

Application note

Amp-Seq One: Making plant and livestock genotyping simple

Introduction

Amp-Seq One is a revolutionary one-step amplicon sequencing workflow designed to accelerate agricultural biotechnology (AgBio) breeding programs. This customised panel kit has a simplified workflow with only one PCR stage that reduces total library preparation time by over 1 hour and doubles throughput compared to 2-stage methods. Additionally, Amp-Seq One eliminates the requirement for liquid handling equipment to transfer material between reaction stages, resulting in reduced consumable costs,

minimisation of any sample cross-contamination and maximising workflow automation.

Here we demonstrate key features of the Amp-Seq One technology including the suitability of Amp-Seq One for plant and livestock species and design compatibility of existing 2-stage panels with the 1-stage protocol. The datasets shown highlight the high quality and accuracy of data that can be generated using Amp-Seq One, with thousands of markers being targeted per reaction.

Application note

Amp-Seq One: Making plant and livestock genotyping simple

Methods

Amp-Seq workflows

Figure 1 illustrates the streamlined workflow of Amp-Seq One, requiring just one PCR stage and no transfer of material between amplification stages, compared to original Amp-Seq.

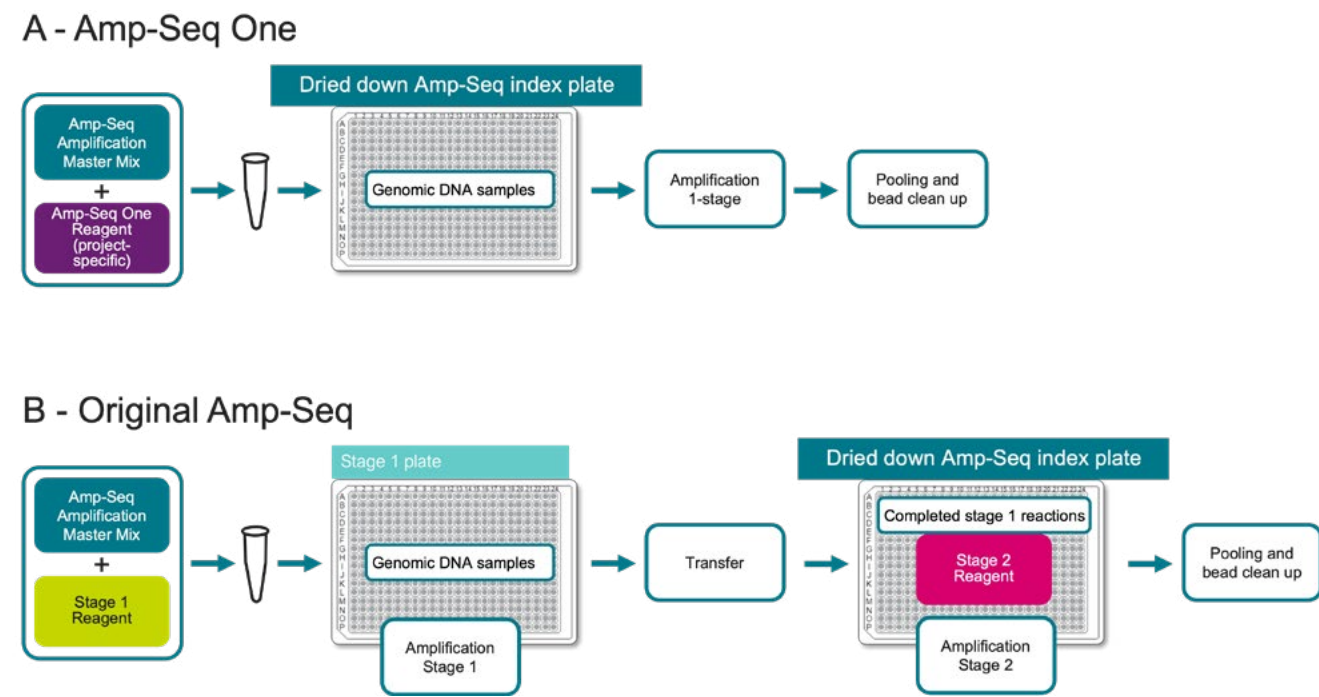


Figure 1. Comparison of Amp-Seq One and original Amp-Seq Reagent System workflows. Amp-Seq One (A) requires only one amplification reaction, fewer reagents and no transfer of materials between PCR stages. Original Amp-Seq (B) requires two amplification steps, transfer of completed stage 1 reactions into the stage 2 plate, and more individual reagents.

