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REPORT

Assessment of Putative Natural Hybridization between the Freshwater Turtles *Chelodina colliei* and *C. steindachneri* in Western Australia

Report prepared for Dr Colin Johnson, Biodiversity Conservation Project Manager (Invasive Species), Central Regional TAFE, Batavia Coast Maritime Institute, Separation Point WA 6530

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T +61 (0)418866741
E arthur.georges@canberra.edu.au
W georges@biomatix.org

Institute for Applied Ecology
University of Canberra
Locked Bag 1
ACT 2601 Australia

Copies of this report is available from:

Wildlife Genetics Laboratory
The Institute for Applied Ecology
University of Canberra ACT 2601
Australia

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Brief

Specimens obtained in Western Australia of *Chelodina colliei* and *Chelodina steindachner*i indicated the possibility of hybridization where the two come into contact. The brief was to use SNP markers to identify any hybridization and introgression between the two species.

Methods

Specimens obtained from Batavia Coast Maritime Institute were combined with those already in the wildlife tissue collection at the University of Canberra.

SNP Genotyping

DNA was extracted, sequenced and informative SNP markers were identified by Diversity Arrays Technologies (DArT Pty Ltd, Canberra, Australia, www.diversityarrays.com) (Kilian *et al.* 2012). Briefly, sequencing for SNP genotyping was done using a combination of complexity reduction using restriction enzymes, implicit fragment size selection and next generation sequencing (Kilian *et al.* 2012). The restriction enzyme combination of PstI (recognition sequence 5'-CTGCA|G-3') and SphI (5'-GCATG|C-3') was selected for the complexity reduction via double digestion. Sequences were processed using proprietary DArT analytical pipelines (Kilian *et al.* 2012) to yield SNP markers polymorphic within the set of samples. Calling quality was assured by high average read depth per locus (mean *ca* 34.5x, after filtering). In addition, approximately one third of samples were processed twice from DNA to allelic calls as technical replicates. Scoring consistency (repeatability) was used as the main selection criteria for high quality/low error rate markers.

The dataset obtained from DArT Pty Ltd contained the SNP genotypes and various associated metadata of which CloneID (unique identity of the sequence tag for a locus), repAvg (proportion of technical replicate assay pairs for which the marker score is identical), avgPIC (polymorphism information content averaged over the reference and alternate SNPs), SnpPosition (position in the sequence tag at which the defined SNP variant base occurs), and CallRate (the frequency with which a locus could be called for a SNP) were used in additional filtering and analyses.

Additional SNP Filtering

The SNP data and associated locus metadata were read into a genlight object ({adegenet}, Jombart 2008) to facilitate processing with package dartR 1.1.6 (Gruber et al. 2018). Only loci with repeatability (repAvg) of 1.0 and no missing values (CallRate) were chosen for subsequent analysis. Because of the high read depth, most "missing data" are not being called during genotyping because of a mutation at one or both of the restriction enzyme recognition sites. Individual GK_K-4[ChsteYarrYarra] was removed from the analysis because it had a call rate less than 60%. Loci were filtered if the read depth (rdepth) did not fall in the range 8-60x. Finally, we filtered out secondary SNPs where they occurred in a single sequenced tag, retaining only one SNP at random. Any monomorphic loci arising as a result of the removal of individuals or populations were also deleted. Given the low within-population sample sizes ($n < 10$), we did not filter loci for departures from

Hardy-Weinberg Equilibrium (HWE) or Linkage Disequilibrium. The data remaining after this additional filtering are regarded as highly reliable and were used in our data analyses.

Visualization

Genetic similarity of individuals and populations was visualized using ordination (Principal Coordinates Analysis or PCoA, Gower 1966). A scree plot of eigenvalues (Cattell 1966) provided an indication of the number of informative axes to examine, taken in the context of the average percentage variation explained by the original variables.

