

A targeted SNP panel for ship rat (*Rattus rattus*) population genetics in New Zealand

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Summary

Project and client

The Department of Conservation (DOC) and Predator Free 2050 contracted the Bioeconomy Science Institute Maiangi Taiao (BSI) ¹ to develop genetic tools for improved management of ship rats (*Rattus rattus*) in New Zealand (NZ). Analyses were undertaken in 2024 and 2025, with writeup completed in January 2026.

Objectives

- 1 Assess the likely efficacy of the RapidRat SNP panel for ship rats in NZ.
- 2 If the RapidRat panel is unlikely to have sufficient resolution for management-relevant questions, design and test a new SNP marker panel that is suitable for use on degraded samples.

Methods

- Using previously obtained Genotyping-By-Sequencing (GBS) data from ship rats on the Miramar Peninsula, Wellington, we compared subsets of the data with equivalent variability to the RapidRat panel to the results from the full GBS data set to determine its relative resolution.
- We assessed GBS data from 282 ship rats from 58 locations across NZ, that were obtained through collaborators to determine the nation-wide genetic population structure.
- We then selected the SNP markers from this data set that provided an optimal average resolution across the six defined populations in New Zealand for assessing relatedness and individual identification anywhere in the country.
- We then amplified and sequenced 240 of these optimised markers for 94 individuals comprising 86 ship rats, plus 4 kiore (*Rattus exulans*) and 4 Norway (*Rattus norvegicus*) rats as controls.
- We assessed amplification and accuracy for all markers to generate a final marker panel.
- We assessed the utility of the marker panel to answer management relevant questions including rat sex and species identification, population assignment, individual identification, and landscape genomics.

Results and discussion

- The RapidRat SNP panel lacked resolution to answer relatedness and landscape genomic questions due to low variability across the majority of the markers in NZ.
- A new targeted SNP panel was created including 175 SNP markers, optimised for ship rats across NZ.
- The new panel performed well for all tasks including sex and species identification (ID), population assignment, individual ID, and landscape genomics.

¹ On 01 July 2025 Landcare Research New Zealand Ltd became the New Zealand Institute for Bioeconomy Science Ltd; Manaaki Whenua – Landcare Research operates as an internal group within this Institute, which is less formally known as the Bioeconomy Science Institute Maiangi Taiao or Bioeconomy Science Institute (BSI).

- Pairwise relatedness values calculated between rats using this novel panel of 175 markers were comparable to the values calculated using thousands of genetic markers from GBS data.
- Individual heterozygosity calculated using the panel was also comparable to larger panels created through GBS, and as marker variability was specifically maximised for the panel design even inbred island populations of rats maintained reasonably high measured variability.
- It was possible to determine if two samples are from the same individual or from different individuals with significant redundancy (missing markers), even for smaller more inbred island populations.
- Population assignment was possible even in situations with small numbers of samples, which represents a real-world scenario where DOC is determining if a small number of samples are survivors from an eradication or reinvaders.
- Both kiore (*Rattus exulans*) and Norway rats (*Rattus norvegicus*) had characteristic marker drop-out patterns so that if they are accidentally sequenced using this panel they will be identified automatically.
- The sex of all known-sex individuals was accurately determined using the panel.

Conclusions and applications

- This novel targeted SNP panel shows considerable promise to answer various management questions (e.g. on sex, species and individual identification from trace samples; population assignment, relatedness, and landscape genomics).
- Some further development is required in terms of assessing error rates for different sample types (e.g. scat) and testing the optimised panel and pipeline on a wider range of rat samples from across the country.
- Further testing can be done on samples and on projects as the need arises, with the caveat that this panel is still in development and may require further refinement.
- As more samples are processed with this SNP panel a database can be built up to understand the origins of ship rats with increasing spatial resolution.

1 Introduction

DOC and Predator Free 2050 contracted the Bioeconomy Science Institute² to help develop genetic tools for improved management questions regarding ship rats (*Rattus rattus*) in New Zealand (NZ). The primary goal of the work has been to improve detection tools for identifying rat re-invaders versus survivors. Knowledge of whether a predator detection in an area already assumed to be cleared of that species represents a residual individual or a new invader is critical in assessing management effectiveness and in defining the focus of an appropriate response.

The secondary goal has been to increased cost-effectiveness of rat detection using trace samples (scat). Identifying individuals from trace samples such as scat saves money during an incursion response in that the number and distribution of individuals can then be ascertained. Once all individuals that have been identified from these trace samples have been removed, the incursion response has good evidence to stop, reducing the cost of further monitoring.

The aim of this specific project was to create a targeted SNP panel for ship rats in NZ. To do this, we first tested the RapidRat panel for efficacy in answering management questions and then used the available data from two Genotyping-By-Sequencing (GBS studies) to create a more effective panel. (The two studies were i) from 74 ship rats on the Miramar Peninsula; ii) from a study of 282 ship rats from 58 locations across NZ).

Once developed, a targeted SNP panel should be able to identify individuals from scat/hair/saliva, assess relatedness between samples, and define the origins of an individual (if spatial sampling has been adequate). Identifying individuals from trace samples means that studies can be undertaken of their spatial use, and it will allow managers to confirm when all known individuals have been eliminated thus reducing costs around incursion responses.

2 Background

Rats are among the most widespread mammals on Earth, thriving on every continent except Antarctica and accompanying humans across millennia of migration, trade, and settlement (Atkinson 1985; Drake & Hunt 2009). Globally, *Rattus* species exert enormous ecological and economic impacts, from damaging crops and infrastructure to driving extinctions of native wildlife on islands. Nowhere are these effects more profound than in NZ, where three invasive rat species kiore (*Rattus exulans*), ship rats (*R. rattus*), and Norway rats (*R. norvegicus*), have transformed ecosystems that evolved without terrestrial mammals. Introduced in successive waves from the arrival of kiore with Polynesian voyagers in the 13th century through to the arrival of Norway rats with European colonisation from the 1770s, and ship rats from the 1870s, these rats have become dominant predators in forests, wetlands, and coastal habitats, preying on birds, lizards, invertebrates, and seeds (Bellingham et al. 2010). Their impacts underpin much of the country's modern biodiversity crisis and remain central to national conservation and Predator Free 2050 strategies (Parkes et al. 2017; Murphy et al. 2019; Innes et al. 2024). Of the three rat species in New Zealand, ship rats are the most ubiquitous, and they have the greatest negative effects on bird species (King & Forsyth 2019).

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There have been multiple previous studies of rat genetics in NZ using microsatellite markers to assess population connectivity and survivor/reinvader questions (e.g. Abdelkrim et al. 2010; Fewster et al. 2011; King et al. 2011; Robins et al. 2016; Yarita et al. 2023). Microsatellites are not easily replicated between labs, are labour-intensive (because they have limited capacities for multiplexing), and there can have high drop-out rates (where one or both alleles are not recorded) for both degraded tissue samples and trace samples such as scat. Currently, when scat is detected and if it is fresh enough individual identification is possible using microsatellite markers. These often fail to amplify well and require considerable replication and manual curation to ensure data quality.

Single Nucleotide Polymorphisms (SNPs) have the benefit that many more markers can be obtained simultaneously, and depending on the method used to generate the data, SNPs can be more effective for degraded samples. Identifying individuals from trace samples such as scat saves money during an incursion response by allowing the number and distribution of individuals to be ascertained. Once all individuals identified from these trace samples have been removed, the incursion response has good evidence to stop, which reduces the cost of further monitoring.

Genotyping-By-Sequencing (GBS) or similar reduced representation sequencing techniques generate thousands to tens of thousands of SNP markers. This provides excellent resolution to evaluate the genetic relationships between individuals. However, a challenge with GBS is that it requires relatively high quality and quantities of DNA, generally sourced from tissues. In addition, GBS samples generally have to be pooled and the analysis pipelines are complex, so the turnaround times are considerable. For management purposes, the ideal SNP genotyping technique is one that can work well on degraded samples such as scat, and be undertaken with a more rapid turnaround so that information can flow back in a management relevant timeframe.

Genotyping-By-Thousands (GT-Seq) is one technique that can be used to amplify hundreds to thousands of SNP markers, and it can be used on degraded samples. This technique involves designing multiple primer pairs that each surround a target SNP, with all of these primers designed to be compatible with one another. These primers are then all combining into one large polymerase chain reaction (PCR reaction), and the resulting mixture is sequenced using Illumina sequencing. The proportion of reads for each allele is then counted to determine the genotype of each individual.

Targeted GT-Seq SNP panels have been developed for use on both *R. norvegicus* and *R. rattus*, specifically to answer questions related to population connectivity and to identify invaders on the islands of Haida Gwaii in Canada (Sjodin et al. 2020). In Sjodin et al.'s study, the researchers were successfully able to use their targeted 'RapidRat' SNP panel to identify the origin of several incursions using a panel of 443 SNPs. However, their targeted SNP panel was designed and optimised for the genetic diversity present across these specific Canadian islands. The rats in NZ have a different history and ancestry, so the efficacy of this panel for addressing similar questions in NZ remains uncertain.

Previous work conducted by EcoGene® (see <https://www.landcareresearch.co.nz/partner-with-us/ecogene-dna-based-diagnostics>) used the RapidRat panel to genotype 79 ship rats from 4 locations around NZ. This data set which was kindly shared with the researchers on the current project showed that most markers included in the RapidRat panel were not variable in NZ, and those that were variable generally had low minor allele frequencies (MAFs). (A minor allele frequency is the frequency of the less common allele for a marker, with SNPs only having two alleles. This means that the minor allele frequency ranges from 0 – 0.5. Markers with low MAFs provide low power and add little extra information for questions relating to individual identification and to assess relationships between individuals: two animals sharing extremely common alleles in

a population don't have to be related.) In the EcoGene® study of the RapidRat panel, only 11 of the markers had MAFs over 0.35, which would indicate a high information content.

3 Objectives

- 1 Assess the likely efficacy of the RapidRat SNP panel for ship rats in NZ.
- 2 If the RapidRat panel is unlikely to have sufficient resolution for management-relevant questions, design and test a new SNP marker panel for rats that is suitable for use on degraded samples

4 Methods

4.1 Determining the efficacy of the RapidRat SNP panel in New Zealand

To calculate the efficacy of the RapidRat panel in NZ, I simulated data by sub-selected loci from the GBS data from Miramar, Wellington that had the same MAFs as each of the loci in the RapidRat panel and did this three times as replicates. This Miramar ship rat genetic dataset was described in more detail in Veale (2024). So, if locus A had a MAF of 0.12 I included a locus from the GBS with a MAF of 0.12 (and did this across all loci, matching them one-for-one). This was replicated three times to check how they compared to one another. Thus, each of these simulated data sets used real loci recorded in *real* samples, with *each locus* having identical characteristics to the RapidRat panel (assuming the MAFs are the same as recorded in the Miramar study as they are averaged across other sites assessed).

Each of the three replicates performed poorly for consistent relatedness estimates and spatial structure and poorly matched the relatedness values obtained from the larger GBS data set. This was a useful exercise to understand the requirements for panel development: creating subsets of the data in various ways selecting markers with different allele frequencies and seeing how well they match larger data sets.

I then created simulated panels of different sizes and found that with 96 markers the correlation between pairwise relatedness values calculated between individuals calculated from the complete GBS data set was $R^2 = 0.6$; and with 192 markers the $R^2 = 0.8$. With 192 highly variable markers (MAF > 0.35) it is possible to obtain sufficient resolution for most spatial questions, and for relatedness.

4.2 Creating a targeted SNP panel for a novel species

Construction of a novel targeted GT-Seq style panel requires the following steps:

- 1 Conduct a population genomics study across all populations of interest to identify a large (10,000+) SNP marker set for robust analyses that can be subsequently filtered.
- 2 Align these sequences to the species' genome to identify longer flanking sequences around the SNPs.
- 3 Filter the SNP panel to retain a set of optimal SNPs to address specific question(s) of interest. For individual identification from trace samples, optimal markers will be those with the highest minor allele frequency (MAF). For geographic studies markers should have strongly divergent frequencies across the landscape at the relevant scale.
- 4 Design in-silico primer pairs around a selection of target SNPs that will amplify at the same temperatures and that do not interact with each other.
- 5 Test these primers on samples from the first study with known SNP genotypes to evaluate the amplification and accuracy of the primer panel.
- 6 Remove markers that did not work to create a reduced marker panel and optimise laboratory and bioinformatic analyses.
- 7 Test optimised methods on trace samples of various types (and levels of degradation) to determine the error rates associated with each sample type.

For a NZ-wide ship rat data set I obtained a ship rat SNP data set from Dr Florian Pichlmüller and Professor James Russell that contained GBS data from $n = 282$ ship rats from $n = 58$ locations across New Zealand. This GBS data set was then used to discover new markers with sufficient variation, and to create primers for these markers. The loci were aligned to the ship rat genome produced by CSIRO of a ship rat sourced from Auckland (GenBank Accession: GCA_011064425.1).

4.2.1 Investigating population structure across New Zealand

First, I assessed population structure across the NZ ship rats, using Principal Coordinate Analyses (PCoAs), FastStructure, and heatmaps of genetic similarity (all conducted in R using Adegenet (Jombard et al. 2013) and Dart.R (Gruber et al. 2018)). Ship rats in NZ have a complex invasion history and population genetic structure (Figure 1, Figure 2). The optimal number of clusters using FastStructure and find.cluster in Adegenet (based on AIC) was approximately 20. This high number of clusters is largely a consequence of each small island becoming its own cluster due to their reduced genetic diversity and inbreeding.

I then combined these results with the various analyses with the mitochondrial data from Russell et al. 2019, along with discussing the unpublished results from whole genome sequencing conducted at the University of Otago, NZ (by Catherine Collins). From all this, I chose an optimal number of $n = 6$ populations to design the targeted SNP panel.

These six populations match the geographic mitochondrial patterns found in Russell et al. 2019. These are: 1) Auckland and Northland, 2) Great Barrier Island (hereafter Aotea), 3) the rest of North Island, 4) South Island (Northern & Central), 5) Southland, and 6) Stewart Island/Rakiura (hereafter 'Stewart Island').

There is added complexity to the population genetic structure of ship rats in New Zealand beyond there just being six genetic clusters. These extra complexities include Dunedin being slightly divergent from other southern populations sampled, and some mixing between the South Island (Southern) population, and the South Island (Northern and Central) population across a transition zone, and small islands being inbred isolated clusters. These patterns can be seen in figures 1 and 2, with intermediate individuals always at the geographic boundaries between adjacent populations. Along with these complexities the well sampled and highly differentiated Rakitu Island (Arid Island) ship rat population east of Great Barrier Island (Aotea Island) was ignored for SNP panel design given that it is no longer extant.

This project's aim was to create an optimal SNP panel that took account of the broader population structure, rather than to describe the population structure in detail. The simplified generalisation of six populations means that across each of these broad regions the marker panel should be evenly optimised, which should lead to a marker panel that can be used in most situations in New Zealand.

