Summary

Sequences containing a set of 495 SNP loci and one sex-linked marker were provided for GT-seq primer design. Primer sets meeting the physical properties for GT-seq and filtered for expected performance in multiplex PCR (N = 332) were designed. Primers for 15 “neutral” markers with lengths greater than 60bp were excluded to reduce primer costs (Nmarkers = 317). The initial test library returned an average on-target rate of 31% and 28 loci were identified that contributed to most of the off-target sequences. Primers for the 28 loci producing primer artifacts were omitted from a new primer pool and a validation library was prepared using the remaining ~~304~~ 289 primer sets. The overall on-target rate for the validation library was 61% among the samples that performed well. Further analysis of the dataset identified a few other primers contributing to the off-target signal.

Primer Design

In order to retain as many of the few high FST markers selected for the GT-seq panel, these markers were preferentially retained when our multiplex PCR primer design algorithm found a conflict with another designed primer. Other marker types labeled as “spatial” and “neutral” represented a larger proportion of the selected markers and were considered more expendable when there were conflicts. Of the initial 495 target markers, 332 ~~369~~ primer sets were ordered for testing.

Library Testing and Validation

The initial test library was created using all 332 ~~369~~ target specific primers that were ordered. This first test library only included a read 1 data and the MiSeq run was not set up to collect read 2. As such, our initial analysis of the first test library did not included a primer-analysis step and we relied on internal probe sequences in the amplicons to determine an on-target rate for all the primer sets. Analysis of the initial test library identified 28 loci that were contributing to the majority of the off-target sequences in the dataset. The validation library was created using a primer pool without the primers from the offending 28 markers identified in test 1. Analysis of this library showed a much improved on-target rate of 61%. Further analysis was conducted to identify the sources of the remaining off-target sequences. The total number of markers that seem to be producing high quality genotype data in the validation library is ~237.

Sex ID marker

The initial results for this marker are that all samples produce a heterozygote signal using these primers. Assuming all samples are not of the same genotypic sex, the primer set doesn’t seem to discriminate by sex. However, multiple samples are producing a co-amplified product that does seem to present in only about half the samples. Further analysis will be required to determine if this can be made into a viable sex determination marker.