Hybridization

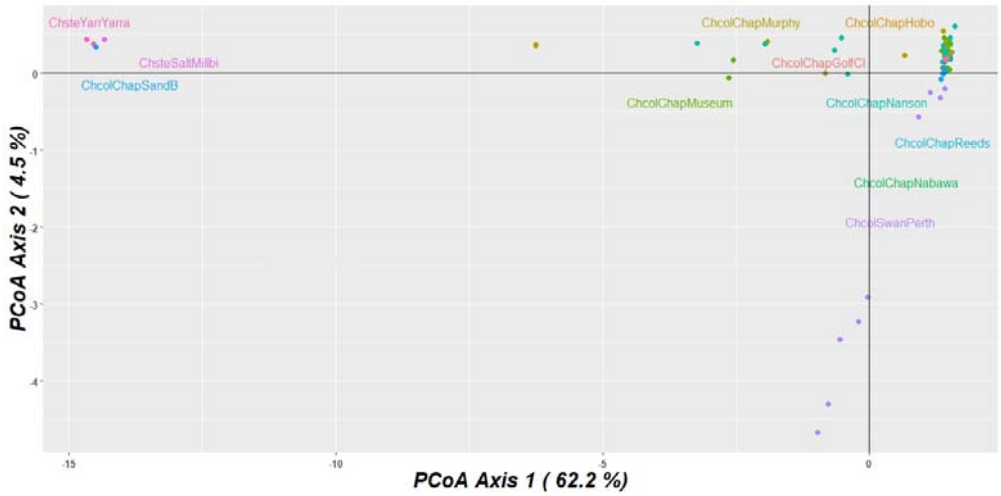
Evidence of hybridization and introgression between species was initially qualitatively assessed by examining the relationships among individuals, identified to population, in PCoA plots. Contemporary admixture was evident as individuals occupying intermediate positions between clearly divergent aggregations of populations. Suspected instances of contemporary admixture of individuals were then assessed using NewHybrids (Anderson & Thompson, 2002). NewHybrids is limited to ca 200 loci. A set of 200 loci were selected at random from loci that were fixed and different when comparing two nominated parental populations. NewHybrids was run with default parameter settings, the Jeffreys Prior for θ, and 10,000 sweeps. F1 hybrids were confirmed by manually examining genotypes at loci that were fixed and different between the two parental populations – F1 status is confirmed by heterozygosity at those loci.

Results

A total of 99,720 polymorphic SNP loci were scored for 79 individuals of the *Chelodina colliei* from 9 waterbodies of southwestern Western Australia, and 5 individuals from two waterbodies for *C. steindachneri* from two waterbodies. After filtering, the dataset comprised 1,175 loci scored for the 79 individuals of *C. colliei*, and 4 individuals of *C. steindachneri* from two waterbodies.

Preliminary examination of the data using Principal Coordinates Analysis (PCoA) gave a strong indication of some level of admixture between the two species.

Figure 1. A principal coordinates analysis summarizing the genetic relationships among individuals of *Chelodina colliei* and *C. steindachneri*. Not that Axis 2 represents only 4.5% of variation, and so dispersion in that direction should be largely ignored.



Parental populations were allocated for *Chelodina colliei* ("ChcolSwanPerth") and *C. steindachneri* ("ChsteSaltMillbi","ChsteYarrYarra") as the basis for identifying putative hybrids and introgressed individuals using New Hybrids.

Table 1. Results of the New Hybrids analysis applied to populations of *Chelodina colliei* and *C. steindachneri*. The body of the table contains posterior probabilities of assignment to bins corresponding to the two parental populations, F1 hybrids and associated backcrosses to the parentals, and F2 hybrids. Sample sizes are given in column headed N.

Population	Chstei	Chcol	F1	F2	back2Chstei	back2Chcol	N
ChsteYarrYarra	1	0	0	0	0	0	**2**
ChcolChapSandB	1	0	0	0	0	0	**1**
ChsteSaltMillbi	1	0	0	0	0	0	**1**
ChcolChapMuseum	0	0	0	0	0	1	**2**
ChcolChapMurphy	0	0	0	0	0	1	**2**
ChcolChapNanson	0	0	0	0	0	1	**5**
ChcolChapMurphy	0	0	1	0	0	0	**2**
ChcolChapGolfCl	0	1	0	0	0	0	**3**
ChcolChapHobo	0	1	0	0	0	0	**4**
ChcolChapMurphy	0	1	0	0	0	0	**16**
ChcolChapMuseum	0	1	0	0	0	0	**8**
ChcolChapNabawa	0	1	0	0	0	0	**1**
ChcolChapNanson	0	1	0	0	0	0	**15**
ChcolChapReeds	0	1	0	0	0	0	**1**
ChcolChapSandB	0	1	0	0	0	0	**9**
ChcolSwanPerth	0	1	0	0	0	0	**10**

Two putative F1 hybrids were identified at location ChapMurphy, together with a further two individuals with profiles consistent with being backcrosses between F1 individuals and *Chelodina colliei*. A further five individuals from ChapNanson and two individuals from ChapMuseum had such backcross profiles.

