Genotyping discordances? Empirical comparison of base-selective adaptors impact in 2b-RAD studies

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Abstract

Population genomic studies are increasing in the last decade, showing great potential to understand the evolutionary patterns in a great variety of organisms, mostly relying on RAD sequencing techniques to obtain reduced representations of the genomes. Among them, 2b-RAD can provide further secondary reduction to adjust study costs by using base-selective adaptors, although its impact on genotyping is unknown. Here we provide empirical comparisons on genotyping and genetic differentiation when using fully degenerate and base-selective adaptors and assess the impact of missing data. We built libraries with the two types of adaptors for the same individuals and generated independent and combined datasets with different missingness filters according to their presence (100%, 75% and 50%). Exploring locus-by-locus, we found 92% of identical genotypes between both libraries of the same individual when using loci present in 100% of the samples, which decreased to 35% when working with loci present in at least 50% of them. We show that missing data is a major source of individual genetic differentiation. The loci discordant by genotyping were in low frequency (7.67%) in all filtered files. Only 0.96% were directly attributable to base-selective adaptors, and 6.44% underestimated heterozygosity in NN libraries, of which ca. 70% had <10 reads per locus indicating that sufficient read depth should be ensured for a correct genotyping. Our work confirms that 2b-RAD libraries using base-selective adaptors are a robust tool to use in population genomics of species with large genome sizes.

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