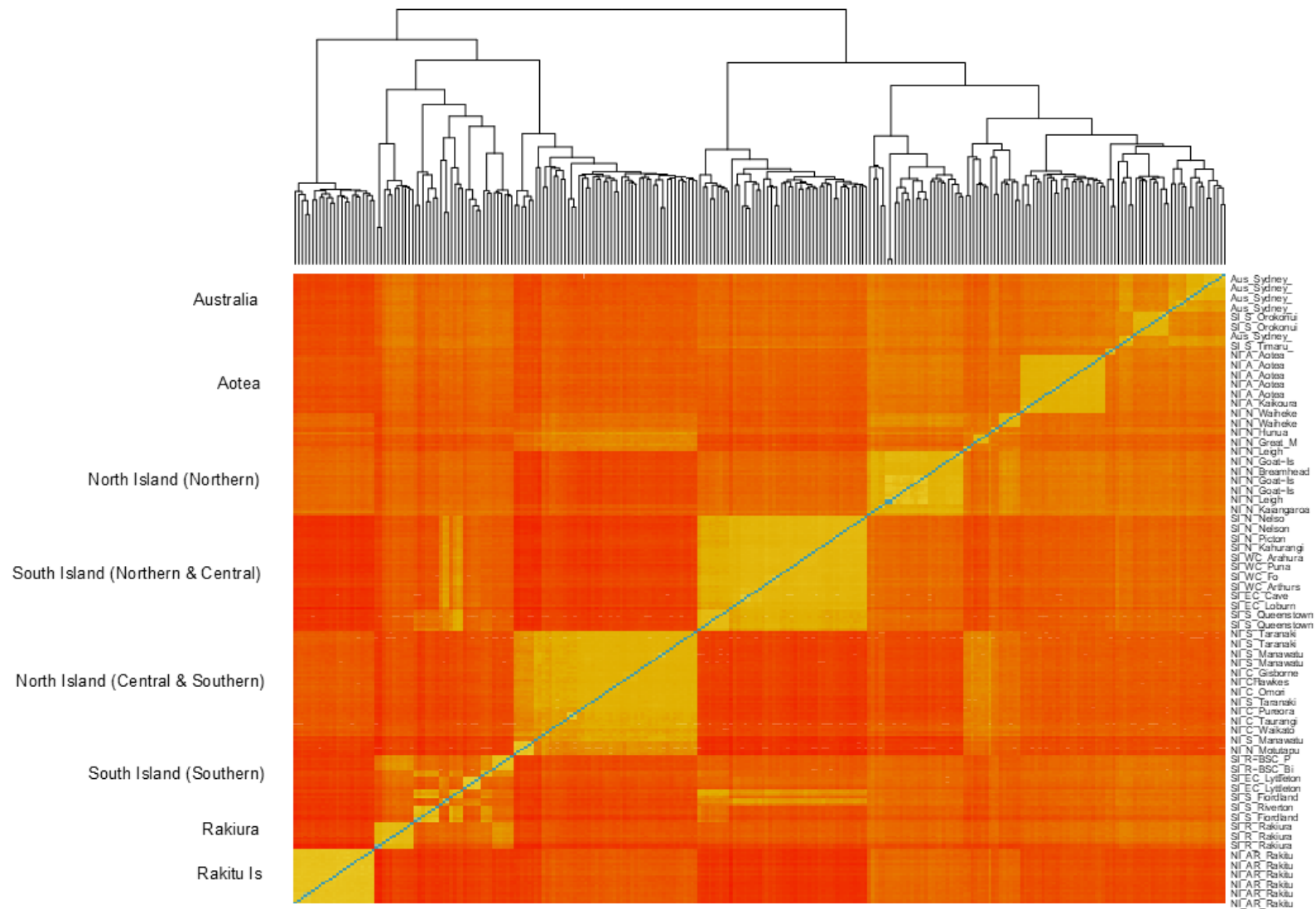


Figure 1. Heatmap and neighbour-joining tree showing pairwise genetic distances between ship rats sequenced with DART-Seq for 8 populations of ship rats (left side of plot). The diagonal is ‘self-relatedness’. Yellow shows higher relatedness whereas red shows lower relatedness. The generalised groups are described to the left with some sample names shown on the right.

Each of the six populations had different allele frequencies, and a genetic panel optimised for one would probably not be optimised for other populations and would therefore lose some power to assess relatedness in these other populations.

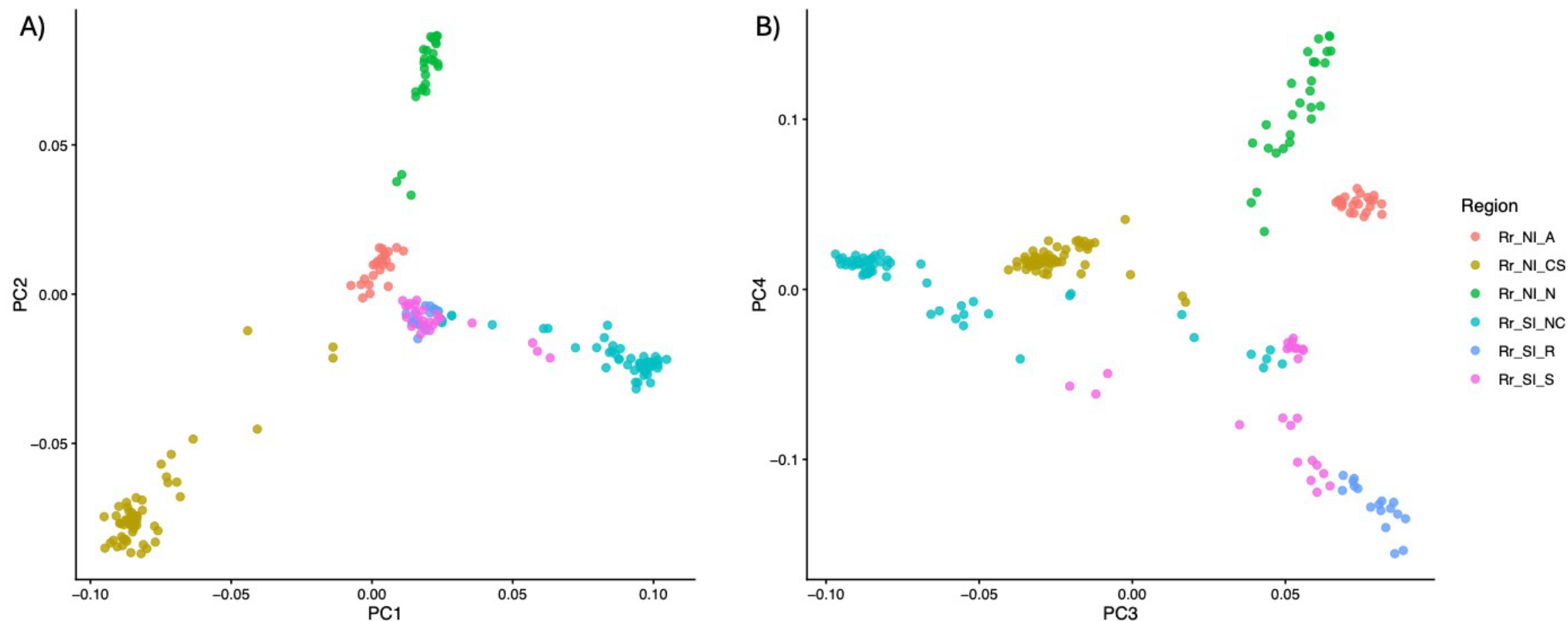


Figure 2. Principal Coordinate Analysis (PCoA) of ship rat genetic similarity. Colours correspond to geographic populations: NI_N = North Island Northland/Auckland, NI_CS = North Island Central & Southern, NI_A = Great Barrier (Aotea), SI_NC = South Island North & Central, SI_S = South Island Southern, SI_R = Rakiura (i.e. Stewart Island/Rakiura).



Figure 3. Map of New Zealand showing sample locations (tags) and approximate locations (dotted red circles) of each of the six ship rat populations the targeted SNP panel was designed for in this study. Blue tag = Rathap1, Red tag = Rathap2, Olive tag = Rathap4, Yellow tag = Rathap7.

4.2.2 Filtering, optimising and evaluating markers

I filtered the SNPs in the New Zealand data set for depth, quality and reproducibility. I calculated the MAF for each of the six populations, and deviations from Hardy Weinberg were also calculated. I then selected the loci with the highest MAFs averaged across populations as potential markers for a targeted SNP panel. These markers on average provide the most information across all populations. I conducted further filtering to remove any markers that were within 1 MB of each other, and then sequences for all loci were individually visually inspected to check for microsatellites that might cause primer binding issues. For this step I included only markers on the autosomal chromosomes (i.e. no sex-linked markers) as including markers on the X-chromosome can create challenges for analyses. Markers for sex determination were added back for the final panel after these autosomal markers were selected.

Along with the markers optimised for individual identification, we included a small number of markers for their resolution at large spatial scales. These were markers with frequencies that are highly differentiated between the six identified populations. From these it would be easier to assess location of origin for unknown samples.

The best 500 sequences were then evaluated in FastPCR (Kalendar et al. 2009) to design a compatible multiplex. This program assesses primer combinations that have similar binding temperatures and that do not interact with each other (which would inhibit amplification). Along with these novel markers, a sex-linked marker on the Y-chromosome from a previously published study was included to provide sex categorisation.

4.3 SNP marker selection from GBS data

Loci identified from the assembly were first filtered to retain only those with the highest information content (highest minor allele frequency), low missingness, and high quality (details of filtering are in Appendix 1). The flanking sequences then had PCR primers designed, ensuring that these were compatible with each other for a single multiplex PCR (details in Appendix 2).

4.4 Targeted SNP panel amplification

Our laboratory methods were based on Campbell et al. 2015, including the modifications recommended by Schmidt et al. 2020. Details in Appendix 3.

The ship rat samples amplified with the panel were chosen for specific tasks and involved the following numbers of individuals and tests, which total 96 amplifications:

- $n = 48$ Wellington ship rats
- $n = 8$ replicates of the Miramar individuals
- $n = 8$ Kawau Island ship rats
- $n = 8$ Waiheke Island ship rats
- $n = 8$ Hunua ship rats
- $n = 6$ Arapaoa Island ship rats
- $n = 4$ Arapaoa Island kiore
- $n = 4$ Wellington Norway rats
- $n = 2$ negative controls

The $n = 48$ Wellington rats had already been GBS sequenced and used for a landscape genomics study (Veale et al. 2023). The results from the targeted SNP panel in this current study were compared to the results obtained from this previous work to confirm whether this SNP panel had the resolution to resolve fine-scale relatedness values, and/or be used for landscape genetics.

The $n = 8$ replicates of the Miramar rats were included to provide an assessment of the error rates of amplification. All laboratory and bioinformatic analyses were conducted separately on each of these replicates, and the level of identity between the replicates was assessed.

The Kawau Island ('Kawau' hereafter), Waiheke Island ('Waiheke' hereafter), and Hunua ship rats ($n = 8$ of each) were included as a real-world example of population assignment using limited samples and conducted on (probably) lower diversity populations. We conducted pairwise population assignment using Geneplots (McMillan & Fewster 2017). This method has been used for answering survivor/reinvader questions using microsatellite data for rats DOC and Auckland Council. Performing this analysis with a 'leave-one-out' assignment method meant that it was possible for us to assess whether the panel provided sufficient resolution to answer these questions. Attempting to perform this analysis with a small number of samples from each population demonstrates a similar scenario to that which regularly occurs on islands where limited samples are available.

The Arapaoa Island ship rats were included to assess another island from a different parent population (South Island Northern & Central). This was to determine the number of loci required to separate individuals, again in a more challenging population different to the other northern islands.

The Arapaoa kiore and Wellington Norway rats were included to provide baseline data for these two species because both species can sometimes be mistaken for ship rats in samples sent for sequencing. When dealing with trace samples such as scat, saliva, or hair this confusion would be even more likely. If the panel is used on such samples we would then have a way to identify these species rather than including them in downstream analyses.

4.5 Targeted panel SNP calling and outputs

We created a novel bioinformatic pipeline based on the GT-Seq pipeline using Perl, Python and R, and based on the pipeline of Campbell et al. (2015) (see <https://github.com/GTseq/GTseq-Pipeline>). This pipeline demultiplexes the samples (i.e. separates each individual), counts the reads of each allele for each locus, and outputs a variety of quality metrics as well as the genotypes for each individual. Details of the pipeline to call SNPs from the sequencing data are included as Appendix 4.

4.6 Species identification pipeline development

For both SNP panels a novel metric was created to determine which rat species a sample came from (for situations where a trace sample is accidentally included from a different species than the intended target species). This was based on the results that some markers in each panel amplify for each non-target, while others did not. Counting the reads for the markers that consistently do amplify and comparing this number to the reads that consistently did not provides a metric to identify species: details in Appendix 5.

4.7 SNP panel evaluation

The data obtained from the targeted SNP panel were evaluated in terms of their rates for missing data, rates for identity comparing the replicated samples, their power to identify if two samples were from the same individual, and the similarity in relatedness scores was compared between the Miramar rats and the new targeted panel, and for the Dart-Seq rats and a reduced panel (in silico), containing only the target SNPs. These relatedness values were calculated using vcftools (Danecek et al. 2011).

Population assignment was conducted both at a regional scale using the final panel loci subset of the original Dart-Seq data, and using the novel data generated on the new samples. These analyses were conducted using Geneplots (McMillan & Fewster 2017). This method has been used for answering survivor/reinvader questions using microsatellite data for rats DOC and Auckland Council. Performing this analysis with a 'leave-one-out' assignment method meant that it was possible for us to assess whether the panel provided sufficient resolution to answer these questions. Attempting to perform this analysis with a small number of samples from each population demonstrates a similar scenario to that which regularly occurs on islands where limited samples are available.

To assess the individual discriminatory power of our SNP panels, we calculated the probability of identity ($P(ID)$) and the probability of identity among siblings ($P(ID)_{sibs}$). These are the probabilities that two randomly selected individuals in the population will be identical across the markers in the panel, or that two full siblings would be identical. These are standard measures of the power of a panel to differentiate samples. Details in Appendix 6.

5 Results and discussion

Primers for 240 markers were amplified for the 96 individuals selected for evaluation (Section 4.4 – 4.7, Figure 4) to create amplicons. The median amplicon length was 86 base pairs (bp), with the maximum length 117 bp. In our experience, amplicons below c. 120 bp in length are required for degraded samples. These short amplicons should therefore be optimal for degraded samples such as hair, scat, or saliva. The average distance on the genome between markers is 8.7 mega bases (MB), and the median is 6.5 MB. Anything over 1 MB is inherited independently, therefore all markers can be considered independent for this panel.

Of the 240 markers we amplified, 175 passed all quality control (QC) and showed consistent amplification (details in Appendix 5). This includes the sex-linked marker which worked well across all the known-sex samples. This success rate of 73% passing QC was higher than the contingency planned for, meaning the desired number of markers were achieved. Neither of the negative controls had any genotypes called.

Eight Miramar individuals had their genotyping replicated in our laboratory to estimate the error rates for each locus. One of these individuals fully failed to amplify (very low quality and quantity of DNA), but the other seven amplified well. Of these, five individuals were fully identical between replicates. Two individuals had genotyping errors with different genotypes between the two replicates, but this included only a single difference for one individual, and four differences for the other (which had lower amplification than the others across all loci). This was a genotyping error rate for DNA extracted from tissue of 0.23%, which will easily be able to be accounted for in analyses. With the current pipeline there was 12% missing data across these individuals.

We evaluated each locus individually to see if there were any issues regarding biased amplification of one allele or the other, the heterozygosity rates, the rates of missing data, and the genotype accuracy compared to the original GBS data. Figure 4 and Figure 5 show output examples and analyses for each locus to demonstrate what the data looks like, and to show the pipeline outputs.