The profiles of the two putative hybrids were compared to those of the parentals for loci that were fixed and different for the parentals. The theoretical expectation for the putative hybrids is that they will be homozygous for all loci that show fixed differences between the parentals. However, there is an error rate associated with fixed differences (see Georges *et al.* 2018), so a small number of homozygous loci for the F1 individuals is tolerable. There were 11 such homozygous loci for the reference allele, and one for the alternate allele, out of 254 loci scored for the two F1 individuals. This was considered an acceptable error rate.

One specimen identified as *Chelodina colliei* (S02 from ChapSandB) had a profile consistent with it being *Chelodina steindachneri*. The identity of the specimen and/sample should be confirmed.

References

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Gower, J. C. (1966). Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika* **53**, 325–338.

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Appendix A
R script used to generate the results

```
# HOUSEKEEPING

  rm(list=ls())
  # install.packages("dartR")
  library(dartR)

# Set working directory
  setwd("C:/workspace/R_analysis/R_chelodina")

# DATA PREPARATION -- SKIP THIS, GO TO STEP WHERE YOU LOAD Chelodina_stein.gl

# Read data into adegenet genlight format
  gl <- gl.read.dart.2row(datafile="Report_DFwt15-1827_plus_SNP_2row.csv", topskip=5, nmetavar=26, nas="-",
        ind.metafile="Chelodina_metadata.csv" )
  gl <- gl.recode.ind(gl,ind.recode="Chelodina_ind_recode.csv",mono.rm=TRUE,recalc=FALSE,v=3)

  nInd(gl)
  nLoc(gl)
  nPop(gl)
  table(pop(gl))

  # 934 individuals
  # 189816 loci
  # 220 Pop Levels

  saveRDS(gl,file="Chelodina.gl")

  # Steindachneri--colliei
    # START HERE
      gl <- readRDS("Chelodina.gl")
    # Select relevant populations
      ch.ste.coll <- gl.keep.pop(gl, pop.list=c(
        "ChcolChapGolfCl","ChcolChapHobo","ChcolChapMurphy","ChcolChapMuseum","ChcolChapNabawa",
        "ChcolChapNanson","ChcolChapReeds","ChcolChapSandB","ChcolSwanPerth","ChsteSaltMillbi",
        "ChsteYarrYarra"),recalc=TRUE,mono.rm=TRUE,v=3)
    # Interrogate resultant gl
      nInd(ch.ste.coll)
      nLoc(ch.ste.coll)
      nPop(ch.ste.coll)
      table(pop(ch.ste.coll))
    # Apply relevant filters (after checking with corresponding report functions)
      gl2 <- gl.filter.callrate(ch.ste.coll,threshold=1,v=3)
