



RapidGenomics
Genotyping Solutions



Application note: Applying the LGC Amp-Seq One ICAR+ panel with 44 Farms samples and Illumina MiSeq i100 for accelerated bovine genotyping

Introduction

Amp-Seq One is LGC's one-step targeted amplicon sequencing technology for agricultural biotechnology breeding programmes. By combining amplification and indexing into a single PCR stage, Amp-Seq One simplifies library preparation, reduces hands-on workflow complexity and supports higher-throughput genotyping, enabling faster turnaround time and higher throughput in-house or outsourced genotyping.

In this application note, we evaluate the [Amp-Seq One industry standard bovine ICAR+ 0.6K panel](#) as a service workflow for bovine parentage, identity and trait-marker screening using customer samples from 44 Farms and sequencing on the Illumina MiSeq™ i100 Plus System.

The Amp-Seq One ICAR+ panel is based on the globally recognised ICAR marker set for parentage verification and SNP selection and extends this foundation by combining ICAR parentage markers with additional targets for commercially relevant traits, including milk quality, coat colour, horned/poled status and known genetic conditions.

44 Farms/Prime Pursuits

44 Farms is a premier cattle operation, known for being leading producer of elite Black Angus genetics and the purveyor of high quality all-natural beef. Through their Prime Pursuits supply chain, 44 Farms is facilitating the use of genetic tools to support ranchers in meeting the market demand for high choice Black Angus beef through their McClaren Farms brand at Walmart.

For this study, 44 Farms/Prime Pursuits provided samples from related animals, with a mix of different ancestries, that included parents and offspring.



“At Prime Pursuits, we are interested in utilizing efficient and reliable methods to generate high quality genomic data to support breeding and management decisions. The Amp-Seq One + MiSeq i100 workflow delivered consistent call rates and sequencing quality that is needed to effectively evaluate parentage. LGC and Illumina offer a compelling approach to accessible, cost-effective and scalable targeted genotyping by sequencing technologies to support small and large genotyping programs.”

- Dr. Sydney Reese, Director of Research & Performance at Prime Pursuits



**Diagnostics
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Illumina

With the MiSeq i100 Series, Illumina advances simple, accurate, and fast benchtop sequencing. Breakthrough improvements in system design, XLEAP-SBS™ chemistry, and integrated data analysis deliver enhanced usability, high data accuracy, and exceptional speed. As part of an end-to-end NGS solution, the MiSeq i100 Series is able to provide rapid genotyping by sequencing results.



Methods

LGC collaborated with 44 Farms and Illumina to evaluate the Amp-Seq One ICAR+ panel in a commercial cattle breeding population. A total of 768 samples, including parental controls, were tested to support parentage confirmation and assessment of key trait-marker presence.

Samples were processed using the Amp-Seq One service workflow to assess panel performance and sequencing compatibility on the Illumina MiSeq i100 platform.

Amp-Seq One Amplification Master Mix was combined with Amp-Seq One Reagent, dispensed into dried-down indexing plates, and combined with DNA samples. A single 50-minute amplification step was performed, followed by pooling and bead clean-up according to the standard Amp-Seq One protocol. Quantification and quality checks were carried out on the generated library on the Tapestation. A concentration of 3.44ng/ul was measured with an optimal library size peaking at 400 bp.

Temperature

Temperature	Time	Cycles
95 °C	2 minutes	1 cycle
95 °C	15 seconds	24 cycles
66 °C***	1 minute	
4 °C	hold	

Table 1. Amp-Seq One cycling conditions. *Optimal temperature may vary due to thermal cycler specifics or the individual Amp-Seq One ICAR+ panel. Expected range 66–68 °C.

