightarrow{ }^{\prime} \mathrm{K}^{\prime} \text {, } \\
& \text { 'AAG' } \Rightarrow{ }^{\prime} \mathrm{K}^{\prime} \text {, } \\
& \text { 'AAR' } \Rightarrow{ }^{\prime} \mathrm{K}^{\prime} \text {, } \\
& { }^{\prime} \text { TTT' } \Rightarrow{ }^{\prime} \mathrm{F}^{\prime} \text {, } \\
& \text { 'TTC' } \Rightarrow{ }^{\prime} \mathrm{F}^{\prime} \text {, } \\
& \text { 'TTY' } \Rightarrow{ }^{\prime} \text { 'F', } \\
& { }^{\prime} \mathrm{CCT}^{\prime} \Rightarrow{ }^{\prime} \mathrm{P}^{\prime} \text {, } \\
& { }^{\prime} \mathrm{CCC}{ }^{\prime} \Rightarrow{ }^{\prime} \mathrm{P}^{\prime} \text {, } \\
& \text { 'CCA' } \Rightarrow{ }^{\prime} \mathrm{P}^{\prime} \text {, } \\
& { }^{\prime} \mathrm{COG}^{\prime} \Rightarrow{ }^{\prime} \mathrm{P}^{\prime} \text {, } \\
& { }^{\prime} \mathrm{CCN}{ }^{\prime} \Rightarrow{ }^{\prime} \mathrm{P}^{\prime} \text {, } \\
& \text { 'COW' } \Rightarrow{ }^{\prime} \mathrm{P}^{\prime} \text {, } \\
& \text { 'CCS' } \Rightarrow{ }^{\prime} \mathrm{P}^{\prime} \text {, } \\
& \text { 'OCM' } \Rightarrow{ }^{\prime} \mathrm{P}^{\prime} \text {, } \\
& \text { 'CCK' } \Rightarrow{ }^{\prime} \mathrm{P}^{\prime} \text {, } \\
& \text { 'CCR' } \Rightarrow{ }^{\prime} \mathrm{P}^{\prime} \text {, }
\end{aligned}
\]
\[
\begin{aligned}
& \text { 'CCY' } \Rightarrow{ }^{\prime} \mathrm{P}^{\prime} \text {, } \\
& { }^{\prime} \mathrm{CCB}{ }^{\prime} \Rightarrow{ }^{\prime} \mathrm{P}^{\prime} \text {, } \\
& \text { 'CCD' } \Rightarrow{ }^{\prime} \mathrm{P}^{\prime} \text {, } \\
& \text { 'CCH' } \Rightarrow{ }^{\prime} \mathrm{P}^{\prime} \text {, } \\
& { }^{\prime} \mathrm{CCV}{ }^{\prime} \Rightarrow{ }^{\prime} \mathrm{P}^{\prime} \text {, } \\
& \text { 'TCT' } \Rightarrow \text { ' } \mathrm{S} \text { ', } \\
& \text { 'TCC' } \Rightarrow{ }^{\prime} \mathrm{S}^{\prime} \text {, } \\
& \text { 'TCA' } \Rightarrow \text { 'S', } \\
& \text { 'TOG' } \Rightarrow \text { 'S ', } \\
& \text { 'AGT' } \Rightarrow \text { ' }{ }^{\prime} \text {, } \\
& { }^{\prime} \text { AGC' } \Rightarrow{ }^{\prime} S^{\prime} \text {, } \\
& { }^{\prime} \mathrm{AGY}^{\prime} \Rightarrow{ }^{\prime} \mathrm{S}^{\prime} \text {, } \\
& \text { 'TCN' } \Rightarrow \text { 'S ', } \\
& { }^{\prime} \text { TCW' } \Rightarrow \text { ' }{ }^{\prime} \text {, } \\
& \text { 'TCS' } \Rightarrow \text { 'S', } \\
& \text { 'TCM' } \Rightarrow \text { 'S', } \\
& \text { 'TCK' } \Rightarrow \text { 'S', } \\
& { }^{\prime} \mathrm{TCR} \text { ' } \Rightarrow \text { ' } \mathrm{S} \text { ', } \\
& \text { 'TCY' } \Rightarrow \text { 'S', } \\
& { }^{\prime} \mathrm{TCB}{ }^{\prime} \Rightarrow{ }^{\prime} \mathrm{S}^{\prime} \text {, } \\
& \text { 'TCD' } \Rightarrow \text { 'S ', } \\
& { }^{\prime} \mathrm{TCH} \text { ' } \Rightarrow \text { 'S ', } \\
& \text { 'TCV' } \Rightarrow \text { 'S ', } \\
& \text { 'ACT' } \Rightarrow{ }^{\prime} \mathrm{T}^{\prime} \text {, } \\
& { }^{\prime} \mathrm{ACC}{ }^{\prime} \Rightarrow{ }^{\prime} \mathrm{T}^{\prime} \text {, } \\
& { }^{\prime} \mathrm{ACA}{ }^{\prime} \Rightarrow \mathrm{T}^{\prime} \text { ', } \\
& { }^{\prime} A C G \text { ' } \Rightarrow{ }^{\prime} \mathrm{T}^{\prime} \text {, } \\
& { }^{\prime} \mathrm{ACN}{ }^{\prime} \Rightarrow \mathrm{T}^{\prime} \text {, } \\
& \text { 'ACW' } \Rightarrow{ }^{\prime} \mathrm{T}^{\prime} \text {, } \\
& { }^{\prime} \mathrm{ACS} \text { ' } \Rightarrow{ }^{\prime} \mathrm{T}^{\prime} \text {, } \\
& \text { 'ACM' } \Rightarrow \text { ' } \mathrm{T}^{\prime} \text {, } \\
& \text { 'ACK' } \Rightarrow{ }^{\prime} \mathrm{T}^{\prime} \text {, }
\end{aligned}
\]
\[
\begin{aligned}
& \text { 'ACR' } \Rightarrow{ }^{\prime} \mathrm{T}^{\prime} \text {, } \\
& \text { 'ACY' } \Rightarrow{ }^{\prime} \mathrm{T}^{\prime} \text {, } \\
& \text { ' } \mathrm{ACB} \text { ' } \Rightarrow{ }^{\prime} \mathrm{T}^{\prime} \text {, } \\
& \text { 'ACD' } \Rightarrow{ }^{\prime} \mathrm{T}^{\prime} \text {, } \\
& \text { 'ACH' } \Rightarrow{ }^{\prime} \mathrm{T} \text { ', } \\
& \text { 'ACV' } \Rightarrow{ }^{\prime} \mathrm{T}^{\prime} \text {, } \\
& \text { 'TGG' } \Rightarrow \text { 'W', } \\
& \text { 'TAT' } \Rightarrow{ }^{\prime} \mathrm{Y}^{\prime} \text {, } \\
& \text { 'TAC' } \Rightarrow{ }^{\prime} \mathrm{Y}^{\prime} \text {, } \\
& \text { 'TAY' } \Rightarrow{ }^{\prime} Y^{\prime} \text {, } \\
& \text { 'GTT' } \Rightarrow{ }^{\prime} \mathrm{V}^{\prime} \text {, } \\
& \text { 'GTC' } \Rightarrow{ }^{\prime}{ }^{\prime} V^{\prime} \text {, } \\
& \text { 'GTA' } \Rightarrow{ }^{\prime} \mathrm{V} \text { ', } \\
& \text { 'GTG' } \Rightarrow{ }^{\prime} \mathrm{V}^{\prime} \text {, } \\
& \text { 'GTN' } \Rightarrow{ }^{\prime} \mathrm{V} \text { ', } \\
& \text { 'GIW' } \Rightarrow{ }^{\prime} \mathrm{V} \text { ', } \\
& \text { 'GTS' } \Rightarrow{ }^{\prime} V^{\prime} \text {, } \\
& \text { 'GTM' } \Rightarrow{ }^{\prime} \mathrm{V}^{\prime} \text {, } \\
& \text { 'GTK' } \Rightarrow \text { 'V', } \\
& \text { 'GTR' } \Rightarrow{ }^{\prime} \text { 'V', } \\
& \text { 'GTY' } \Rightarrow{ }^{\prime} \mathrm{V} \text { ', } \\
& \text { 'GTB' } \Rightarrow{ }^{\prime} \mathrm{V}^{\prime} \text {, } \\
& \text { 'GTD' } \Rightarrow{ }^{\prime} \mathrm{V}^{\prime} \text {, } \\
& \text { 'GTH' } \Rightarrow{ }^{\prime} \mathrm{V} \text { ', } \\
& \text { 'GTV' } \Rightarrow{ }^{\prime} \mathrm{V} \text { ', } \\
& \text { 'TAA' } \Rightarrow{ }^{\prime}{ }^{*} \text {, } \\
& \text { 'TGA' } \Rightarrow{ }^{\prime}{ }^{\prime} \text { ', } \\
& \text { 'TAG' } \Rightarrow{ }^{\prime}{ }^{*} \text { ', } \\
& \text { 'TAR' } \Rightarrow{ }^{\prime}{ }^{\prime} \text { ', } \\
& \text { 'TRA' } \Rightarrow{ }^{\prime}{ }^{\prime}{ }^{\prime}
\end{aligned}
\]
```

);
return %g;
}

```
sub six_frame_translate\{
    my \$aa_return ;
    my \(\$\) nt_return;
    \#process our record into a header and sequence and our reverse
        compliment
    (my \$header , my @tmp) = split (/\n/,shift @_);
my \$sequence = uc join ', , @tmp ;
my \(\$\) r_sequence \(=\) reverse \(\$\) sequence; \(\#\) reverse
\$r_sequence \(=\sim \operatorname{tr} /\) ATGC/TACG/; \#compliment
my @starts \(=([0],[1],[2],[0],[1],[2]) ;\) \# declare our array of arrays;
my @stops =([],[],[],[],[],[]); \#declare our array of arrays;
my \(\$\) nt_length \(=\$\) orf_size \(* 3+3\); \(\#\) add three for the stop codon
    if (\$header \(\left.=^{\sim} /(\backslash \mathrm{d}+) /\right)\{\)
    \$header \(=\$ 1\); \#\# if our header has a number in it (ie scaffold
        number we'll use it)
        \}
if (\$informatic_stops \(=0)\{\)
while (\$sequence \(=^{\sim} /\) ATG/ig) \(\{\)
    push (@starts[(pos(\$sequence)\%3)], pos (\$sequence)-3);
    \}
while (\$sequence \(=^{\sim} /\) TAG|TAA|TAR|TRA|TGA/ig) \(\{\)
    push (@stops[(pos(\$sequence)\%3)], pos (\$sequence));
    \}
while (\$r_sequence \(=^{\sim} /\) ATG/ig) \(\{\)
    push (@starts \(\left[\left(\operatorname{pos}\left(\$ r_{\text {_sequence }}\right) \% 3\right)+3\right]\), pos \((\$ r\) _sequence \(\left.)-3\right)\);
    \}
while (\$r_sequence \(=^{\sim} /\) TAG|TAA|TAR|TRA|TGA/ig) \(\{\)
    push (@stops [(pos(\$r_sequence)\%3) +3] , pos (\$r_sequence));
```

foreach (0..2){
push (@stops[(length($sequence)-$_)%3], length($sequence)-$_);
push (@stops[(length($sequence)-$_)%3+3], length($sequence)-$_
)
}
foreach my \$frame (0,1,2){
my $current_start = shift @{$starts[\$frame]};
my $current_stop = shift @{$stops[$frame]};
    while (@{$starts[$frame]}){ # while I have starts
        if(!(@{$stops[$frame]})){ # check if I have any stops, if
            no more stops clear out starts
            @{$starts[\$frame]} =();
}
my $length = ($current_stop - $current_start);
        if ($length >= \$nt_length){ \#our current start/stop pair are
far enough apart
\#print out our sequences to the correct files;
my $translated = translate(substr($sequence,
\$current_start, \$length));
$nt_return .= ">scaf_$header"."_frame_". ($frame+1) . "
                _start_".$current_start."_stop_".$current_stop."\n".
                substr($sequence, \$current_start, \$length)."\n";
$aa_return .= ">scaf_$header"."_frame_". ($frame+1) . "
                _start_".$current_start."_stop_".\$current_stop."\n".
$translated."\n";
            while ($current_start < \$current_stop){ \# while my start
is in front of my stop

```
```

        if (!@{$starts[$frame]}){ # if I'm out of start
        codons
            $current_start = $current_stop +1; # make a fake
                start that's beyond my current stop
            }
                else{
                    $current_start = shift @{$starts[$frame]}; #
                    other wise get the next start codon
                    }
                }
            }
    elsif (($length < $nt_length) && ($length >= 3)){ ## too
        short
        $current_start = shift @{$starts[$frame]};
        }
    elsif (($length <= 0)){
        $current_stop = shift @{$stops[$frame]};
        }
    }
    }
    
## now the reverse side

foreach my \$frame ( 3,4,5){
my $current_start = shift @{$starts[\$frame]};
my $current_stop = shift @{$stops[$frame]};
    while (@{$starts[$frame]}){ # while I have starts
    if(!(@{$stops[$frame]})){ # check if I have any stops, if
        no more stops clear out starts
            @{$starts[\$frame]} =();
}
my $length = ($current_stop-\$current_start);

```
```

if (\$length >= \$nt_length){ \#our current start/stop pair are
far enough apart
\#print out our sequences to the correct files;
my $translated = translate(substr($r_sequence,
$current_start,$length) , \$-[2]);
$nt_return .= ">scaf_"."$header" . "_frame_". ($frame+1)
    ."_start_".(length($r_sequence)-$current_start
    +1) . " _stop_" . (length($r_sequence)-$current_stop
    +1) ."\n".substr($r_sequence,$current_start,$length)
."\n";
$aa_return .= ">scaf_"."$header" ."_frame_". ($frame+1)
    ."_start_" . (length($r_sequence)-$current_start
    +1) . " -stop_" . (length($r_sequence)-\$current_stop
+1) ."\n". \$translated ."\n";

```
\#\# this loop bleeds off start codons so we dont get
    multiple sub strings
while (\$current_start < \$current_stop) \(\left\{\begin{array}{l}\text { \# while my start }\end{array}\right.\)
    is in front of my stop
    if (!@\{\$starts[\$frame]\}) \{ \# if I'm out of start
        codons
            \$current_start \(=\$\) current_stop +1 ; \# make a fake
                start that's beyond my current stop
            \}
    else \{
            \$current_start \(=\) shift @\{\$starts[\$frame]\}; \#
                other wise get the next start codon
            \}
    \}
\}
```

            elsif (($length < $nt_length) && ($length >= 3)){ ## too
                    short
                        $current_start = shift @{$starts[$frame]};
                }
            elsif (($length<= 0)){
                $current_stop = shift @{$stops[$frame]};
                }
                }
        }
    return ($nt_return , $aa_return);
    }
if(\$informatic_stops=1){
}
}
sub translate{
\#accepts a nucleotide sequence and returns an AA
my \$sequence_in = $_ [0];
    my @DNA = split(//,$sequence_in);
my \$protein;
while (@DNA) {
my $codon = join( "",(splice(@DNA,0,3)));
    if (exists ($g{\$codon })){
\$protein .= $g{$codon };
}
else{
$protein . = 'X';
            }
    }
    return($protein);