      gl2 <- gl.filter.repavg(gl2,t=0.1, v=3)
      gl2 <- gl.filter.secondaries(gl2,v=3)
      gl2 <- utils.recalc.rdepth(gl2)
      gl2 <- gl.filter.rdepth(x=gl2,upper = 60,lower = 4,v = 3)
    # Interrogate resultant gl
      nInd(gl2)
      nLoc(gl2)
      nPop(gl2)
      table(pop(gl2))
    # Save the gl
      gl.stei.coll <- gl2
      saveRDS(gl.stei.coll,file="Chelodina_stein_coll.Rdata")
      write.csv(gl.stei.coll,file="Chelodina_stein_coll.csv")

# ANALYSIS

      gl.stei.coll <- readRDS("Chelodina_stein_coll.Rdata")
    # Bit of tidying up
      gl.stei.coll <- gl.drop.ind(gl.stei.coll,"GK_K-4") # Abberant individual?
      gl.stei.coll <- gl.drop.ind(gl.stei.coll,"ATP503") # Abberant individual?
    # Principal Coordinates Analysis
      pcoa <- gl.pcoa(gl.stei.coll,nfactors=5)
      gl.pcoa.scree((pcoa))
      gl.pcoa.plot(pcoa,gl.stei.coll)

      gl.pcoa.plot(pcoa,gl.stei.coll,labels="interactive")
      library(plotly)
      ggplotly()
    # New Hybrids Analysis
      # Set the parentals
        p0 <- c("ChsteSaltMillbi","ChsteYarrYarra")
        p1 <- "ChcolSwanPerth"
      # Run New Hybrids
        setwd("C:/workspace/R_analysis/R_chelodina")
        nhy <- gl.nhybrids(gl=gl.stei.coll, nhyb.directory = "C:/workspace/R/NewHybsPC", method="random", p0=p0, p1=p1,
              BurnIn = 10000, sweeps = 10000, outpath=getwd(), outfile="deleteme.txt")
      # Separate out the F1's and parentals for a check on error rate (F1 should be het for all loci, assuming no error rate
        on fixed differences -- but samples are small)
        nhy2 <- gl.keep.ind(nhy,c("MY04","MY05", "ATP501","ATP502",
              "UC_0238","UC_0227","UC_0228","UC_0230","UC_0231"),mono.rm=FALSE)
      # Convert to a matrix and invert
        tmat <- t(as.matrix(nhy2))
      # Tally homozygous ref, heterozygotes and homozygous alternate for the F1s (the homozygotes should be zero,
        theoretically)
        table(c(tmat[,"MY04"],tmat[,"MY05"]))
      # Calculate the false Error rate
      (table(c(tmat[,"MY04"],tmat[,"MY05"]))[1]+table(c(tmat[,"MY04"],tmat[,"MY05"]))[3])*100/
              sum(table(c(tmat[,"MY04"],tmat[,"MY05"])))
        # There are 11 homozygotes ref and 1 homozygote alternate, total 12 = 4.7%, regarded within the acceptable error rate
          for fixed differences.
      # Output the scores
          write.csv(t(as.matrix(nhy2)),file="ch_stei_coll_loci.csv")

# ENDE
```

