Genome analysis

SONiCS: PCR stutter noise correction in genome-scale microsatellites

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Abstract

Motivation: Massively parallel capture of short tandem repeats (STRs, or microsatellites) provides a strategy for population genomic and demographic analyses at high resolution with or without a reference genome. However, the high Polymerase Chain Reaction (PCR) cycle numbers needed for target capture experiments create genotyping noise through polymerase slippage known as PCR stutter.

Results: We developed SONiCS—Stutter mONte Carlo Simulation—a solution for stutter correction based on dense forward simulations of PCR and capture experimental conditions. To test SONiCS, we genotyped a 2499-marker STR panel in 22 humpback dolphins (Sousa sahulensis) using target capture, and generated capillary-based genotypes to validate five of these markers. In these 110 comparisons, SONiCS showed a 99.1% accuracy rate and a 98.2% genotyping success rate, mis-calling a single allele in a marker with low sequence coverage and rejecting another as un-callable.

Availability and implementation: Source code and documentation for SONiCS is freely available at https://github.com/kzkedzierska/sonics. Raw read data used in experimental validation of SONiCS have been deposited in the Sequence Read Archive under accession number SRP135756.

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Supplementary information: Supplementary data are available at Bioinformatics online.

1 Introduction

For species lacking reference genomes, reduced representation sequencing methods such as target capture (Gnirke et al., 2009), RAD-seq (Baird et al., 2008), and genotyping-by-sequencing (Elshire et al., 2011) can yield efficient datasets suitable for a wide range of genomic applications. These methods are typically used to develop sets of single nucleotide polymorphisms (SNPs), but target capture of genome-wide short tandem repeats (STRs) has recently emerged as a strategy for generating massively parallel datasets with rapid rates of evolution (Kistler et al., 2017). STR capture circumvents the workflow bottlenecks of traditional STR development and genotyping while enhancing resolution in population genomic contexts compared with strictly SNP-based approaches. Furthermore, analyses of linked and co-phased SNPs and STRs allows for control of homoplasy that is frequently observed at STR loci, which can be identical by state without being identical by descent due to their high mutability (Ellegren, 2004).

A key challenge for STR capture is polymerase chain reaction (PCR) stutter—the physical slippage of DNA polymerase on the template strand causing molecules with different number of repeats
of the motif sequence to be synthesized (Schlötzerer and Tautz, 1992). Stutter is a well-known obstacle for traditional STR genotyping based on amplicon size, and it remains confounding when using genomic STR methods (Gymrek et al., 2012; Kistler et al., 2017). In target capture experiments, a library amplification step is used both before and after probe–library hybridization totaling 20 or more PCR cycles (SOM), increasing opportunities for stutter-based alleles to appear and propagate through successive PCR steps. To address this issue, we developed a method for fitting the best diploid STR genotype to a set of raw allele counts by comparing the results of dense forward simulations.

2 Materials and methods

The complete SONiCS method is described in SOM, and outlined in Figure 1. Briefly, the user provides a set of raw reads supporting an unknown genotype and a starting pool of simulated molecules is generated using two independent alleles selected from the set of all possible genotypes present. A set of reaction parameters including the efficiency of amplification, efficiency of capture and the probability of polymerase slippage modeled separately for insertions and deletions are then drawn from weak uniform priors based on experimental observations or user inputs. The complete PCR and capture process is then modeled in-silico under the chosen parameters, and we calculate the likelihood of observing the input dataset from the product of the simulated starting genotype. After running a large number of independent simulations, we compare the distributions of the log likelihoods between all possible pairs of genotype calls that could be made. We then call a genotype on basis of a Bonferroni-corrected Mann–Whitney U test P-value and the likelihood ratios between the best fitting and second best fitting genotype.

SONiCS accepts either a single genotype provided at the command line or a VCF file including ALLREADS, MOTIF and REF fields as produced by allelotype, a component of lobSTR (Gymrek et al., 2012). SONiCS writes a summary genotype file including the Mann–Whitney U test results, likelihood ratios for best and third quartile lnL values between alternative genotypes and the number of trials conducted. Optionally, SONiCS can also report the verbose parameters and results of each individual simulation for under all tested genotypes. This functionality allows completely flexible interrogation of simulation results and alternative filtering schemes for genotype selection according to the specific needs of stringency and experimental design. In order to optimize performance, large parts of the code for SONiCS are written in Cython (Behnel et al., 2011), a superset of the Python programming language designed to give C-like performance with code mostly written in Python. The software is capable of using multiple processors and uses the ‘multithreading’ package from Python. On a single processor, SONiCS calculates 6–9 genotypes per minute on average. The signal to noise ratio at an STR locus depends on the coverage at the locus, the extent of the stutter, the repeat motif, the distribution of biological alleles and several other factors. Based on sub-sampling at validated loci (Supplementary Fig. S5, Supplementary Methods), we recommend using SONiCS on loci with a minimal coverage of 45 reads as a conservative threshold.

