

## Axiom® Buffalo Genotyping Array

### Highest density array for genotyping water buffalo across multiple species and breeds

Axiom® Buffalo Genotyping Array provides the highest genome-wide coverage of polymorphic SNPs across multiple species and breeds of the water buffalo (*Bubalus bubalis bubalis* and *Bubalus bubalis carabanensis*), one of the most important beef and dairy animals in Italy, Brazil, and South Asia.

This array is the only commercially available high-density buffalo genotyping tool.

#### Highlights

- **Comprehensive content** – 90,000 common and rare markers selected from sequencing initiatives of the water buffalo (*B. bubalis bubalis*)
- **Multiple breeds represented** – Mediterranean, Murrah, Jaffarabadi, and Nili-Ravi
- **Robust assay performance** – SNPs demonstrate a  $\geq 99\%$  call rate and  $\geq 85\%$  conversion rate on Murrah and Mediterranean samples

#### Applications

##### Molecular breeding:

- Genomic biomarker discovery
- Marker-assisted selection
- Routine screening
- DNA fingerprinting for animal tracking

##### Complex trait research:

- High-resolution mapping of genetic loci in complex traits such as milk production, percentage of milk fat, and feed conversion efficiency
- Discovery of genes and pathways underlying simple and complex traits

##### Conservation and biodiversity:

- Population diversity studies in river and swamp buffalo
- Genomic marker-assisted conservation strategies



#### Array design

Axiom Buffalo Genotyping Array was designed through the Expert Design Program, facilitated by Affymetrix. The array was developed in collaboration with the International Buffalo Genome Consortium, which included Fondazione Parco Tecnologico Padano in Italy, Iowa State University, and the Agricultural Research Service, United States Department of Agriculture (USDA).

#### SNP discovery

1. The genome of one Mediterranean female water buffalo (*B. bubalis bubalis*) was assembled at  $>100\times$  genome sequence coverage.
2. Sequence contigs and paired end reads were aligned from 86 other buffaloes representing 8 breeds and 2 subspecies, including river and swamp types.
3. The sequence reads were aligned to the *Bos taurus* reference genome (UMD3.1).
4. Starting with a list of 16.7 million SNPs and indels, markers that did not have another SNP within 10 bases and had a base pair quality score  $>10$  were included for calculation of minor allele frequency (MAF).
5. A total of 5.8 million SNPs that were polymorphic in at least one breed were selected as candidate SNPs for Axiom Buffalo Genotyping Array.

#### Marker selection

1. SNPs that met the threshold criteria for predicting reproducibility of SNPs based on Affymetrix' *in silico* design scores were included.
2. Sequences that were highly repetitive in the genome and contained ambiguities were removed.
3. The resulting SNPs were selected for uniform spacing across the genome.

The final array contains 90,000 SNPs, 7,583 probes for sample QC, and 6,853 gender calling probes. The Mediterranean, Murrah, Jaffarabadi, and Nili-Ravi water buffalo breeds are represented in the ratio 30:30:20:20.

#### Experimental results

A total of 1,056 customer samples representing two different river buffalo breeds (Mediterranea Italiana and Murrah) were genotyped on the array. The samples were prepared with automated target preparation and processed on GeneTitan® MC Instrument.

The genotyping analysis on the data including clustering was automated using Affymetrix Genotyping Console™ Software and Affymetrix SNPolar™ package. The SNPs were filtered as per the *Best Practice Supplement to Axiom® Genotyping Solution Data Analysis* (P/N 703083).

Array performance was measured in 89,988 SNPs across 1,036 samples that passed the sample QC thresholds. A total of 12 Y SNPs were excluded in this analysis. SNPolar package refined the clustering based on six genotype cluster classifications, which are listed in Table 1 with summaries of the results for each classification.

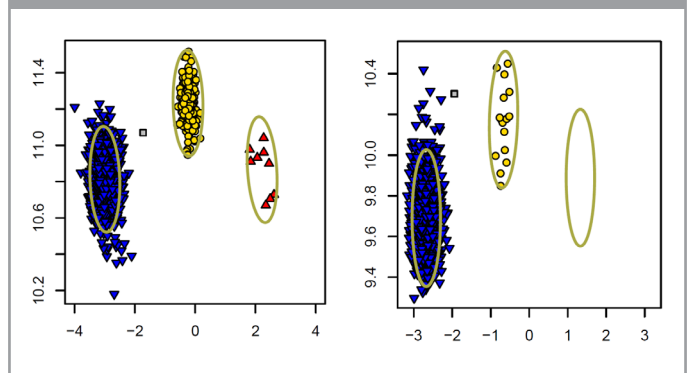
**Table 1. Genotyping performance on commercial samples**

Genotype cluster category	Percentage of markers	No. of markers
Polymorphic high-resolution	74.8%	67,330
Monomorphic high-resolution	10.3%	9,229
No minor homozygous	1.7%	1,494
Off-target variants	0.1%	83
SNPs with call rate below threshold	4.1%	3,668
Other	9.1%	8,184
<b>Total</b>	<b>100.0%</b>	<b>89,988</b>

The polymorphic high-resolution marker category has a sample call rate of 99.75% and the average sample reproducibility is 99.96%.

Cluster plots representative of two of the classification categories from the analysis of Axiom® Buffalo Genotyping Array are shown in Figure 1.

**Figure 1:** The cluster plot on the left is an example of a marker in the “polymorphic high-resolution” category that demonstrates good cluster resolution and at least two examples of the minor allele. The plot on the right shows a marker in the “no minor homozygous” category, which represents markers with two clusters with no representation of the minor allele.



## Ordering information

Part number	Description	Details
550431	Axiom® Buffalo Genotyping Array	Contains one plate with 96 arrays. Reagents and GeneTitan® MC consumables must be ordered separately.
901606	Axiom® GeneTitan® Consumables Kit	Contains all GeneTitan® Instrument consumables required to process one array plate
901758	Axiom® 2.0 Reagent Kit	Includes all reagents (except isopropanol) for processing 96 DNA samples

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