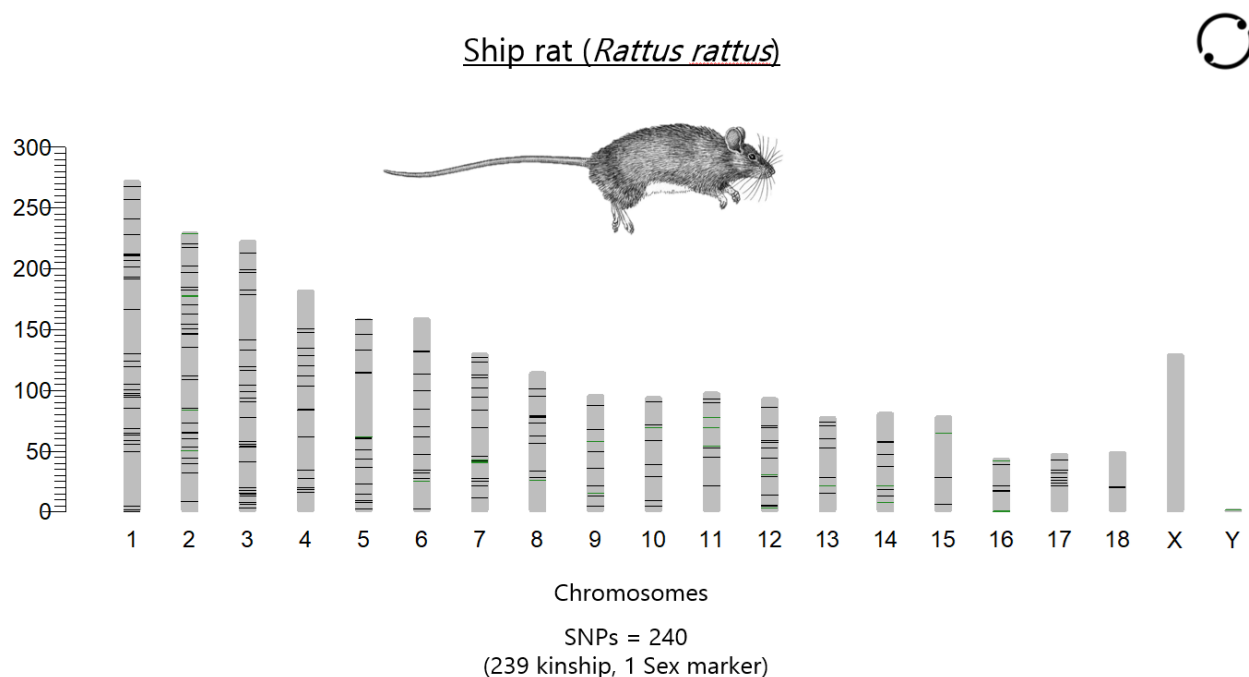


Figure 4. Locations (lines) on each of the chromosomes of the final SNP markers included in the ship rat panel. X-axis – chromosome number; y-axis – Chromosome length in MB

For each SNP locus, the locus identifier contains all the details required to describe it, and this can be extracted automatically by the analysis pipeline. For example, here is a locus with the identifier

Rr_Chr3_23285043_A-G

Rr = *Rattus rattus*

Chr# = chromosome #

= position on chromosome.

X-X = Allele 1 – Allele 2

For the above example, this is *Rattus rattus*, Chromosome 3, position 23285043 on this chromosome, Allele 1 = A, Allele 2 = G.

All loci were evaluated individually to determine the accuracy of calls and the patterns of amplification. All loci retained in the final panels looked like the locus allele frequency plot example below (Figure 5), with clear genotype scoring and high heterozygosity.

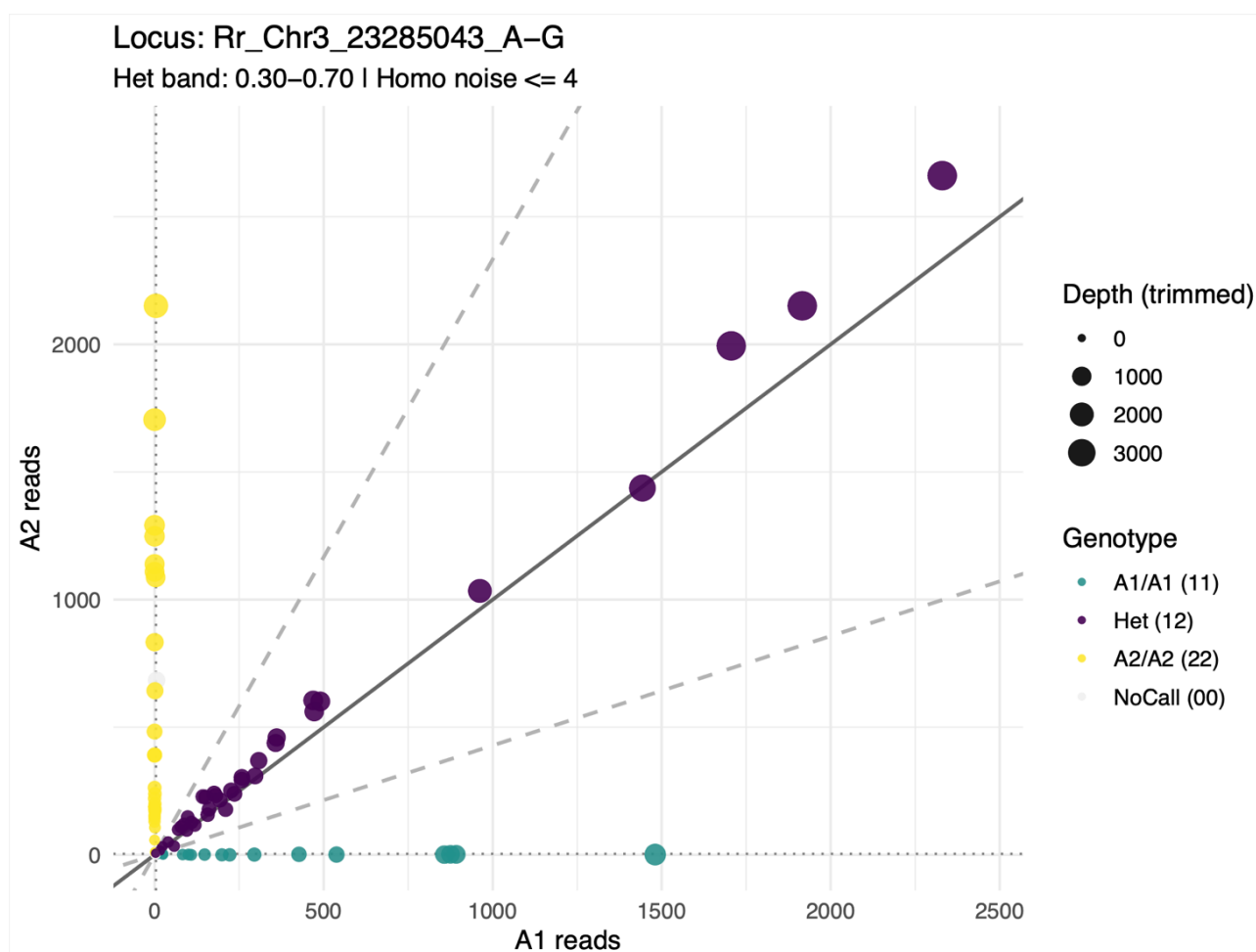


Figure 5. An example of the pipeline output showing the read depth (number of reads recorded) for each allele of the locus Rr_Chr3_23285043_A-G for each individual ship rat. Allele 1 is A, while Allele 2 is G at this position.

5.1 Relatedness

Ascertainment bias occurs when genetic markers are selected based on their characteristics (such as variability) in one population, but then used in a different population with different allele frequencies. This can often cause issues with analyses such as under or over estimating parameters like relatedness in this other population. The SNP panel designed in this study was explicitly designed to minimise ascertainment bias across the country, so that ideally analyses based on the data are consistent and comparable from any location.

The primary inputs for the fine-scale landscape genetics conducted on Miramar previously (Veale 2024) were a matrix of pairwise relatedness values. In this study, equivalent pairwise relatedness values were calculated within each ship rat population from the New Zealand-wide DART-Seq data, and for a reduced marker set containing only the loci retained in the final targeted SNP panel. These pairwise relatedness values matched closely between the full and reduced data sets (Figure 6) – and the relationships were similar among the populations. This indicates that there was minimal ascertainment bias towards one population over another in terms of resolution for calculating relatedness when using these markers.

Figure 6. Relatedness values between rats calculated within six ship rat populations the targeted SNP panel was designed for in this study using the complete filtered DART-Seq GBS data set (8,519 SNPs) compared to the reduced final GT-Seq panel (175 SNPs) of all rat pairs within each population.

We also calculated the pairwise relatedness values between matching pairs of individuals within the Miramar ship rat population using the data from (Veale et al. 2024). These relatedness values were also strongly correlated ($R^2 = 5.6$) but there was a greater scatter than in the larger regional data sets (Figure 7). However, note that for these calculations, some of the Miramar rats ($n = 48$) had higher levels of missing data (due to some now-identified laboratory issues) which may explain this slightly greater scatter.

Figure 7. Relatedness values between rats calculated within six ship rat populations the targeted SNP panel was designed for in this study using the complete filtered GBS data set for Miramar (15,110 SNPs) compared to the reduced final GT-Seq panel for Miramar (175 SNPs) for all rat pairs that were sequenced with both methods.

5.2 Heterozygosity and inbreeding

Individual average heterozygosity was high across all populations from which the panel was designed (Figure 8). It was also high in the deliberately chosen lower diversity populations assessed on islands (Figure 9). The Hunua population heterozygosity we measured was comparable to that for the North Island (Central & Southern, i.e. NI_CS,) which is the larger population that it is located in. The Miramar rats sequenced were at the lower end of the distribution for the North Island (NI_CS) population, which was also to be expected as this was a relatively isolated population sampled after considerable suppression efforts by Predator Free Wellington. The three island populations (Arapaoa, Kawau, and Waiheke) all had lower observed heterozygosity than their larger parent populations, but all still had average heterozygosity values over 0.2. The least heterozygous population (Arapaoa) can serve as an example of a low heterozygosity population for other calculations (most challenging scenario).

Figure 8. Individual average heterozygosity for SNP panel for each of the six ship rat populations the targeted SNP panel was designed for in this study. Populations included are NI_A = Aotea, NI_CS = North Island (Central & Southern), NI_N = North Island (Northern), SI_NC = South Island (Northern & Central), SI_R = Rakiura, SI_S = South Island (Southern).

Figure 9. Individual average heterozygosity for SNP panel for each of the six ship rat populations the targeted SNP panel was designed for in this study. Populations included are NI_Hu = Hunua, NI_KI = Kawau Is., NI_We = Miramar, NI_WI = Waiheke Is., SI_AP = Arapaoa Is.

The distribution of Inbreeding coefficients (F) we calculated using the SNP panel were comparable across ship rat populations (Figure 10). We note that the Aotea population included a number of samples from the now eradicated Rakitu Island ship rat population, which was particularly inbred, giving the bimodal distribution for this population.

Figure 10. Violin and raincloud plots of the inbreeding coefficient (F) measured in each ship rat population using the SNP panel. Populations include Australia, NI_A = Aotea (i.e. Great Barrier (Aotea Island), NI_cs = North Island (Central & Southern), NI_n = North Island (Northern), SI-cs- South Island (Northern & Central), SI_R = Rakiura, SI_s = South Island (Southern)

5.3 Population assignment

While population assignment was not the primary design aim of the SNP panel, it also worked well across the populations sampled. Using the subset data from the DART-Seq GBS (including only the markers retained in the final SNP panel), population assignment was highly accurate across most pairwise comparisons. For each of these plots the distance from the diagonal line represents the assignment probability from GeneClass2 (Piry et al. 2004), with the adjacent parallel lines in being 95% confidence in assignment (see Figure 11). In only one of these comparisons were any individuals ‘misassigned’, with three South Island (southern) rats assigned to the South Island (Northern & Central) population, and one South Island (Northern & Central) assigned to the South Island (Southern) population (Figure 11 D). These ‘misassigned’ individuals were all sourced from locations near the known transition zone between these populations. As discussed previously, these populations are not fully separate, and there is mixing in these zones, so having ‘misassigned’ individuals is unsurprising. Similar patterns will likely be observed for rats sourced from around the transition zone around Auckland between the North Island (Northern) population, and the North Island (Central & Southern) population.

The real-world SNP data approximating an incursion or a survivor/reinvade management scenario showed that population assignment is also possible with fewer samples and with more missing data. In all cases all individuals were correctly assigned (using a take-one-out procedure). This included comparing just 7 Kawau Island rats with 8 Waiheke Island rats, or comparing either of these islands with 6 mainland rats from the Hunua Ranges (Figure 12). That is a real-world scenario equivalent to when a rat appears on an island and DOC wants to determine where it came from with limited samples available from each possible source population.

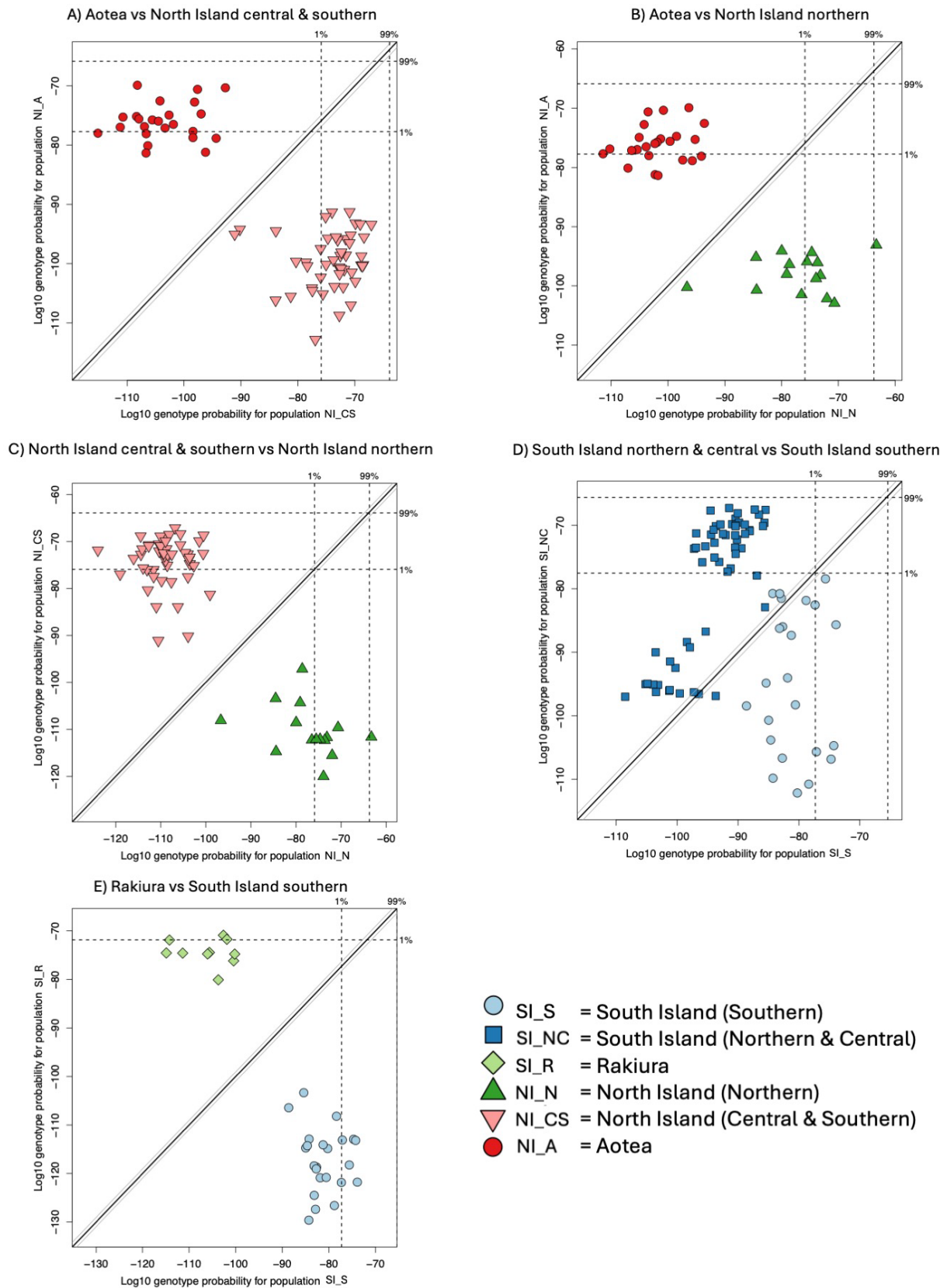


Figure 11. Geneplot pairwise assignment plots showing the probability of assignment for each individual to each of the six ship rat populations the targeted SNP panel was designed for in this study using a take-one-out procedure. Individuals above the diagonal line are assigned to the population on the y-axis while individuals on below the diagonal line are assigned to the population on the x-axis. Only one comparison had some misassigned individuals (between the Southern South Island [SI-S]) and the rest of the South Island), and there was population mixing at the border between populations.