Table 1 provides a summary of the protocol parameters for Amp-Seq One and original Amp-Seq, illustrating the savings in library preparation time and consumables.

Protocol parameter	Targeted GBS technology		
	Amp-Seq One	Original Amp-Seq	Next fastest targeted GBS method
Amplification time	50 minutes	100 minutes	180+ minutes
Protocol turnaround time*	120 minutes	180 minutes	235+ minutes
Sample transfer**	None	Required	Required
Number of plates used per sample	1	2	2

Table 1. Summary of protocol parameters for Amp-Seq One, original Amp-Seq and the next fastest amplicon-based targeted GBS method.
*Turnaround time defined by processing one plate from starting materials to sequencing-ready library as prepared on standardly available liquid handling platforms.
**referring to sample transfer from PCR 1 plate to PCR 2 plate.

Application note

Amp-Seq One: Making plant and livestock genotyping simple

All samples utilised in this study underwent targeted genotyping by sequencing using both original Amp-Seq and Amp-Seq One workflows to facilitate direct comparisons between sequencing data generated using these methods with the exception of the dataset for the maize 5,200 panel that was generated using Amp-Seq One only.

For original Amp-Seq, the process was performed as per the Amp-Seq reagent system manual, and data analysis was performed using BiosearchCaller, as per the Amp-Seq data analysis software manual; both manuals can be requested using the form at the bottom of [this webpage](#). For Amp-Seq One, Amp-Seq Amplification Master Mix was combined with Amp-Seq One Reagent and then added to the dried down indexing plate, alongside DNA samples. A single 50 min amplification step was performed (as per table 2) before proceeding to pooling and bead clean up as per original Amp-Seq.

Temperature	Time	Cycles
95 °C	2 minutes	1 cycle
95 °C	15 seconds	24 cycles
67 °C***	1 minute	
4 °C	hold	

Table 2. Amp-Seq One cycling conditions.

*** Optimal temperature may vary due to thermal cycler specifics or the individual Amp-Seq panel. Expected range 66-68 °C.

Sequencing was performed on an Illumina® sequencer. A range of parameters were measured to facilitate comparisons between data obtained from original Amp-Seq and Amp-Seq One. These included uniformity, numbers of reads mapped to target, percentage of markers called, and concordance of genotype calls between the two technologies. Here, uniformity is defined as the percentage of

markers that are covered at a depth of $\geq 0.2X$ the average coverage depth.

Samples and Amp-Seq panels

Maize 1,920 panel

Bulk DNA was purified from maize seed, and data generated using a 1,920 marker panel for maize (B73, unmodified 2-stage panel). DNA input amount was 20 ng for both libraries. Optimised primer pool concentrations were used for library preparation, and this panel utilised an annealing temperature of 64 °C in the amplification step. Sequencing was performed to a depth of 200X.

Soy 1,100 panel

DNA was purified from 384 individual soy leaf punch samples using [sbeadex™ plant chemistry](#). Library preparation was performed using optimised primer concentrations. The soy 1,100 panel was an unmodified panel designed for original Amp-Seq. Average input DNA amounts were 2 ng (Amp-Seq One) and 1 ng (original Amp-Seq). For the 2-stage protocol, three bead clean ups were performed whilst for Amp-Seq One two bead clean ups were sufficient. Sequencing was performed to a depth of 200X.

Bovine 199 panel

DNA was isolated from bovine blood samples (n=22), originating from a range of different breeds. Library preparation was performed using optimised primer concentrations, and DNA input amount per sample was 1 ng. The dataset was generated using a 199 marker panel, developed to the International Society for Animal Genetics' (ISAG) recommended SNPs. Sequencing was performed to a depth of 200X, and two bead clean ups were performed.