```

\section*{Appendix F}

\section*{RxLR and CRN Node Descriptions}

\section*{F. 1 RxLR Node Descriptions}

PHYCAScaffold_25 DSRXLR CDS 323402323764 . + . ID=name"RXLR_0"
PHYCAScaffold_8 DSRXLR CDS 247029247274 . - . ID=name"RXLR_1"
PHYCAScaffold_18 DSRXLR CDS 388085388681 . + . ID=name"RXLR_2"
PHYCAScaffold_48 DSRXLR CDS 137619 139427 . - . ID=name"RXLR_3"
PHYCAScaffold_6 DSRXLR CDS 13033071303645 . + . ID=name"RXLR_4"
PHYCAScaffold_17 DSRXLR CDS 419015420544 . - . ID=name"RXLR_5"
PHYCAScaffold_32 DSRXLR CDS 390473 390856 . + . ID=name"RXLR_6"
PHYCAScaffold_40 DSRXLR CDS 393862394293 . - . ID=name"RXLR_7"
PHYCAScaffold_55 DSRXLR CDS 246914 249292 . - . ID=name"RXLR_8"
PHYCAScaffold_41 DSRXLR CDS 225282 225803 . - . ID=name"RXLR_9"
PHYCAScaffold_24 DSRXLR CDS 612902613150 . + . ID=name"RXLR_10"
PHYCAScaffold_37 DSRXLR CDS 175084 176634. - . ID=name"RXLR_11"
PHYCAScaffold_3 DSRXLR CDS 1504049 1508131 . - . ID=name"RXLR_12"
PHYCAScaffold_92 DSRXLR CDS 7176372062 . - . ID=name"RXLR_13"
PHYCAScaffold_106 DSRXLR CDS 7283173556 . + . ID=name"RXLR_14"
PHYCAScaffold_38 DSRXLR CDS 229809230123 . - . ID=name"RXLR_15"

PHYCAScaffold_431 DSRXLR CDS 26542914 . - . ID=name"RXLR_16" PHYCAScaffold_53 DSRXLR CDS 4779348248 . - . ID=name"RXLR_17" PHYCAScaffold_28 DSRXLR CDS 357593360748 . + . ID=name"RXLR_18" PHYCAScaffold_64 DSRXLR CDS 3497136383 . + . ID=name"RXLR_19" PHYCAScaffold_10 DSRXLR CDS 11416691142448 . - . ID=name"RXLR_20" PHYCAScaffold_68 DSRXLR CDS 117067117285 . - . ID=name"RXLR_21" PHYCAScaffold_31 DSRXLR CDS 505883506356 . + . ID=name"RXLR_22" PHYCAScaffold_103 DSRXLR CDS 129318 129734 . + . ID=name"RXLR_23" PHYCAScaffold_2 DSRXLR CDS 323335324462 . + . ID=name"RXLR_24" PHYCAScaffold_60 DSRXLR CDS 162527 164635 . - . ID=name"RXLR_25" PHYCAScaffold_21 DSRXLR CDS 666958 668031 . + . ID=name"RXLR_26" PHYCAScaffold_13 DSRXLR CDS 1004702 1008061 . + . ID=name"RXLR_27" PHYCAScaffold_5 DSRXLR CDS \(378003378584 .+\). ID=name"RXLR_28" PHYCAScaffold_36 DSRXLR CDS 601248602570 . + . ID=name"RXLR_29" PHYCAScaffold_8 DSRXLR CDS 20972474 . - . ID=name"RXLR_30" PHYCAScaffold_19 DSRXLR CDS 637850639442 . + . ID=name"RXLR_31" PHYCAScaffold_108 DSRXLR CDS 44392 46107 . - . ID=name"RXLR_32" PHYCAScaffold_62 DSRXLR CDS 271547 272839 . + . ID=name"RXLR_33" PHYCAScaffold_10 DSRXLR CDS 716479716982 . + . ID=name"RXLR_34" PHYCAScaffold_11 DSRXLR CDS 8799388256 . + . ID=name"RXLR_35" PHYCAScaffold_16 DSRXLR CDS 449616452552 . + . ID=name"RXLR_36" PHYCAScaffold_50 DSRXLR CDS 102935 103612 . - . ID=name"RXLR_37" PHYCAScaffold_40 DSRXLR CDS 445614446174 . - . ID=name"RXLR_38" PHYCAScaffold_70 DSRXLR CDS 118220 118888 . - . ID=name"RXLR_39" PHYCAScaffold_40 DSRXLR CDS 247798248127 . - . ID=name"RXLR_40" PHYCAScaffold_19 DSRXLR CDS 460491462089 . - . ID=name"RXLR_41" PHYCAScaffold_27 DSRXLR CDS 691216692610 . + . ID=name"RXLR_42" PHYCAScaffold_87 DSRXLR CDS 4072 4518 . - . ID=name"RXLR_43" PHYCAScaffold_2 DSRXLR CDS 1312901 1313387 . - . ID=name"RXLR_44"

PHYCAScaffold_2 DSRXLR CDS 1212053 1212397 . - . ID=name"RXLR_45" PHYCAScaffold_1 DSRXLR CDS 629240629965 . - . ID=name"RXLR_46" PHYCAScaffold_7 DSRXLR CDS 169457 169729 . + . ID=name"RXLR_47" PHYCAScaffold_13 DSRXLR CDS 314756315094 . + . ID=name"RXLR_48" PHYCAScaffold_18 DSRXLR CDS 882491882712 . + . ID=name"RXLR_49" PHYCAScaffold_19 DSRXLR CDS 724778725227 . + . ID=name"RXLR_50" PHYCAScaffold_53 DSRXLR CDS 170437173070 . + . ID=name"RXLR_51" PHYCAScaffold_2 DSRXLR CDS 315335315790 . - . ID=name"RXLR_52" PHYCAScaffold_23 DSRXLR CDS 345130347379 . - . ID=name"RXLR_53" PHYCAScaffold_20 DSRXLR CDS 354427354654 . + . ID=name"RXLR_54" PHYCAScaffold_30 DSRXLR CDS 2819629137 . + . ID=name"RXLR_55" PHYCAScaffold_29 DSRXLR CDS 321243 321641 . - . ID=name"RXLR_56" PHYCAScaffold_21 DSRXLR CDS 423578423841 . + . ID=name"RXLR_57" PHYCAScaffold_4 DSRXLR CDS 355630357201 . - . ID=name"RXLR_58" PHYCAScaffold_5 DSRXLR CDS 997091997765 . - . ID=name"RXLR_59" PHYCAScaffold_104 DSRXLR CDS 7573776348 . - . ID=name"RXLR_60" PHYCAScaffold_39 DSRXLR CDS 272413272784 . - . ID=name"RXLR_61" PHYCAScaffold_11 DSRXLR CDS 221929222540 . + . ID=name"RXLR_62" PHYCAScaffold_8 DSRXLR CDS 599681600712 . - . ID=name"RXLR_63" PHYCAScaffold_52 DSRXLR CDS 6763468023 . - . ID=name"RXLR_64" PHYCAScaffold_49 DSRXLR CDS 304665306644 . - . ID=name"RXLR_65" PHYCAScaffold_2 DSRXLR CDS 1499362 1499717. - . ID=name"RXLR_66" PHYCAScaffold_96 DSRXLR CDS 13378 14031 . + . ID=name"RXLR_67" PHYCAScaffold_2 DSRXLR CDS 317937318323 . + . ID=name"RXLR_68" PHYCAScaffold_57 DSRXLR CDS 325662325874 . - . ID=name"RXLR_69" PHYCAScaffold_21 DSRXLR CDS 90428 93247 . - . ID=name"RXLR_70" PHYCAScaffold_6 DSRXLR CDS 905322905768 . - . ID=name"RXLR_71" PHYCAScaffold_80 DSRXLR CDS 3709737423 . - . ID=name"RXLR_72" PHYCAScaffold_48 DSRXLR CDS 6500368284 . + . ID=name"RXLR_73"

PHYCAScaffold_2 DSRXLR CDS 157778 158170. - . ID=name"RXLR_74" PHYCAScaffold_42 DSRXLR CDS 2152421796 . + . ID=name"RXLR_75" PHYCAScaffold_4 DSRXLR CDS 4370044113 . - . ID=name"RXLR_76" PHYCAScaffold_2 DSRXLR CDS 156932157300 . - . ID=name"RXLR_77" PHYCAScaffold_9 DSRXLR CDS 684380685951 . - . ID=name"RXLR_78" PHYCAScaffold_7 DSRXLR CDS 881413881802 . - . ID=name"RXLR_79" PHYCAScaffold_38 DSRXLR CDS 498080498781 . - . ID=name"RXLR_80" PHYCAScaffold_87 DSRXLR CDS 6614466452 . - . ID=name"RXLR_81" PHYCAScaffold_12 DSRXLR CDS 813376818256 . + . ID=name"RXLR_82" PHYCAScaffold_6 DSRXLR CDS 13179371318254 . - . ID=name"RXLR_83" PHYCAScaffold_33 DSRXLR CDS 276962 278917. - . ID=name"RXLR_84" PHYCAScaffold_29 DSRXLR CDS 346894348741 . - . ID=name"RXLR_85" PHYCAScaffold_1 DSRXLR CDS \(773946774881 .+\). ID=name"RXLR_86" PHYCAScaffold_30 DSRXLR CDS 6070461219 . - . ID=name"RXLR_87" PHYCAScaffold_16 DSRXLR CDS 320249321379 . - . ID=name"RXLR_88" PHYCAScaffold_29 DSRXLR CDS 607577 608101 . + . ID=name"RXLR_89" PHYCAScaffold_12 DSRXLR CDS 192776 193192 . + . ID=name"RXLR_90" PHYCAScaffold_50 DSRXLR CDS 361564361791 . - . ID=name"RXLR_91" PHYCAScaffold_4 DSRXLR CDS 237951238235 . - . ID=name"RXLR_92" PHYCAScaffold_536 DSRXLR CDS 2814 3098. - . ID=name"RXLR_93" PHYCAScaffold_2 DSRXLR CDS 588637588933 . + . ID=name"RXLR_94" PHYCAScaffold_14 DSRXLR CDS 851114851455 . + . ID=name"RXLR_95" PHYCAScaffold_72 DSRXLR CDS 25279 25596 . + . ID=name"RXLR_96" PHYCAScaffold_47 DSRXLR CDS 197853 198869 . + . ID=name"RXLR_97" PHYCAScaffold_3 DSRXLR CDS 1266965 1268998 . - . ID=name"RXLR_98" PHYCAScaffold_43 DSRXLR CDS 4418744411 . + . ID=name"RXLR_99" PHYCAScaffold_86 DSRXLR CDS 45554 46108 . - . ID=name"RXLR_100" PHYCAScaffold_48 DSRXLR CDS \(407397407654 .+\). ID=name"RXLR_101" PHYCAScaffold_27 DSRXLR CDS 256683 258155 . + . ID=name"RXLR_102"

PHYCAScaffold_18 DSRXLR CDS 930295930546 . + . ID=name"RXLR_103" PHYCAScaffold_1 DSRXLR CDS 12401141240698 . - . ID=name"RXLR_104" PHYCAScaffold_7 DSRXLR CDS 787456787866 . - . ID=name"RXLR_105" PHYCAScaffold_46 DSRXLR CDS 238173238667 . - . ID=name"RXLR_106" PHYCAScaffold_29 DSRXLR CDS 263020263517 . + . ID=name"RXLR_107" PHYCAScaffold_25 DSRXLR CDS 329087 329506 . - . ID=name"RXLR_108" PHYCAScaffold_20 DSRXLR CDS \(832772833359 .+\). ID=name"RXLR_109" PHYCAScaffold_14 DSRXLR CDS \(10051471008800 .+\). ID=name"RXLR_110" PHYCAScaffold_26 DSRXLR CDS 244038244904 . - . ID=name"RXLR_111" PHYCAScaffold_37 DSRXLR CDS 221168 222031 . - . ID=name"RXLR_112" PHYCAScaffold_3 DSRXLR CDS 256453256878 . - . ID=name"RXLR_113" PHYCAScaffold_65 DSRXLR CDS 135948 136790 . - . ID=name"RXLR_114" PHYCAScaffold_1 DSRXLR CDS 154552154764 . + . ID=name"RXLR_115" PHYCAScaffold_37 DSRXLR CDS 451785454598 . - . ID=name"RXLR_116" PHYCAScaffold_7 DSRXLR CDS 608505609764 . + . ID=name"RXLR_117" PHYCAScaffold_104 DSRXLR CDS 6562966234 . - . ID=name"RXLR_118" PHYCAScaffold_3 DSRXLR CDS 533356534846 . - . ID=name"RXLR_119" PHYCAScaffold_2 DSRXLR CDS 13224821323174 . + . ID=name"RXLR_120" PHYCAScaffold_55 DSRXLR CDS \(8208482464 .+\). ID=name"RXLR_121" PHYCAScaffold_81 DSRXLR CDS 103693 104391 . - . ID=name"RXLR_122" PHYCAScaffold_40 DSRXLR CDS 335532336290 . - . ID=name"RXLR_123" PHYCAScaffold_17 DSRXLR CDS 839188839529 . - . ID=name"RXLR_124" PHYCAScaffold_11 DSRXLR CDS 396067 396351 . + . ID=name"RXLR_125" PHYCAScaffold_14 DSRXLR CDS 758513758863 . + . ID=name"RXLR_126" PHYCAScaffold_51 DSRXLR CDS 405525407660 . + . ID=name"RXLR_127" PHYCAScaffold_2 DSRXLR CDS 705106706329 . + . ID=name"RXLR_128" PHYCAScaffold_12 DSRXLR CDS 1116105 1116563 . - . ID=name"RXLR_129" PHYCAScaffold_7 DSRXLR CDS 865807866112 . - . ID=name"RXLR_130" PHYCAScaffold_21 DSRXLR CDS 183799 185793 . - . ID=name"RXLR_131"

PHYCAScaffold_3 DSRXLR CDS 697798 699396 . - . ID=name"RXLR_132" PHYCAScaffold_38 DSRXLR CDS 467057468412 . - . ID=name"RXLR_133" PHYCAScaffold_9 DSRXLR CDS 730927731655 . - . ID=name"RXLR_134" PHYCAScaffold_74 DSRXLR CDS 5400754294 . - . ID=name"RXLR_135" PHYCAScaffold_102 DSRXLR CDS 8562186358 . + . ID=name"RXLR_136" PHYCAScaffold_87 DSRXLR CDS 161140161775 . + . ID=name"RXLR_137" PHYCAScaffold_86 DSRXLR CDS 7581976898 . + . ID=name"RXLR_138" PHYCAScaffold_12 DSRXLR CDS 706366706833 . - . ID=name"RXLR_139" PHYCAScaffold_12 DSRXLR CDS \(931251931553 .+\). ID=name"RXLR_140" PHYCAScaffold_4 DSRXLR CDS 390259 390531. - . ID=name"RXLR_141" PHYCAScaffold_37 DSRXLR CDS 498072 498491 . + . ID=name"RXLR_142" PHYCAScaffold_4 DSRXLR CDS 11418431142124 . + . ID=name"RXLR_143" PHYCAScaffold_46 DSRXLR CDS 234864235526 . + . ID=name"RXLR_144" PHYCAScaffold_3 DSRXLR CDS 539108541129 . + . ID=name"RXLR_145" PHYCAScaffold_32 DSRXLR CDS 251798255175 . - . ID=name"RXLR_146" PHYCAScaffold_15 DSRXLR CDS 801631802014 . + . ID=name"RXLR_147" PHYCAScaffold_5 DSRXLR CDS 1108202 1108510 . - . ID=name"RXLR_148" PHYCAScaffold_1 DSRXLR CDS 491235491879 . - . ID=name"RXLR_149" PHYCAScaffold_6 DSRXLR CDS 11167181117008 . + . ID=name"RXLR_150" PHYCAScaffold_17 DSRXLR CDS 94628 95146 . + . ID=name"RXLR_151" PHYCAScaffold_7 DSRXLR CDS 125582125848 . + . ID=name"RXLR_152" PHYCAScaffold_36 DSRXLR CDS 110317 110766 . - . ID=name"RXLR_153" PHYCAScaffold_90 DSRXLR CDS 118929 119171 . - . ID=name"RXLR_154" PHYCAScaffold_27 DSRXLR CDS 348452348694 . - . ID=name"RXLR_155" PHYCAScaffold_6 DSRXLR CDS 633546634535 . - . ID=name"RXLR_156" PHYCAScaffold_105 DSRXLR CDS \(3833240374 .+\) ID=name"RXLR_157" PHYCAScaffold_46 DSRXLR CDS 6510065543 . - . ID=name"RXLR_158" PHYCAScaffold_37 DSRXLR CDS 178093 180252 . - . ID=name"RXLR_159" PHYCAScaffold_34 DSRXLR CDS 450662455890 . + . ID=name"RXLR_160"