Appendix B
Full Results of the New Hybrids Analysis

id	pop	Chste	Chcol	F1	F2	F1xChste	F1xChcoll
ATP502	ChsteYarrYarra	1	0	0	0	0	0
ATP501	ChsteYarrYarra	1	0	0	0	0	0
UC_0238	ChsteSaltMillbi	1	0	0	0	0	0
S02	ChcolChapSandB	1	0	0	0	0	0
MY02	ChcolChapMurphy	0	0	0	0	0	1
NH17	ChcolChapNanson	0	0	0	0	0	1
M07	ChcolChapMuseum	0	0	0	0	0	1
M08	ChcolChapMuseum	0	0	0	0	0	1
NH11	ChcolChapNanson	0	0	0	0	0	1
NH19	ChcolChapNanson	0	0	0	0	0	1
NH05	ChcolChapNanson	0	0	0	0	0	1
NH13	ChcolChapNanson	0	0	0	0	0	1
MY07	ChcolChapMurphy	0	0	0	0	0	1
MY04	ChcolChapMurphy	0	0	1	0	0	0
MY05	ChcolChapMurphy	0	0	1	0	0	0
UC_0755	ChcolSwanPerth	0	1	0	0	0	0
UC_0228	ChcolSwanPerth	0	1	0	0	0	0
UC_0754	ChcolSwanPerth	0	1	0	0	0	0
UC_0227	ChcolSwanPerth	0	1	0	0	0	0
UC_0751	ChcolSwanPerth	0	1	0	0	0	0
UC_0398	ChcolSwanPerth	0	1	0	0	0	0
UC_0394	ChcolSwanPerth	0	1	0	0	0	0
UC_0389	ChcolSwanPerth	0	1	0	0	0	0
UC_0231	ChcolSwanPerth	0	1	0	0	0	0
UC_0230	ChcolSwanPerth	0	1	0	0	0	0
MY01	ChcolChapMurphy	0	1	0	0	0	0
MY09	ChcolChapMurphy	0	1	0	0	0	0
MY17	ChcolChapMurphy	0	1	0	0	0	0
M05	ChcolChapMuseum	0	1	0	0	0	0
C04	ChcolChapSandB	0	1	0	0	0	0
CHGC46	ChcolChapGolfCl	0	1	0	0	0	0
NY01	ChcolChapNabawa	0	1	0	0	0	0
NH08	ChcolChapNanson	0	1	0	0	0	0
NH16	ChcolChapNanson	0	1	0	0	0	0
MY10	ChcolChapMurphy	0	1	0	0	0	0
MY18	ChcolChapMurphy	0	1	0	0	0	0
M06	ChcolChapMuseum	0	1	0	0	0	0
C05	ChcolChapSandB	0	1	0	0	0	0
CHHL39	ChcolChapHobo	0	1	0	0	0	0
NH01	ChcolChapNanson	0	1	0	0	0	0
NH09	ChcolChapNanson	0	1	0	0	0	0
MY03	ChcolChapMurphy	0	1	0	0	0	0
MY11	ChcolChapMurphy	0	1	0	0	0	0
MY19	ChcolChapMurphy	0	1	0	0	0	0
C02	ChcolChapSandB	0	1	0	0	0	0
CHHL40	ChcolChapHobo	0	1	0	0	0	0
NH02	ChcolChapNanson	0	1	0	0	0	0
NH10	ChcolChapNanson	0	1	0	0	0	0
NH18	ChcolChapNanson	0	1	0	0	0	0
MY12	ChcolChapMurphy	0	1	0	0	0	0
MY20	ChcolChapMurphy	0	1	0	0	0	0
CHCO37	ChcolChapSandB	0	1	0	0	0	0
CHHL41	ChcolChapHobo	0	1	0	0	0	0
NH03	ChcolChapNanson	0	1	0	0	0	0
MY13	ChcolChapMurphy	0	1	0	0	0	0
M01	ChcolChapMuseum	0	1	0	0	0	0
M09	ChcolChapMuseum	0	1	0	0	0	0
CHCO38	ChcolChapSandB	0	1	0	0	0	0
CHHL42	ChcolChapHobo	0	1	0	0	0	0
NH04	ChcolChapNanson	0	1	0	0	0	0
NH12	ChcolChapNanson	0	1	0	0	0	0
NH20	ChcolChapNanson	0	1	0	0	0	0
MY06	ChcolChapMurphy	0	1	0	0	0	0
MY14	ChcolChapMurphy	0	1	0	0	0	0
M02	ChcolChapMuseum	0	1	0	0	0	0
M10	ChcolChapMuseum	0	1	0	0	0	0
CHCO36	ChcolChapSandB	0	1	0	0	0	0
CHBR43	ChcolChapReeds	0	1	0	0	0	0
MY15	ChcolChapMurphy	0	1	0	0	0	0
M03	ChcolChapMuseum	0	1	0	0	0	0
C01	ChcolChapSandB	0	1	0	0	0	0
CHGC44	ChcolChapGolfCl	0	1	0	0	0	0
S01	ChcolChapSandB	0	1	0	0	0	0
NH06	ChcolChapNanson	0	1	0	0	0	0
NH14	ChcolChapNanson	0	1	0	0	0	0
MY08	ChcolChapMurphy	0	1	0	0	0	0
MY16	ChcolChapMurphy	0	1	0	0	0	0
M04	ChcolChapMuseum	0	1	0	0	0	0
C03	ChcolChapSandB	0	1	0	0	0	0
CHGC45	ChcolChapGolfCl	0	1	0	0	0	0
NH07	ChcolChapNanson	0	1	0	0	0	0
NH15	ChcolChapNanson	0	1	0	0	0	0

Appendix C
Sample genotypes used for ascertaining F1 hybrid status for MY04 and MY05

