SNP genotyping of markers with nearby secondary polymorphisms using Axiom genotyping assays

Abstract

Single nucleotide polymorphisms (SNPs) in highly polymorphic organisms of commercial value pose unique challenges for microarray-based genotyping. For instance, the eucalyptus genome contains up to one SNP per 16 bp [1], and there is one SNP per 29–40 bp in the potato genome [2].

Applied Biosystems™ Axiom™ genotyping assays typically utilize 30-mer oligonucleotide probes that are complementary to the genomic sequence near the SNP to be genotyped [3]. Following hybridization of the amplified target DNA, on-array enzymatic ligation is carried out using hapten-labeled solution probes to interrogate the polymorphic base (Figure 1). The multicolor signal complex that forms on the array surface is then detected. In principle, the presence of a secondary polymorphism in a 30-mer probe sequence introduces a destabilizing base-pair mismatch that can interfere with hybridization and ligation of the oligonucleotide probe. Interference by secondary SNPs can thus change the genotyping intensity and clustering properties of the primary SNP.

The performance of human SNPs with known secondary polymorphisms is measured, and the clustering positions of wheat SNPs interrogated with all secondary polymorphic alleles are compared to simulated data.

Since the surface of the microarray is tiled with 30-mer oligonucleotide probes, the overall stringency of the universal hybridization and wash procedure for Axiom genotyping assays is somewhat permissive of secondary polymorphisms. The Axiom platform can thus accommodate nearby secondary polymorphisms. With its broad sample compatibility, high-throughput processing capability, and advanced genotyping algorithms for polyploid genomes, the Axiom genotyping platform addresses the unmet needs of genotype–trait association studies and marker-assisted selection in plant and animal breeding programs.

Analytical specificity of the Axiom genotyping assay

Amplified whole-genome target DNA hybridizes with Axiom probes, which are synthesized on the microarray and tethered at the 3′ end. This is followed by ligation of the differentially labeled oligonucleotide solution probes that are used to query the base at the ligation junction.

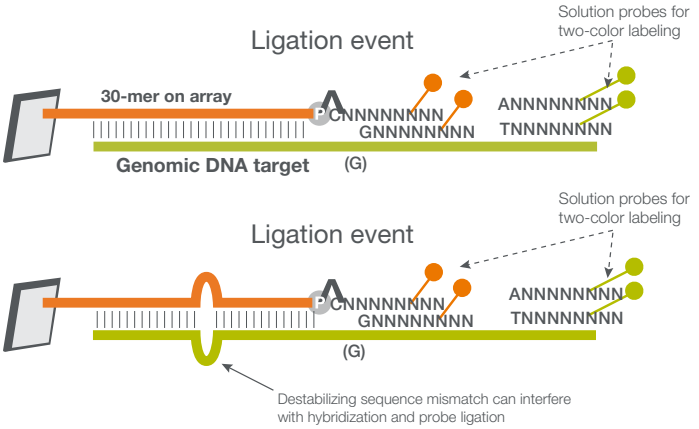

Figure 1. Schematic illustration of Axiom chemistry in the absence of a secondary polymorphism (above) and in the presence of a secondary polymorphism (below).

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The Axiom genotype calling algorithm follows a Bayesian procedure that dynamically adapts to the observed data for prior estimated genotype cluster positions. SNP conversion requires evidence of polymorphism and high-quality clustering based on the call rate, cluster separation, and position (Figure 2).

Genotype cluster simulation

Assume that a secondary polymorphism is associated with one allele of a target SNP but not the other. The array intensity produced by allelic loci that do not have the secondary polymorphism will be higher than that of allelic loci that do. Assign an uppercase A or B and 100 intensity units to the allele without the secondary polymorphism (Figure 3). Assign a lowercase a or b and 30 intensity units to the allele with the secondary polymorphism, and add a background value of 100 to each signal.

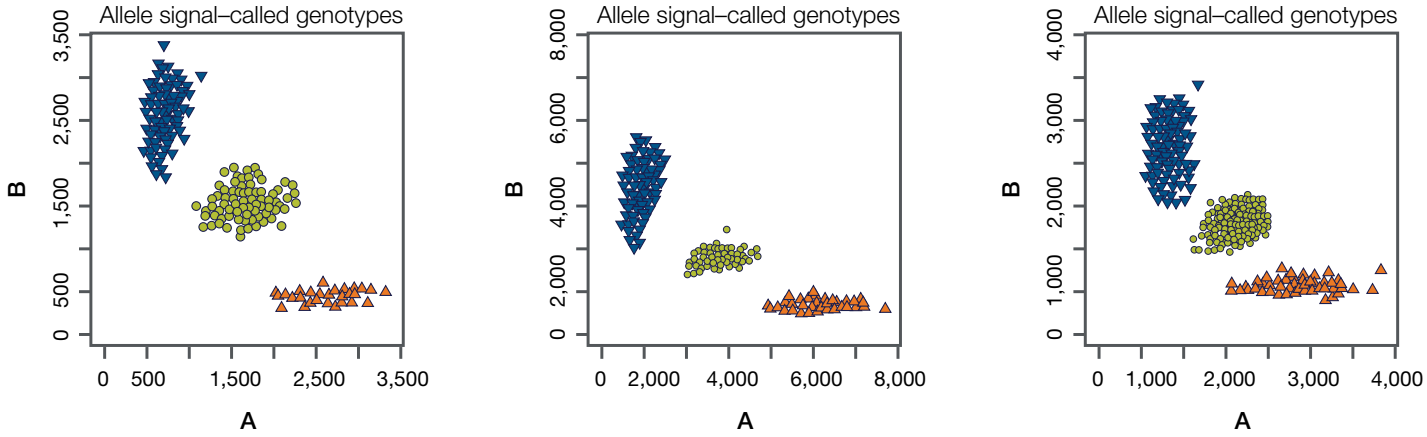

Figure 2. Converted SNP cluster plots.