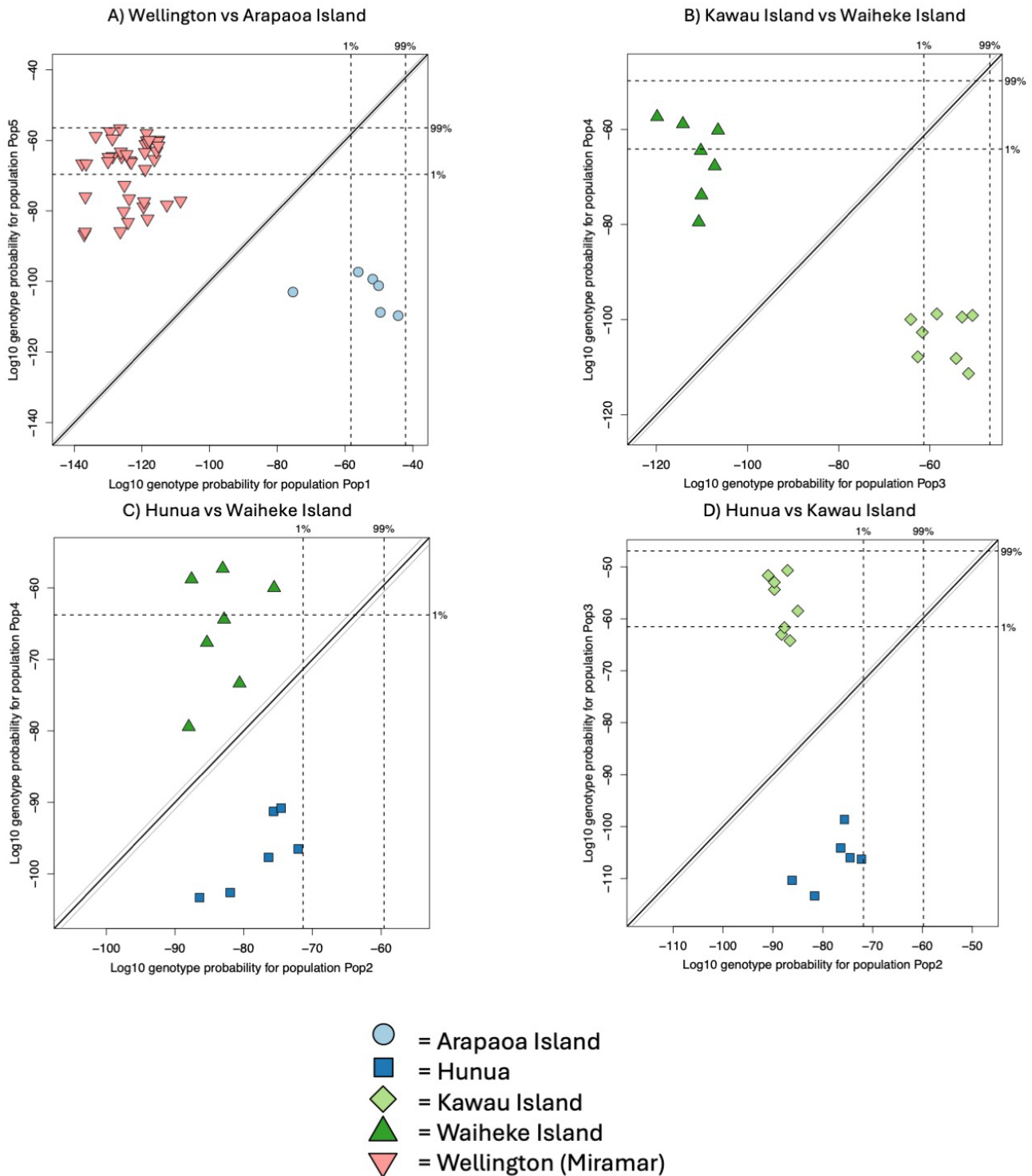


Figure 12. Geneplot pairwise assignment plots showing the probability of assignment for each individual to each population listed in the key above using a take-one-out procedure. Individuals above the diagonal line are assigned to the population on the y axis while individuals below the diagonal line are assigned to the population on the x axis.

5.4 Probability of identity

One of the primary metrics for the usefulness of the targeted SNP panels is the probability of identity $P(ID)$ and the probability of identity for full siblings $P(ID)sibs$. Our panel had effectively identical metrics for these statistics across all populations examined, even in the less variable rat populations on islands. Across all populations tested, fewer than 1% of full siblings will be identical at 9 loci from either of these panels (Figures 13 & 14). The graph shows the statistics for the least variable ship rat population measured for this panel so far (Arapaoa). Fewer than 0.1% of random individuals from the New Zealand population will be identical at 10 loci, and under 0.01% of random individuals will be identical at 15 loci. Fewer than 0.01% of full siblings will be identical at 27 loci. Therefore, if at least 27 markers amplify well for a sample, this sample will be able to be reliably matched with another sample that amplifies the same markers. Twenty-seven markers is less than 16% of each of the 175 SNPs included in our final panel, so there is a considerable level of contingency for identifying samples using this panel.

Figure 13. The cumulative probability of identity [$P(ID)$], and the cumulative probability of identity for siblings [$P(ID)sib$] for ship rats on Arapaoa Island amplified with the SNP panel.

Figure 14. The cumulative probability of identity [P(ID)], and the cumulative probability of identity for siblings [P(ID)sib] on Arapaoa Island amplified with the SNP panel on log scale showing the number of loci to reach specific thresholds.

5.5 Species identification

We sequenced the four Norway rats (from Wellington) and four kiore samples (from Arapaoa Island) for a 556 bp fragment of the mitochondrial control region to confirm species identity. These sequences were identical to the GenBank sequences available for each of these species (GenBank Accession: KY814721.1 for kiore; MH751483 for Norway rat).

Of the $n = 175$ loci retained in the final panel, $n = 62$ did not produce any genotypes for any kiore sequenced, and $n = 89$ did not produce any genotypes for the Norway rats sequenced (given the SNP-calling parameters used). These marker drop-outs could be because the primers did not amplify these loci for these species. Alternatively, they could be because the probes (in silico) did not exactly match the sequences amplified for these species. All loci that did amplify for both species had no variation detected for these SNPs in these other species. The allele that did amplify for each of these species is listed in Table A5.1 (Appendix 5).

Using these differences in marker amplification it was possible to assign individuals to the correct species with ratios of the number of reads for those markers that are likely to amplify well for a given species, versus those that are unlikely to amplify for that species. This species classification was automated as part of the pipeline so that if, in future, any samples are accidentally included from either of these species, the pipeline will automatically highlight them as a different species.

6 General discussion – lessons and adjustments

All measures so far indicate that the targeted SNP panel for ship rats in New Zealand that we designed and tested will be useful for answering most management-relevant questions. This more general discussion section will now briefly discuss some lessons and adjustments that came up during the research. In the next section I will outline the questions it can address and the strengths and weaknesses of the panel.

The data showed that there were issues with the indexes on some of the primers, meaning that some samples failed to amplify. This occurred in rows of 8 and this pattern is easily identifiable. This will be resolved for future laboratory work when using this panel. Fortunately, this just lowered the sample size of the Wellington rats that amplified, and doesn't affect the overall conclusions.

We deliberately did not undertake any sample or amplicon normalisation steps, so as to understand the importance of these steps, and to provide a range of quality values for the samples. This did result in quite a large range of read depths per sample, with some amplifying poorly by comparison. For any future use of this panel, it is strongly recommended that sample standardisation is applied to ensure that any strongly amplifying samples do not dominate the sequencing. Again, this did not affect the evaluations of the markers to date, and in the next batch of sequencing with the reduced marker set this standardisation will be conducted.

Amplification rates are likely to be higher for future runs of this panel (assuming other inputs remain constant) in that the number of primers will be reduced to those that worked well, so there will be less extraneous DNA amplified and sequenced. Also, loci that amplified particularly well (such as the Y-chromosome marker) will have their concentrations reduced, while those that amplified comparatively poorly will be increased. It is an iterative process to get the right concentrations. Despite being a non-optimised run, all metrics so far were very promising for all questions thus far.

7 Conclusions and applications

The RapidRat panel had insufficient power to address questions of rat relatedness and individual identification in NZ due to low marker variability.

Genetic markers from a new NZ-wide study of ship rats indicated there are six different generalised populations, which each have different allele frequencies. We selected 240 novel markers from this data set, with primers designed for a GT-Seq style panel to amplify these. Some 175 of these met all QC metrics and were retained for a final panel.

Our targeted SNP panel (Rara_RASP)³ was optimised for individual identification and relatedness calculations of ship rats from across NZ. All analyses so far indicate that it has reasonably even resolution across the country and can be used to answer management-relevant questions.

³ Rara_RASP stands for '*Rattus rattus* Aotearoa SNP Panel'.

7.1 Applications

Sections 7.1.1–7.1.6 cover the potential management questions that could be relevant for the use of this panel, and an assessment of whether it is appropriate for each of them.

7.1.1 Sex identification

Our panel functions well to identify the sex of individuals genetically. This worked for all known-sex samples included, for all three rat species. This primer could also be used independently for a sex determination test. This locus amplified considerably more than most other loci (when present) so will have to be decreased in concentration for future versions of the panel.

7.1.2 Species identification

Our panel will identify any of the other *Rattus* species known to be in NZ. The pipeline has considerable contingency built in so that even if there is some extra variance in the amplification ratios it should be robust to these. If any new *Rattus* species arrive, they will look unusual but will not be specifically identified using this panel. There are other cheaper methods to do genetic species identification (e.g. Veale et al. in prep), but if any samples from additional species are amplified with this primer set by mistake the pipeline will identify this as an output.

7.1.3 Individual identification from trace samples

Our panel was designed to work on trace samples by only allowing amplicons below 120 base pairs in length. We have not yet amplified trace samples with our panel, and therefore the error rates and missingness rates have yet to be assessed. Both rates could be substantially higher than was observed for the tissue samples but will still yield strong results for these calculations. Further work will be required to ascertain error rates and finalise this application. Other similarly designed panels are known to work on trace samples (Sjodin et al. 2020) and there is considerable contingency even in inbred island populations, providing confidence that our new panel will work on trace samples.

7.1.4 Population assignment

Our panel shows considerable power for assigning samples to populations. Using only small number of samples from Kawai, Waiheke, and the Auckland mainland (Hunua) we were able to assign all samples to their population of origin. These results were similar (or stronger) than similar tests using microsatellites (e.g. Russell et al. 2010; Fewster 2017). The number of SNP markers included in this panel greatly exceeds the recommended number required for population assignment for natural populations (Morin et al. 2009). It is possible that some populations will not be separated using this panel, but our early results are very promising. In addition, because the panel was designed to work across all of NZ there should not be a regional bias in results.

7.1.5 Pairwise relatedness

Across all populations analysed there was a strong correlation between relatedness values assessed with thousands of markers using GBS and the much smaller targeted SNP data set. In average populations it should be possible to determine basic categories of relatedness using the SNP panel (close relatives, vs distant relatives, vs unrelated individuals). It remains possible that such measurements in particularly inbred populations may be challenging, but that will be the case for any genetic marker. All pairwise relatedness measures need to be calculated within a particular

reference population, so having enough samples from the region is essential to answer this question.

In a recent study on parentage analyses using SNPs (Flanagan & Jones 2018), the authors state: 'Virtually all studies have concluded that a relatively small number of SNP markers, from 60 to 200, usually provides resolving power equal to or better than that provided by the available microsatellite markers for the species under consideration. The exact number of SNPs required will depend on a number of factors, including the minor allele frequencies of the SNPs, linkage disequilibrium among SNPs, the frequency of null alleles and genotyping errors, the number of parental pairs, the distribution of offspring numbers per family and the mating design.' For this novel ship rat SNP panel, the minor allele frequencies were specifically optimised for high resolution, and linkage disequilibrium was accounted for by selecting markers with sufficient spacing on the genome. This makes it highly likely that in a well-designed study parentage should be able to be accurately assessed with this panel.

For simplicity the pipeline for calling alleles was based on the older GT-Seq pipeline that just uses primer and probe sequences to find a single SNP per locus, but this project also trialled the newer one available (see https://github.com/GTseq/gtseq_microhap) which finds further microhaplotypes. Many loci in the panel did contain this extra information. If very fine-scale relatedness is required for a management question then this SNP calling pipeline is also an option to add further resolution.

7.1.6 Landscape genomics

Broadscale landscape genomics like population assignment so far appear to work well as discussed above (section 7.14). Applying the panel to clarify fine-scale landscape genomics based on pairwise relatedness values appears promising given the comparison with the previous Miramar ship rat GBS data. It should be possible to conduct a similar study (Veale et al., 2024) using this new panel, and it is likely that new immigrants into an area can be identified in such a study. This will need further data sets under a range of conditions to verify the strength of this method. For such a study having as high a proportion of animals in the population sampled, and having broad geographic coverage will always be critical for any landscape genomics study.

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Appendix 1 – SNP filtering and primer design

SNP loci were filtered using VCFtools (Danecek et al., 2011) using the following parameters: maxminDP = 40, maxmissing = 0.8, HWE = 0.001, maf = 0.35, minDP = 5. These parameters mean that loci with unusually high coverage (which may therefore be misassembled repetitive regions) are removed (maxminDP), at least two individuals must have the same SNP (mac), at least 80% of individuals must have data at the locus (maxmissing), the locus must be in Hardy-Weinberg equilibrium (again to avoid repetitive regions and regions under selection), each individual requires at least 5 reads to have that locus called (minDP), and the minor allele frequency must be at least 35%, ensuring maximum information content per marker.

Loci on the X-chromosome were removed because these are always hemizygous in males and thus would require extra analytical steps to interpret.

For each species I selected 400 loci from each filtered data set, ensuring that all markers were separated by at least 0.5 MB (to ensure that they were distributed across the genome and so that each marker provides independent information). We then produced VCFs for each data set and used a custom Python script to extract a 200bp flanking sequence surrounding each of the SNPs from each respective genome assembly.

From the flanking sequences we then used the program FastPCR (Kalendar et al. 2017)⁵ to find compatible primer pairs for a multiplex PCR using all these flanking sequences as inputs. Given the large number of sequences, it was likely that not all loci could have compatible primers generated for a single PCR, so an excess of sequences was used. Once these compatible marker sets were determined, these primers were modified by adding Illumina sequencing primer tags, specifically the 'small RNA sequencing primer' and 'read 2 sequencing primer' for use in PCR1. 240 markers were chosen for laboratory testing. These primers were ordered from IDT (www.idtdna.com) in plate format.

Appendix 2 – PCR1 primer details

These are the PCR1 primer details for the GT-Seq library. Rr = *Rattus rattus*, Ch = Chromosome, ##### = position on chromosome, F or R = Forward or Reverse.