To test the accuracy of SONiCS, we used BaitSTR (Kistler et al., 2017) and targeted resequencing to generate a set of 2499 STRs in a set of 22 Australian humpback dolphins (Sousa sahulesis; target regions provided as Supplementary Dataset S1). We then used lobSTR (Gymrek et al., 2012) to align reads and summarize raw read support for target STRs and SONiCS to calculate resulting STR genotypes. We used traditional PCR and capillary genotyping to analyze five of the captured STR loci as a truth-set for comparison with SONiCS results ( Supplementary Table S1). Complete genotyping procedures are described in SOM. Tissue samples were originally
collected under permits from the Queensland Department of Environment and Heritage protection (WISP16457615) and combined permit of the Great Barrier Reef Marine Park Authority and Queensland Parks and Wildlife Service (G10/33405.1), with animal ethics committee approval from Southern Cross University.

3 Results

We recovered 95.2% of the complete STR panel across individuals through target capture—52,325 out of 54,978 possible STRs called—including all five markers overlapping capillary calls in all 22 samples. Across samples, a median 216 independent reads covered each marker, with between 13.0% and 18.9% of all reads per sample overlapping target STRs. After duplicate removal, this on-target proportion of reads equates to an effective 551-fold median enrichment of the target regions compared with whole genome shotgun sequencing data (range 393- to 807-fold). After SONiCS genotype calculation, we miscalled the capillary-validated genotype in only one instance—a 99.1% accuracy rate. SONiCS correctly rejected one additional genotype for failing the validation filters, yielding 108 genotypes for downstream analysis. The single miscalled locus was a heterozygous tetramer containing 10 and 11 repeats (Supplementary Tables S1 and S3). Coverage of the miscalled locus was in the 11th percentile of all markers (48x), and visual inspection confirms that the majority of reads (n = 37) supported the 11-repeat allele (Supplementary Table S2). Thus this specific locus is a difficult-to-resolve case where manual calling would suggest a noisy homozygote—consistent with SONiCS—and where allelic dropout and capture biases in the presence of low coverage may have confounded the underlying genotype.

4 Discussion

Existing methods for stutter correction in genomic sequence data primarily involve training a noise model on a large haploid subset of experimental data, such as a human Y-chromosome (Gymrek et al., 2012). This approach provides an effective and replicable genotype likelihood rescaling method for whole-genome STR datasets, but is not typically applicable to STR capture datasets: First, species without a chromosome-level reference genome or lacking large haploid chromosomes—most plants, for example—cannot make use of this haploid training approach. Indeed a major advantage of the BaitSTR method (Kistler et al., 2017) used here for marker development is that no genome assembly is required, and this benefit is incompatible with the lobSTR training framework. Second, even given a large haploid genomic region, reduced representation experiments would need to devote a large proportion of probe sets to training markers that may not be applicable to broader research priorities, and the training process itself might be confounded by the extreme depth and variation in coverage typical in capture experiments. Finally, STR makeup and base composition in flanking regions (e.g. GC content) have the potential to influence capture efficiency, PCR uptake and polymerase slippage during STR target capture. These variables would be difficult to unravel using a model-based noise correction method, but their influence is absorbed into the Monte Carlo approach using only weak uniform priors to constrain simulations.

For these reasons, we aimed to develop a stutter correction method that (i) can be used without a reference genome or an a priori noise model, and (ii) is robust to the idiosyncratic coverage variation and potential inter-locus biases of a target capture experiment. The statistical framework of SONiCS allows genotype selection in a strictly local context with only weak constraints on the parameters of STR fidelity and therefore confers both independence from any reference genome and tolerance to highly variable genomic representation in a target panel. Genotype calls from SONiCS matched our truth-set of capillary STRs in all but one of the cases, experimentally demonstrating that the SONiCS approach is effective for de-noising sequence-based STRs containing PCR stutter.

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References