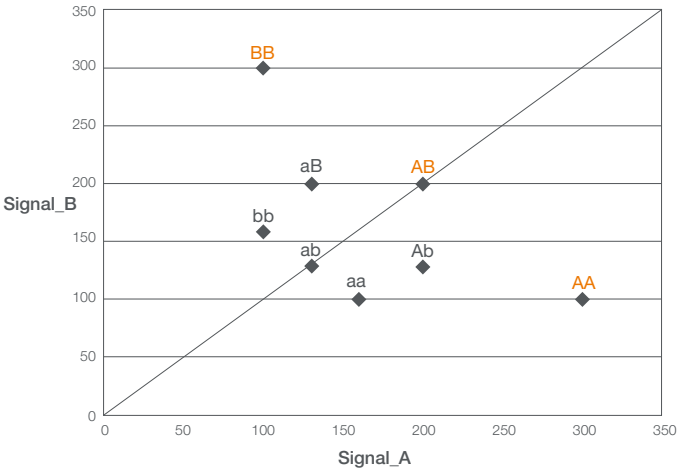

Figure 3. Simulated cluster positions. The red clusters indicate the positions of alleles that lack interference by secondary polymorphisms. Signal_A=#A*100+#a*30+100. Signal_B=#B*100+#b*30+100.

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Data

Empirical data for the allohexaploid wheat genome have shown that Axiom probe sets designed for all alleles with secondary polymorphisms enable successful genotyping of SNPs with secondary polymorphisms in a probe-dependent manner. This is true even when a clear cluster split phenotype is induced by secondary polymorphism (Figure 4).

Results

While common secondary polymorphisms (MAF >5%) affect SNP probe set conversion to a greater extent than rare secondary polymorphisms (MAF <5%), secondary polymorphisms located more than 20 bp from the target SNP are increasingly unlikely to impact SNP probe set conversion. The Axiom genotype calling algorithm is capable of genotyping SNPs, even in the presence of secondary polymorphisms (Figure 5).

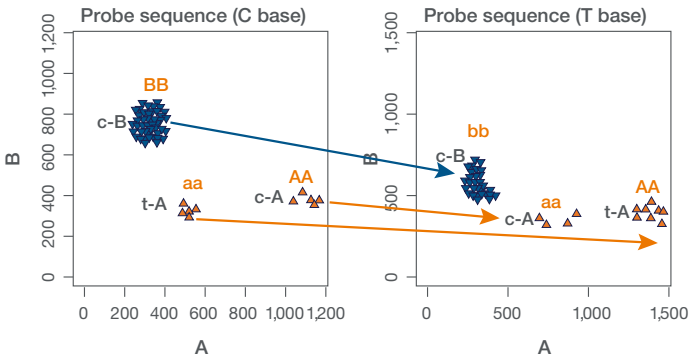

Figure 4. Cluster plots for a SNP with a secondary polymorphism (C/T) 17 bases from the target SNP. The probe sequence in the left plot contains the base C. The sequence in the right plot contains the base T. The secondary polymorphism causes the AA cluster to split into two clusters, AA and aa. The clusters are positioned as expected based on the simulation, and the Axiom genotype calls agree with calls made with KBioscience™ KASPar™ assays.

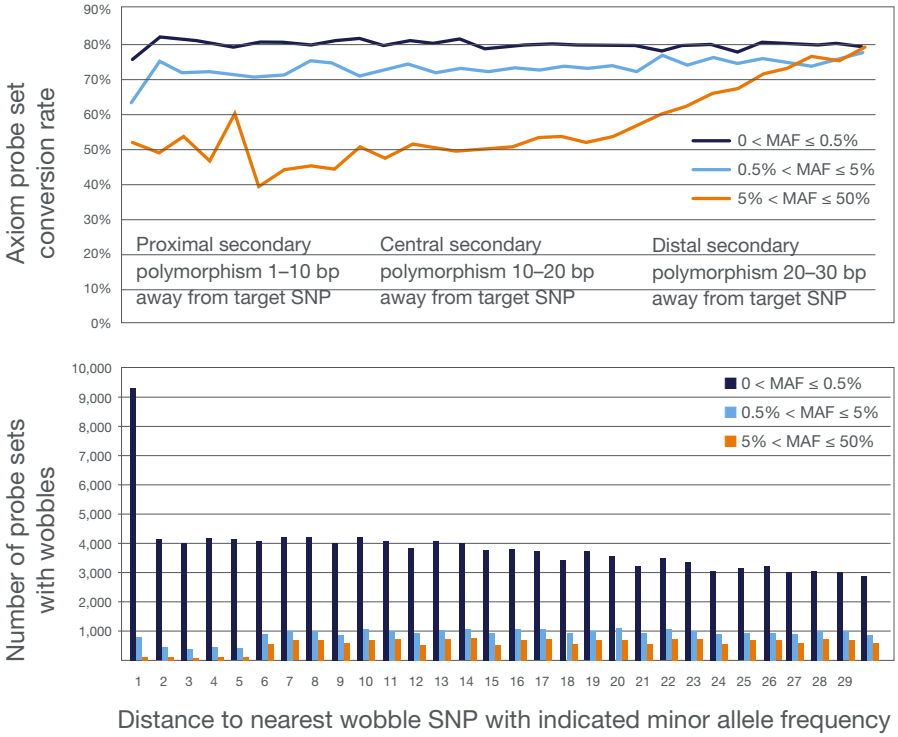

Figure 5. Axiom probe set conversion rate as a function of proximity and the MAFs of secondary polymorphisms in a human dataset.

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