PrimerID	Complete
Rr_Ch_1_454369_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTCCCAGAATGCCTCCTTTCT
Rr_Ch_1_454369_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTACAGGGTTGCTGAGGTCT
Rr_Ch_1_1983692_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAAAGATCTGGGTGTAGGGGT
Rr_Ch_1_1983692_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGTACCATCAAGTCCTTCCA
Rr_Ch_1_4427464_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACATCCACATCTAAGGCCCAT
Rr_Ch_1_4427464_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCGTTTAATGGGAGCAGTCTC
Rr_Ch_1_41189327_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCCTTATCTGGGCTTGTACTGT
Rr_Ch_1_41189327_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCTCTTCTAAACCTCCAGCAA
Rr_Ch_1_49755867_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGCTCCGGTATCGGTACTAAA
Rr_Ch_1_49755867_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTGATTGACAAGACTCTGTCTGC
Rr_Ch_1_55944582_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCTTCCTGTCTTACCTGGT
Rr_Ch_1_55944582_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAACATGGAGGAAGCTAAGTAGC
Rr_Ch_1_58646718_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTGAGCAAGCTGGAGAAGC
Rr_Ch_1_58646718_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGATCACAAGCCTTAGCAGGA
Rr_Ch_1_63561669_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCTTGTGTTCTGAAATCACTGCA
Rr_Ch_1_63561669_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTCTATGAACTCTCTTTCAGCA
Rr_Ch_1_64937336_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCCATCATCAGAAAAGCTTCCTC
Rr_Ch_1_64937336_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGAGTGTTCAGGTTTCTCACT
Rr_Ch_1_68798228_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTGCAGGATTAATCCATGGAGC
Rr_Ch_1_68798228_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCATGGACACACATGTCAC
Rr_Ch_1_85127962_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGAGTCTTTTGGTATGCTGAGC
Rr_Ch_1_85127962_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCTTCTCAGGACACTAAAACC
Rr_Ch_1_90215858_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACAGGATCAGGTTGTCCACTC

PrimerID	Complete
Rr_Ch_1_90215858_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGGTAAGTTTACCCAGATGTGC
Rr_Ch_1_94380113_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTTTACAGCCATGAGGGATGT
Rr_Ch_1_94380113_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTCAAGAGTTTAAATGTGAACAGC
Rr_Ch_1_96123523_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTTATCTGACCAAGGACCCCT
Rr_Ch_1_96123523_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAGGACATTTTCTGGTTGGTT
Rr_Ch_1_97706965_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATGTCTGCAGGAACTCAGCA
Rr_Ch_1_97706965_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCACAGCTATCTAACTACAGTGT
Rr_Ch_1_100626118_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGACAGTACTTGCGAACTGCAA
Rr_Ch_1_100626118_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGAAAGACTCGCTACTTAAACTC
Rr_Ch_1_104789417_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGAGACTTCTAAGTCCCTCAGTGT
Rr_Ch_1_104789417_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCCAGTCACAAAATGGAGAGTT
Rr_Ch_1_119469489_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCACAGGGGAAATGGAGACTAGAC
Rr_Ch_1_119469489_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCCCATTTCATGCAGTTCTCA
Rr_Ch_1_124172715_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGATGGGAAAACAGCTGCTAACA
Rr_Ch_1_124172715_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATGCTGGTTTCCAAGAATCTGT
Rr_Ch_1_130233726_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACAACAAAGCAGTCCAATGTTCT
Rr_Ch_1_130233726_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGCATGCATTTGAGATGTTCAA
Rr_Ch_1_148919649_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCAGCAATATGCCAAAACGTGC
Rr_Ch_1_148919649_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTATGCATCCGTTCCATGGAGAA
Rr_Ch_1_166414097_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCTCTACCACTGAGCTAAGTCC
Rr_Ch_1_166414097_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAAGTCCGAGTCTAGCACAC
Rr_Ch_1_193044213_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCACTGGGTCTGCAGTAAGTCA
Rr_Ch_1_193044213_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAGGAGGTGGGTGATGTAAT
Rr_Ch_1_201884788_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTGTTGTGAGGTAGGAGCCAA
Rr_Ch_1_201884788_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCAGATGTCCCTTTTCTGA
Rr_Ch_1_207159406_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCACACTTGATGATTACTGTTGC
Rr_Ch_1_207159406_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGAACATACACATGACTGTCATAGA
Rr_Ch_1_211038962_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTCTGCAGGTTCTCAGACCA

PrimerID	Complete
Rr_Ch_1_211038962_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCAACGAGGCTGACATTGAA
Rr_Ch_1_212059018_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAAAGGAATTGGTAGCAAGCCA
Rr_Ch_1_212059018_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAGTCTGGCTGGGATATTATTGT
Rr_Ch_1_227953352_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTCAAGATAAACCCGTGTTTCCT
Rr_Ch_1_227953352_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCAACAGAGACCTTTTGTCAAA
Rr_Ch_1_240918269_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTGCAGAGGACACTTACCCTA
Rr_Ch_1_240918269_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTTTGAGTGCTTTAGAGGGAGAA
Rr_Ch_1_249540742_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCTCATCCTTTCCAGAAGACTC
Rr_Ch_1_249540742_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAACACTGCTGAACCTTGAGCA
Rr_Ch_1_267539751_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATGTGCTGCAGGTGTGAAC
Rr_Ch_1_267539751_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGACAGCAAAGGTTTTTCTGGT
Rr_Ch_2_8571143_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCTGTGGAACCCCTTGTTAA
Rr_Ch_2_8571143_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAGCAGCTGAGGAAAGT
Rr_Ch_2_39735328_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAAGAGTAAGCGGAGGGAAACA
Rr_Ch_2_39735328_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAATCATTCCACTGGCGGTCA
Rr_Ch_2_43975779_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGATCATCGCTCTGAACACGA
Rr_Ch_2_43975779_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTAGAGAAATGTGGAGGAACTGC
Rr_Ch_2_50501597_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGGTGGTACCAAAATGTCTGC
Rr_Ch_2_50501597_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTCTTAGTCATTGTGTTAGCATCT
Rr_Ch_2_53167838_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCAGATTTCTTTCTCCTAGCGTC
Rr_Ch_2_53167838_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTTCTTGTCTCTGAGGGCA
Rr_Ch_2_60047239_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGAGTGTGTATCTGCCTAGC
Rr_Ch_2_60047239_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCAACCATGTGAGACTAAGC
Rr_Ch_2_65195009_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCATTTAGATGGCATGGCCAAT
Rr_Ch_2_65195009_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTCTTGCCCTAATCCATAGC
Rr_Ch_2_72937716_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGAACACAGAGGACAATCAGC
Rr_Ch_2_72937716_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTTACCAGTTGGTCCATGA
Rr_Ch_2_83555439_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTAACCGACAAGAACTCCCCTT

PrimerID	Complete
Rr_Ch_2_83555439_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGATTAGCGCCCTACCTTTCA
Rr_Ch_2_97953956_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCCTCTCCTATAGTTCTTTGTCC
Rr_Ch_2_97953956_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAGATGGAGCTCTTGAGGTA
Rr_Ch_2_109021025_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTTCTGCAGCCTGGATCTGAA
Rr_Ch_2_109021025_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTTAGACAGTTCCTTTGAGGTC
Rr_Ch_2_111637504_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTCTTGAGGGCCTTACCTTGT
Rr_Ch_2_111637504_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGGCTCTGAGGACATTCAAAT
Rr_Ch_2_135677418_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTAACGCTTAAACACTGCAGCT
Rr_Ch_2_135677418_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTTGCTCAAAGCATGCCTTT
Rr_Ch_2_145705956_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTTTAGCGGTCACTTGACCAC
Rr_Ch_2_145705956_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAAGCTACTGATACCACAGGGA
Rr_Ch_2_146798458_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTCAATTCATCTTGAGCTGGCT
Rr_Ch_2_146798458_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGTGAGCTTTGGGACAGGA
Rr_Ch_2_150865520_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGTTTCTTATCCCCAGCTCT
Rr_Ch_2_150865520_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAACTGAGAGCCCCAGAGA
Rr_Ch_2_154459517_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTGGTCCAGCACCTTTCAT
Rr_Ch_2_154459517_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTATGGAACGTACTTACCCGGAA
Rr_Ch_2_160973837_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATAAGGAAGGGGTCACTGTCC
Rr_Ch_2_160973837_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTAGGGCTTCTTCTGACTCC
Rr_Ch_2_162540831_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTGCTCATCATTTCCGGGAGC
Rr_Ch_2_162540831_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGTGTCTCTAAAGTACCTTCTC
Rr_Ch_2_170143844_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTTAGAACCCAGCTTCCAGGA
Rr_Ch_2_170143844_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCTACACATGCTTCTCATCCT
Rr_Ch_2_182591444_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGCTTATGCAACTCTGAGCTCT
Rr_Ch_2_182591444_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCTTATGACCCAGGATTTTGT
Rr_Ch_2_184831413_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACTGCCTGCAGCAGACATTAA
Rr_Ch_2_184831413_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTTTTCCTTGCCGAGCCATT
Rr_Ch_2_196945467_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCACCTAACTGGAGATGCTGA

PrimerID	Complete
Rr_Ch_2_196945467_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTATTTCTGGTGCACCCTCCTTT
Rr_Ch_2_202589002_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCACCCTGAAGGGCTGTAGATT
Rr_Ch_2_202589002_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGGTATCCCAAGCATGTCCA
Rr_Ch_2_212905971_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTCTGCAGTATTTGGTGTCTCT
Rr_Ch_2_212905971_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGGGAAAGATGTGACAGAAGT
Rr_Ch_2_217381408_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCATCTATGTCTGCAGTCTTGCT
Rr_Ch_2_217381408_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCGTGTAAACAAAATGTGCCATCA
Rr_Ch_2_220745396_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTCTCCCCAGAGCTGAAGTAA
Rr_Ch_2_220745396_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCTGTAGAATAATATGCCTCCA
Rr_Ch_2_227682084_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTTACCTCTGAACGTTGCA
Rr_Ch_2_227682084_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTATCTCAGCAGCCTTCATGTCC
Rr_Ch_2_229034355_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGAGGCTAATCTTTCTGATGGAC
Rr_Ch_2_229034355_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTATGCATAGGGGCAAAGTAGTCT
Rr_Ch_3_3398644_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCTGCAGATCACTGCATCCTA
Rr_Ch_3_3398644_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAGTTGGCATTCTCAAAAGACA
Rr_Ch_3_5970996_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGACAGATGGAGCCACATGAA
Rr_Ch_3_5970996_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCAGACATATCTTGGGTGGAT
Rr_Ch_3_7796605_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCATCAGGCGTGCATGTAATA
Rr_Ch_3_7796605_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCACTGTGTGATACTCCAAGAT
Rr_Ch_3_12930630_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTTTCGCCTTTAATAAACCCAGCA
Rr_Ch_3_12930630_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGTGCAGGTGGGACTAGAAA
Rr_Ch_3_19931350_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCCTAAAGGGTTTCATTTCATTCC
Rr_Ch_3_19931350_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTGAAGGATTTCCCTCATGT
Rr_Ch_3_23285043_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTACTCCTGCAGTAGTTCCCT
Rr_Ch_3_23285043_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCACTCCACTACTGAGCAA
Rr_Ch_3_41215428_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCACCTTTGGTAAAGCACAT
Rr_Ch_3_41215428_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACATATTCTGGGTTTGAAAGCATTT
Rr_Ch_3_53440504_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAACACATCCTGCAGTAGCACA

PrimerID	Complete
Rr_Ch_3_53440504_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTACTGTGCATGCTCCCCAAA
Rr_Ch_3_54465081_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGACCAATCTGAGGTGACTGT
Rr_Ch_3_54465081_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAATTTAGAGTGCTTGGCAACACA
Rr_Ch_3_55735166_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTTCAATACGAGTTTGTCTGCA
Rr_Ch_3_55735166_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAACCTCTGGATCTGTTCTGTT
Rr_Ch_3_58172475_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCATGGTTAGACTGAGTCTGCA
Rr_Ch_3_58172475_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTCTTTCAGACCACCTAGGTT
Rr_Ch_3_78045204_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGCTCTTTTCATGCCCTCCTA
Rr_Ch_3_78045204_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGACCCCTAATATTGGTAGCAGA
Rr_Ch_3_90506797_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTGCATCCAGGACATACCACA
Rr_Ch_3_90506797_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGACTAGCAAAGGGCAAATCTC
Rr_Ch_3_93422569_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGGAATCCAGGTTGAAGAGGT
Rr_Ch_3_93422569_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTTGGCTAATCTCACACAGTCT
Rr_Ch_3_98614586_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGGTCTCCAGAGGGAATCAGA
Rr_Ch_3_98614586_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTCACTCTTTAGTCTCTGA
Rr_Ch_3_104367713_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGGCTTATTTTGGTTACTGGTCA
Rr_Ch_3_104367713_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGATACCTCATGTGAACAATGGA
Rr_Ch_3_116324132_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGCAATTACCAACTGCAGTAACA
Rr_Ch_3_116324132_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAATCAGGGCCTGCTGTTTACA
Rr_Ch_3_119352954_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAAATTCTGTAATGCTTGGTAATGGA
Rr_Ch_3_119352954_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTCAAACAGCTCTGAATA
Rr_Ch_3_132927847_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCTAAGTTACAGCCTCAAATCGT
Rr_Ch_3_132927847_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAAGCAAATTTTCATCTTCTGTCTTT
Rr_Ch_3_137668896_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAACTGGGAACATTCATCAACCT
Rr_Ch_3_137668896_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGCCTTTTCATGCCTGTTTAC
Rr_Ch_3_141508165_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACCAGGGTAGAGGCATTTTAGTC
Rr_Ch_3_141508165_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGAGTTCCAATGAGAAGAGGT
Rr_Ch_3_174058792_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGAGAGTGATTGGTCTTAAACA

PrimerID	Complete
Rr_Ch_3_174058792_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCTGGCACTGTTTGCACAC
Rr_Ch_3_179060364_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTTTACCACAGGTCTTTGGACT
Rr_Ch_3_179060364_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAAGCAATCTTACATGACATTTTGC
Rr_Ch_3_182742805_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTGCAGAGCTGATGTACCTA
Rr_Ch_3_182742805_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTCAGGGATGCATGCTTTCA
Rr_Ch_3_196978091_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGAAGCCAACAGGAAGTAGCT
Rr_Ch_3_196978091_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTATACCTGAATTCTGTGGAGAAGC
Rr_Ch_3_199563048_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCAGCAGGAAAAGACGCTCTC
Rr_Ch_3_199563048_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTTTGCAGGAGTCCGTTCTC
Rr_Ch_4_18733340_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTCTGGCCAGGCTGATAATGA
Rr_Ch_4_18733340_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGAATGCTTGTGAGCACAGC
Rr_Ch_4_19887402_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGACCACAACCTAGCTTCTGC
Rr_Ch_4_19887402_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGGCAGTACAGAGTGTGCA
Rr_Ch_4_27244494_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACTTGATGCTCATTGAGCTGC
Rr_Ch_4_27244494_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTATTCCCTGATGCCCAAACAGT
Rr_Ch_4_34490490_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCAATTGATAAGAACGAGGAGCCA
Rr_Ch_4_34490490_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTCAACCACTGCCTTTATAGC
Rr_Ch_4_61472646_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCAGCATGTTCCACAATCTGT
Rr_Ch_4_61472646_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTTGAGAGGACATGTGGTGTT
Rr_Ch_4_83741628_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTTTCTGGTCTCACCCGATGA
Rr_Ch_4_83741628_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAAGGAGAGGAGTGTGGTCC
Rr_Ch_4_103514149_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGGGTGAGTAACTGAGCGTTA
Rr_Ch_4_103514149_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCATGAGATGGTCTGGAGTTACT
Rr_Ch_4_112093358_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTGCTCTTCTGCAGGCTTAATT
Rr_Ch_4_112093358_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAATGTGCCTGAACGATACGGT
Rr_Ch_4_128388100_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGACTTTAAGTTACTGAGGCCAGA
Rr_Ch_4_128388100_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGCTAGAATAAATCCCTCACCT
Rr_Ch_4_134526370_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCACATGTAGTCAACCCAAGC

