Animal Biology

Genotyping-by-Sequencing (GBS) of large amphibian genomes: a comparative study of two non-model species endemic to Italy

Valentina Rovelli^{1,2,*}, Aritz Ruiz-González^{3,4,5}, Leonardo Vignoli², Daniele Macale⁶, Vincenzo Buono², Francesca Davoli⁵, David R. Vieites⁷, Nadav Pezaro¹ and Ettore Randi^{5,8}

¹ Department of Evolutionary and Environmental Biology, Faculty of Natural Sciences, Institute of Evolution,
University of Haifa, Haifa, Israel

² Department of Science, Roma Tre University, Viale Marconi 446, 00146 Rome, Italy

³ Department of Zoology and Animal Cell Biology, University of the Basque Country UPV/EHU, C/ Paseo de la Universidad 7, 01006, Vitoria-Gasteiz, Spain

⁴ Systematics, Biogeography and Population Dynamics Research Group, Lascaray Research Center, University of the Basque Country (UPV/EHU), Avda. Miguel de Unamuno, 3, 01006, Vitoria-Gasteiz, Spain

⁵ Conservation Genetics Laboratory, National Institute for Environmental Protection and Research (ISPRA), Via Cà Fornacetta 9, 40064, Ozzano dell'Emilia, Bologna, Italy

⁶ Fondazione Bioparco di Roma, Viale del Giardino Zoologico 20, 00197 Rome, Italy

Museo Nacional de Ciencias Naturales — Consejo Superior de Investigaciones Científicas (CSIC), C/ José Gutiérrez Abascal 2, 28006, Madrid, Spain

8 Department 18/ Section of Environmental Engineering, Aalborg University, Sohngårdsholmsvej 57, 9000, Aalborg, Denmark

Submitted: September 10, 2018. Final revision received: November 11, 2018. Accepted: November 12, 2018

Abstract

Next Generation Sequencing (NGS) and related technologies have revolutionized the field of conservation and population genetics, providing novel tools and the capacity to discover thousands of new Single Nucleotide Polymorphisms (SNPs) for the analysis of population parameters. However, gathering NGS data for organisms with very large genomes, such as amphibians, remains challenging because it is still unclear how the current methods perform. Here, we use the Genotyping-by-Sequencing (GBS) approach to generate SNP data for the genotyping of two amphibian species that are of conservation concern, the Sardinian brook salamander (*Euproctus platycephalus*) and the Italian stream frog (*Rana italica*). Both *E*.

platycephalus and R. italica have very large genomes (5.53 Gb and >20 Gb, respectively) so genomic data are not available for either of them. We used 95 individual samples and one Illumina lane for each species, with an additional lane for E. platycephalus. After filtering, we obtained 961 and 854 high-coverage SNPs for E. platycephalus and R. italica, respectively. Our results suggest that GBS can serve as a reliable and cost-effective method for genotyping large amphibian genomes, including non-model species.

Keywords

Conservation genomics; frog; newt; Next Generation Sequencing; SNPs; whole genome duplications

*) Corresponding author; e-mail: valentinarovelli.bio@gmail.com

Supplementary material

Text file listing the 96 individual barcodes used during the PCR reaction.

AACGCACATT, AACGTGCCT, AACTGG, AAGACGCT, AATAACCAA, AATGAACGA, AATTAG, ACAACCAACT, ACAACT, ACAGT, ACCAGGA, ACGCGCG, ACGGTACT, ACTGCGAT, ACTGCT, ATAGAT, ATATAA, ATATCGCCA, ATCCG, ATCTGT, ATGAGCAA, ATGGCAA, ATTAT, CAACCACACA, CAAGT, CACCA, CAGAGGT, CAGATA, CAGTGCCATT, CATAT, CATCTGCCG, CCACTCA, CCGAACA, CCTCG, CCTTGCCATT, CGCAACCAGT, CGCACCAATT, CGTCGCCACT, CGTGGACAGT, CGTGTCA, CGTTCA, CTAAGCA, CTCAT, CTCGCGG, CTCGTCG, CTCTA, CTCTCGCAT, CTTAG, CTTGA, GAAGCA, GAAGTG, GAATGCAATA, GAGCGACAT, GCAAGCCAT, GCCAACAAGA, GCCTACCT, GCGCCG, GCGCTCA, GCGTACAAT, GCGTCCT, GCTCCGA, GGAACGA, GGAAGACAT, GGACAG, GGACAG, GGAGTCAAG, GGATA, GGCTTA, GGTATA, GGTGCACATT, GGTGT, GTCGCCT, GTGACACAT, TAGATGA, TAGCAG, TAGCCAA, TAGCGGAT, TATCA, TATGT, TATTCGCAT, TCACGGAAG, TCACTG, TCAGAGAT, TCCGAG, TCTTGG, TGAAT, TGACGCCA, TGCAGA, TGCCGCAT, TGCTT, TGGCAACAGA, TGGCACAGA, TGGCCACAG, TTATTACAT, TTCGTT, TTGCTG