PHYCAScaffold_25 DSRXLR CDS 705132705512 . + . ID=name"RXLR_161" PHYCAScaffold_76 DSRXLR CDS 9857199077 . + . ID=name"RXLR_162" PHYCAScaffold_11 DSRXLR CDS 295183 297129 . + . ID=name"RXLR_163" PHYCAScaffold_18 DSRXLR CDS 856163856591 . - . ID=name"RXLR_164" PHYCAScaffold_5 DSRXLR CDS 5402 5824 . - . ID=name"RXLR_165" PHYCAScaffold_26 DSRXLR CDS 109723110064 . + . ID=name"RXLR_166" PHYCAScaffold_640 DSRXLR CDS 18102082 . - . ID=name"RXLR_167" PHYCAScaffold_53 DSRXLR CDS 4445444855 . + . ID=name"RXLR_168" PHYCAScaffold_121 DSRXLR CDS 5051950785 . + . ID=name"RXLR_169" PHYCAScaffold_592 DSRXLR CDS 32663478 . + . ID=name"RXLR_170" PHYCAScaffold_47 DSRXLR CDS 216197 216628. - . ID=name"RXLR_171" PHYCAScaffold_8 DSRXLR CDS 1124521 1125948. - . ID=name"RXLR_172" PHYCAScaffold_4 DSRXLR CDS 1083030 1083308 . - . ID=name"RXLR_173" PHYCAScaffold_11 DSRXLR CDS 1063702 1065099 . - . ID=name"RXLR_174" PHYCAScaffold_35 DSRXLR CDS \(413375415471 .+\). ID=name"RXLR_175" PHYCAScaffold_36 DSRXLR CDS 242708 244201 . + . ID=name"RXLR_176" PHYCAScaffold_30 DSRXLR CDS 328116328367 . - . ID=name"RXLR_177" PHYCAScaffold_4 DSRXLR CDS 1470348 1470599 . - . ID=name"RXLR_178" PHYCAScaffold_4 DSRXLR CDS 12858421286090 . + . ID=name"RXLR_179" PHYCAScaffold_100 DSRXLR CDS 8028580593 . - . ID=name"RXLR_180" PHYCAScaffold_2 DSRXLR CDS 479198479608 . - . ID=name"RXLR_181" PHYCAScaffold_5 DSRXLR CDS 844151845401 . + . ID=name"RXLR_182" PHYCAScaffold_40 DSRXLR CDS 242698 244503 . + . ID=name"RXLR_183" PHYCAScaffold_9 DSRXLR CDS 1035173 1035751 . + . ID=name"RXLR_184" PHYCAScaffold_3 DSRXLR CDS 535599537218 . - . ID=name"RXLR_185" PHYCAScaffold_1 DSRXLR CDS 929114929813 . + . ID=name"RXLR_186" PHYCAScaffold_18 DSRXLR CDS 788069788467 . + . ID=name"RXLR_187" PHYCAScaffold_1 DSRXLR CDS 2010182 2011090 . - . ID=name"RXLR_188" PHYCAScaffold_2 DSRXLR CDS 10370991037365 . + . ID=name"RXLR_189"

PHYCAScaffold_21 DSRXLR CDS 8148182236 . + . ID=name"RXLR_190" PHYCAScaffold_25 DSRXLR CDS 771328771894 . - . ID=name"RXLR_191" PHYCAScaffold_1 DSRXLR CDS 20274702027745 . + . ID=name"RXLR_192" PHYCAScaffold_37 DSRXLR CDS 104105 104998 . + . ID=name"RXLR_193" PHYCAScaffold_9 DSRXLR CDS 955046955810 . + . ID=name"RXLR_194" PHYCAScaffold_15 DSRXLR CDS 443355446510 . - . ID=name"RXLR_195" PHYCAScaffold_82 DSRXLR CDS 118308 118961 . - . ID=name"RXLR_196" PHYCAScaffold_25 DSRXLR CDS \(356900357550 .+\). ID=name"RXLR_197" PHYCAScaffold_111 DSRXLR CDS 28885 29109 . + . ID=name"RXLR_198" PHYCAScaffold_111 DSRXLR CDS 7485975383 . + . ID=name"RXLR_199" PHYCAScaffold_7 DSRXLR CDS 171968 172378 . - . ID=name"RXLR_200" PHYCAScaffold_27 DSRXLR CDS 2195126951 . + . ID=name"RXLR_201" PHYCAScaffold_41 DSRXLR CDS 223367223744 . + . ID=name"RXLR_202" PHYCAScaffold_48 DSRXLR CDS 420624421199 . + . ID=name"RXLR_203" PHYCAScaffold_8 DSRXLR CDS 1156246 1160307 . - . ID=name"RXLR_204" PHYCAScaffold_29 DSRXLR CDS 257568257807 . + . ID=name"RXLR_205" PHYCAScaffold_2 DSRXLR CDS 541581542585 . + . ID=name"RXLR_206" PHYCAScaffold_1 DSRXLR CDS 439295439609 . + . ID=name"RXLR_207" PHYCAScaffold_19 DSRXLR CDS 596651 598480 . - . ID=name"RXLR_208" PHYCAScaffold_53 DSRXLR CDS 334297335220 . + . ID=name"RXLR_209" PHYCAScaffold_46 DSRXLR CDS \(254174254767 .+\). ID=name"RXLR_210" PHYCAScaffold_12 DSRXLR CDS 473192474175 . - . ID=name"RXLR_211" PHYCAScaffold_13 DSRXLR CDS 312245312583 . - . ID=name"RXLR_212" PHYCAScaffold_42 DSRXLR CDS 486648487049 . - . ID=name"RXLR_213" PHYCAScaffold_20 DSRXLR CDS 3288533142 . + . ID=name"RXLR_214" PHYCAScaffold_7 DSRXLR CDS 10577761058249 . + . ID=name"RXLR_215" PHYCAScaffold_77 DSRXLR CDS 135242 135538 . - . ID=name"RXLR_216" PHYCAScaffold_37 DSRXLR CDS 214948 215397 . - . ID=name"RXLR_217" PHYCAScaffold_57 DSRXLR CDS 5641557692 . + . ID=name"RXLR_218"

PHYCAScaffold_7 DSRXLR CDS 876213876800 . - . ID=name"RXLR_219" PHYCAScaffold_17 DSRXLR CDS 531552534035 . + . ID=name"RXLR_220" PHYCAScaffold_65 DSRXLR CDS 121117 121545 . + . ID=name"RXLR_221" PHYCAScaffold_6 DSRXLR CDS 12011651201413 . + . ID=name"RXLR_222" PHYCAScaffold_104 DSRXLR CDS 102289 102708 . - . ID=name"RXLR_223" PHYCAScaffold_14 DSRXLR CDS 363216363869 . + . ID=name"RXLR_224" PHYCAScaffold_36 DSRXLR CDS \(646254646724 .+\). ID=name"RXLR_225" PHYCAScaffold_21 DSRXLR CDS 494368497733 . - . ID=name"RXLR_226" PHYCAScaffold_1 DSRXLR CDS 20520382052655 . - . ID=name"RXLR_227" PHYCAScaffold_21 DSRXLR CDS 523642525390 . + . ID=name"RXLR_228" PHYCAScaffold_5 DSRXLR CDS 6471665867 . - . ID=name"RXLR_229" PHYCAScaffold_37 DSRXLR CDS 8915590048 . - . ID=name"RXLR_230" PHYCAScaffold_10 DSRXLR CDS 432741433787 . + . ID=name"RXLR_231" PHYCAScaffold_22 DSRXLR CDS 378423 379244 . - . ID=name"RXLR_232" PHYCAScaffold_17 DSRXLR CDS 219024219305 . - . ID=name"RXLR_233" PHYCAScaffold_26 DSRXLR CDS \(335958336992 .+\) ID=name"RXLR_234" PHYCAScaffold_46 DSRXLR CDS 177459 177860 . - . ID=name"RXLR_235" PHYCAScaffold_92 DSRXLR CDS 4962 5408 . + . ID=name"RXLR_236" PHYCAScaffold_10 DSRXLR CDS 320141320359 . + . ID=name"RXLR_237" PHYCAScaffold_9 DSRXLR CDS 207320209557 . + . ID=name"RXLR_238" PHYCAScaffold_1 DSRXLR CDS 2019607 2020503 . - . ID=name"RXLR_239" PHYCAScaffold_1 DSRXLR CDS 11520111152448 . + . ID=name"RXLR_240" PHYCAScaffold_99 DSRXLR CDS 138321 138686 . - . ID=name"RXLR_241" PHYCAScaffold_22 DSRXLR CDS 570874571125 . + . ID=name"RXLR_242" PHYCAScaffold_35 DSRXLR CDS 91394 91693 . - . ID=name"RXLR_243" PHYCAScaffold_1 DSRXLR CDS 20538942054235 . + . ID=name"RXLR_244" PHYCAScaffold_10 DSRXLR CDS 718749719252 . + . ID=name"RXLR_245" PHYCAScaffold_42 DSRXLR CDS 400887404054 . - . ID=name"RXLR_246" PHYCAScaffold_13 DSRXLR CDS 1026619 1026975 . - . ID=name"RXLR_247"

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PHYCAScaffold_48 DSRXLR CDS 231650231919 . + . ID=name"RXLR_277" PHYCAScaffold_6 DSRXLR CDS 128730129938 . + . ID=name"RXLR_278" PHYCAScaffold_66 DSRXLR CDS 9168292188 . + . ID=name"RXLR_279" PHYCAScaffold_1 DSRXLR CDS 16282971628743 . + . ID=name"RXLR_280" PHYCAScaffold_25 DSRXLR CDS 290208293318 . + . ID=name"RXLR_281" PHYCAScaffold_27 DSRXLR CDS 190021 190326 . + . ID=name"RXLR_282" PHYCAScaffold_2 DSRXLR CDS 316258316668 . - . ID=name"RXLR_283" PHYCAScaffold_57 DSRXLR CDS \(165135165380 .+\) ID=name"RXLR_284" PHYCAScaffold_237 DSRXLR CDS 5079 5366 . - . ID=name"RXLR_285" PHYCAScaffold_73 DSRXLR CDS 194639 194995 . + . ID=name"RXLR_286" PHYCAScaffold_15 DSRXLR CDS 6902369595 . + . ID=name"RXLR_287" PHYCAScaffold_6 DSRXLR CDS 180633 182348 . - . ID=name"RXLR_288" PHYCAScaffold_7 DSRXLR CDS 12006511200869 . + . ID=name"RXLR_289" PHYCAScaffold_70 DSRXLR CDS 91553 91867 . - . ID=name"RXLR_290" PHYCAScaffold_48 DSRXLR CDS 6981270858 . - . ID=name"RXLR_291" PHYCAScaffold_3 DSRXLR CDS 447447448238 . - . ID=name"RXLR_292" PHYCAScaffold_11 DSRXLR CDS \(265467266468 .+\). ID=name"RXLR_293" PHYCAScaffold_15 DSRXLR CDS 873937874362 . - . ID=name"RXLR_294" PHYCAScaffold_13 DSRXLR CDS 929397929690 . + . ID=name"RXLR_295" PHYCAScaffold_3 DSRXLR CDS 476612477079 . + . ID=name"RXLR_296" PHYCAScaffold_10 DSRXLR CDS 151315 151572 . - . ID=name"RXLR_297" PHYCAScaffold_1 DSRXLR CDS 19594351960472 . - . ID=name"RXLR_298" PHYCAScaffold_41 DSRXLR CDS 133567136524 . - . ID=name"RXLR_299" PHYCAScaffold_25 DSRXLR CDS 528104528502 . + . ID=name"RXLR_300" PHYCAScaffold_7 DSRXLR CDS 140077140460 . - . ID=name"RXLR_301" PHYCAScaffold_2 DSRXLR CDS 191960 192295 . - . ID=name"RXLR_302" PHYCAScaffold_5 DSRXLR CDS 704414704839 . - . ID=name"RXLR_303" PHYCAScaffold_44 DSRXLR CDS 380323381774 . + . ID=name"RXLR_304" PHYCAScaffold_1 DSRXLR CDS 1165829 1166266 . + . ID=name"RXLR_305"

PHYCAScaffold_42 DSRXLR CDS 445832446422 . + . ID=name"RXLR_306" PHYCAScaffold_47 DSRXLR CDS 266980267321 . - . ID=name"RXLR_307" PHYCAScaffold_31 DSRXLR CDS 322357322941 . + . ID=name"RXLR_308" PHYCAScaffold_79 DSRXLR CDS 52802 54565 . - . ID=name"RXLR_309" PHYCAScaffold_3 DSRXLR CDS 531393532706 . - . ID=name"RXLR_310" PHYCAScaffold_8 DSRXLR CDS 150384 150710 . - . ID=name"RXLR_311" PHYCAScaffold_17 DSRXLR CDS 7533676355 . + . ID=name"RXLR_312" PHYCAScaffold_3 DSRXLR CDS 1223861 1224076 . + . ID=name"RXLR_313" PHYCAScaffold_33 DSRXLR CDS \(440460440966 .+\). ID=name"RXLR_314" PHYCAScaffold_6 DSRXLR CDS 613234615537 . + . ID=name"RXLR_315" PHYCAScaffold_19 DSRXLR CDS \(698053701541 .+\). ID=name"RXLR_316" PHYCAScaffold_53 DSRXLR CDS \(4535445794 .+\). ID=name"RXLR_317" PHYCAScaffold_101 DSRXLR CDS \(5118951614 .+\) ID=name"RXLR_318" PHYCAScaffold_6 DSRXLR CDS 463977464804 . - . ID=name"RXLR_319" PHYCAScaffold_106 DSRXLR CDS 3663836886 . - . ID=name"RXLR_320" PHYCAScaffold_494 DSRXLR CDS \(7451578 .+\). ID=name"RXLR_321" PHYCAScaffold_6 DSRXLR CDS 1135732 1135953 . - . ID=name"RXLR_322" PHYCAScaffold_26 DSRXLR CDS 489888491075 . + . ID=name"RXLR_323" PHYCAScaffold_29 DSRXLR CDS 247983 248936 . + . ID=name"RXLR_324" PHYCAScaffold_6 DSRXLR CDS 13086241308920 . - . ID=name"RXLR_325" PHYCAScaffold_17 DSRXLR CDS \(403482403775 .+\). ID=name"RXLR_326" PHYCAScaffold_14 DSRXLR CDS 891564892550 . + . ID=name"RXLR_327" PHYCAScaffold_495 DSRXLR CDS 40334467 . - . ID=name"RXLR_328" PHYCAScaffold_5 DSRXLR CDS 768937769353 . + . ID=name"RXLR_329" PHYCAScaffold_50 DSRXLR CDS 316199 316621 . - . ID=name"RXLR_330" PHYCAScaffold_39 DSRXLR CDS 403827405947 . - . ID=name"RXLR_331" PHYCAScaffold_11 DSRXLR CDS 395392395670 . + . ID=name"RXLR_332" PHYCAScaffold_1 DSRXLR CDS 535596 535826 . - . ID=name"RXLR_333" PHYCAScaffold_79 DSRXLR CDS 7601379453 . - . ID=name"RXLR_334"