PrimerID	Complete
Rr_Ch_4_134526370_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAATTGCTTCTAGAAATGGGAGCT
Rr_Ch_4_136250768_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGAGCTAAACTCTCCGATCAGT
Rr_Ch_4_136250768_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAAAATCCACTCACCATAAAGC
Rr_Ch_4_147669350_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGTTTTTCATTGCTGACTTGCA
Rr_Ch_4_147669350_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTATGTGCATGCGACAACATTCC
Rr_Ch_4_150846801_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCTCACTCATCCTTTCACATATGCT
Rr_Ch_4_150846801_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAGGTATGAATGGCTAGGAAGT
Rr_Ch_4_152715179_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGCCTCTGATTTGCAGGGTTT
Rr_Ch_4_152715179_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTATTCAAGGTCTGTCCCTGACTC
Rr_Ch_4_178477453_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTCACAGAGTCCTGCAGATCA
Rr_Ch_4_178477453_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTTCTGTGTCTGCATGCTCA
Rr_Ch_5_7706649_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCCATATGGAGCTGTCAACCT
Rr_Ch_5_7706649_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGGTATATGGGCGACTTTCTGTT
Rr_Ch_5_9641003_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCTTCTGCAGTGAAGAACTCA
Rr_Ch_5_9641003_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCTGAGATCTAGACACATCAGA
Rr_Ch_5_14492440_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATAAACATTTGCCTGCAGCTCT
Rr_Ch_5_14492440_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCAAGATGGCAGTGTTATCCCTT
Rr_Ch_5_23029472_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGAAGCACAGGTGACTTGCA
Rr_Ch_5_23029472_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCTGTAGACGGTCCAGGTAA
Rr_Ch_5_36892468_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGATCTTAGGGCAAATGAACCAGT
Rr_Ch_5_36892468_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTACACTTCTGCTCTTAACCAC
Rr_Ch_5_43676760_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTAGATCACTGCAGGCTTTGGA
Rr_Ch_5_43676760_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGCATGCTTAAGGGAAAACTGA
Rr_Ch_5_48228583_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCATAAAGATGCTTACGGACTGCA
Rr_Ch_5_48228583_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCCATTAACCATCCCCTGTAAA
Rr_Ch_5_51430819_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCAGTCTCCCTGTGAGTCTTT
Rr_Ch_5_51430819_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAAGAAATTACCCCCAGGGAA
Rr_Ch_5_60660123_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGGGTGCAGAATTGTTGGACT

PrimerID	Complete
Rr_Ch_5_60660123_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACAGAGGTATTGTTCACTGGCA
Rr_Ch_5_61891445_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCTAAGAACTACACTTCCCAGC
Rr_Ch_5_61891445_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCGGTTTCCATTGATCAGTT
Rr_Ch_5_63976278_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCATCTGCAGAAACCAAACCA
Rr_Ch_5_63976278_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCCTCTCAGGAGACAGCTAT
Rr_Ch_5_71229155_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAAGGCTTTACGTCTCCCTTCC
Rr_Ch_5_71229155_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGAGCAATCTGTATGGTCATTT
Rr_Ch_5_80983281_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATGATGAGAGCACCGGTTTCT
Rr_Ch_5_80983281_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCATGTGAGTGCTAAGAATTGA
Rr_Ch_5_95651073_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCTATGGTTTTCAACAGAGCACA
Rr_Ch_5_95651073_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGGTAGAACACGTGAGAGATCA
Rr_Ch_5_114599777_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCCTACCAGAATTCATCTGACAC
Rr_Ch_5_114599777_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAATGTCTCAGGGGCTCTAGAT
Rr_Ch_5_132932432_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGGTTTTTCAGGCCCATCTCAA
Rr_Ch_5_132932432_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTGCATGCTGAGATGACAGGT
Rr_Ch_5_146167601_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTCTGAGCTTCTTATTCTGTGCA
Rr_Ch_5_146167601_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTAGGGACGCTTTGCTACGAT
Rr_Ch_5_151533106_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCAGAGAGAAGGCTTCTGCCA
Rr_Ch_5_151533106_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGATCCAAGAGGAACACAGC
Rr_Ch_5_158352255_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCAGCCACTAGAGTCTTACTGT
Rr_Ch_5_158352255_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGACATGGCTGCATAGGACTA
Rr_Ch_6_2694267_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTAGCAAGAGATCGATGCCTGC
Rr_Ch_6_2694267_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGAGAGGATTGGAACACTGCT
Rr_Ch_6_23324793_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATGAGCAGTCTCAAGGGTAGC
Rr_Ch_6_23324793_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGAGAAATCTGTGATCAGGAGT
Rr_Ch_6_25105159_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCTCTAGTTATTTCTCCTCTGTTCA
Rr_Ch_6_25105159_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTATTGTCCCTGGTACGATGGA
Rr_Ch_6_32461173_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCTGATTACCACAGTTGACTGC

PrimerID	Complete
Rr_Ch_6_32461173_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTATCCAAGACAGGCGGTAAACA
Rr_Ch_6_34606797_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACTTCTCTGACCACACAAGCT
Rr_Ch_6_34606797_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTAGACTGAGAAAGTCTTTCCA
Rr_Ch_6_47459856_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGTTCCTGTTATATAGGACGGAC
Rr_Ch_6_47459856_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCTGAGTTCTCAACAATCCCT
Rr_Ch_6_61913826_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATGCAGCACTTCTGGAAAGTAAA
Rr_Ch_6_61913826_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTATCTTTATTTTATGTGGGTGCTGT
Rr_Ch_6_70398993_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGACATATGCTCTGTTTTCTGCT
Rr_Ch_6_70398993_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAATGTTCCCTCATGACTTCCCAC
Rr_Ch_6_84324213_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGAAAATGGTGCAACAGAATTGC
Rr_Ch_6_84324213_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGAGCAAAGAACAGGAGCCTT
Rr_Ch_6_87792941_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTCTGCAGTGCTGGCACTA
Rr_Ch_6_87792941_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAGATCACTGCAAGGTAAACA
Rr_Ch_6_90413868_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATCTCAACTGGCCAGGAACT
Rr_Ch_6_90413868_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTGATATATTTAGTCCCCCAGC
Rr_Ch_6_108291273_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTACTTCTTAGTCGCCACTGCT
Rr_Ch_6_108291273_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAGCAGTCATTAACATTGCAAACT
Rr_Ch_6_113546702_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGCAATTTACGACTGGACTAACA
Rr_Ch_6_113546702_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGTCTCTGAGGGCACTTATCT
Rr_Ch_6_132017539_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGGCCAAGCATCCAGAACTT
Rr_Ch_6_132017539_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGGCAAGCACTACCAATAACCTA
Rr_Ch_7_11849482_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTCCAGCATCAGGAATGGAA
Rr_Ch_7_11849482_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTATGCACACAGAAGCATTTCT
Rr_Ch_7_21834432_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAAGAGAAAGGGCTGCAGTACC
Rr_Ch_7_21834432_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGAGTAGCATGCTCCAAC
Rr_Ch_7_25297836_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTCACCTCCATATTCAGACTCC
Rr_Ch_7_25297836_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGTGAAGCATCTATGTAGGTGT
Rr_Ch_7_27610315_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTTCCTCCTCAGAGTGTCTTAA

PrimerID	Complete
Rr_Ch_7_27610315_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGTATCCGAGTGTTTCATGGA
Rr_Ch_7_34766069_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTCGTCTCTGGACTGCAGATA
Rr_Ch_7_34766069_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGACACTTGAGAGCAAGACAGTA
Rr_Ch_7_40818312_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATATTCCAGAAACCTCCGAACCT
Rr_Ch_7_40818312_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTCTGACACATGCCTCCATAT
Rr_Ch_7_42203627_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTATCTCTTCGCCCCGACTTT
Rr_Ch_7_42203627_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGATTGCCCCAACTTTTGCT
Rr_Ch_7_45913562_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTTCTGCTAAGCCCTATACAACC
Rr_Ch_7_45913562_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTCAGGTTACTAGATTTGTGCT
Rr_Ch_7_69369644_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTGCAGATGAGAGTCTAAAAGCA
Rr_Ch_7_69369644_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGGACATAAGTGATAAAGCAGC
Rr_Ch_7_84002804_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCTGAGGAAAAAGTCAGAGAACT
Rr_Ch_7_84002804_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTGTGAGGACGCATAGCT
Rr_Ch_7_92113511_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGACTATTCAAGTCCAGGCTCT
Rr_Ch_7_92113511_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCAGAAGATGGTGTGTCTGT
Rr_Ch_7_94758590_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGACGCCTGCAGCTTATTAGC
Rr_Ch_7_94758590_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCAGACATCTTCACTGTCTACCA
Rr_Ch_7_101723370_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTTGCCAGGAAAGGAAACTGA
Rr_Ch_7_101723370_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGACAGTCTTCAAGTCCACCT
Rr_Ch_7_110578220_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCCAGCAATAAACCCCATCTGAA
Rr_Ch_7_110578220_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAACATGCTTGTTGAGGAGGAT
Rr_Ch_7_112739698_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGAGTCAAGAACTGCAACACAA
Rr_Ch_7_112739698_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGAGCACAGTTCATCTGTTGTT
Rr_Ch_7_123041574_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGTTTATGCAGCAGCGCTTAA
Rr_Ch_7_123041574_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTACCCGCTTTCTGTTTCAGA
Rr_Ch_8_23766757_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGACAATGAATGTGGCTGAGCA
Rr_Ch_8_23766757_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTGAGGAAAATTCTCAAGAGCCT
Rr_Ch_8_25072556_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGATCTCTGTTATTCTGGCTCTT

PrimerID	Complete
Rr_Ch_8_25072556_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTCCAAGTCTCATCCCTTAGT
Rr_Ch_8_28252058_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACCATGAGCTACATAAACCACTC
Rr_Ch_8_28252058_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCCGTTTGTAAACATTTGGCTTT
Rr_Ch_8_33831435_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTGCTGCAGTGGAAGAATCT
Rr_Ch_8_33831435_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACAGACTGAACCTTGAGAGAAGA
Rr_Ch_8_57170399_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCCTGGCTCTCTGCAGTTA
Rr_Ch_8_57170399_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACAGTGGAGAATAGATGACGCA
Rr_Ch_8_62383454_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCATTCTGCTCCCTTTGAACA
Rr_Ch_8_62383454_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTTTTCATTAGCCACACGGT
Rr_Ch_8_73791420_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTATACAGGTCCTGCAGTCGT
Rr_Ch_8_73791420_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTGAGCACTACACCAGATGA
Rr_Ch_8_78139592_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTCAGTTTTTGTACCTACCTTCTA
Rr_Ch_8_78139592_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGGAGCAGAGGAACTCAGTT
Rr_Ch_8_79610079_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTAATCGAGCTGCTTTTAGCCA
Rr_Ch_8_79610079_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTTCTAGGATTTCTAGTGTGATGT
Rr_Ch_8_85349591_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCAGAGAAGCTGCTGATTTGT
Rr_Ch_8_85349591_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGACAACAGTTTGAATGCTCCTC
Rr_Ch_8_86451934_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCTGCAGGAGACACAGTAAGT
Rr_Ch_8_86451934_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGGCTAATGTCTAGGGTAATCCA
Rr_Ch_8_92407169_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGAAGGGGAATTCTGACTGAGA
Rr_Ch_8_92407169_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAACTGAGGGAAACCGGATCTT
Rr_Ch_8_95329726_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGATCTCCAGGTCTACCACAT
Rr_Ch_8_95329726_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAACTTACTGAGCAGTTGAGACTC
Rr_Ch_8_101279096_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTCTTGTACCAAGGCATGTC
Rr_Ch_8_101279096_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCTTGTGGTCACCAAGAAA
Rr_Ch_8_111599785_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGAAAAGCTTCCTGGGGTCTGA
Rr_Ch_8_111599785_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGATCAGGGCACTGACAGA
Rr_Ch_9_4649581_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTATCACACCACTGTCCCTGC

PrimerID	Complete
Rr_Ch_9_4649581_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCATTTAAAGAGCCAAGTACA
Rr_Ch_9_5930255_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCCTGTCGGAAGTGTGGAAA
Rr_Ch_9_5930255_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAAGTGGGGTCTCTGTCACT
Rr_Ch_9_13159644_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATACTCTCTCATCACAGGTCCT
Rr_Ch_9_13159644_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAAAGGAAGTTTGAGTACAGCA
Rr_Ch_9_17203083_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCTATGGGCTGACTTGAGGAA
Rr_Ch_9_17203083_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGAGAGAGACATAGAGGCATTAAGT
Rr_Ch_9_21439554_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGATGGTACCCTAGAATATCTGC
Rr_Ch_9_21439554_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTATGCCTGAAAGGAGGTATGGA
Rr_Ch_9_29777423_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCAGGCACTCCAAGTCAATGTT
Rr_Ch_9_29777423_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCTCCTTTGGTTTGCTGAGC
Rr_Ch_9_38773024_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTGTGGCGCTCTACTGTCTTA
Rr_Ch_9_38773024_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCATGGTATTTGCACAGAGTCT
Rr_Ch_9_49845457_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGGCGTTAGGGTTTGAATTCC
Rr_Ch_9_49845457_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGGTGAGGAGAGCTGAA
Rr_Ch_9_87563377_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCAGGAGCCTGACTCAAACAAT
Rr_Ch_9_87563377_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTAATCCGAAATAGTAGCCTGA
Rr_Ch_10_4768724_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTGCTGCTGTGAGGGAACATA
Rr_Ch_10_4768724_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACACAGTTAGGCACTCACTC
Rr_Ch_10_9606239_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGATGAGCTGGTTTGTCTGCA
Rr_Ch_10_9606239_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGAGTCAGCTCCGACCTACTA
Rr_Ch_10_23694451_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCAAGGTGCAATTCCAAGATGC
Rr_Ch_10_23694451_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTCCACAGTGTAAGTCAGGGT
Rr_Ch_10_38981793_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCAAGTGATAGCAGGTGATGAGTT
Rr_Ch_10_38981793_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCTAGAGCAGGACACAGTAAAA
Rr_Ch_10_58820656_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTCCAGTCATCAGGCCAACTC
Rr_Ch_10_58820656_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTGGAGGTGGACATTGTTGA
Rr_Ch_10_71470606_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATCTGAGAGGCCTTGATTGGA

PrimerID	Complete
Rr_Ch_10_71470606_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGACTAGCAACTCCCTTCTCT
Rr_Ch_10_90345201_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACCAGTGTGTCAGCACTTACT
Rr_Ch_10_90345201_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTCGATTTTACAGAGCAGTCT
Rr_Ch_11_3108252_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTACCAGCTACGCACCGT
Rr_Ch_11_3108252_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGTACCTGCTGTGCACTGA
Rr_Ch_11_21446045_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTATATATGCGCGTGGCTGCA
Rr_Ch_11_21446045_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTAGCTGACAACCAGCTGAT
Rr_Ch_11_42367322_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCTAGGTAGTATGGTGTCTGCA
Rr_Ch_11_42367322_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACACAGCTGTTGTGAGTTTGTAT
Rr_Ch_11_45224978_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGAAGAATAGTGAGGCCAACTGT
Rr_Ch_11_45224978_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTACTGCATATTGAATTGCCCAGT
Rr_Ch_11_52873477_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAAGAATGGGAACACAAAGCAAGA
Rr_Ch_11_52873477_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGGTAACCTTGTTTAACCCA
Rr_Ch_11_68657565_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGGATATAGCGACATGAATCCAC
Rr_Ch_11_68657565_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAATTCTACGGGAGAAAGGAGC
Rr_Ch_11_77736670_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCAAAACAGGCTGTATCCCTT
Rr_Ch_11_77736670_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAGGAGTGTCTTACTGAAGGTACC
Rr_Ch_11_87739718_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCAGACCCTCAGTACTCATAGA
Rr_Ch_11_87739718_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTATTCGGTATTAATTGGGCCT
Rr_Ch_11_89975056_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACCCTTACTGATACAGCAGACTT
Rr_Ch_11_89975056_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGATAAAACCAAAGAGCAATTCAGT
Rr_Ch_11_92962885_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTAATCTTACAGTCAGCATGGGT
Rr_Ch_11_92962885_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTATCATCTCTTGGCAGGACCTT
Rr_Ch_12_3054167_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATTTGAGCCCTCTGCCTATGT
Rr_Ch_12_3054167_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCAGGTAAACATGAGTACCCA
Rr_Ch_12_28892321_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGAGGAGTTTATGGAGCCTCAGA
Rr_Ch_12_28892321_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGAACATAGCAAACCTTACCAGATGA
Rr_Ch_12_30891222_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTGCAGTTCACCTCTCCTAGTTCC

