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Genetic diversity of Japanese quails from wild button quails based on alignment with reference (chicken) genome

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Abstract

Japanese quail farming is a very popular small-scale enterprise booming in various parts of our country. Though Japanese quails are from a different geographic location and not native to India there is a common notion that these birds are similar to native quails found in our jungles. Native quails are also phenotypically similar and so based on Wildlife Protection act, 1972, farming or domestication of these birds is prohibited. In this study, we deploy next generation sequencing technology to sequence DNA of native and Japanese quail to find the percentage match between the SSR enriched genomes and match the sequence with reference chicken genome.

Keywords: Japanese quails, NGS, reads, contigs and sequence alignment

1. Introduction

Diversified poultry production other than chicken viz. ducks, Japanese quails, turkeys, guinea fowls and emu birds are very popular but not commercially undertaken by corporate sector in most countries and still remains a farmer's small scale venture. Quails are broadly classified as Japanese (*Coturnix japonica*) and the common quail (*Coturnix coturnix*); the two are morphologically very similar with very little variation. The distribution patterns of both the species are separate; the common quail is a migratory bird, whereas the Japanese is not (Guyomarch *et al.* 1998) ^[1]. As per IUCN Red List, native quails are listed under threatened category due to possible hunting and habitat destruction.

In the current study we have analyzed sequence from repeat enriched (SSRs) dataset from next-generation sequencing from both Japanese (*Coturnix japonica*) and wild quails (*Turnix sylvaticus*) and compared the sequences with chicken genome via. bioinformatics tool to analyse percent match between the two genomes.

2. Materials and Methods

2.1. Species identification

Prior to NGS species confirmation of the study birds captured from jungles was carried out using 12S rRNA sequence analysis as described by Li Yang *et al.* (2014) ^[5]. Universal primers reported by Kocher *et al.* (1989) ^[2] were used for amplification of the partial segment of 12S rRNA gene.

2.2. Ion torrent sequencing

Blood was used as source of genomic DNA. For DNA isolation conventional phase separation using phenol-chloroform was followed. Quality and quantity of DNA were assessed using Nanodrop (Thermo Scientific) and electrophoresis on a 0.7% agarose gel. Double stranded genomic library was prepared following Ion Xpress™ Plus Fragment Library Kit (Life Technologies). Fragments of 350-500 bp were size selected using E-Gel® SizeSelect™ Gels (Invitrogen) and quantified using autoanalyzer (Agilent 2100, Agilent Technologies). Barcoded adapters from Life technologies were ligated to the size selected genomic fragments.

The captured genomic fragments were amplified on beads through emulsion PCR and DNA-positive beads from emulsion PCR were recovered, enriched and subjected to Ion torrent PGM sequencer (314 chip) following manufacturer's protocol for 260 cycles for 5 hours. The obtained reads were assembled into singletons and contigs using CLC genomics workbench 4.90.

3. Results and Discussion

De novo assembly was used to cluster and assemble sequence reads that passed minimum quality standards. Total number of sequence, average read length, percent singletons and contigs from the sequence are presented in Table 1. A total of sequences 2,33,963 sequences from Japanese and 7,03,447 sequences from wild quails were generated via Ion Torrent run. Raw reads obtained were assembled into singletons and contigs using CLC genomics 4.90.

The average read length obtained in this study was lower than previous studies for ESTs in non-model organisms like Glanville fritillary (197 bp Roche 454) (Vera *et al.* 2008) [6], giant freshwater prawn (311 bp Roche 454) (Jung *et al.* 2011) [4] and channel catfish (292 bp Roche 454) (Jiang *et al.* 2011) [3]. Compared to the time required to develop some tens of microsatellite loci by traditional approaches, this approach from DNA isolation till sequencing (after repeat enrichment) can be very well completed within a 2-week period. Ion torrent platform remains to be currently one of the shortest run time and fastest speed (Lomen *et al.*, 2012) and lowest cost next generation sequencers capable of multi-million read level outputs.

Table 1: Summary of Ion-Torrent sequences for Japanese and wild quail

	Japanese Quail	Wild Quail
Total number of bases (Mb)	41,808,978	118,502,834
Total singleton reads	233,963	7,03,447
Average read length (bp)	175	160
Total contigs	5,257	25,914
Average contig read length (bp)	401	349

Percent similarity of the assembled sequence obtained via ion torrent sequencing to the reference genome (Chicken genome) is presented in table 2 and figure1. In Japanese Quail, a total of 1,29,556 raw reads *i.e.* 55.37% aligned with reference and 1,04,407 (44.63%) did not align to the reference genome.

Table 2: Percent similarity of the assembled sequence

	Raw Reads		Assembled data			
			Singletons		Contigs	
	JQ	WQ	JQ	WQ	JQ	WQ
Matched to reference%	55.37	15.24	58.77	12.39	66.48	10.95
Unmatched to reference%	44.63	84.76	41.23	87.61	33.52	89.05

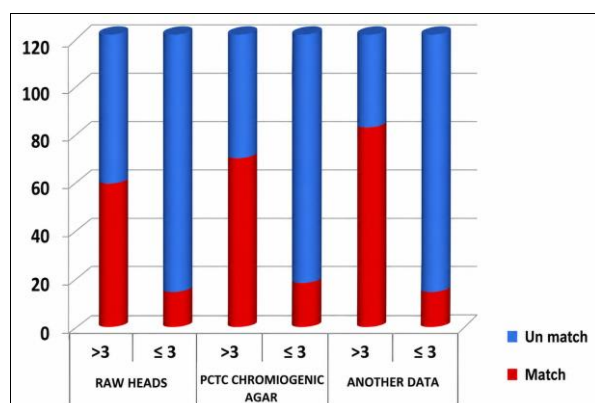


Fig 2: Percent match with reference genome

Whereas in wild quail only 15.24% of the raw reads showed similarity to the reference genome and remaining 84.76% did not show any sequence similarity to the reference genome. The same trend was noticed in the assembled singleton reads in Japanese quail (58.77% matched and 41.23% unmatched) and wild quail (12.39% matched and 87.61% unmatched). Among the contigs 66.48% of the reads matched to reference in Japanese quail and 10.95% reads showed similarity with respect to wild quail.

Percent match in the SSR sequences between Japanese quail and chicken, also wild quail and chicken indicates the wide variation between the two genomes may be due to the geographical distance between the habitats where they occur. Further study using common markers will help to identify private alleles in each of the species which will be a valuable tool for differentiation of the two species.

To conclude, wide sequence differences from genome libraries enriched for microsatellite rich regions between our study birds were evident. Based on the result we could report that Japanese quail genome is very distinct and highly diverse from our native quail.

Conflict of Interest

Not available

Financial Support

Not available

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