PHYCAScaffold_22 DSRXLR CDS 8476287242 . - . ID=name"RXLR_335" PHYCAScaffold_36 DSRXLR CDS 499212500405 . - . ID=name"RXLR_336" PHYCAScaffold_32 DSRXLR CDS 330137330724 . + . ID=name"RXLR_337" PHYCAScaffold_11 DSRXLR CDS 720322720876 . - . ID=name"RXLR_338" PHYCAScaffold_81 DSRXLR CDS 63248 64018 . + . ID=name"RXLR_339" PHYCAScaffold_1 DSRXLR CDS 2049539 2050138 . - . ID=name"RXLR_340" PHYCAScaffold_2 DSRXLR CDS 155819 156760 . - . ID=name"RXLR_341" PHYCAScaffold_36 DSRXLR CDS 191571 191981 . - . ID=name"RXLR_342" PHYCAScaffold_40 DSRXLR CDS 165289 165822 . + . ID=name"RXLR_343" PHYCAScaffold_100 DSRXLR CDS 2564026584 . + . ID=name"RXLR_344" PHYCAScaffold_1 DSRXLR CDS 1673888 1674106 . + . ID=name"RXLR_345" PHYCAScaffold_77 DSRXLR CDS \(7734877680 .+\). ID=name"RXLR_346" PHYCAScaffold_25 DSRXLR CDS 337072 339201 . - . ID=name"RXLR_347" PHYCAScaffold_8 DSRXLR CDS 952194952886 . + . ID=name"RXLR_348" PHYCAScaffold_29 DSRXLR CDS \(537904538428 .+\). ID=name"RXLR_349" PHYCAScaffold_37 DSRXLR CDS 128309 128578 . - . ID=name"RXLR_350" PHYCAScaffold_8 DSRXLR CDS 1118960 1123651 . + . ID=name"RXLR_351" PHYCAScaffold_12 DSRXLR CDS 455256457640 . + . ID=name"RXLR_352" PHYCAScaffold_6 DSRXLR CDS 151997 152389 . - . ID=name"RXLR_353" PHYCAScaffold_25 DSRXLR CDS \(607387607794 .+\). ID=name"RXLR_354" PHYCAScaffold_2 DSRXLR CDS 455597456496 . - . ID=name"RXLR_355" PHYCAScaffold_2 DSRXLR CDS 675198675470 . + . ID=name"RXLR_356" PHYCAScaffold_25 DSRXLR CDS 574602574991 . + . ID=name"RXLR_357" PHYCAScaffold_11 DSRXLR CDS 107696 108046 . - . ID=name"RXLR_358" PHYCAScaffold_214 DSRXLR CDS 10325 10717 . - . ID=name"RXLR_359" PHYCAScaffold_7 DSRXLR CDS 1323081 1323536 . - . ID=name"RXLR_360" PHYCAScaffold_42 DSRXLR CDS 144827 146983 . + . ID=name"RXLR_361" PHYCAScaffold_83 DSRXLR CDS \(9289693348 .+\). ID=name"RXLR_362" PHYCAScaffold_17 DSRXLR CDS 830340830729 . + . ID=name"RXLR_363"

PHYCAScaffold_7 DSRXLR CDS 255303255686 . + . ID=name"RXLR_364" PHYCAScaffold_10 DSRXLR CDS 424815425039 . + . ID=name"RXLR_365" PHYCAScaffold_87 DSRXLR CDS 112320 112583 . + . ID=name"RXLR_366" PHYCAScaffold_50 DSRXLR CDS 317280317615 . + . ID=name"RXLR_367" PHYCAScaffold_9 DSRXLR CDS 418797419675 . + . ID=name"RXLR_368" PHYCAScaffold_7 DSRXLR CDS 468940469299 . - . ID=name"RXLR_369" PHYCAScaffold_81 DSRXLR CDS 112786113352 . + . ID=name"RXLR_370" PHYCAScaffold_21 DSRXLR CDS 129668 137668 . + . ID=name"RXLR_371" PHYCAScaffold_74 DSRXLR CDS 157008 157532 . + . ID=name"RXLR_372" PHYCAScaffold_1 DSRXLR CDS 16658311668404 . + . ID=name"RXLR_373" PHYCAScaffold_5 DSRXLR CDS 13675651369100 . + . ID=name"RXLR_374" PHYCAScaffold_6 DSRXLR CDS 1373286 1374137 . + . ID=name"RXLR_375" PHYCAScaffold_73 DSRXLR CDS 202049 204064 . - . ID=name"RXLR_376" PHYCAScaffold_5 DSRXLR CDS 15466971547119 . + . ID=name"RXLR_377" PHYCAScaffold_71 DSRXLR CDS 5217152668 . + . ID=name"RXLR_378" PHYCAScaffold_31 DSRXLR CDS 633947634723 . - . ID=name"RXLR_379" PHYCAScaffold_5 DSRXLR CDS 960759 961346 . + . ID=name"RXLR_380" PHYCAScaffold_1 DSRXLR CDS 20131532016263 . - . ID=name"RXLR_381" PHYCAScaffold_57 DSRXLR CDS 180136181104 . - . ID=name"RXLR_382" PHYCAScaffold_7 DSRXLR CDS 10775081077870 . - . ID=name"RXLR_383" PHYCAScaffold_8 DSRXLR CDS 421213422073 . - . ID=name"RXLR_384" PHYCAScaffold_6 DSRXLR CDS 118835119218 . + . ID=name"RXLR_385" PHYCAScaffold_47 DSRXLR CDS 344225344566 . - . ID=name"RXLR_386" PHYCAScaffold_2 DSRXLR CDS 14359651443761 . + . ID=name"RXLR_387" PHYCAScaffold_24 DSRXLR CDS 190809 191573 . + . ID=name"RXLR_388" PHYCAScaffold_155 DSRXLR CDS 19091 19480 . - . ID=name"RXLR_389" PHYCAScaffold_10 DSRXLR CDS 141378 141932 . + . ID=name"RXLR_390" PHYCAScaffold_52 DSRXLR CDS 268778 269188 . + . ID=name"RXLR_391" PHYCAScaffold_43 DSRXLR CDS 381859382140 . + . ID=name"RXLR_392"

PHYCAScaffold_22 DSRXLR CDS 715037716266 . + . ID=name"RXLR_393" PHYCAScaffold_27 DSRXLR CDS 195639 198104 . - . ID=name"RXLR_394" PHYCAScaffold_3 DSRXLR CDS 1404461 1405141 . - . ID=name"RXLR_395" PHYCAScaffold_62 DSRXLR CDS 270142 270417. - . ID=name"RXLR_396" PHYCAScaffold_17 DSRXLR CDS 830393835510 . - . ID=name"RXLR_397" PHYCAScaffold_18 DSRXLR CDS 9460096036 . + . ID=name"RXLR_398" PHYCAScaffold_16 DSRXLR CDS \(456414456776 .+\). ID=name"RXLR_399" PHYCAScaffold_24 DSRXLR CDS 220066220437 . + . ID=name"RXLR_400" PHYCAScaffold_13 DSRXLR CDS 4071942299 . - . ID=name"RXLR_401" PHYCAScaffold_25 DSRXLR CDS 581645 582073 . + . ID=name"RXLR_402" PHYCAScaffold_7 DSRXLR CDS 10956701096011 . + . ID=name"RXLR_403" PHYCAScaffold_18 DSRXLR CDS 869239871011 . - . ID=name"RXLR_404" PHYCAScaffold_31 DSRXLR CDS 584263 584586 . + . ID=name"RXLR_405" PHYCAScaffold_46 DSRXLR CDS 214487 215467. + . ID=name"RXLR_406" PHYCAScaffold_22 DSRXLR CDS 239210239485 . - . ID=name"RXLR_407" PHYCAScaffold_90 DSRXLR CDS 20149 24063 . + . ID=name"RXLR_408" PHYCAScaffold_51 DSRXLR CDS 304401305465 . + . ID=name"RXLR_409" PHYCAScaffold_37 DSRXLR CDS 2577826926 . + . ID=name"RXLR_410" PHYCAScaffold_53 DSRXLR CDS 175264175482 . + . ID=name"RXLR_411" PHYCAScaffold_95 DSRXLR CDS 8551886036 . - . ID=name"RXLR_412" PHYCAScaffold_29 DSRXLR CDS 501158502588 . + . ID=name"RXLR_413" PHYCAScaffold_22 DSRXLR CDS 470726471247 . + . ID=name"RXLR_414" PHYCAScaffold_48 DSRXLR CDS 426041426532 . + . ID=name"RXLR_415" PHYCAScaffold_8 DSRXLR CDS 155537 156238 . - . ID=name"RXLR_416" PHYCAScaffold_399 DSRXLR CDS 28193337 . - . ID=name"RXLR_417" PHYCAScaffold_55 DSRXLR CDS 462045462446 . - . ID=name"RXLR_418" PHYCAScaffold_9 DSRXLR CDS 1014381 1014806 . - . ID=name"RXLR_419" PHYCAScaffold_3 DSRXLR CDS 266960267367 . - . ID=name"RXLR_420" PHYCAScaffold_84 DSRXLR CDS 9726597540 . - . ID=name"RXLR_421"

PHYCAScaffold_38 DSRXLR CDS 380578 381003 . + . ID=name"RXLR_422" PHYCAScaffold_65 DSRXLR CDS 7909679410 . - . ID=name"RXLR_423" PHYCAScaffold_10 DSRXLR CDS 827135827668 . + . ID=name"RXLR_424" PHYCAScaffold_3 DSRXLR CDS 10446571045211 . + . ID=name"RXLR_425" PHYCAScaffold_2 DSRXLR CDS 1324371 1324862 . + . ID=name"RXLR_426" PHYCAScaffold_1 DSRXLR CDS 1821926 1822186 . - . ID=name"RXLR_427" PHYCAScaffold_9 DSRXLR CDS 717213717629 . - . ID=name"RXLR_428" PHYCAScaffold_14 DSRXLR CDS 959521 960381 . - . ID=name"RXLR_429" PHYCAScaffold_11 DSRXLR CDS 831732834227 . - . ID=name"RXLR_430" PHYCAScaffold_58 DSRXLR CDS 162409 162747 . + . ID=name"RXLR_431" PHYCAScaffold_48 DSRXLR CDS 194739195029 . + . ID=name"RXLR_432" PHYCAScaffold_33 DSRXLR CDS 260464 263148 . + . ID=name"RXLR_433" PHYCAScaffold_100 DSRXLR CDS 8544185713 . + . ID=name"RXLR_434" PHYCAScaffold_6 DSRXLR CDS 8427084815 . + . ID=name"RXLR_435" PHYCAScaffold_62 DSRXLR CDS 4235342727 . - . ID=name"RXLR_436" PHYCAScaffold_76 DSRXLR CDS 9171691937 . + . ID=name"RXLR_437" PHYCAScaffold_29 DSRXLR CDS 5647556885 . + . ID=name"RXLR_438" PHYCAScaffold_51 DSRXLR CDS 246203246499 . + . ID=name"RXLR_439" PHYCAScaffold_6 DSRXLR CDS 501553502341 . - . ID=name"RXLR_440" PHYCAScaffold_32 DSRXLR CDS 503592 503816 . + . ID=name"RXLR_441" PHYCAScaffold_52 DSRXLR CDS 166596 166838. - . ID=name"RXLR_442" PHYCAScaffold_27 DSRXLR CDS 537016 537813 . - . ID=name"RXLR_443" PHYCAScaffold_51 DSRXLR CDS 5833858580 . + . ID=name"RXLR_444" PHYCAScaffold_12 DSRXLR CDS 28369 29088 . + . ID=name"RXLR_445" PHYCAScaffold_6 DSRXLR CDS 537553537828 . + . ID=name"RXLR_446" PHYCAScaffold_23 DSRXLR CDS 551650 551907 . + . ID=name"RXLR_447" PHYCAScaffold_8 DSRXLR CDS 769165769440 . - . ID=name"RXLR_448" PHYCAScaffold_24 DSRXLR CDS 184601 184981 . + . ID=name"RXLR_449" PHYCAScaffold_20 DSRXLR CDS 733911735221 . + . ID=name"RXLR_450"

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PHYCAScaffold_8 DSRXLR CDS 688533688883 . - . ID=name"RXLR_480" PHYCAScaffold_13 DSRXLR CDS 565047 566540 . + . ID=name"RXLR_481" PHYCAScaffold_16 DSRXLR CDS 713083719214 . - . ID=name"RXLR_482" PHYCAScaffold_62 DSRXLR CDS 36477 36698 . + . ID=name"RXLR_483" PHYCAScaffold_102 DSRXLR CDS 78719 78943 . - . ID=name"RXLR_484" PHYCAScaffold_57 DSRXLR CDS 217147 217638 . - . ID=name"RXLR_485" PHYCAScaffold_7 DSRXLR CDS 821872822168 . - . ID=name"RXLR_486" PHYCAScaffold_119 DSRXLR CDS 2811428680 . - . ID=name"RXLR_487" PHYCAScaffold_1 DSRXLR CDS 77862 78131 . - . ID=name"RXLR_488" PHYCAScaffold_39 DSRXLR CDS 293501293779 . + . ID=name"RXLR_489" PHYCAScaffold_23 DSRXLR CDS 627147627527 . - . ID=name"RXLR_490" PHYCAScaffold_10 DSRXLR CDS 708802709305 . - . ID=name"RXLR_491" PHYCAScaffold_18 DSRXLR CDS 926495926719 . + . ID=name"RXLR_492" PHYCAScaffold_11 DSRXLR CDS 397054 397323 . + . ID=name"RXLR_493" PHYCAScaffold_12 DSRXLR CDS \(705857706135 .+\). ID=name"RXLR_494" PHYCAScaffold_12 DSRXLR CDS 11100161110474 . + . ID=name"RXLR_495" PHYCAScaffold_4 DSRXLR CDS 14538521454454 . + . ID=name"RXLR_496" PHYCAScaffold_3 DSRXLR CDS 605129605926 . + . ID=name"RXLR_497" PHYCAScaffold_23 DSRXLR CDS 521955 522569 . - . ID=name"RXLR_498" PHYCAScaffold_9 DSRXLR CDS 796209796430 . + . ID=name"RXLR_499" PHYCAScaffold_22 DSRXLR CDS 9146291755 . - . ID=name"RXLR_500" PHYCAScaffold_10 DSRXLR CDS 880172882019 . - . ID=name"RXLR_501" PHYCAScaffold_6 DSRXLR CDS 1350393 1350773 . + . ID=name"RXLR_502" PHYCAScaffold_6 DSRXLR CDS 542035542400 . + . ID=name"RXLR_503" PHYCAScaffold_22 DSRXLR CDS 454513454779 . - . ID=name"RXLR_504" PHYCAScaffold_50 DSRXLR CDS 8729088102 . + . ID=name"RXLR_505" PHYCAScaffold_46 DSRXLR CDS 209453 210058 . + . ID=name"RXLR_506" PHYCAScaffold_37 DSRXLR CDS 428027428245 . + . ID=name"RXLR_507" PHYCAScaffold_79 DSRXLR CDS 43669 45057 . - . ID=name"RXLR_508"