PrimerID	Complete
Rr_Ch_12_30891222_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGATCATGTGCAACTGTATCAAGT
Rr_Ch_12_44090291_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATCTGCAGTGGTCCTAATAATCC
Rr_Ch_12_44090291_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCACAGAGCATCAATTCCAAT
Rr_Ch_12_58750229_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTCTGACCTGAGACTATGAACCA
Rr_Ch_12_58750229_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACGTGTTTGACCATAAGGATGA
Rr_Ch_12_69561184_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGACACTAAAGTCTCTGAACCT
Rr_Ch_12_69561184_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTGGAAAAGTGACCTTGGAAT
Rr_Ch_12_80920327_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGCCCTTGCCTTATGTCCCT
Rr_Ch_12_80920327_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAAGAATCTTGCTCTTTACGCT
Rr_Ch_12_86283114_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCAAGGCTGGCTTCAAATCCAT
Rr_Ch_12_86283114_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTATTGGAAGGCAGAACTTGGA
Rr_Ch_12_87671903_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACCATGTGGGTGCTAGGAATT
Rr_Ch_12_87671903_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGTCAATGTTGGCTTAGAGGT
Rr_Ch_12_89753539_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTTAAAGCCACTTTGGAGCCT
Rr_Ch_12_89753539_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGAGTCTTGGGGATTGCACTC
Rr_Ch_13_1244044_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCAGCCACAAACAAGTCTACACT
Rr_Ch_13_1244044_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTCAGGAGCAATGGGAGAACC
Rr_Ch_13_15521548_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTGTCCCCAAGAATTGGATAGT
Rr_Ch_13_15521548_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTGTCAGCAGCTATGCAC
Rr_Ch_13_18346350_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCCATAGGATTTGACCAGGACT
Rr_Ch_13_18346350_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGGCATCTGACTATACTGTGAC
Rr_Ch_13_21231419_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTTCTCCCTAAGAGTGGGGTT
Rr_Ch_13_21231419_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGGCAGACAAGTAGTACTCAGA
Rr_Ch_13_28705307_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGACAGTGGGAATGAAGGA
Rr_Ch_13_28705307_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCTGCCTTAACCTCCATTAGT
Rr_Ch_13_38422560_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGATCCTGCTTTGTTCTGATTGA
Rr_Ch_13_38422560_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAACCTGCTTTACAAGGCTTCC
Rr_Ch_13_52631554_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTTGGAATGCAGAGCGAAACT

PrimerID	Complete
Rr_Ch_13_52631554_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGCAAGAGTCCTTCCAGACA
Rr_Ch_13_59962832_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGGATTTGTATTCCCCTAAGAGC
Rr_Ch_13_59962832_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTCTGACTTTACGGGGAAA
Rr_Ch_13_70523682_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGTCATATTGAGACCTAGAGCA
Rr_Ch_13_70523682_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTTTGTCCATGCAGTAACTCT
Rr_Ch_13_73674045_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTCTCTCATCCAGGGTGTACA
Rr_Ch_13_73674045_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCTGTGATGTCCATTAAGGTCA
Rr_Ch_14_8168805_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCTGCAGGCTTTTGCATTTACA
Rr_Ch_14_8168805_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTATAGTAATTGAGCAGAAAGGCCA
Rr_Ch_14_12979632_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCATAAAGCCAAGTCCTGACACT
Rr_Ch_14_12979632_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTAGGAGACAGCCATACTAGGA
Rr_Ch_14_18694834_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGCTTAGCTTATATCTCCAGGCT
Rr_Ch_14_18694834_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCCTAAACTGACTGAGGACCT
Rr_Ch_14_21445015_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGACAGCAGAAGGGAACACAT
Rr_Ch_14_21445015_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTGTTCTCAGTGTTACAACGT
Rr_Ch_14_37133206_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCAAAGGACTCTGCAGGTGACT
Rr_Ch_14_37133206_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCACTGTTTTGACAACCTCGT
Rr_Ch_14_47687384_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGCACTCACTTATACTTGTCTGA
Rr_Ch_14_47687384_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCGTGTTGCATGCTGAATATGA
Rr_Ch_14_57581430_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGACATTTGATCCTACCAACTGC
Rr_Ch_14_57581430_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTGTACCATGGGAGTTACATCC
Rr_Ch_15_2501333_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACAAAAATGAAGAAGAGCCCGA
Rr_Ch_15_2501333_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAATAAGAGTTTCAGGCCTTGGA
Rr_Ch_15_6420545_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCAAGCACATGTATGTAGTGCCT
Rr_Ch_15_6420545_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCAGATCCTGCAACTTCAATAA
Rr_Ch_15_10486151_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCCTTTTGTAATCCAGTTGCA
Rr_Ch_15_10486151_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTATATGCTTGTGTACCCTGTGCAT
Rr_Ch_15_26919614_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTCCTTTTTTCCAGTTGGACCT

PrimerID	Complete
Rr_Ch_15_26919614_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCTGACCTCACATCTAAGTGA
Rr_Ch_15_47184844_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGACACTATGTGGTCTTCTACT
Rr_Ch_15_47184844_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTAGGGATGGCTATCCTGGA
Rr_Ch_15_64566165_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGAGGTTTGCAGGAGTGAACAC
Rr_Ch_15_64566165_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAGAACATAGTGACAGCCCCA
Rr_Ch_16_656794_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCATTTGTGACTCCCTACACATCT
Rr_Ch_16_656794_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATTCTGTATTCCTCAGAAGGCT
Rr_Ch_16_14708916_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGCATTGCTGCAGTCATTACT
Rr_Ch_16_14708916_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTCTCCTCTGGCATGCAGT
Rr_Ch_16_18397681_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTGAACTCTGGTTCTCCACCT
Rr_Ch_16_18397681_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTAGCATGCTGGAGTCCAT
Rr_Ch_16_21188809_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGTTCTGACTGCAGAGCAGAT
Rr_Ch_16_21188809_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTCATTGACTCAGTGTCCT
Rr_Ch_16_39190655_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCCCCAATAAATGTTTAGACCTGC
Rr_Ch_16_39190655_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACTGTGAGGTAGAACATGATCC
Rr_Ch_16_42186366_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAAGCCCTTTACTCTCTGCCT
Rr_Ch_16_42186366_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCGTTTACTGGCACCAGTTGA
Rr_Ch_16_43759652_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGCATCAAAGAAGCCAACGAT
Rr_Ch_16_43759652_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGAAGGTTGTGCGTAACAGC
Rr_Ch_17_26284209_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCAGATTCAGATAGAATCCAAAACC
Rr_Ch_17_26284209_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCACCGTTTCAAAGAATGGTTGA
Rr_Ch_17_28075943_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTGCAAAGAAGCCACTCAGT
Rr_Ch_17_28075943_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTATTCATGTCAGCACCCAGCA
Rr_Ch_17_32479381_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCATATACTAAAGGGACTGGTGT
Rr_Ch_17_32479381_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCCATAAATATATGCTGTAGAGCT
Rr_Ch_17_34489837_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACCGACTCTAGAACTCAGGCT
Rr_Ch_17_34489837_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGGTAACCTCTCTCTGGAGTAGA
Rr_Ch_17_42827761_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGATAGAGGGGCTTTCCGGCT

PrimerID	Complete
Rr_Ch_17_42827761_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTAGCTGTGATCCTCACTGTGA
Rr_Ch_18_18832671_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTCTTTAGCTGGCAACTAACAGT
Rr_Ch_18_18832671_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAAGTTGGATGCAGTCTCACC
Rr_Ch_18_20930993_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCTCAGCAGAGATGAGAATTACC
Rr_Ch_18_20930993_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGAAGTAAGAAGGAGAGGACTTTTA
Rr_Ch_18_26339617_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTGATGCACAGAGTGTCAGC
Rr_Ch_18_26339617_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTCAGGTGAACCCCAGAAAGTC
Rr_Ch_18_45550133_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCACTGGGACTGTGACATCATT
Rr_Ch_18_45550133_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTAAAGCCTGGCTTCAACACTT
Rr_Ch_y_1927253_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCGCCCCATGAATGCATT
Rr_Ch_y_1927253_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGATTCTGTTGAGCCAACTTGC

Appendix 3 – Targeted SNP panel amplification laboratory methods

These laboratory methods were based on Campbell et al. 2015, including the modifications recommended by Schmidt et al. 2020.

For each SNP panel, all primers were mixed in equal proportions and diluted to a stock concentration of 250 nM per primer to create a PCR1 pooled primer mix.

The first round PCR (PCR1) was used to amplify the target loci, while round 2 (PCR2) incorporated individual indexes. The reaction mix for multiplex PCR amplification of target loci was 2 µL of DNA extract, 3.5 µL of Qiagen Plus multiplex master mix and 1.5 µL pooled primer mix (final volume = 7 µL; final primer concentration at each locus 54 nM). Thermal cycling was conducted in 96-well PCR plates with the following conditions for PCR1: 95 °C – 15 min; 5 cycles [95 °C – 30 s, 5% ramp down to 57 °C – 30 s, 72 °C – 2 min]; 10 cycles [95 °C – 30 s, 65 °C – 30 s, 72 °C – 30 s]; 4 °C hold.

Following PCR1, the amplified samples were diluted 10-fold, and 3 µL of PCR product was transferred to a new 96-well PCR plate. For PCR2, 1 µL of 10 M well-specific i5 tag primers were then added to the amplified samples, and another 1 µL of plate-specific tagging primer was then added. Five microlitres of Qiagen Plus multiplex master mix (Q + MM) was then added to each well, bringing the final reaction volume to 10 µL. Thermal cycling conditions for PCR2 were 95 °C – 15 min; 10 cycles [98 °C – 10 s; 65 °C – 30 s; 72 °C – 30 s]; 72 °C – 5 min; 4 °C hold.

After PCR2 Ampure XP beads were used to clean-up each reaction, removing the primers for sequencing with a 1.1 : 1 ratio. PCR products were then pooled within each species, and each pooled library was examined with a Bioanalyzer for quality control and then sequenced on one lane of an Illumina Miseq 2x150 bp paired-end sequencing.

Appendix 4 – Rara_RASP SNP panel bioinformatic pipeline

GT-Seq library processing and genotype calling

GT-Seq amplicon libraries were demultiplexed using dual-indexed Illumina identifiers exported from the instrument sample sheet. Raw FASTQ files were first converted to Unix format, and reads corresponding to each sample were extracted with BBMap's `demuxbyname.sh`, using exact i5+i7 barcode combinations as matching keys. Forward and reverse reads were retained as unmerged pairs to avoid loss of loci with non-overlapping amplicons.

For each sample, allele-specific read counts were generated using a custom Python probe-counter script, which performs high-throughput exact-string matching against locus-specific probe sequences. The script operates in parallel, dividing each FASTQ file into chunks and counting matches for allele 1 (A1) and allele 2 (A2) probes independently. Only exact matches (0 mismatches) were allowed to maintain assay specificity, given that all loci should be separated by only a single base.

The output for each sample consists of per-locus counts of allele 1 and allele 2 reads, total depth, and inferred genotype, alongside locus-level and sample-level QC statistics (e.g. call rate, on-target percentage, allele balance).

Genotype collation and export to standard formats

Allele-count matrices (A1 and A2 counts across all samples) and genotype calls were merged using a custom R pipeline. Locus names followed the convention *Species_chr_Position_Allele1–Allele2*, enabling automated parsing of genomic coordinates and alleles. For each locus and sample, genotypes were translated into PLINK- and VCF-compatible formats. PLINK `.map` and `.ped` files were generated with alleles coded directly as the biological nucleotides rather than numeric surrogates, allowing seamless downstream population genetic analysis.

A full multi-sample VCF was constructed with per-sample GT:AD:DP fields, where GT reflects phased genotype (0/0, 0/1, 1/1), AD is the A1 and A2 read counts, and DP is the total depth. This export step ensures that GT-Seq data can be analysed alongside SNP array, RADseq, or whole-genome data sets using standard bioinformatic workflows

Together, the steps in this pipeline provide a reproducible, high-resolution genotyping framework suitable for population assignment, hybrid identification, kinship inference, and conservation management applications.

Appendix 5 – Output metrics for each locus retained in each SNP panel.

Table A5.1 Metrics for loci retained in the final Rara_RASP GT-Seq panel. Hets = number of heterozygous loci, het rat = rate of heterozygosity. The allele that did amplify for each of the alternative Rattus species (if one did)

Note, for these loci that did amplify for these two alternative species, not all loci amplified well, but when one allele did amplify for any individual it is listed

Locus	n	calls	call_rate	hets	het_rate	mean_depth	R exulans	R norvegicus
Rr_Ch1_454369_A-G	67	62	0.93	15	0.24	397.99	G	A
Rr_Ch1_4427464_T-G	67	60	0.90	18	0.30	291.73	T	T
Rr_Ch1_41189327_C-G	67	67	1.00	13	0.19	247.24	C	C
Rr_Ch1_55944582_G-C	67	66	0.99	19	0.29	147.18	G	
Rr_Ch1_63561669_G-T	67	61	0.91	29	0.48	139.19	G	G
Rr_Ch1_68798228_T-C	67	64	0.96	20	0.31	267.37	C	
Rr_Ch1_85127962_T-G	67	66	0.99	15	0.23	162.21		
Rr_Ch1_90215858_C-T	67	65	0.97	15	0.23	355.09		
Rr_Ch1_94380113_T-C	67	64	0.96	18	0.28	244.31	T	T
Rr_Ch1_96123523_A-G	67	58	0.87	20	0.34	765.37	A	A
Rr_Ch1_100626118_G-T	67	51	0.76	13	0.25	62.93	G	
Rr_Ch1_104789417_C-A	67	66	0.99	21	0.32	181.93	C	C
Rr_Ch1_119469489_T-G	67	47	0.70	23	0.49	214.66	T	T
Rr_Ch1_148919649_G-T	67	67	1.00	24	0.36	324.34		
Rr_Ch1_193044213_C-T	67	65	0.97	22	0.34	113.87	C	C
Rr_Ch1_201884788_G-A	67	65	0.97	25	0.38	357.52	A	A
Rr_Ch1_207159406_A-G	67	41	0.61	17	0.41	74.57		
Rr_Ch1_227953352_C-T	67	43	0.64	9	0.21	56.73		C
Rr_Ch1_249540742_T-G	67	55	0.82	13	0.24	122.07	T	
Rr_Ch2_8571143_G-A	67	58	0.87	13	0.22	106.58	A	G
Rr_Ch2_39735328_G-A	67	62	0.93	20	0.32	119.79		
Rr_Ch2_43975779_T-C	67	63	0.94	12	0.19	184.22		C
Rr_Ch2_50501597_G-C	67	62	0.93	17	0.27	155.25		