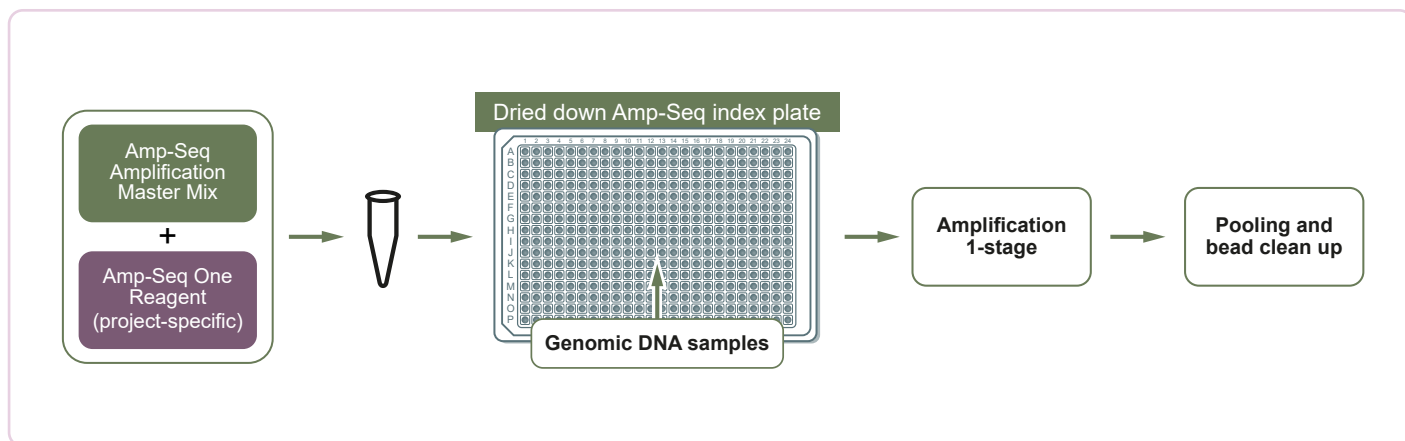


Figure 1. Schematic of the Amp-Seq One Reagent System workflow

Sequencing was performed on the MiSeq i100 Plus System with the MiSeq i100 Series 100M Reagent Kit (300 cycles) (Illumina, Catalog no. 20141599) using the 2 x 150 bp run configuration with 8 bp dual indexing. Library pool was loaded using a final concentration of 100 pM along with a 2% PhiX Control spike-in. Data analysis was performed using the DRAGEN BCL Convert app for demultiplexing results. Using LGC's BiosearchCaller software, the demultiplexed data was aligned and genotyped, producing VCF and tab-separated output files and summary statistics. Panel performance was assessed using marker call rate, uniformity, percentage of reads mapped to target and average coverage depth. Uniformity was defined as the percentage of markers covered at a depth of $\geq 0.2X$ the average coverage depth. A schematic of the Amp-Seq One workflow is shown in Figure 1.

Results and discussion

The ICAR+ Amp-Seq One service workflow generated high-quality targeted genotyping data across the 768-sample 44 Farms bovine cohort. The results support the use of the panel for parentage confirmation, identity testing and trait-marker assessment in a commercial breeding population. Sequencing on the Illumina MiSeq i100 Plus System delivered a 98.07% marker call rate and 94.34% uniformity, indicating robust genotype recovery and consistent target representation across the sample set. In addition, 78.30% of reads mapped to target and average coverage depth reached 247X, supporting confident target assignment for practical breeding applications.

	Call rate	Uniformity	Coverage
Illumina MiSeq i100	98.07%	94.34%	247X

Table 2. Sequencing performance metrics for the Amp-Seq One ICAR+ panel on the Illumina MiSeq i100.

Together, these results demonstrate that Amp-Seq One can deliver reliable, targeted genotyping data while simplifying service delivery at commercial scale. For customers such as 44 Farms, the workflow enables parentage, identity and trait-associated data to be generated from a single targeted panel, reducing operational complexity while supporting actionable breeding decisions.

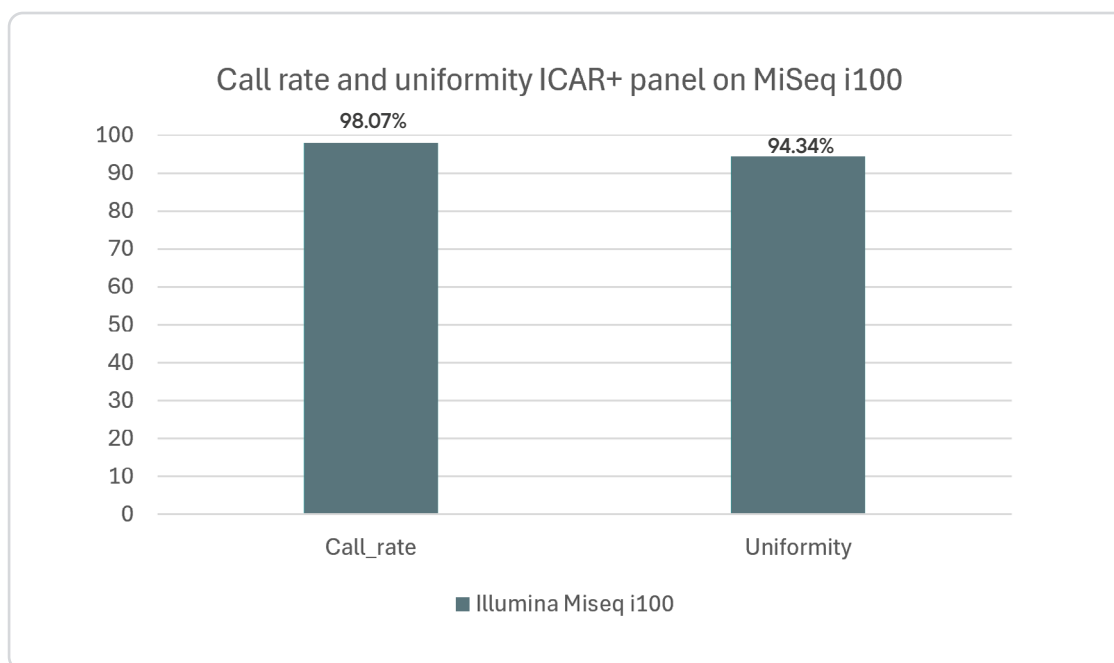


Figure 2. Call rate and uniformity metrics for the Amp-Seq One ICAR+ panel across 768 44 Farms cattle samples sequenced on the Illumina MiSeq i100.

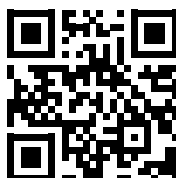
Summary

This evaluation demonstrates the value of the Amp-Seq One ICAR+ service for bovine genotyping in a commercial breeding context. Across 768 samples from 44 Farms, the panel delivered a 98.07% marker call rate, 94.34% uniformity, 78.30% reads mapped to target and an average coverage depth of 247X on the Illumina MiSeq i100.

By combining amplification and indexing into a one-step workflow, Amp-Seq One simplifies targeted genotyping from DNA sample to sequencing-ready library and supports scalable service delivery for breeding programmes that require reliable parentage and trait-marker data.

The combination of an industry-standard ICAR+ panel, Amp-Seq One service workflow and Illumina MiSeq i100 sequencing provides a practical route to faster, simpler and more actionable bovine genotyping at commercial scale.

If you want to speak to an expert, [contact us today](#):



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