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PHYCAScaffold_22 DSRXLR CDS 98161 99498. - . ID=name"RXLR_567" PHYCAScaffold_16 DSRXLR CDS 713763714302 . - . ID=name"RXLR_568" PHYCAScaffold_1 DSRXLR CDS 341813342148 . - . ID=name"RXLR_569" PHYCAScaffold_3 DSRXLR CDS 10842971084599 . + . ID=name"RXLR_570" PHYCAScaffold_30 DSRXLR CDS 589685 590131 . + . ID=name"RXLR_571" PHYCAScaffold_5 DSRXLR CDS 663492663896 . + . ID=name"RXLR_572" PHYCAScaffold_40 DSRXLR CDS 110202110480 . + . ID=name"RXLR_573" PHYCAScaffold_13 DSRXLR CDS 486647486973 . + . ID=name"RXLR_574" PHYCAScaffold_1 DSRXLR CDS 20592482059589 . + . ID=name"RXLR_575" PHYCAScaffold_157 DSRXLR CDS 3601836398 . + . ID=name"RXLR_576" PHYCAScaffold_95 DSRXLR CDS 85498917 . - . ID=name"RXLR_577" PHYCAScaffold_27 DSRXLR CDS 6750568644 . - . ID=name"RXLR_578" PHYCAScaffold_43 DSRXLR CDS 9888599469 . - . ID=name"RXLR_579" PHYCAScaffold_117 DSRXLR CDS 49312 51222. - . ID=name"RXLR_580" PHYCAScaffold_207 DSRXLR CDS 70047372 . + . ID=name"RXLR_581" PHYCAScaffold_4 DSRXLR CDS 1224629 1224904 . - . ID=name"RXLR_582" PHYCAScaffold_42 DSRXLR CDS 428965429489 . - . ID=name"RXLR_583" PHYCAScaffold_14 DSRXLR CDS 723533727678 . - . ID=name"RXLR_584" PHYCAScaffold_623 DSRXLR CDS 1396 2103 . + . ID=name"RXLR_585" PHYCAScaffold_22 DSRXLR CDS 166813 167070 . + . ID=name"RXLR_586" PHYCAScaffold_8 DSRXLR CDS 811193812689 . - . ID=name"RXLR_587" PHYCAScaffold_14 DSRXLR CDS 775321775641 . + . ID=name"RXLR_588" PHYCAScaffold_21 DSRXLR CDS 908122909801 . + . ID=name"RXLR_589" PHYCAScaffold_24 DSRXLR CDS 481306482007 . - . ID=name"RXLR_590" PHYCAScaffold_42 DSRXLR CDS 392232392669 . - . ID=name"RXLR_591" PHYCAScaffold_17 DSRXLR CDS 972551973963 . - . ID=name"RXLR_592" PHYCAScaffold_52 DSRXLR CDS 148311 148682 . - . ID=name"RXLR_593" PHYCAScaffold_44 DSRXLR CDS 527477 527746 . - . ID=name"RXLR_594" PHYCAScaffold_186 DSRXLR CDS 38246349 . - . ID=name"RXLR_595"

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PHYCAScaffold_46 DSRXLR CDS 461468462502 . + . ID=name" RXLR_625" PHYCAScaffold_25 DSRXLR CDS 683541684143 . + . ID=name"RXLR_626" PHYCAScaffold_18 DSRXLR CDS 953526 958943 . - . ID=name"RXLR_627" PHYCAScaffold_68 DSRXLR CDS 126115 126525 . - . ID=name"RXLR_628" PHYCAScaffold_29 DSRXLR CDS 588650588952 . + . ID=name"RXLR_629" PHYCAScaffold_85 DSRXLR CDS 164159 164539 . - . ID=name"RXLR_630" PHYCAScaffold_34 DSRXLR CDS 234354236336 . - . ID=name"RXLR_631" PHYCAScaffold_100 DSRXLR CDS \(8296484664 .+\) ID=name"RXLR_632" PHYCAScaffold_1 DSRXLR CDS 19230841924382 . + . ID=name"RXLR_633" PHYCAScaffold_17 DSRXLR CDS 655773664340 . - . ID=name"RXLR_634" PHYCAScaffold_5 DSRXLR CDS 110327 110737 . - . ID=name"RXLR_635" PHYCAScaffold_7 DSRXLR CDS 938487940319 . - . ID=name"RXLR_636" PHYCAScaffold_57 DSRXLR CDS \(175969177828 .+\). ID=name"RXLR_637" PHYCAScaffold_29 DSRXLR CDS 374705375061 . - . ID=name"RXLR_638" PHYCAScaffold_68 DSRXLR CDS \(320566320928 .+\). ID=name"RXLR_639" PHYCAScaffold_73 DSRXLR CDS 4090341238 . + . ID=name"RXLR_640" PHYCAScaffold_52 DSRXLR CDS 183444184430 . - . ID=name"RXLR_641" PHYCAScaffold_20 DSRXLR CDS 615716616936 . + . ID=name"RXLR_642" PHYCAScaffold_6 DSRXLR CDS 139967140284 . - . ID=name"RXLR_643" PHYCAScaffold_73 DSRXLR CDS 222910223233 . - . ID=name"RXLR_644" PHYCAScaffold_5 DSRXLR CDS 1474762 1475307 . - . ID=name"RXLR_645" PHYCAScaffold_4 DSRXLR CDS 572132578179 . - . ID=name"RXLR_646" PHYCAScaffold_4 DSRXLR CDS 13051851305406 . + . ID=name"RXLR_647" PHYCAScaffold_4 DSRXLR CDS 260883264296 . - . ID=name"RXLR_648" PHYCAScaffold_11 DSRXLR CDS 257536 264279 . + . ID=name"RXLR_649" PHYCAScaffold_29 DSRXLR CDS 316852317196 . + . ID=name"RXLR_650" PHYCAScaffold_23 DSRXLR CDS 259640 261895 . - . ID=name"RXLR_651" PHYCAScaffold_41 DSRXLR CDS 141714142079 . + . ID=name"RXLR_652" PHYCAScaffold_17 DSRXLR CDS 718777722196 . + . ID=name"RXLR_653"

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PHYCAScaffold_40 DSRXLR CDS 431226431573 . + . ID=name"RXLR_712" PHYCAScaffold_4 DSRXLR CDS 383970384212 . - . ID=name"RXLR_713" PHYCAScaffold_4 DSRXLR CDS 529945530559 . + . ID=name"RXLR_714" PHYCAScaffold_20 DSRXLR CDS 283136283426 . + . ID=name"RXLR_715" PHYCAScaffold_13 DSRXLR CDS 649574 651946 . - . ID=name"RXLR_716" PHYCAScaffold_51 DSRXLR CDS 123069 124409 . - . ID=name"RXLR_717" PHYCAScaffold_66 DSRXLR CDS 6479665119 . - . ID=name"RXLR_718" PHYCAScaffold_23 DSRXLR CDS 537260537472 . + . ID=name"RXLR_719" PHYCAScaffold_29 DSRXLR CDS 459320459679 . - . ID=name"RXLR_720" PHYCAScaffold_100 DSRXLR CDS 9682 10131 . - . ID=name"RXLR_721" PHYCAScaffold_118 DSRXLR CDS 62148 62753 . - . ID=name"RXLR_722" PHYCAScaffold_21 DSRXLR CDS 853035854840 . - . ID=name"RXLR_723" PHYCAScaffold_2 DSRXLR CDS 339154339393 . + . ID=name"RXLR_724" PHYCAScaffold_30 DSRXLR CDS 534647 534901 . - . ID=name"RXLR_725" PHYCAScaffold_72 DSRXLR CDS \(2656727448 .+\). ID=name"RXLR_726" PHYCAScaffold_11 DSRXLR CDS 590527 590973 . + . ID=name"RXLR_727" PHYCAScaffold_24 DSRXLR CDS 138930 139262 . - . ID=name"RXLR_728" PHYCAScaffold_3 DSRXLR CDS 12691891269464 . + . ID=name"RXLR_729" PHYCAScaffold_44 DSRXLR CDS 193992 194327. + . ID=name"RXLR_730" PHYCAScaffold_50 DSRXLR CDS 194691 195113 . + . ID=name"RXLR_731" PHYCAScaffold_20 DSRXLR CDS 713975714832 . - . ID=name"RXLR_732" PHYCAScaffold_225 DSRXLR CDS 5283 5636 . - . ID=name"RXLR_733" PHYCAScaffold_23 DSRXLR CDS 686311686790 . + . ID=name"RXLR_734" PHYCAScaffold_30 DSRXLR CDS 3834639146 . - . ID=name"RXLR_735" PHYCAScaffold_3 DSRXLR CDS 170214170963 . + . ID=name"RXLR_736" PHYCAScaffold_48 DSRXLR CDS \(351654351890 .+\). ID=name"RXLR_737" PHYCAScaffold_52 DSRXLR CDS 440539440877 . - . ID=name"RXLR_738" PHYCAScaffold_47 DSRXLR CDS \(381599382078 .+\). ID=name"RXLR_739" PHYCAScaffold_29 DSRXLR CDS 6179262115 . + . ID=name"RXLR_740"

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PHYCAScaffold_66 DSRXLR CDS 109837 110337 . + . ID=name"RXLR_770" PHYCAScaffold_40 DSRXLR CDS 391599392018 . - . ID=name"RXLR_771" PHYCAScaffold_25 DSRXLR CDS 689118689684 . - . ID=name"RXLR_772" PHYCAScaffold_107 DSRXLR CDS 9238192803 . + . ID=name"RXLR_773" PHYCAScaffold_52 DSRXLR CDS 5858658882 . - . ID=name"RXLR_774" PHYCAScaffold_5 DSRXLR CDS 672614673183 . + . ID=name"RXLR_775" PHYCAScaffold_2 DSRXLR CDS 17437261744520 . + . ID=name"RXLR_776" PHYCAScaffold_9 DSRXLR CDS 157873 158331 . + . ID=name"RXLR_777" PHYCAScaffold_15 DSRXLR CDS 606511606969 . - . ID=name"RXLR_778" PHYCAScaffold_81 DSRXLR CDS 176252 177679 . - . ID=name"RXLR_779" PHYCAScaffold_1 DSRXLR CDS 20043892004607 . + . ID=name"RXLR_780" PHYCAScaffold_305 DSRXLR CDS 25072773 . + . ID=name"RXLR_781" PHYCAScaffold_52 DSRXLR CDS 149233 149607 . - . ID=name"RXLR_782" PHYCAScaffold_5 DSRXLR CDS 893767894570 . + . ID=name"RXLR_783" PHYCAScaffold_96 DSRXLR CDS 10737 10952 . + . ID=name"RXLR_784" PHYCAScaffold_100 DSRXLR CDS 5417954526 . + . ID=name"RXLR_785" PHYCAScaffold_61 DSRXLR CDS 229809230045 . + . ID=name"RXLR_786" PHYCAScaffold_34 DSRXLR CDS 243775243990 . + . ID=name"RXLR_787" PHYCAScaffold_47 DSRXLR CDS 4133042250 . + . ID=name"RXLR_788" PHYCAScaffold_27 DSRXLR CDS 686765687229 . - . ID=name"RXLR_789" PHYCAScaffold_32 DSRXLR CDS 244593245015 . + . ID=name"RXLR_790" PHYCAScaffold_4 DSRXLR CDS 10030591005497 . + . ID=name"RXLR_791" PHYCAScaffold_29 DSRXLR CDS 259530260222 . + . ID=name"RXLR_792" PHYCAScaffold_32 DSRXLR CDS 305082306866 . - . ID=name"RXLR_793" PHYCAScaffold_27 DSRXLR CDS 266719 267087 . + . ID=name"RXLR_794" PHYCAScaffold_438 DSRXLR CDS 904 1398 . - . ID=name"RXLR_795" PHYCAScaffold_32 DSRXLR CDS 489908490210 . - . ID=name"RXLR_796" PHYCAScaffold_29 DSRXLR CDS 539609540103 . + . ID=name"RXLR_797" PHYCAScaffold_11 DSRXLR CDS \(871880872260 .+\). ID=name"RXLR_798"

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PHYCAScaffold_37 DSRXLR CDS 110618 110923 . + . ID=name"RXLR_828" PHYCAScaffold_83 DSRXLR CDS 50154 50396 . - . ID=name"RXLR_829" PHYCAScaffold_104 DSRXLR CDS 111228 111578 . - . ID=name" RXLR_830" PHYCAScaffold_16 DSRXLR CDS 446246448993 . + . ID=name"RXLR_831" PHYCAScaffold_7 DSRXLR CDS 572430572711 . - . ID=name"RXLR_832" PHYCAScaffold_18 DSRXLR CDS 800464800745 . + . ID=name"RXLR_833" PHYCAScaffold_1 DSRXLR CDS 1417667 1418146 . - . ID=name"RXLR_834" PHYCAScaffold_43 DSRXLR CDS 410852411220 . + . ID=name"RXLR_835" PHYCAScaffold_53 DSRXLR CDS 293624293968 . - . ID=name"RXLR_836" PHYCAScaffold_17 DSRXLR CDS 124154 124603 . - . ID=name"RXLR_837" PHYCAScaffold_13 DSRXLR CDS 689150690370 . - . ID=name"RXLR_838" PHYCAScaffold_1 DSRXLR CDS 1919760 1921748 . - . ID=name"RXLR_839" PHYCAScaffold_71 DSRXLR CDS 94224 94865 . - . ID=name"RXLR_840" PHYCAScaffold_18 DSRXLR CDS 860804861229 . - . ID=name"RXLR_841" PHYCAScaffold_3 DSRXLR CDS 662698663237 . + . ID=name"RXLR_842" PHYCAScaffold_63 DSRXLR CDS 145590 145826 . - . ID=name"RXLR_843" PHYCAScaffold_81 DSRXLR CDS 100602 101795 . - . ID=name"RXLR_844" PHYCAScaffold_3 DSRXLR CDS 351635351859 . + . ID=name"RXLR_845" PHYCAScaffold_68 DSRXLR CDS 323426323848 . - . ID=name"RXLR_846" PHYCAScaffold_26 DSRXLR CDS 469290471011 . - . ID=name"RXLR_847" PHYCAScaffold_9 DSRXLR CDS 1010008 1010943 . - . ID=name"RXLR_848" PHYCAScaffold_7 DSRXLR CDS 355223355588 . - . ID=name"RXLR_849" PHYCAScaffold_2 DSRXLR CDS 549735551720 . + . ID=name"RXLR_850" PHYCAScaffold_49 DSRXLR CDS 465996467165 . - . ID=name"RXLR_851" PHYCAScaffold_77 DSRXLR CDS 139392 139688 . - . ID=name"RXLR_852" PHYCAScaffold_46 DSRXLR CDS 58557 59000 . - . ID=name"RXLR_853" PHYCAScaffold_13 DSRXLR CDS 10278311028313 . - . ID=name"RXLR_854" PHYCAScaffold_46 DSRXLR CDS 6092661369 . - . ID=name"RXLR_855" PHYCAScaffold_3 DSRXLR CDS 203980204324 . - . ID=name"RXLR_856"