Locus	n	calls	call_rate	hets	het_rate	mean_depth	R exulans	R norvegicus
Rr_Chr2_53167838_A-G	67	65	0.97	30	0.46	326.33	G	G
Rr_Chr2_65195009_T-C	67	62	0.93	14	0.23	123.18		
Rr_Chr2_72937716_T-G	67	65	0.97	19	0.29	176.55	G	T
Rr_Chr2_97953956_C-T	67	60	0.90	23	0.38	215.58	C	C
Rr_Chr2_109021025_T-C	67	66	0.99	12	0.18	170.67	T	T
Rr_Chr2_111637504_C-T	67	61	0.91	16	0.26	136.40		
Rr_Chr2_146798458_G-A	67	65	0.97	31	0.48	533.67	G	G
Rr_Chr2_150865520_C-T	67	66	0.99	28	0.42	223.46	C	C
Rr_Chr2_154459517_G-A	67	60	0.90	15	0.25	216.00		
Rr_Chr2_162540831_C-T	67	67	1.00	27	0.40	202.34	C	
Rr_Chr2_170143844_A-G	67	51	0.76	6	0.12	178.25	A	A
Rr_Chr2_182591444_C-T	67	53	0.79	2	0.04	64.18		
Rr_Chr2_184831413_C-T	67	62	0.93	16	0.26	119.04	C	C
Rr_Chr2_196945467_C-G	67	67	1.00	26	0.39	305.48	G	G
Rr_Chr2_202589002_A-G	67	57	0.85	8	0.14	98.34		G
Rr_Chr2_212905971_C-G	67	55	0.82	14	0.25	91.34		
Rr_Chr2_220745396_A-G	67	64	0.96	20	0.31	165.31	A	A
Rr_Chr2_227682084_C-A	67	63	0.94	19	0.30	199.39	C	
Rr_Chr2_229034355_C-T	67	34	0.51	0	0.00	53.03	C	
Rr_Chr3_3398644_C-A	67	55	0.82	13	0.24	79.87	C	
Rr_Chr3_5970996_A-G	67	65	0.97	14	0.22	154.34	A	
Rr_Chr3_7796605_T-C	67	60	0.90	20	0.33	109.25	C	
Rr_Chr3_19931350_A-G	67	60	0.90	9	0.15	116.43		
Rr_Chr3_23285043_A-G	67	66	0.99	32	0.48	739.90	A	A
Rr_Chr3_41215428_G-A	67	49	0.73	15	0.31	73.55		
Rr_Chr3_55735166_C-T	67	59	0.88	14	0.24	116.16		
Rr_Chr3_90506797_A-T	67	67	1.00	33	0.49	269.93		A
Rr_Chr3_93422569_G-A	67	67	1.00	32	0.48	236.88	A	A

Locus	n	calls	call_rate	hets	het_rate	mean_depth	R exulans	R norvegicus
Rr_Chr3_98614586_A-C	67	56	0.84	12	0.21	251.45	C	C
Rr_Chr3_104367713_C-T	67	63	0.94	16	0.25	163.82	C	C
Rr_Chr3_116324132_G-A	67	66	0.99	29	0.44	180.67	G	G
Rr_Chr3_141508165_T-C	67	62	0.93	21	0.34	101.31	T	T
Rr_Chr3_174058792_A-C	67	48	0.72	12	0.25	124.73	A	A
Rr_Chr3_179060364_G-A	67	47	0.70	1	0.02	75.13	G	
Rr_Chr3_182742805_C-T	67	55	0.82	11	0.20	83.27	C	
Rr_Chr3_196978091_T-C	67	64	0.96	16	0.25	268.48	C	
Rr_Chr4_18733340_T-C	67	67	1.00	21	0.31	185.55	C	T
Rr_Chr4_34490490_G-A	67	50	0.75	0	0.00	308.69		A
Rr_Chr4_61472646_C-A	67	62	0.93	22	0.35	203.12		
Rr_Chr4_103514149_T-A	67	60	0.90	0	0.00	183.42	A	
Rr_Chr4_112093358_G-T	67	65	0.97	20	0.31	390.46	G	G
Rr_Chr4_134526370_A-T	67	66	0.99	19	0.29	533.66	A	A
Rr_Chr4_136250768_G-A	67	63	0.94	16	0.25	142.84		
Rr_Chr4_147669350_G-A	67	67	1.00	12	0.18	320.88		A
Rr_Chr4_150846801_C-T	67	66	0.99	17	0.26	220.33		C
Rr_Chr4_178477453_C-G	67	64	0.96	23	0.36	176.13		C
Rr_Chr5_9641003_G-A	67	47	0.70	11	0.23	93.78		
Rr_Chr5_14492440_G-T	67	67	1.00	26	0.39	323.54		T
Rr_Chr5_23029472_G-A	67	64	0.96	25	0.39	481.75	A	G
Rr_Chr5_36892468_G-A	67	66	0.99	20	0.30	227.22	G	G
Rr_Chr5_43676760_A-G	67	65	0.97	25	0.38	225.96		
Rr_Chr5_48228583_A-T	67	66	0.99	27	0.41	393.31	A	
Rr_Chr5_51430819_C-T	67	65	0.97	11	0.17	203.51		T
Rr_Chr5_60660123_T-G	67	66	0.99	25	0.38	657.73	G	G
Rr_Chr5_80983281_A-G	67	55	0.82	9	0.16	94.64	A	
Rr_Chr5_132932432_G-A	67	63	0.94	22	0.35	399.34	G	

Locus	n	calls	call_rate	hets	het_rate	mean_depth	R exulans	R norvegicus
Rr_Ch5_146167601_A-G	67	62	0.93	14	0.23	244.52	A	A
Rr_Ch5_158352255_A-T	67	65	0.97	14	0.22	233.81	A	A
Rr_Ch6_23324793_C-G	67	66	0.99	14	0.21	340.27	C	
Rr_Ch6_25105159_G-A	67	65	0.97	10	0.15	148.79		A
Rr_Ch6_32461173_G-A	67	65	0.97	25	0.38	247.54	G	G
Rr_Ch6_34606797_T-C	67	58	0.87	9	0.16	119.33	C	T
Rr_Ch6_47459856_T-C	67	64	0.96	23	0.36	261.69	C	T
Rr_Ch6_61913826_A-G	67	65	0.97	14	0.22	149.88		
Rr_Ch6_70398993_C-A	67	67	1.00	15	0.22	348.07	C	C
Rr_Ch6_84324213_C-T	67	67	1.00	32	0.48	320.36	T	T
Rr_Ch6_87792941_C-A	67	64	0.96	16	0.25	138.51		
Rr_Ch6_90413868_A-G	67	61	0.91	27	0.44	737.16	G	G
Rr_Ch6_108291273_T-C	67	63	0.94	18	0.29	129.34	T	T
Rr_Ch6_113546702_T-C	67	55	0.82	8	0.15	80.96	C	
Rr_Ch7_21834432_C-T	67	65	0.97	21	0.32	316.45	C	C
Rr_Ch7_27610315_G-A	67	49	0.73	8	0.16	78.33	G	G
Rr_Ch7_34766069_G-A	67	51	0.76	8	0.16	97.78	G	G
Rr_Ch7_42203627_T-C	67	67	1.00	10	0.15	379.55	T	C
Rr_Ch7_45913562_G-A	67	54	0.81	2	0.04	133.87	A	
Rr_Ch7_69369644_A-C	67	63	0.94	16	0.25	120.79	A	
Rr_Ch7_84002804_G-A	67	66	0.99	15	0.23	155.28	G	G
Rr_Ch7_92113511_C-A	67	46	0.69	8	0.17	64.43	C	C
Rr_Ch7_94758590_A-C	67	47	0.70	8	0.17	67.55	C	C
Rr_Ch7_101723370_G-A	67	67	1.00	16	0.24	154.58		
Rr_Ch7_110578220_T-C	67	64	0.96	17	0.27	145.06	C	
Rr_Ch8_23766757_A-G	67	65	0.97	29	0.45	178.81	G	G
Rr_Ch8_25072556_T-C	67	67	1.00	28	0.42	226.60	T	
Rr_Ch8_28252058_C-T	67	64	0.96	22	0.34	143.58	C	C

Locus	n	calls	call_rate	hets	het_rate	mean_depth	R exulans	R norvegicus
Rr_Chr8_33831435_C-T	67	44	0.66	16	0.36	76.57	C	C
Rr_Chr8_57170399_A-G	67	65	0.97	33	0.51	243.39		
Rr_Chr8_62383454_C-T	67	44	0.66	16	0.36	42.19	C	
Rr_Chr8_73791420_C-G	67	63	0.94	27	0.43	137.33	C	
Rr_Chr8_78139592_A-G	67	43	0.64	15	0.35	60.52		
Rr_Chr8_79610079_T-C	67	40	0.60	6	0.15	60.25	T	C
Rr_Chr8_86451934_A-G	67	63	0.94	26	0.41	146.48	A	
Rr_Chr8_92407169_A-T	67	62	0.93	18	0.29	210.66	T	
Rr_Chr8_95329726_C-T	67	55	0.82	0	0.00	102.10		
Rr_Chr8_101279096_T-C	67	62	0.93	13	0.21	143.93		C
Rr_Chr9_4649581_C-T	67	65	0.97	19	0.29	222.57	C	
Rr_Chr9_5930255_C-T	67	65	0.97	15	0.23	205.55	C	C
Rr_Chr9_17203083_A-T	67	42	0.63	13	0.31	35.25	T	
Rr_Chr10_9606239_C-G	67	49	0.73	0	0.00	329.66	C	G
Rr_Chr10_23694451_G-A	67	51	0.76	2	0.04	224.76	G	
Rr_Chr10_38981793_T-G	67	60	0.90	19	0.32	158.49	G	
Rr_Chr10_58820656_C-T	67	52	0.78	0	0.00	182.01	C	C
Rr_Chr10_71470606_C-T	67	65	0.97	19	0.29	235.54		C
Rr_Chr10_90345201_A-G	67	52	0.78	12	0.23	68.78		
Rr_Chr11_3108252_C-T	67	67	1.00	6	0.09	96.69		C
Rr_Chr11_21446045_A-G	67	60	0.90	7	0.12	264.51		
Rr_Chr11_45224978_C-T	67	55	0.82	21	0.38	705.79	T	
Rr_Chr11_52873477_A-G	67	52	0.78	14	0.27	102.34	G	G
Rr_Chr11_68657565_C-G	67	66	0.99	36	0.55	191.94		
Rr_Chr11_77736670_C-A	67	41	0.61	2	0.05	103.19	C	
Rr_Chr11_87739718_A-G	67	67	1.00	5	0.07	331.37	G	G
Rr_Chr11_89975056_A-G	67	52	0.78	18	0.35	90.16	A	
Rr_Chr11_92962885_G-A	67	57	0.85	22	0.39	332.54	G	G

Locus	n	calls	call_rate	hets	het_rate	mean_depth	R exulans	R norvegicus
Rr_Ch12_3054167_A-G	67	39	0.58	0	0.00	151.63	A	A
Rr_Ch12_30891222_C-T	67	64	0.96	16	0.25	158.04	C	C
Rr_Ch12_44090291_C-T	67	61	0.91	6	0.10	142.82	C	C
Rr_Ch12_58750229_G-C	67	42	0.63	0	0.00	67.91		
Rr_Ch12_69561184_A-G	67	59	0.88	17	0.29	134.45	A	A
Rr_Ch12_80920327_C-T	67	65	0.97	20	0.31	347.21	C	
Rr_Ch12_86283114_C-T	67	66	0.99	20	0.30	309.21	T	T
Rr_Ch12_89753539_A-G	67	60	0.90	16	0.27	285.06	A	
Rr_Ch13_1244044_T-C	67	64	0.96	12	0.19	158.51		T
Rr_Ch13_15521548_G-C	67	62	0.93	17	0.27	147.81		
Rr_Ch13_18346350_A-G	67	61	0.91	17	0.28	311.93	A	A
Rr_Ch13_21231419_C-T	67	58	0.87	27	0.47	58.48		T
Rr_Ch13_38422560_C-A	67	53	0.79	8	0.15	110.87	A	
Rr_Ch13_52631554_C-A	67	65	0.97	14	0.22	163.75		
Rr_Ch13_59962832_A-C	67	66	0.99	14	0.21	572.60	A	A
Rr_Ch13_70523682_G-A	67	66	0.99	22	0.33	214.28		A
Rr_Ch13_73674045_A-G	67	66	0.99	29	0.44	221.76	G	
Rr_Ch14_12979632_G-A	67	63	0.94	21	0.33	196.36	G	
Rr_Ch14_18694834_T-A	67	47	0.70	0	0.00	220.15		
Rr_Ch14_21445015_T-G	67	50	0.75	3	0.06	133.81		
Rr_Ch14_37133206_G-A	67	62	0.93	21	0.34	497.88	G	G
Rr_Ch14_57581430_C-G	67	67	1.00	28	0.42	274.07	C	C
Rr_Ch15_2501333_G-A	67	60	0.90	12	0.20	134.34		
Rr_Ch15_6420545_G-A	67	62	0.93	21	0.34	128.81	G	
Rr_Ch15_10486151_C-T	67	67	1.00	19	0.28	141.79		
Rr_Ch15_47184844_G-A	67	64	0.96	10	0.16	112.00	A	
Rr_Ch15_64566165_C-T	67	62	0.93	39	0.63	492.48	T	C
Rr_Ch16_14708916_A-G	67	65	0.97	26	0.40	113.13	G	G

Locus	n	calls	call_rate	hets	het_rate	mean_depth	R exulans	R norvegicus
Rr_Ch16_18397681_A-G	67	55	0.82	1	0.02	184.45		G
Rr_Ch16_21188809_A-G	67	66	0.99	20	0.30	144.24	G	G
Rr_Ch16_39190655_G-A	67	55	0.82	0	0.00	121.30	G	A
Rr_Ch16_42186366_T-C	67	52	0.78	0	0.00	199.73	C	C
Rr_Ch16_43759652_T-A	67	66	0.99	13	0.20	429.46	A	A
Rr_Ch17_28075943_T-C	67	65	0.97	23	0.35	759.88		
Rr_Ch17_32479381_G-A	67	64	0.96	22	0.34	145.81	G	G
Rr_Ch17_42827761_T-C	67	66	0.99	12	0.18	364.34	T	
Rr_Ch18_18832671_T-C	67	66	0.99	29	0.44	298.04	T	T
Rr_Ch18_26339617_G-C	67	40	0.60	0	0.00	155.33		C
Rr_Ch18_45550133_T-A	67	52	0.78	0	0.00	164.55		
Rr-Ch-y-1927253_y	67	31	0.46	0	0.00	349.61	present	present

Appendix 6 – Probability of identity calculations

Genotypes were first exported from VCF format to PLINK raw additive format (.raw) using PLINK v1.9 (Purcell et al. 2007).⁴ For each locus, allele frequencies were estimated directly from the observed genotype counts, and locus-specific $P(ID)$ and $P(ID)_{sibs}$ values were computed following standard forensic formulations (Waits et al. 2001). Multi-locus $P(ID)$ and $P(ID)_{sibs}$ were obtained as the cumulative product across loci. To visualise the incremental contribution of each SNP, we generated cumulative plots on both linear and log10 scales, including horizontal forensic thresholds ($P(ID)=10^{-4}$, $P(ID)=10^{-6}$, $P(ID)_{sibs}=10^{-2}$) and marking intersection points. Per-locus information gain ($\Delta P(ID)$) was quantified as the reduction in $P(ID)$ when each locus was added to the ordered panel. All calculations and visualisations were performed in R using custom scripts.

⁴ References for Appendices 1–6 are included in the main reference list, rather than in each appendix.