Locus	Chelodina colliei				Chelodina steindachneri			F1 Hybrids	
	UC_0228	UC_0227	UC_0231	UC_0230	ATP502	ATP501	UC_0238	MY04	MY05
6612280|22-A/G	0	0	0	0	2	2	2	1	1
31650684|63-A/G	0	0	0	0	2	2	2	0	0
31633021|29-T/A	0	0	0	0	2	2	2	1	1
6612107|8-C/A	0	0	0	0	2	2	2	1	1
15957734|34-C/G	0	0	0	0	2	2	2	1	1
4137453|67-C/T	0	0	0	0	2	2	2	1	1
6625850|50-T/G	0	0	0	0	2	2	2	0	0
20131346|13-C/A	2	2	2	2	0	0	0	1	1
20243850|38-T/G	2	2	2	2	0	0	0	1	1
20136918|36-C/G	2	2	2	2	0	0	0	1	1
6232365|13-G/A	0	0	0	0	2	2	2	1	1
6617022|15-T/C	0	0	0	0	2	2	2	0	1
6629565|8-C/G	0	0	0	0	2	2	2	1	1
19263605|20-C/T	0	0	0	0	2	2	2	1	1
31591367|38-A/G	0	0	0	0	2	2	2	1	1
4164249|5-T/C	0	0	0	0	2	2	2	1	1
6620715|22-T/C	0	0	0	0	2	2	2	1	1
20239573|6-G/A	0	0	0	0	2	2	2	1	1
4149994|15-G/A	0	0	0	0	2	2	2	1	1
6605964|6-G/A	2	2	2	2	0	0	0	1	1
4141190|52-G/A	0	0	0	0	2	2	2	1	1
6617219|42-T/C	0	0	0	0	2	2	2	1	1
24903094|5-C/T	0	0	0	0	2	2	2	0	0
15949765|44-A/T	0	0	0	0	2	2	2	1	1
15950646|17-G/A	0	0	0	0	2	2	2	1	1
6621581|7-C/T	0	0	0	0	2	2	2	1	1
20262804|6-G/A	0	0	0	0	2	2	2	1	1
4148702|15-C/T	2	2	2	2	0	0	0	1	1
6613118|22-C/T	0	0	0	0	2	2	2	1	1
15265472|29-C/G	2	2	2	2	0	0	0	1	1
4137678|23-T/C	2	2	2	2	0	0	0	1	2
31591168|57-C/T	0	0	0	0	2	2	2	1	1
24899028|20-T/C	2	2	2	2	0	0	0	1	1
19264325|34-A/T	0	0	0	0	2	2	2	1	1
20136176|37-A/G	2	2	2	2	0	0	0	1	1
11355472|13-T/G	2	2	2	2	0	0	0	1	1
31591683|64-T/C	2	2	2	2	0	0	0	1	1
6229703|23-C/T	0	0	0	0	2	2	2	1	1
15951139|7-A/C	0	0	0	0	2	2	2	1	0
24901975|67-A/G	0	0	0	0	2	2	2	0	0
6606179|24-C/G	2	2	2	2	0	0	0	1	1
31606891|36-C/T	2	2	2	2	0	0	0	1	1
31650950|6-G/A	0	0	0	0	2	2	2	1	1
31612888|62-G/T	2	2	2	2	0	0	0	1	1
20137045|14-A/G	2	2	2	2	0	0	0	1	1
6612444|33-C/A	0	0	0	0	2	2	2	1	1
20140384|26-A/G	2	2	2	2	0	0	0	1	1
31631843|13-G/T	2	2	2	2	0	0	0	1	1
31630991|8-G/C	2	2	2	2	0	0	0	1	1
24902728|54-A/G	0	0	0	0	2	2	2	1	1
6615315|29-C/T	2	2	2	2	0	0	0	1	1
6604418|22-A/G	2	2	2	2	0	0	0	1	1
11352155|7-C/T	2	2	2	2	0	0	0	1	1
31590979|27-T/A	0	0	0	0	2	2	2	1	1
15954099|18-G/A	0	0	0	0	2	2	2	1	1
19263730|47-C/T	2	2	2	2	0	0	0	1	1
16929624|34-C/T	0	0	0	0	2	2	2	1	1
4142381|56-T/A	0	0	0	0	2	2	2	1	1
4156714|16-A/G	0	0	0	0	2	2	2	1	1
6604521|33-C/T	0	0	0	0	2	2	2	1	1
15950620|24-T/C	0	0	0	0	2	2	2	1	1
4164904|36-T/A	0	0	0	0	2	2	2	1	1