Application note

Amp-Seq One: Making plant and livestock genotyping simple

Maize 5,200 panel

Bulk DNA was purified from maize seed, and data generated using a 5,200 marker panel for maize (B73, unmodified 2-stage panel). DNA input amount was 20 ng and optimised primer pool concentrations were used for library preparation. Sequencing was performed to depth of 200X and two bead clean ups were performed.

Results and discussion

Across the plant and livestock panels in this study, design compatibility is clearly demonstrated with panels developed for original Amp-Seq performing comparably

when utilised with Amp-Seq One. The datasets also illustrate that Amp-Seq One works with diverse input sample types, as shown with the biologically diverse soy leaf discs and bovine blood samples. The four high-quality sequencing datasets demonstrate typical genotyping call rates for plant and livestock species of greater than 90% and typical genotyping efficiencies of greater than 95% (on target genotyping rate). The ability to use Amp-Seq One to genotype large numbers of SNPs is also demonstrated with the data for the high-density maize 5,200 panel. The individual datasets will now be discussed in more detail.

Amp-Seq One sequencing data quality	Panel			
	Maize 1,920	Soy 1,100	Bovine 199	Maize 5,200
Reads mapped	98.7%	97.3%	98.1%	97.3%
Reads on target	96.6%	95.5%	95.3%	97.1%
Markers called	96.9%	92.6%	99.2%	99.0%
Uniformity	89.1%	89.5%	95.3%	95.0%

Table 3. Summary of Amp-Seq One sequencing data quality values for the four panels in this study.

For the 1920 maize panel, sequencing data quality values demonstrate comparable performance between original Amp-Seq and Amp-Seq One (figure 2). For both the original protocol and the Amp-Seq One protocol, percentage of reads mapped to the reference genome was greater than 98.5%, and the percentage of reads mapped to the target SNP location was greater than 95%. Genotyping call rates and uniformity (read depth consistency across markers) were also comparable between both methods.

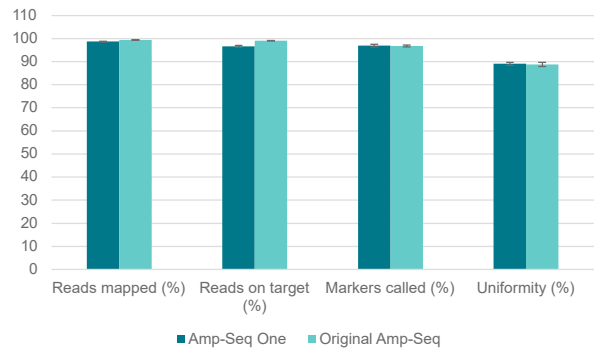


Figure 2. Dataset for 1,920 marker panel for maize, illustrating comparable performance between Amp-Seq One and original Amp-Seq.

Application note

Amp-Seq One: Making plant and livestock genotyping simple

Results obtained with the 1,100 soy panel with diverse biological samples demonstrate almost identical performance between the two technologies, with no loss of sequencing data quality when using the streamlined Amp-Seq One protocol. Figure 3 illustrates the sequencing data quality values for original Amp-Seq and Amp-Seq One for the 1,100 soy panel. Percentage of reads mapped to the reference genome was greater than 97% and the percentage of reads mapped to the target SNP location was greater than 95%. Genotyping call rates (92%) and uniformity (89%) were also identical between both methods. Genotyping concordance for this panel, between genotypes called using Amp-Seq One and original Amp-Seq, was 98.82%.

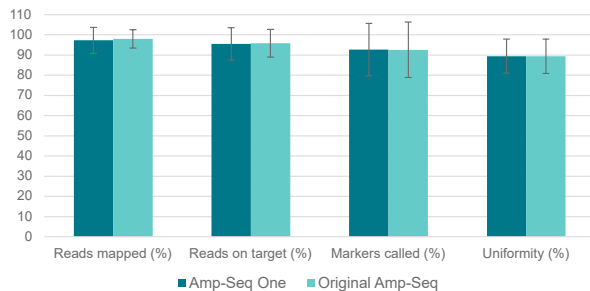


Figure 3. Dataset for 1,100 marker panel for soy, illustrating almost identical performance between Amp-Seq One and original Amp-Seq.

