



Automated genomic DNA extraction and high-throughput SSR/SNP genotyping sample preparation using apricot[®] liquid handling systems

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Summary

Marker-assisted breeding programs in agriculture rely on high-throughput genotyping, which in turn depends on consistent, high-quality genomic DNA and reproducible, error-free PCR set-up. In this application note, ACSEN Agriscience and SPT Labtech describe a semi-automated workflow that pairs the apricot[®] S1 liquid handling system for genomic DNA extraction from plant leaf tissue with the apricot[®] S3 liquid handling system for downstream PCR sample preparation and post-PCR processing ahead of SSR and SNP genotyping on a ZAG Fragment Analyzer. The workflow reduces manual pipetting, standardizes reagent dispensing across plates, and supports the throughput required for routine molecular marker analysis.

Introduction

Simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) genotyping are core tools in modern plant breeding, underpinning marker-assisted selection, germplasm characterization, and trait mapping. As breeding programs scale up sample numbers, the manual steps involved in DNA extraction, normalization, and PCR set-up become a bottleneck, introducing variability and increasing the risk of pipetting error.

To address this, ACSEN Agriscience implemented an automated liquid handling workflow spanning two stages of the genotyping pipeline. The apricot S1 automates repetitive liquid handling steps in plant genomic DNA extraction, including buffer dispensing, reagent addition, mixing, lysate transfer, precipitation reagent addition, wash buffer dispensing, and DNA elution. Downstream, the apricot S3 liquid handling system automates DNA aliquoting, normalization, PCR master mix dispensing, DNA dispensing into 96- and 384-well PCR plates, and post-PCR dilution ahead of fragment analysis, supporting SSR genotyping, SNP genotyping, and fragment analysis workflows.

This application note outlines the automated protocols used for both stages of the workflow and presents representative DNA quality and yield data generated from plant leaf samples processed using the apricot S1 and S3.



Materials and methods

1. Automated genomic DNA extraction from plant leaf tissue (apricot S1)

Principle

The apricot S1 automates repetitive liquid-handling steps in the plant genomic DNA extraction workflow, including:

- Buffer dispensing
- Reagent addition
- Mixing
- Transfer of lysate
- DNA precipitation reagent addition
- Wash buffer dispensing
- DNA elution

Protocol

1. Add 10–15 mg of plant leaf tissue into DNA blocks and add Buffer A.
2. Grind the plant tissue using a tissue lyser and centrifuge at 3,000 rpm for 5 min.
3. Add Buffer B to the ground tissue.
4. Incubate the ground tissue in a hot air oven at 65°C for 50 min.
5. Add organic solvent for purification and mix the contents.
6. Centrifuge at 10,000 rpm for 15 min.
7. Carefully transfer the upper aqueous layer to a new DNA block.

8. Add an equal volume of precipitating buffer to the aqueous layer and mix gently.
9. Incubate at –20°C for 30 min, then at room temperature for 10 min.
10. Mix the total volume and centrifuge at 10,000 rpm for 15 min.
11. Discard the supernatant.
12. Add washing buffer and centrifuge at 10,000 rpm for 10 min.
13. Discard the supernatant.
14. Add washing buffer and centrifuge at 10,000 rpm for 10 min.
15. Discard the supernatant.
16. Incubate the tubes at 65°C for 10 min to dry the DNA pellet.
17. Add elution buffer to the DNA pellet and incubate the tubes at 65°C for 10 min.
18. Store the eluted DNA at 5°C for immediate use or at –20°C for long-term storage.

2. DNA quantification and quality assessment

Extracted DNA (n = 15 plant leaf samples) was quantified spectrophotometrically. Absorbance readings at 260 nm, 280 nm, and 320 nm were used to calculate the 260/280 purity ratio and DNA concentration for each sample (Table 1).