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PHYCAScaffold_96 DSRXLR CDS 137119137749 . + . ID=name"RXLR_1002" PHYCAScaffold_37 DSRXLR CDS 226057226899 . - . ID=name"RXLR_1003" PHYCAScaffold_78 DSRXLR CDS 171168 171401 . + . ID=name"RXLR_1004" PHYCAScaffold_96 DSRXLR CDS 8050780746 . - . ID=name"RXLR_1005" PHYCAScaffold_81 DSRXLR CDS 4412044689 . + . ID=name"RXLR_1006" PHYCAScaffold_11 DSRXLR CDS 9946699747 . + . ID=name"RXLR_1007" PHYCAScaffold_49 DSRXLR CDS 98264 100132 . - . ID=name"RXLR_1008" PHYCAScaffold_98 DSRXLR CDS 3926 4306 . - . ID=name"RXLR_1009" PHYCAScaffold_25 DSRXLR CDS 524607525011 . - . ID=name"RXLR_1010" PHYCAScaffold_2 DSRXLR CDS 854153854449 . + . ID=name"RXLR_1011" PHYCAScaffold_184 DSRXLR CDS 10199 10726 . - . ID=name"RXLR_1012" PHYCAScaffold_25 DSRXLR CDS 230650230997 . + . ID=name"RXLR_1013" PHYCAScaffold_12 DSRXLR CDS 307439309022 . - . ID=name"RXLR_1014" PHYCAScaffold_3 DSRXLR CDS 1170492 1170887 . - . ID=name"RXLR_1015" PHYCAScaffold_38 DSRXLR CDS 453671454222 . + . ID=name"RXLR_1016" PHYCAScaffold_95 DSRXLR CDS 8844389705 . + . ID=name"RXLR_1017" PHYCAScaffold_77 DSRXLR CDS 107711 108052 . - . ID=name"RXLR_1018" PHYCAScaffold_2 DSRXLR CDS 17960931796770 . + . ID=name"RXLR_1019" PHYCAScaffold_25 DSRXLR CDS 556556556954 . + . ID=name"RXLR_1020" PHYCAScaffold_44 DSRXLR CDS 339704340381 . + . ID=name"RXLR_1021" PHYCAScaffold_4 DSRXLR CDS 463963465624 . - . ID=name"RXLR_1022" PHYCAScaffold_50 DSRXLR CDS 7510475439 . - . ID=name"RXLR_1023" PHYCAScaffold_335 DSRXLR CDS 57576074 . + . ID=name"RXLR_1024" PHYCAScaffold_35 DSRXLR CDS 542903543199 . - . ID=name"RXLR_1025" PHYCAScaffold_49 DSRXLR CDS 426088427068 . - . ID=name"RXLR_1026" PHYCAScaffold_42 DSRXLR CDS 279826 280128 . - . ID=name"RXLR_1027" PHYCAScaffold_25 DSRXLR CDS 8754588864 . - . ID=name"RXLR_1028" PHYCAScaffold_97 DSRXLR CDS 9129191980 . - . ID=name"RXLR_1029" PHYCAScaffold_41 DSRXLR CDS 247229247612 . + . ID=name"RXLR_1030"

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PHYCAScaffold_44 DSRXLR CDS 447467447802 . + . ID=name"RXLR_1060" PHYCAScaffold_143 DSRXLR CDS 5896 6138. - . ID=name"RXLR_1061" PHYCAScaffold_21 DSRXLR CDS 319104319454 . + . ID=name"RXLR_1062" PHYCAScaffold_9 DSRXLR CDS 626254626478 . + . ID=name"RXLR_1063" PHYCAScaffold_4 DSRXLR CDS 1035146 1035364 . - . ID=name"RXLR_1064" PHYCAScaffold_44 DSRXLR CDS 243687 244067. - . ID=name"RXLR_1065" PHYCAScaffold_31 DSRXLR CDS 110907111629 . - . ID=name"RXLR_1066" PHYCAScaffold_2 DSRXLR CDS 592438592650 . + . ID=name"RXLR_1067" PHYCAScaffold_6 DSRXLR CDS 1420586 1421032 . - . ID=name"RXLR_1068" PHYCAScaffold_2 DSRXLR CDS 779335779568 . + . ID=name"RXLR_1069" PHYCAScaffold_94 DSRXLR CDS 114157115503 . + . ID=name"RXLR_1070" PHYCAScaffold_86 DSRXLR CDS 123016 124227 . - . ID=name"RXLR_1071" PHYCAScaffold_22 DSRXLR CDS 400935402659 . - . ID=name"RXLR_1072" PHYCAScaffold_3 DSRXLR CDS 14857751488750 . + . ID=name"RXLR_1073" PHYCAScaffold_10 DSRXLR CDS 11691511169453 . - . ID=name"RXLR_1074" PHYCAScaffold_54 DSRXLR CDS 6844668826 . + . ID=name"RXLR_1075" PHYCAScaffold_4 DSRXLR CDS 125862 126134 . - . ID=name"RXLR_1076" PHYCAScaffold_9 DSRXLR CDS 218170218553 . + . ID=name"RXLR_1077" PHYCAScaffold_153 DSRXLR CDS 16000 16245 . + . ID=name"RXLR_1078" PHYCAScaffold_11 DSRXLR CDS 1019254 1020036 . - . ID=name"RXLR_1079" PHYCAScaffold_94 DSRXLR CDS 182681 183256 . + . ID=name"RXLR_1080" PHYCAScaffold_3 DSRXLR CDS 894078894299 . - . ID=name"RXLR_1081" PHYCAScaffold_79 DSRXLR CDS 9256992814 . + . ID=name"RXLR_1082" PHYCAScaffold_42 DSRXLR CDS 479232479456 . + . ID=name"RXLR_1083" PHYCAScaffold_71 DSRXLR CDS 9742898075 . + . ID=name"RXLR_1084" PHYCAScaffold_85 DSRXLR CDS 100722 101459 . - . ID=name"RXLR_1085" PHYCAScaffold_104 DSRXLR CDS 7723577927 . + . ID=name"RXLR_1086" PHYCAScaffold_51 DSRXLR CDS 119061 119924 . - . ID=name"RXLR_1087" PHYCAScaffold_6 DSRXLR CDS 574411575112 . - . ID=name"RXLR_1088"

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PHYCAScaffold_24 DSRXLR CDS 158316158690 . + . ID=name"RXLR_1118" PHYCAScaffold_80 DSRXLR CDS 2247522864 . - . ID=name"RXLR_1119" PHYCAScaffold_46 DSRXLR CDS 402844403119 . + . ID=name"RXLR_1120" PHYCAScaffold_47 DSRXLR CDS 232264232479 . + . ID=name"RXLR_1121" PHYCAScaffold_7 DSRXLR CDS 569249 569530 . + . ID=name"RXLR_1122" PHYCAScaffold_25 DSRXLR CDS 459945460388 . - . ID=name"RXLR_1123" PHYCAScaffold_55 DSRXLR CDS 58774 59382 . - . ID=name"RXLR_1124" PHYCAScaffold_47 DSRXLR CDS 4303844210 . + . ID=name"RXLR_1125" PHYCAScaffold_12 DSRXLR CDS 1093672 1095585 . - . ID=name"RXLR_1126" PHYCAScaffold_47 DSRXLR CDS 398527401088 . - . ID=name"RXLR_1127" PHYCAScaffold_24 DSRXLR CDS 761670766400 . - . ID=name"RXLR_1128" PHYCAScaffold_129 DSRXLR CDS 25815 26162.-. ID=name"RXLR_1129" PHYCAScaffold_6 DSRXLR CDS 410370410873 . + . ID=name"RXLR_1130" PHYCAScaffold_57 DSRXLR CDS 83258654 . - . ID=name"RXLR_1131" PHYCAScaffold_81 DSRXLR CDS 7660977481 . + . ID=name"RXLR_1132" PHYCAScaffold_24 DSRXLR CDS 627009627587 . - . ID=name"RXLR_1133" PHYCAScaffold_20 DSRXLR CDS 338320341118 . + . ID=name"RXLR_1134" PHYCAScaffold_67 DSRXLR CDS 232580237610 . - . ID=name"RXLR_1135" PHYCAScaffold_64 DSRXLR CDS 2671727610 . - . ID=name"RXLR_1136" PHYCAScaffold_26 DSRXLR CDS 728333728956 . - . ID=name"RXLR_1137" PHYCAScaffold_29 DSRXLR CDS 150553 151962 . - . ID=name"RXLR_1138" PHYCAScaffold_25 DSRXLR CDS 687391687729 . - . ID=name"RXLR_1139" PHYCAScaffold_6 DSRXLR CDS 13105561315202 . + . ID=name"RXLR_1140" PHYCAScaffold_29 DSRXLR CDS 164999 165355 . + . ID=name"RXLR_1141" PHYCAScaffold_4 DSRXLR CDS 3653939190 . - . ID=name"RXLR_1142" PHYCAScaffold_28 DSRXLR CDS 125421 125744. - . ID=name"RXLR_1143" PHYCAScaffold_86 DSRXLR CDS 156986 157333 . + . ID=name"RXLR_1144" PHYCAScaffold_77 DSRXLR CDS 121977 122273 . + . ID=name"RXLR_1145" PHYCAScaffold_10 DSRXLR CDS 603882605429 . + . ID=name"RXLR_1146"

PHYCAScaffold_30 DSRXLR CDS 487604489778 . + . ID=name"RXLR_1147" PHYCAScaffold_15 DSRXLR CDS 576084 576536 . - . ID=name"RXLR_1148" PHYCAScaffold_15 DSRXLR CDS 392005393702 . - . ID=name"RXLR_1149" PHYCAScaffold_111 DSRXLR CDS 2525425841 . - . ID=name"RXLR_1150" PHYCAScaffold_46 DSRXLR CDS 520896 521141 . - . ID=name"RXLR_1151" PHYCAScaffold_19 DSRXLR CDS 175068 175466 . + . ID=name"RXLR_1152" PHYCAScaffold_11 DSRXLR CDS \(421616422014 .+\). ID=name"RXLR_1153" PHYCAScaffold_42 DSRXLR CDS 141260 141985 . + . ID=name"RXLR_1154" PHYCAScaffold_6 DSRXLR CDS 8267882992 . - . ID=name"RXLR_1155" PHYCAScaffold_11 DSRXLR CDS 307766 308056 . - . ID=name"RXLR_1156" PHYCAScaffold_4 DSRXLR CDS 777667777960 . - . ID=name"RXLR_1157" PHYCAScaffold_29 DSRXLR CDS 602093602542 . - . ID=name"RXLR_1158" PHYCAScaffold_6 DSRXLR CDS 222423223112 . - . ID=name"RXLR_1159" PHYCAScaffold_4 DSRXLR CDS 455477456871 . + . ID=name"RXLR_1160" PHYCAScaffold_16 DSRXLR CDS 355615356106 . + . ID=name"RXLR_1161" PHYCAScaffold_15 DSRXLR CDS 335443335724 . + . ID=name"RXLR_1162" PHYCAScaffold_9 DSRXLR CDS 213694214200 . - . ID=name"RXLR_1163" PHYCAScaffold_9 DSRXLR CDS 241885242148 . - . ID=name"RXLR_1164" PHYCAScaffold_35 DSRXLR CDS 482279484687 . + . ID=name"RXLR_1165" PHYCAScaffold_11 DSRXLR CDS \(676667678328 .+\). ID=name"RXLR_1166" PHYCAScaffold_6 DSRXLR CDS 599206601446 . + . ID=name"RXLR_1167" PHYCAScaffold_46 DSRXLR CDS 256905257810 . + . ID=name"RXLR_1168" PHYCAScaffold_29 DSRXLR CDS 638127638417 . - . ID=name"RXLR_1169" PHYCAScaffold_5 DSRXLR CDS 171504172301 . - . ID=name"RXLR_1170" PHYCAScaffold_8 DSRXLR CDS 59296804 . + . ID=name"RXLR_1171" PHYCAScaffold_30 DSRXLR CDS 546447 546701 . - . ID=name"RXLR_1172" PHYCAScaffold_2 DSRXLR CDS 353401354267 . + . ID=name"RXLR_1173" PHYCAScaffold_22 DSRXLR CDS 5624556457 . + . ID=name"RXLR_1174" PHYCAScaffold_6 DSRXLR CDS 133609 133833 . - . ID=name"RXLR_1175"

PHYCAScaffold_28 DSRXLR CDS 387432388652 . - . ID=name"RXLR_1176" PHYCAScaffold_48 DSRXLR CDS 187283 188161 . - . ID=name"RXLR_1177" PHYCAScaffold_5 DSRXLR CDS 688240688665 . - . ID=name"RXLR_1178" PHYCAScaffold_4 DSRXLR CDS 14780231478826 . - . ID=name"RXLR_1179" PHYCAScaffold_22 DSRXLR CDS 189866 191233 . - . ID=name"RXLR_1180" PHYCAScaffold_22 DSRXLR CDS 511259 511507 . - . ID=name"RXLR_1181" PHYCAScaffold_11 DSRXLR CDS 762580762801 . - . ID=name"RXLR_1182" PHYCAScaffold_13 DSRXLR CDS 536085 538082 . - . ID=name"RXLR_1183" PHYCAScaffold_360 DSRXLR CDS 19172261 . - . ID=name"RXLR_1184" PHYCAScaffold_443 DSRXLR CDS \(33253831 .+\). ID=name"RXLR_1185" PHYCAScaffold_61 DSRXLR CDS 227609227977 . - . ID=name"RXLR_1186" PHYCAScaffold_7 DSRXLR CDS 856840859101 . - . ID=name"RXLR_1187" PHYCAScaffold_25 DSRXLR CDS 577741578130 . + . ID=name"RXLR_1188" PHYCAScaffold_12 DSRXLR CDS 350946 351764 . - . ID=name"RXLR_1189" PHYCAScaffold_12 DSRXLR CDS 868181868678 . + . ID=name"RXLR_1190" PHYCAScaffold_50 DSRXLR CDS 138059 138391 . + . ID=name"RXLR_1191" PHYCAScaffold_10 DSRXLR CDS 838302838808 . - . ID=name"RXLR_1192" PHYCAScaffold_47 DSRXLR CDS 486903487325 . + . ID=name"RXLR_1193" PHYCAScaffold_2 DSRXLR CDS 9110791394 . + . ID=name"RXLR_1194" PHYCAScaffold_3 DSRXLR CDS 212284212649 . + . ID=name"RXLR_1195" PHYCAScaffold_10 DSRXLR CDS 339903341219 . - . ID=name"RXLR_1196" PHYCAScaffold_48 DSRXLR CDS 260199260915 . + . ID=name"RXLR_1197" PHYCAScaffold_49 DSRXLR CDS 120723 121133 . - . ID=name"RXLR_1198" PHYCAScaffold_47 DSRXLR CDS 434721434933 . + . ID=name"RXLR_1199" PHYCAScaffold_41 DSRXLR CDS 508181508741 . + . ID=name"RXLR_1200" PHYCAScaffold_18 DSRXLR CDS 801800802237 . + . ID=name"RXLR_1201" PHYCAScaffold_35 DSRXLR CDS 165225 165587 . - . ID=name"RXLR_1202" PHYCAScaffold_25 DSRXLR CDS \(448216448584 .+\). ID=name"RXLR_1203" PHYCAScaffold_9 DSRXLR CDS \(843133843501 .+\) ID=name"RXLR_1204"