For the bovine 199 panel, high quality sequencing data was obtained from 22 samples of various breeds for both original Amp-Seq and Amp-Seq One technologies (figure 4). Over 98% of reads were mapped to the reference genome and over 95% of reads were mapped to the target SNP location. Uniformity was comparable between both methods and overall genotyping call rates were higher for Amp-Seq One than for original Amp-Seq. Genotyping concordance for this panel, between genotypes called using Amp-Seq One and original Amp-Seq, was 99.42%.

Concordance with ISAG genotypes was also >98.9% for both Amp-Seq One and original Amp-Seq.

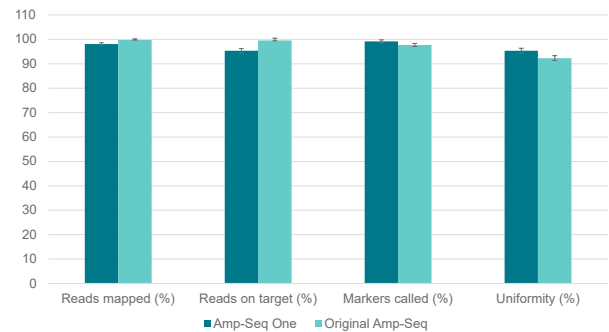


Figure 4. Dataset for 199 marker panel for bovine, illustrating comparable performance between Amp-Seq One and original Amp-Seq.

The high density (5,200) panel for maize generated high quality sequencing data as illustrated in figure 5. Overall genotyping call rate for this panel was over 99% and 97% of reads were mapped to the reference genome.

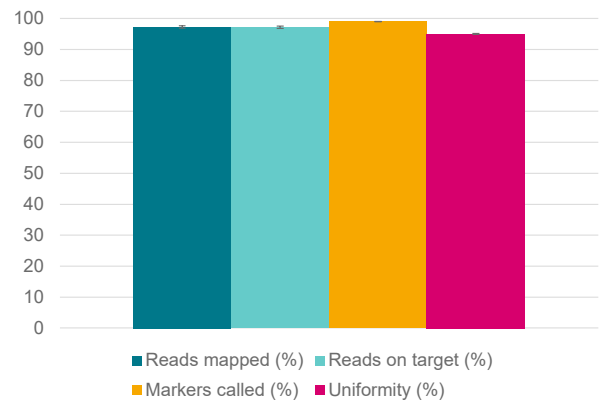


Figure 5. Dataset for 5,200 marker panel for maize, illustrating high quality sequencing results obtained with Amp-Seq One and a high density panel.

LGC have an expert panel design team who work on each panel to maximise design rates and to address the panels' unique design challenges. For the four panels presented, the *in-silico* design to function assay conversion rates were all high.

Application note

Amp-Seq One: Making plant and livestock genotyping simple

Summary

These datasets demonstrate comparable performance of Amp-Seq One technology with original Amp-Seq on plant and livestock samples, presenting high-quality genotyping data generated through ultra-high-throughput NGS targeted sequencing. Panels developed for original Amp-Seq have design compatibility with Amp-Seq One, and the Amp-Seq One technology works with diverse input sample types. In addition, Amp-Seq One technology is compatible with high density panels, as the high-quality data for the maize 5,200 panel demonstrates.

Our Amp-Seq One protocol has a simplified workflow that is highly automatable and requires just one PCR stage. This offers the benefit of doubling throughput whilst reducing consumable costs. This ultra efficient approach uses room temperature stable reagents, cuts library preparation time to an industry leading 120 minutes, and enables same-day progression from DNA sample to library creation and sequencing. This innovative product is the latest addition to LGC Biosearch Technologies' portfolio of genotyping tools that are transforming agriculture.

X f in @LGCBiosearch | biosearchtech.com

All trademarks and registered trademarks mentioned herein are the property of their respective owners. All other trademarks and registered trademarks are the property of LGC and its subsidiaries. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details. No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording or any retrieval system, without the written permission of the copyright holder. © LGC Limited, 2024. All rights reserved. GEN/1197/SW/1224

BIOSEARCH[™]
TECHNOLOGIES