Read #	Sample	Location	260 Raw	280 Raw	320 Raw	260	280	260/280	ng/μL
1	SPL1	B2	0.117	0.076	0.039	0.069	0.032	2.136	68.676
1	SPL2	C2	0.114	0.076	0.040	0.066	0.031	2.114	65.641
1	SPL3	D2	0.110	0.073	0.038	0.063	0.030	2.129	63.206
1	SPL4	E2	0.107	0.071	0.038	0.061	0.028	2.139	60.790
1	SPL5	F2	0.099	0.070	0.041	0.051	0.024	2.080	50.947
1	SPL6	G2	0.098	0.069	0.039	0.051	0.026	2.015	51.397
1	SPL7	H2	0.115	0.078	0.041	0.067	0.032	2.051	66.621
1	SPL8	A3	0.109	0.075	0.040	0.061	0.030	2.022	60.969
1	SPL9	B3	0.092	0.066	0.039	0.046	0.023	2.034	45.893
1	SPL10	C3	0.107	0.072	0.039	0.061	0.029	2.086	60.659
1	SPL11	D3	0.095	0.067	0.038	0.049	0.024	2.052	49.182
1	SPL12	E3	0.084	0.059	0.038	0.039	0.017	2.254	38.730
1	SPL13	F3	0.101	0.070	0.039	0.055	0.026	2.067	54.620
1	SPL14	G3	0.103	0.070	0.038	0.059	0.028	2.080	58.749
1	SPL15	H3	0.098	0.068	0.038	0.053	0.025	2.086	52.704

Table 1. Quantification of genomic DNA isolated from 15 plant leaf samples using the apricot S1 liquid handling system.

DNA integrity was further assessed by agarose gel electrophoresis (Figure 1).

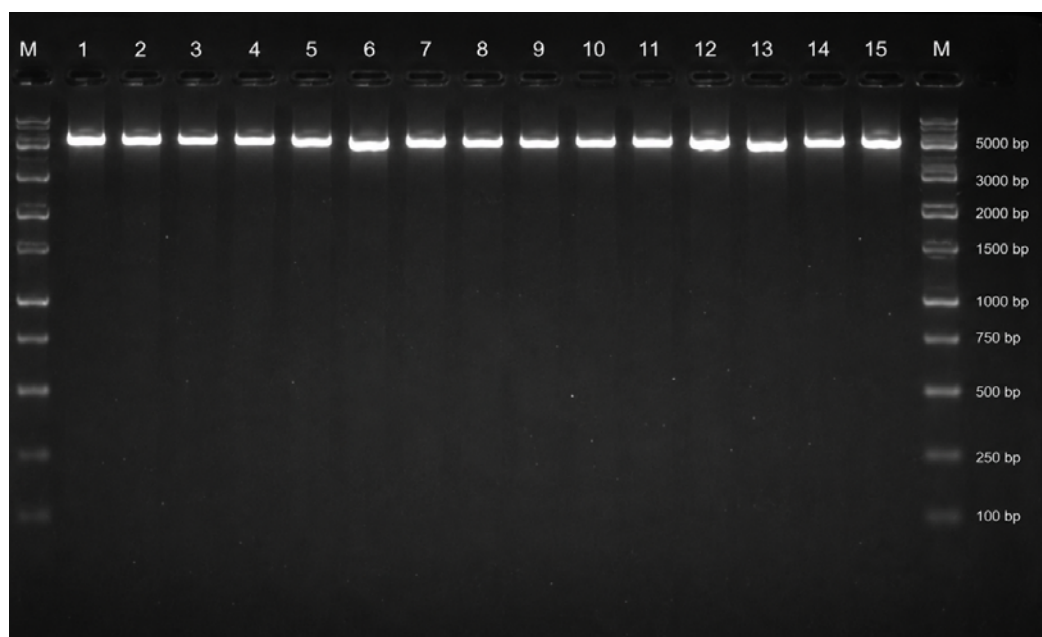


Figure 1. Agarose gel electrophoresis of genomic DNA isolated from 15 plant leaf samples using the apricot S1 liquid handling system.