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PHYCAScaffold_14 DSRXLR CDS 612475612960 . - . ID=name"RXLR_1292" PHYCAScaffold_20 DSRXLR CDS 108931 109383 . + . ID=name"RXLR_1293" PHYCAScaffold_1 DSRXLR CDS 1737035 1737262 . - . ID=name"RXLR_1294" PHYCAScaffold_13 DSRXLR CDS 914080914451 . + . ID=name"RXLR_1295" PHYCAScaffold_24 DSRXLR CDS 281451281741 . + . ID=name"RXLR_1296" PHYCAScaffold_53 DSRXLR CDS 9735297657 . + . ID=name"RXLR_1297" PHYCAScaffold_89 DSRXLR CDS 7940779856 . + . ID=name"RXLR_1298" PHYCAScaffold_107 DSRXLR CDS 5637757189 . + . ID=name"RXLR_1299" PHYCAScaffold_142 DSRXLR CDS 2427 2708 . - . ID=name"RXLR_1300" PHYCAScaffold_54 DSRXLR CDS 71277363 . - . ID=name"RXLR_1301" PHYCAScaffold_46 DSRXLR CDS 518515 518754 . - . ID=name"RXLR_1302" PHYCAScaffold_14 DSRXLR CDS 1020015 1021322 . - . ID=name"RXLR_1303" PHYCAScaffold_1 DSRXLR CDS 1363689 1363916 . + . ID=name"RXLR_1304" PHYCAScaffold_67 DSRXLR CDS 230645230917 . + . ID=name"RXLR_1305" PHYCAScaffold_325 DSRXLR CDS 20582351 . + . ID=name"RXLR_1306" PHYCAScaffold_18 DSRXLR CDS 356564356779 . + . ID=name"RXLR_1307" PHYCAScaffold_5 DSRXLR CDS 15401771540488 . + . ID=name"RXLR_1308" PHYCAScaffold_104 DSRXLR CDS 8150182217 . + . ID=name"RXLR_1309" PHYCAScaffold_8 DSRXLR CDS 325877326851 . - . ID=name"RXLR_1310" PHYCAScaffold_16 DSRXLR CDS 588275588514 . + . ID=name"RXLR_1311" PHYCAScaffold_29 DSRXLR CDS 547987 552303 . - . ID=name"RXLR_1312" PHYCAScaffold_405 DSRXLR CDS 11141545 . + . ID=name"RXLR_1313" PHYCAScaffold_117 DSRXLR CDS \(4403944320 .+\). ID=name"RXLR_1314" PHYCAScaffold_49 DSRXLR CDS 205259205489 . + . ID=name"RXLR_1315" PHYCAScaffold_24 DSRXLR CDS 754199754450 . - . ID=name"RXLR_1316" PHYCAScaffold_51 DSRXLR CDS 412795413199 . - . ID=name"RXLR_1317" PHYCAScaffold_118 DSRXLR CDS \(4668747208 .+\). ID=name"RXLR_1318" PHYCAScaffold_7 DSRXLR CDS 1012698 1014245 . - . ID=name"RXLR_1319" PHYCAScaffold_6 DSRXLR CDS 1101811 1103256 . + . ID=name"RXLR_1320"

PHYCAScaffold_11 DSRXLR CDS 92822 93136 . - . ID=name"RXLR_1321" PHYCAScaffold_7 DSRXLR CDS 11997781200041 . + . ID=name"RXLR_1322" PHYCAScaffold_1 DSRXLR CDS 771928772677 . - . ID=name"RXLR_1323" PHYCAScaffold_3 DSRXLR CDS 824013824288 . - . ID=name"RXLR_1324" PHYCAScaffold_326 DSRXLR CDS \(81068525 .+\). ID=name"RXLR_1325" PHYCAScaffold_686 DSRXLR CDS 1755 . - . ID=name"RXLR_1326" PHYCAScaffold_32 DSRXLR CDS 393625393870 . + . ID=name"RXLR_1327" PHYCAScaffold_30 DSRXLR CDS 635624635899 . - . ID=name"RXLR_1328" PHYCAScaffold_54 DSRXLR CDS 53190 53495 . - . ID=name"RXLR_1329" PHYCAScaffold_43 DSRXLR CDS 5658157582 . + . ID=name"RXLR_1330" PHYCAScaffold_13 DSRXLR CDS 399908400468 . + . ID=name"RXLR_1331" PHYCAScaffold_9 DSRXLR CDS 960513 963197 . + . ID=name"RXLR_1332" PHYCAScaffold_33 DSRXLR CDS 311693312625 . + . ID=name"RXLR_1333" PHYCAScaffold_11 DSRXLR CDS 105930 106256 . - . ID=name"RXLR_1334" PHYCAScaffold_53 DSRXLR CDS 281793282050 . - . ID=name"RXLR_1335" PHYCAScaffold_50 DSRXLR CDS 2181623822 . + . ID=name"RXLR_1336" PHYCAScaffold_78 DSRXLR CDS 3273733099 . + . ID=name"RXLR_1337" PHYCAScaffold_2 DSRXLR CDS 1461558 1461905 . - . ID=name"RXLR_1338" PHYCAScaffold_97 DSRXLR CDS 100933 101193 . - . ID=name"RXLR_1339" PHYCAScaffold_12 DSRXLR CDS 1029334 1029573 . + . ID=name"RXLR_1340" PHYCAScaffold_27 DSRXLR CDS 239182245970 . + . ID=name"RXLR_1341" PHYCAScaffold_42 DSRXLR CDS 56231 57184 . - . ID=name"RXLR_1342" PHYCAScaffold_6 DSRXLR CDS 1377688 1377924 . - . ID=name"RXLR_1343" PHYCAScaffold_10 DSRXLR CDS 701633702136 . - . ID=name"RXLR_1344" PHYCAScaffold_24 DSRXLR CDS 103644 107426 . - . ID=name"RXLR_1345" PHYCAScaffold_30 DSRXLR CDS 7295574037 . + . ID=name"RXLR_1346" PHYCAScaffold_31 DSRXLR CDS 264353264817 . + . ID=name"RXLR_1347" PHYCAScaffold_29 DSRXLR CDS 493530493787 . + . ID=name"RXLR_1348" PHYCAScaffold_13 DSRXLR CDS 489695492583 . + . ID=name"RXLR_1349"

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PHYCAScaffold_462 DSRXLR CDS 20642483 . + . ID=name"RXLR_1408" PHYCAScaffold_13 DSRXLR CDS 1009245 1011677 . + . ID=name"RXLR_1409" PHYCAScaffold_4 DSRXLR CDS 513051515162 . + . ID=name"RXLR_1410" PHYCAScaffold_8 DSRXLR CDS 330523330747 . + . ID=name"RXLR_1411" PHYCAScaffold_9 DSRXLR CDS 952865953701 . - . ID=name"RXLR_1412" PHYCAScaffold_51 DSRXLR CDS 51396 51836 . + . ID=name"RXLR_1413" PHYCAScaffold_35 DSRXLR CDS 108550 108999 . - . ID=name"RXLR_1414" PHYCAScaffold_42 DSRXLR CDS 164154 165233 . + . ID=name"RXLR_1415" PHYCAScaffold_15 DSRXLR CDS 350966351373 . + . ID=name"RXLR_1416" PHYCAScaffold_19 DSRXLR CDS 804574 805071 . - . ID=name"RXLR_1417" PHYCAScaffold_20 DSRXLR CDS 781901782362 . + . ID=name"RXLR_1418" PHYCAScaffold_9 DSRXLR CDS 330893331126 . + . ID=name"RXLR_1419" PHYCAScaffold_16 DSRXLR CDS 358140358418 . + . ID=name"RXLR_1420" PHYCAScaffold_3 DSRXLR CDS 704701705171 . + . ID=name"RXLR_1421" PHYCAScaffold_2 DSRXLR CDS 6078361232 . - . ID=name"RXLR_1422" PHYCAScaffold_2 DSRXLR CDS 14447491445129 . + . ID=name"RXLR_1423" PHYCAScaffold_85 DSRXLR CDS 1255012801 . - . ID=name"RXLR_1424" PHYCAScaffold_12 DSRXLR CDS 736218736472 . + . ID=name"RXLR_1425" PHYCAScaffold_1 DSRXLR CDS 132400133014 . - . ID=name"RXLR_1426" PHYCAScaffold_7 DSRXLR CDS 353286354224 . - . ID=name"RXLR_1427" PHYCAScaffold_8 DSRXLR CDS 11653281165552 . + . ID=name"RXLR_1428" PHYCAScaffold_748 DSRXLR CDS 494943 . - . ID=name"RXLR_1429" PHYCAScaffold_882 DSRXLR CDS 549 1298 . - . ID=name"RXLR_1430" PHYCAScaffold_29 DSRXLR CDS 177687178817 . + . ID=name"RXLR_1431" PHYCAScaffold_19 DSRXLR CDS 901813 902025 . - . ID=name"RXLR_1432" PHYCAScaffold_60 DSRXLR CDS 128053 128331 . + . ID=name"RXLR_1433" PHYCAScaffold_29 DSRXLR CDS 300620300919 . + . ID=name"RXLR_1434"

\section*{F. 2 CRN Node Descriptions}

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\section*{Appendix G}

\section*{RxLR Paralagous Groups}


Figure G.1: Paralagous group 37 of the RxLR effector family.


Figure G.2: Paralagous group 43 of the RxLR effector family.


Figure G.3: Paralagous group 0 of the RxLR effector family.


Figure G.4: Paralagous group 100 of the RxLR effector family.


Figure G.5: Paralagous group 101 of the RxLR effector family.


Figure G.6: Paralagous group 103 of the RxLR effector family.


Figure G.7: Paralagous group 104 of the RxLR effector family.


Figure G.8: Paralagous group 105 of the RxLR effector family.


Figure G.9: Paralagous group 106 of the RxLR effector family.


Figure G.10: Paralagous group 107 of the RxLR effector family.


Figure G.11: Paralagous group 10 of the RxLR effector family.


Figure G.12: Paralagous group 110 of the RxLR effector family.


Figure G.13: Paralagous group 111 of the RxLR effector family.


Figure G.14: Paralagous group 115 of the RxLR effector family.


Figure G.15: Paralagous group 116 of the RxLR effector family.


Figure G.16: Paralagous group 117 of the RxLR effector family.


Figure G.17: Paralagous group 12 of the RxLR effector family.


Figure G.18: Paralagous group 13 of the RxLR effector family.


Figure G.19: Paralagous group 17 of the RxLR effector family.


Figure G.20: Paralagous group 18 of the RxLR effector family.


Figure G.21: Paralagous group 19 of the RxLR effector family.


Figure G.22: Paralagous group 20 of the RxLR effector family.


Figure G.23: Paralagous group 22 of the RxLR effector family.


Figure G.24: Paralagous group 23 of the RxLR effector family.


Figure G.25: Paralagous group 25 of the RxLR effector family.


Figure G.26: Paralagous group 27 of the RxLR effector family.


Figure G.27: Paralagous group 29 of the RxLR effector family.


Figure G.28: Paralagous group 2 of the RxLR effector family.


Figure G.29: Paralagous group 31 of the RxLR effector family.


Figure G.30: Paralagous group 32 of the RxLR effector family.


Figure G.31: Paralagous group 34 of the RxLR effector family.


Figure G.32: Paralagous group 36 of the RxLR effector family.


Figure G.33: Paralagous group 39 of the RxLR effector family.


Figure G.34: Paralagous group 40 of the RxLR effector family.


Figure G.35: Paralagous group 41 of the RxLR effector family.


Figure G.36: Paralagous group 42 of the RxLR effector family.


Figure G.37: Paralagous group 44 of the RxLR effector family.


Figure G.38: Paralagous group 45 of the RxLR effector family.


Figure G.39: Paralagous group 48 of the RxLR effector family.


Figure G.40: Paralagous group 49 of the RxLR effector family.


Figure G.41: Paralagous group 4 of the RxLR effector family.


Figure G.42: Paralagous group 50 of the RxLR effector family.


Figure G.43: Paralagous group 54 of the RxLR effector family.


Figure G.44: Paralagous group 57 of the RxLR effector family.


Figure G.45: Paralagous group 5 of the RxLR effector family.


Figure G.46: Paralagous group 61 of the RxLR effector family.


Figure G.47: Paralagous group 62 of the RxLR effector family.


Figure G.48: Paralagous group 65 of the RxLR effector family.


Figure G.49: Paralagous group 68 of the RxLR effector family.


Figure G.50: Paralagous group 69 of the RxLR effector family.


Figure G.51: Paralagous group 6 of the RxLR effector family.


Figure G.52: Paralagous group 71 of the RxLR effector family.


Figure G.53: Paralagous group 72 of the RxLR effector family.


Figure G.54: Paralagous group 74 of the RxLR effector family.


Figure G.55: Paralagous group 75 of the RxLR effector family.


Figure G.56: Paralagous group 76 of the RxLR effector family.


Figure G.57: Paralagous group 77 of the RxLR effector family.


Figure G.58: Paralagous group 78 of the RxLR effector family.


Figure G.59: Paralagous group 80 of the RxLR effector family.


Figure G.60: Paralagous group 81 of the RxLR effector family.


Figure G.61: Paralagous group 84 of the RxLR effector family.


Figure G.62: Paralagous group 85 of the RxLR effector family.


Figure G.63: Paralagous group 86 of the RxLR effector family.


Figure G.64: Paralagous group 88 of the RxLR effector family.


Figure G.65: Paralagous group 89 of the RxLR effector family.


Figure G.66: Paralagous group 8 of the RxLR effector family.


Figure G.67: Paralagous group 91 of the RxLR effector family.


Figure G.68: Paralagous group 92 of the RxLR effector family.


Figure G.69: Paralagous group 98 of the RxLR effector family.


Figure G.70: Paralagous group 9 of the RxLR effector family.


Figure G.71: Paralagous group 113 of the RxLR effector family.


Figure G.72: Paralagous group 114 of the RxLR effector family.


Figure G.73: Paralagous group 16 of the RxLR effector family.


Figure G.74: Paralagous group 21 of the RxLR effector family.


Figure G.75: Paralagous group 24 of the RxLR effector family.


Figure G.76: Paralagous group 26 of the RxLR effector family.


Figure G.77: Paralagous group 30 of the RxLR effector family.


Figure G.78: Paralagous group 35 of the RxLR effector family.


Figure G.79: Paralagous group 46 of the RxLR effector family.


Figure G.80: Paralagous group 52 of the RxLR effector family.


Figure G.81: Paralagous group 55 of the RxLR effector family.


Figure G.82: Paralagous group 64 of the RxLR effector family.


Figure G.83: Paralagous group 66 of the RxLR effector family.


Figure G.84: Paralagous group 67 of the RxLR effector family.


Figure G.85: Paralagous group 70 of the RxLR effector family.


Figure G.86: Paralagous group 73 of the RxLR effector family.


Figure G.87: Paralagous group 79 of the RxLR effector family.


Figure G.88: Paralagous group 82 of the RxLR effector family.


Figure G.89: Paralagous group 93 of the RxLR effector family.


Figure G.90: Paralagous group 99 of the RxLR effector family.


Figure G.91: Paralagous group 102 of the RxLR effector family.


Figure G.92: Paralagous group 112 of the RxLR effector family.


Figure G.93: Paralagous group 14 of the RxLR effector family.


Figure G.94: Paralagous group 15 of the RxLR effector family.


Figure G.95: Paralagous group 1 of the RxLR effector family.


Figure G.96: Paralagous group 47 of the RxLR effector family.


Figure G.97: Paralagous group 53 of the RxLR effector family.


Figure G.98: Paralagous group 56 of the RxLR effector family.