4151947|15-A/C	0	0	0	0	2	2	2	1	1
6612802|6-G/A	0	0	0	0	2	2	2	1	1
31627408|20-A/C	0	0	0	0	2	2	2	1	1
6612742|6-C/A	0	0	0	0	2	2	2	1	1
31591968|67-T/C	2	2	2	2	0	0	0	1	1
6605796|14-T/G	2	2	2	2	0	0	0	1	1
24898870|17-G/T	0	0	0	0	2	2	2	1	1
4153149|50-C/G	0	0	0	0	2	2	2	1	1
15954769|28-G/A	0	0	0	0	2	2	2	1	1
6605072|16-C/A	2	2	2	2	0	0	0	1	1
4144930|5-C/G	0	0	0	0	2	2	2	1	1
6227510|29-C/T	0	0	0	0	2	2	2	1	1
4144305|46-G/A	2	2	2	2	0	0	0	1	1
4146454|13-A/T	2	2	2	2	0	0	0	1	1
19260080|29-A/G	0	0	0	0	2	2	2	1	1
31611847|68-C/T	2	2	2	2	0	0	0	1	1
11354960|58-A/G	0	0	0	0	2	2	2	1	1
6612086|7-A/C	0	0	0	0	2	2	2	1	1
4138737|22-A/C	0	0	0	0	2	2	2	1	1
31627728|28-C/T	0	0	0	0	2	2	2	1	1
24905978|35-C/T	0	0	0	0	2	2	2	1	1
31552156|12-A/G	0	0	0	0	2	2	2	0	1
20240986|21-C/T	0	0	0	0	2	2	2	1	1
11358350|67-A/G	0	0	0	0	2	2	2	1	1
4135494|44-T/C	2	2	2	2	0	0	0	1	1
6611679|14-T/C	2	2	2	2	0	0	0	1	1
31607327|33-T/A	2	2	2	2	0	0	0	1	1
6236680|19-T/C	2	2	2	2	0	0	0	1	1
6612592|8-C/T	0	0	0	0	2	2	2	1	1
11336762|39-T/A	2	2	2	2	0	0	0	1	1
11364758|36-C/T	2	2	2	2	0	0	0	1	1
6626116|53-T/G	0	0	0	0	2	2	2	1	1
31551582|23-A/G	2	2	2	2	0	0	0	1	1
24902023|6-T/C	2	2	2	2	0	0	0	1	1
4164752|36-C/T	0	0	0	0	2	2	2	1	1
4150338|44-G/A	0	0	0	0	2	2	2	1	1
6610862|32-T/C	0	0	0	0	2	2	2	1	1
15266259|24-T/A	0	0	0	0	2	2	2	1	1
6617133|35-A/C	0	0	0	0	2	2	2	1	1
20139260|27-A/G	0	0	0	0	2	2	2	1	1
4138079|14-G/A	0	0	0	0	2	2	2	1	1
32451257|21-G/T	0	0	0	0	2	2	2	1	1
31571599|30-G/A	2	2	2	2	0	0	0	1	1
24905988|13-G/C	0	0	0	0	2	2	2	1	1
11368792|25-G/A	2	2	2	2	0	0	0	1	1
6610598|12-C/T	0	0	0	0	2	2	2	1	1
31677522|5-A/G	2	2	2	2	0	0	0	1	1
20137227|38-A/T	0	0	0	0	2	2	2	1	1
31717780|5-G/C	0	0	0	0	2	2	2	1	1
11356374|30-C/T	0	0	0	0	2	2	2	1	1
6237227|30-T/C	0	0	0	0	2	2	2	1	1
31708174|6-C/T	0	0	0	0	2	2	2	1	1
4155519|29-C/G	0	0	0	0	2	2	2	1	1
19259973|25-T/A	0	0	0	0	2	2	2	1	1
15265673|9-A/G	2	2	2	2	0	0	0	1	1
31679263|16-T/G	0	0	0	0	2	2	2	1	1
6612514|53-C/G	0	0	0	0	2	2	2	1	1
6611548|26-T/G	0	0	0	0	2	2	2	1	1
6621246|21-G/A	0	0	0	0	2	2	2	1	1
31570622|11-C/T	0	0	0	0	2	2	2	1	1
11367314|29-C/T	2	2	2	2	0	0	0	1	1
6231882|36-T/C	2	2	2	2	0	0	0	1	1
11342427|46-T/A	0	0	0	0	2	2	2	1	1
20137706|10-G/T	2	2	2	2	0	0	0	1	1
6611494|15-G/A	0	0	0	0	2	2	2	1	1