3. High-throughput PCR sample preparation and post-PCR processing (apricot S3)

Principle

The apricot S3 liquid handling system automates repetitive liquid handling tasks involved in PCR set-up and post-PCR sample preparation. The system provides high precision, reproducibility, and minimizes manual pipetting errors during molecular marker analysis, and is routinely used for SSR genotyping, SNP genotyping, and fragment analysis in high-throughput laboratories. The apricot S3 automates:

- DNA aliquoting
- DNA normalization (dilution)
- PCR master mix dispensing
- DNA dispensing into PCR plates
- SNP reaction set-up in 384-well plates
- Post-PCR dilution
- Sample preparation for the ZAG Fragment Analyzer

3.1 Pre-PCR workflow

3.1.1 DNA aliquoting

Principle: Genomic DNA is transferred from DNA storage blocks into PCR plates using the apricot S3.

1. Load DNA storage blocks onto the apricot S3 deck.
2. Place a sterile 96- or 384-well PCR plate on the destination deck.
3. Select the DNA aliquoting protocol.
4. Transfer the required DNA volume into each PCR well.
5. Seal the PCR plate.
6. Store until PCR set-up.

3.1.2 DNA dilution for SNP genotyping

Principle: DNA samples are normalized to the required concentration before SNP genotyping to ensure uniform amplification.

1. Load the DNA plate.
2. Load the TE buffer reservoir.
3. Select the DNA dilution protocol.
4. The apricot S3 dispenses TE buffer.
5. DNA is mixed automatically.
6. Normalized DNA is transferred to the PCR plate.

3.1.3 Master mix dispensing for SSR PCR

Principle: The apricot S3 dispenses equal volumes of PCR master mix into all wells, reducing variation caused by manual pipetting.

1. Prepare the SSR PCR master mix.
2. Load the reagent reservoir.
3. Load the PCR plate.
4. Select the dispensing protocol.
5. Dispense master mix into each well.
6. Seal the plate.

3.1.4 DNA dispensing into 384-well PCR plate

Principle: Normalized DNA is transferred into 384-well PCR plates for high-throughput PCR.

1. Load the DNA source plate.
2. Load the 384-well PCR plate.
3. Select the transfer program.
4. Dispense DNA into PCR wells.
5. Seal the plate.

3.1.5 Master mix dispensing for SNP PCR

Principle: The apricot S3 dispenses SNP PCR master mix uniformly into 384-well PCR plates.

1. Prepare the SNP master mix.
2. Load the reagent reservoir.
3. Load the PCR plate.
4. Dispense master mix.
5. Add DNA samples.
6. Seal the plate.
7. Transfer to the PCR thermal cycler.

3.2 Post-PCR processing for fragment analysis

3.2.1 Preparation of PCR products for the ZAG Fragment Analyzer

Principle: Following PCR amplification, products are diluted automatically using the apricot S3 prior to fragment analysis.

1. Load the PCR plate.
2. Load the buffer reservoir.
3. Dispense 24 μ L sample buffer into a new analysis plate.

4. Transfer 2 μ L PCR product into the buffer.
5. Mix automatically.
6. Seal the plate.
7. Load the plate into the ZAG Fragment Analyzer.

3.2.2 Plate preparation for the ZAG Fragment Analyzer

Principle: The prepared plate is used directly for automated capillary electrophoresis.

1. Verify the plate layout.
2. Centrifuge briefly.
3. Load the plate into the ZAG fragment analyzer.
4. Start electrophoresis.
5. Analyze fragment size and genotype.

Complete workflow

Figure 2 summarizes the full, semi-automated workflow from plant leaf sample to SSR/SNP genotyping result, spanning DNA extraction on the apricot S1 and PCR/post-PCR processing on the apricot S3.