Figure G.99: Paralagous group 59 of the RxLR effector family.


Figure G.100: Paralagous group 60 of the RxLR effector family.


Figure G.101: Paralagous group 7 of the RxLR effector family.


Figure G.102: Paralagous group 90 of the RxLR effector family.


Figure G.103: Paralagous group 95 of the RxLR effector family.


Figure G.104: Paralagous group 96 of the RxLR effector family.


Figure G.105: Paralagous group 108 of the RxLR effector family.


Figure G.106: Paralagous group 38 of the RxLR effector family.


Figure G.107: Paralagous group 3 of the RxLR effector family.


Figure G.108: Paralagous group 97 of the RxLR effector family.


Figure G.109: Paralagous group 63 of the RxLR effector family.


Figure G.110: Paralagous group 94 of the RxLR effector family.


Figure G.111: Paralagous group 87 of the RxLR effector family.


Figure G.112: Paralagous group 109 of the RxLR effector family.


Figure G.113: Paralagous group 51 of the RxLR effector family.


Figure G.114: Paralagous group 58 of the RxLR effector family.


Figure G.115: Paralagous group 11 of the RxLR effector family.


Figure G.116: Paralagous group 28 of the RxLR effector family.


Figure G.117: Paralagous group 33 of the RxLR effector family.


Figure G.118: Paralagous group 83 of the RxLR effector family.

\section*{Appendix H}

\section*{CRN Paralagous Groups}


Figure H.1: Paralagous group 15 of the CRN effector family.


Figure H.2: Paralagous group 10 of the CRN effector family.


Figure H.3: Paralagous group 11 of the CRN effector family.


Figure H.4: Paralagous group 12 of the CRN effector family.


Figure H.5: Paralagous group 14 of the CRN effector family.


Figure H.6: Paralagous group 19 of the CRN effector family.


Figure H.7: Paralagous group 1 of the CRN effector family.


Figure H.8: Paralagous group 3 of the CRN effector family.


Figure H.9: Paralagous group 4 of the CRN effector family.


Figure H.10: Paralagous group 8 of the CRN effector family.


Figure H.11: Paralagous group 9 of the CRN effector family.


Figure H.12: Paralagous group 0 of the CRN effector family.


Figure H.13: Paralagous group 13 of the CRN effector family.


Figure H.14: Paralagous group 17 of the CRN effector family.


Figure H.15: Paralagous group 18 of the CRN effector family.


Figure H.16: Paralagous group 22 of the CRN effector family.


Figure H.17: Paralagous group 2 of the CRN effector family.


Figure H.18: Paralagous group 5 of the CRN effector family.


Figure H.19: Paralagous group 6 of the CRN effector family.


Figure H.20: Paralagous group 21 of the CRN effector family.


Figure H.21: Paralagous group 23 of the CRN effector family.


Figure H.22: Paralagous group 7 of the CRN effector family.


Figure H.23: Paralagous group 20 of the CRN effector family.


Figure H.24: Paralagous group 16 of the CRN effector family.

\section*{Appendix I}

\section*{Calculated Selection Pressures on Paralagous RxLR Groups}


Figure I.1: Calculated codon level selection pressure on paralagous group 0. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 100}


Figure I.2: Calculated codon level selection pressure on paralagous group 100. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 101}


Figure I.3: Calculated codon level selection pressure on paralagous group 101. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 102}


Figure I.4: Calculated codon level selection pressure on paralagous group 102. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 103}


Figure I.5: Calculated codon level selection pressure on paralagous group 103. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 104}


Figure I.6: Calculated codon level selection pressure on paralagous group 104. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 105}


Figure I.7: Calculated codon level selection pressure on paralagous group 105. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 106}


Figure I.8: Calculated codon level selection pressure on paralagous group 106. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 107}


Figure I.9: Calculated codon level selection pressure on paralagous group 107. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 108}


Figure I.10: Calculated codon level selection pressure on paralagous group 108. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 109}


Figure I.11: Calculated codon level selection pressure on paralagous group 109. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 10}


Figure I.12: Calculated codon level selection pressure on paralagous group 10. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 110}


Figure I.13: Calculated codon level selection pressure on paralagous group 110 . Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 111}


Figure I.14: Calculated codon level selection pressure on paralagous group 111. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 112}


Figure I.15: Calculated codon level selection pressure on paralagous group 112. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 113}


Figure I.16: Calculated codon level selection pressure on paralagous group 113. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 114}


Figure I.17: Calculated codon level selection pressure on paralagous group 114. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 115}


Figure I.18: Calculated codon level selection pressure on paralagous group 115. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 116}


Figure I.19: Calculated codon level selection pressure on paralagous group 116. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 117}


Figure I.20: Calculated codon level selection pressure on paralagous group 117. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 11}


Figure I.21: Calculated codon level selection pressure on paralagous group 11. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 12}


Figure I.22: Calculated codon level selection pressure on paralagous group 12. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 13}


Figure I.23: Calculated codon level selection pressure on paralagous group 13. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 14}


Figure I.24: Calculated codon level selection pressure on paralagous group 14. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 15}


Figure I.25: Calculated codon level selection pressure on paralagous group 15. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 16}


Figure I.26: Calculated codon level selection pressure on paralagous group 16. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 17}


Figure I.27: Calculated codon level selection pressure on paralagous group 17. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 18}


Figure I.28: Calculated codon level selection pressure on paralagous group 18. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 19}


Figure I.29: Calculated codon level selection pressure on paralagous group 19. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 1}


Figure I.30: Calculated codon level selection pressure on paralagous group 1. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 20}


Figure I.31: Calculated codon level selection pressure on paralagous group 20. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 21}


Figure I.32: Calculated codon level selection pressure on paralagous group 21. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 22}


Figure I.33: Calculated codon level selection pressure on paralagous group 22. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 23}


Figure I.34: Calculated codon level selection pressure on paralagous group 23. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 24}


Figure I.35: Calculated codon level selection pressure on paralagous group 24. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 25}


Figure I.36: Calculated codon level selection pressure on paralagous group 25. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 26}


Figure I.37: Calculated codon level selection pressure on paralagous group 26. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 27}


Figure I.38: Calculated codon level selection pressure on paralagous group 27. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 28}


Figure I.39: Calculated codon level selection pressure on paralagous group 28. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 29}


Figure I.40: Calculated codon level selection pressure on paralagous group 29. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 2}


Figure I.41: Calculated codon level selection pressure on paralagous group 2. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 30}


Figure I.42: Calculated codon level selection pressure on paralagous group 30 . Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 31}


Figure I.43: Calculated codon level selection pressure on paralagous group 31. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 32}


Figure I.44: Calculated codon level selection pressure on paralagous group 32 . Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 33}


Figure I.45: Calculated codon level selection pressure on paralagous group 33. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 34}


Figure I.46: Calculated codon level selection pressure on paralagous group 34. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 35}


Figure I.47: Calculated codon level selection pressure on paralagous group 35 . Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 36}


Figure I.48: Calculated codon level selection pressure on paralagous group 36 . Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 37}


Figure I.49: Calculated codon level selection pressure on paralagous group 37. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 38}


Figure I.50: Calculated codon level selection pressure on paralagous group 38 . Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 39}


Figure I.51: Calculated codon level selection pressure on paralagous group 39. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 3}


Figure I.52: Calculated codon level selection pressure on paralagous group 3. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 40}


Figure I.53: Calculated codon level selection pressure on paralagous group 40. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 41}


Figure I.54: Calculated codon level selection pressure on paralagous group 41. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 42}


Figure I.55: Calculated codon level selection pressure on paralagous group 42. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 43}


Figure I.56: Calculated codon level selection pressure on paralagous group 43. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 44}


Figure I.57: Calculated codon level selection pressure on paralagous group 44. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 45}


Figure I.58: Calculated codon level selection pressure on paralagous group 45 . Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 46}


Figure I.59: Calculated codon level selection pressure on paralagous group 46. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 47}


Figure I.60: Calculated codon level selection pressure on paralagous group 47. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 48}


Figure I.61: Calculated codon level selection pressure on paralagous group 48. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 49}


Figure I.62: Calculated codon level selection pressure on paralagous group 49. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 4}


Figure I.63: Calculated codon level selection pressure on paralagous group 4. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 50}


Figure I.64: Calculated codon level selection pressure on paralagous group 50 . Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 51}


Figure I.65: Calculated codon level selection pressure on paralagous group 51. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 52}


Figure I.66: Calculated codon level selection pressure on paralagous group 52 . Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 53}


Figure I.67: Calculated codon level selection pressure on paralagous group 53. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 54}


Figure I.68: Calculated codon level selection pressure on paralagous group 54. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 55}


Figure I.69: Calculated codon level selection pressure on paralagous group 55. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 56}


Figure I.70: Calculated codon level selection pressure on paralagous group 56. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 57}


Figure I.71: Calculated codon level selection pressure on paralagous group 57. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 58}


Figure I.72: Calculated codon level selection pressure on paralagous group 58. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 59}


Figure I.73: Calculated codon level selection pressure on paralagous group 59. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 5}


Figure I.74: Calculated codon level selection pressure on paralagous group 5. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 60}


Figure I.75: Calculated codon level selection pressure on paralagous group 60. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 61}


Figure I.76: Calculated codon level selection pressure on paralagous group 61. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 62}


Figure I.77: Calculated codon level selection pressure on paralagous group 62. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 63}


Figure I.78: Calculated codon level selection pressure on paralagous group 63. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 64}


Figure I.79: Calculated codon level selection pressure on paralagous group 64. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 65}


Figure I.80: Calculated codon level selection pressure on paralagous group 65. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 66}


Figure I.81: Calculated codon level selection pressure on paralagous group 66. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 67}


Figure I.82: Calculated codon level selection pressure on paralagous group 67. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 68}


Figure I.83: Calculated codon level selection pressure on paralagous group 68. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 69}


Figure I.84: Calculated codon level selection pressure on paralagous group 69. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 6}


Figure I.85: Calculated codon level selection pressure on paralagous group 6. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 70}


Figure I.86: Calculated codon level selection pressure on paralagous group 70. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 71}


Figure I.87: Calculated codon level selection pressure on paralagous group 71. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 72}


Figure I.88: Calculated codon level selection pressure on paralagous group 72. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 73}


Figure I.89: Calculated codon level selection pressure on paralagous group 73. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 74}


Figure I.90: Calculated codon level selection pressure on paralagous group 74. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 75}


Figure I.91: Calculated codon level selection pressure on paralagous group 75. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 76}


Figure I.92: Calculated codon level selection pressure on paralagous group 76 . Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 77}


Figure I.93: Calculated codon level selection pressure on paralagous group 77. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 78}


Figure I.94: Calculated codon level selection pressure on paralagous group 78. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 79}


Figure I.95: Calculated codon level selection pressure on paralagous group 79. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 7}


Figure I.96: Calculated codon level selection pressure on paralagous group 7. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 80}


Figure I.97: Calculated codon level selection pressure on paralagous group 80. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 81}


Figure I.98: Calculated codon level selection pressure on paralagous group 81. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 82}


Figure I.99: Calculated codon level selection pressure on paralagous group 82. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 83}


Figure I.100: Calculated codon level selection pressure on paralagous group 83. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 84}


Figure I.101: Calculated codon level selection pressure on paralagous group 84. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 85}


Figure I.102: Calculated codon level selection pressure on paralagous group 85. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 86}


Figure I.103: Calculated codon level selection pressure on paralagous group 86. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 87}


Figure I.104: Calculated codon level selection pressure on paralagous group 87. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 88}


Figure I.105: Calculated codon level selection pressure on paralagous group 88 . Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 89}


Figure I.106: Calculated codon level selection pressure on paralagous group 89. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 8}


Figure I.107: Calculated codon level selection pressure on paralagous group 8. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 90}


Figure I.108: Calculated codon level selection pressure on paralagous group 90. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 91}


Figure I.109: Calculated codon level selection pressure on paralagous group 91. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 92}


Figure I.110: Calculated codon level selection pressure on paralagous group 92. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 93}


Figure I.111: Calculated codon level selection pressure on paralagous group 93. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 94}


Figure I.112: Calculated codon level selection pressure on paralagous group 94. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 95}


Figure I.113: Calculated codon level selection pressure on paralagous group 95. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 96}


Figure I.114: Calculated codon level selection pressure on paralagous group 96. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 97}


Figure I.115: Calculated codon level selection pressure on paralagous group 97. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 98}


Figure I.116: Calculated codon level selection pressure on paralagous group 98. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 99}


Figure I.117: Calculated codon level selection pressure on paralagous group 99. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 9}


Figure I.118: Calculated codon level selection pressure on paralagous group 9. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Appendix J}

\section*{Calculated Selection Pressures on Paralagous CRN Groups}

\section*{Selection Pressure on Cluster 0}


Figure J.1: Calculated codon level selection pressure on paralagous group 0. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 10}


Figure J.2: Calculated codon level selection pressure on paralagous group 10. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 11}


Figure J.3: Calculated codon level selection pressure on paralagous group 11. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 12}


Figure J.4: Calculated codon level selection pressure on paralagous group 12. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 13}


Figure J.5: Calculated codon level selection pressure on paralagous group 13. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 14}


Figure J.6: Calculated codon level selection pressure on paralagous group 14. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 15}


Figure J.7: Calculated codon level selection pressure on paralagous group 15. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 16}


Figure J.8: Calculated codon level selection pressure on paralagous group 16. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 17}


Figure J.9: Calculated codon level selection pressure on paralagous group 17. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 18}


Figure J.10: Calculated codon level selection pressure on paralagous group 18. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 1}


Figure J.11: Calculated codon level selection pressure on paralagous group 1. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 20}


Figure J.12: Calculated codon level selection pressure on paralagous group 20. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 21}


Figure J.13: Calculated codon level selection pressure on paralagous group 21. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 22}


Figure J.14: Calculated codon level selection pressure on paralagous group 22. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 23}


Figure J.15: Calculated codon level selection pressure on paralagous group 23. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 2}


Figure J.16: Calculated codon level selection pressure on paralagous group 2. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 3}


Figure J.17: Calculated codon level selection pressure on paralagous group 3. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 4}


Figure J.18: Calculated codon level selection pressure on paralagous group 4. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 5}


Figure J.19: Calculated codon level selection pressure on paralagous group 5. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 6}


Figure J.20: Calculated codon level selection pressure on paralagous group 6. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 7}


Figure J.21: Calculated codon level selection pressure on paralagous group 7. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 8}


Figure J.22: Calculated codon level selection pressure on paralagous group 8. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Selection Pressure on Cluster 9}


Figure J.23: Calculated codon level selection pressure on paralagous group 9. Levels above 0 are correspondent to positive selection pressure and levels below 0 are negative selection pressures. Anything over a selection pressure of \(\pm 0.9\) is considered significant.

\section*{Vita}

Dylan Bobby Storey, only child of Robert and Diane, began his collegiate education at California State University, Fresno where he received a Bachelor of Science in Biology with a concentration in Molecular Cellular and Developmental biology. He then continued his education in the lab of Dr. Jim Prince at the same institution where he received his Masters of Science in Biology for his work on integrating linkage maps of Capsicum annuum.

He entered the University of Tennessee's Genome Science and Technology program in 2009 as an NSF IGERT trainee. In 2012 he was awarded a USDA-NIFA pre-doctoral fellowship for his proposed work on the application of novel graph theory algorithms to RNA-Seq data. His dissertation work while in the lab of Dr. Kurt Lamour has centered around the application of next generation sequencing technologies to assess and measure genomic levels of variation in the plant pathogens P. capsici and P. tropicalis.```