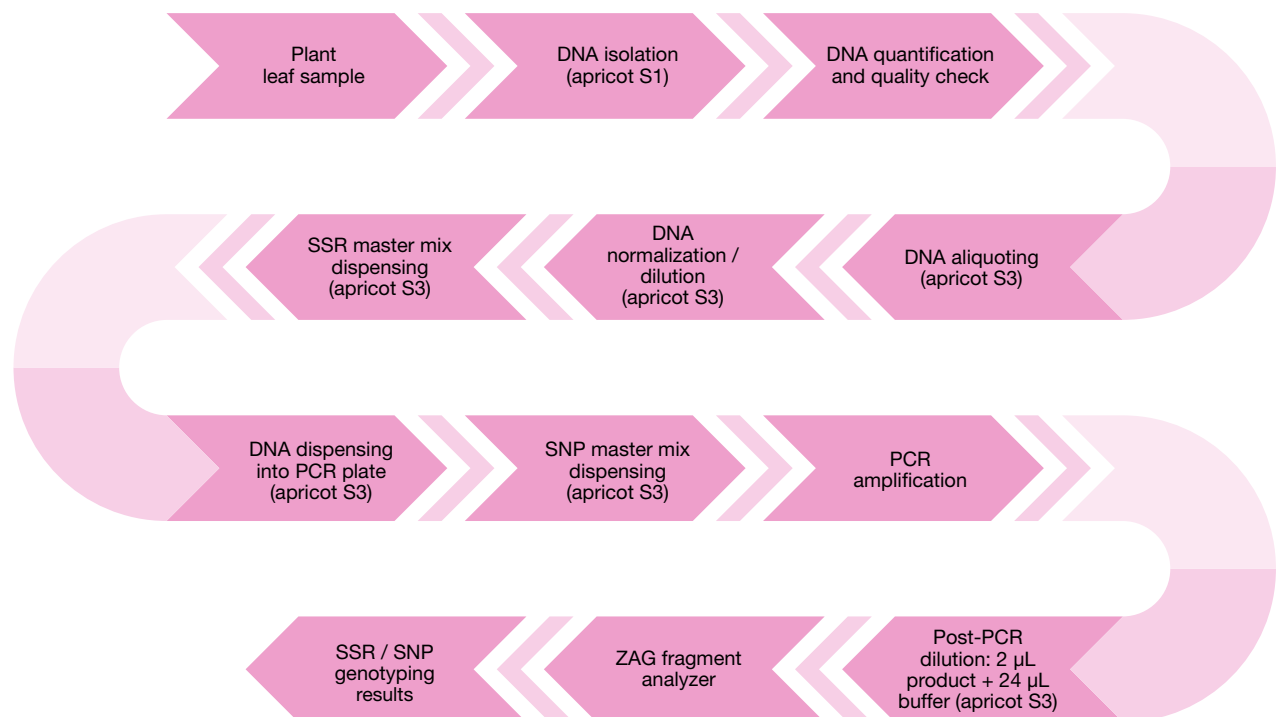


Figure 2. Complete automated workflow from plant leaf sample to SSR/SNP genotyping result.

Results

Genomic DNA extracted from 15 plant leaf samples using the apricot S1 yielded consistent, high-quality DNA suitable for downstream PCR-based marker analysis. Mean DNA concentration across the sample set was approximately 56.6 ng/μL (range 38.7–68.7 ng/μL), with a mean A260/A280 ratio of approximately 2.09 (range 2.02–2.25), indicating minimal protein or reagent carryover (Table 1).

Agarose gel electrophoresis confirmed that all 15 samples produced a single, high-molecular-weight genomic DNA band above 5,000 bp with no visible smearing, consistent with intact, non-degraded DNA (Figure 1).

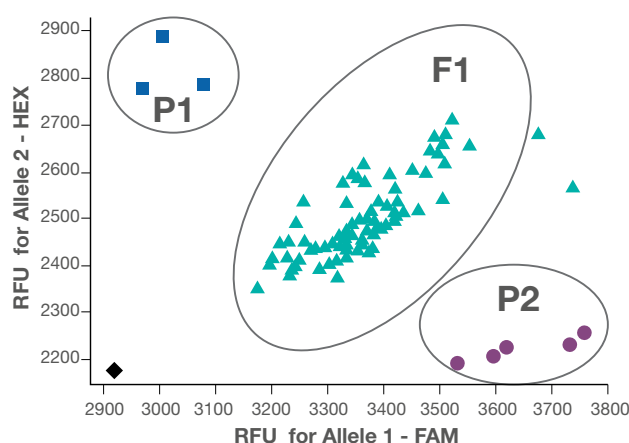


Figure 3. Allelic discrimination plot from a representative SNP genotyping run on DNA prepared using the apricot S3. Blue and purple points: homozygous positive controls; black point: negative control; green points: heterozygous calls.

Downstream, the apricot S3 automated the DNA aliquoting, normalization, PCR master mix dispensing, and post-PCR dilution steps required to prepare SSR and SNP reactions in 96- and 384-well formats ahead of fragment analysis on the ZAG Fragment Analyzer, standardizing reagent volumes across plates and reducing manual pipetting during high-throughput genotyping runs.

SNP genotyping calls generated from DNA prepared using the apricot S3 workflow showed clear allelic discrimination, with sample clusters separating cleanly from the assay controls (Figure 3). Blue and orange points represent the homozygous positive controls, the black point represents the no-template negative control, and green points indicate heterozygous calls.

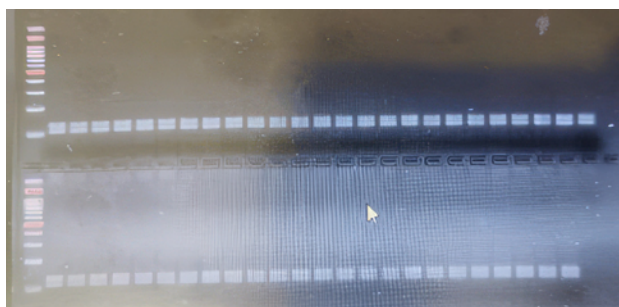


Figure 4. Agarose gel electrophoresis of PCR products amplified from DNA prepared using the apricot S3 liquid handling system.

PCR products amplified from apricot S3-prepared reactions were also verified by agarose gel electrophoresis, confirming consistent, specific amplification across sample wells (Figure 4).

Conclusion

This application note demonstrates a two-stage, semi-automated workflow combining the apricot S1 for plant genomic DNA extraction with the apricot S3 for PCR sample preparation and post-PCR processing. Key advantages of this approach include:

- Consistent, high-quality DNA extraction from plant leaf tissue, evidenced by high 260/280 purity ratios and intact high-molecular-weight DNA on gel electrophoresis.
- Reduced manual pipetting and improved reproducibility across DNA normalization, master mix dispensing, and post-PCR dilution steps.
- Support for high-throughput SSR genotyping, SNP genotyping, and fragment analysis workflows in 96- and 384-well formats.
- A streamlined path from plant leaf sample to genotyping result, integrating directly with the ZAG Fragment Analyzer.

Together, the apricot S1 and apricot S3 liquid handling systems provide ACSen Agriscience with a scalable automation platform for routine molecular marker analysis in plant breeding programs. ■

For more information, please visit:

ACSEN Agriscience: acsenagriscience.com

SPT Labtech: sptlabtech.com

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SPT Labtech makes products that transform the way scientists work. For nearly two decades, our expert scientists, engineers, and business innovators have created innovative solutions for liquid handling, sample preparation and management that help accelerate research and make a real difference to human health. We work collaboratively with our customers, building trusted relationships that enable us to deliver exceptional, personalized experiences designed for real world challenges in the lab.

About ACSEN Agriscience

ACSEN Agriscience, founded in 2009 and formerly known as ACSEN HyVeg, works to improve the lives and livelihoods of Indian farmers and has grown into a global agri-biotech company focused on vegetable seeds and row crops. Guided by its purpose of more profit per acre for more farmers, year after year, the company develops high-yielding, climate-resilient, and pest-resistant seed varieties backed by a large R&D land base and a network of R&D satellite centers.

