

Discovery of Single Nucleotide Polymorphisms in Hexaploid Wheat and Scoring by Invader Operating System

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SNPs in wheat can be scored using Invader operating system.

INTRODUCTION

Single nucleotide polymorphisms (SNPs) are now proven to be a very useful tool in deciphering genetic variations because they are highly abundant and well suited for automated, high throughput genotyping platforms. The wheat genome is large, hexaploid and contains excessive amounts of both repeated DNA and paralogous gene families. These drawbacks present difficulties for the discovery, validation and high throughput scoring of SNP markers. We have applied the Invader operating system to genotype wheat varieties using SNPs as markers.

MATERIALS & METHODS

Wheat cDNA libraries

↓ Select unique expressed sequences (using CAP3 assembly program).

↓ CAP3 singleton expressed nuclear genes (325 genes)

↓ Design PCR primers (at the more polymorphic 3'-end of the genes).

↓ PCR primer pairs (325 primer pairs)

↓ Sequence 8 selected wheat varieties for each gene.

↓ Sequence alignment of 8 wheat varieties (Figure 1a)

↓ Identify putative SNPs and Indels (Insertion/Deletions).

↓ Design SNaPshot primers.

SNaPshot primers

↓ Run SNaPshot kit.

↓ The SNaPshot primer is extended at the 3'-end by just one fluorescently labeled nucleotide (ddNTPs).

↓ Read SNaPshot Assays on ABI 3100 Genetic Analyzer.

SNaPshot Results

↓ Compare the extended single base with that of the original sequences.

↓ Validated SNPs by SNaPshot (Figure 1b)

↓ Design and synthesize the Invader and signal (primary) probes for Invader assays.

Invader Kit

↓ Perform Invader assays with genomic DNAs. (Figure 2)

↓ Compare the Invader results with SNaPshot results.

↓ Validated SNPs by Invader Assay (Figure 1c)

RESULTS AND DISCUSSION

For 15 Canadian wheat varieties/genotypes, 114 confirmed SNPs among these varieties were identified by utilizing DNA sequence alignment (Figure 1a) and SNaPshot assays. This yielded an average of one SNP per 370 bp. Forward and

reverse SNaPshot primers were designed to interrogate the same SNP and both primers were extended by the expected single base at the SNP position (Figure 1b). This primer extension assay can distinguish all 4 possible SNP alleles simultaneously, at a single SNP site using the 4-color nucleotides and capillary electrophoresis on ABI3100. At many SNP sites some varieties showed two alleles reflecting the hexaploid nature of wheat.

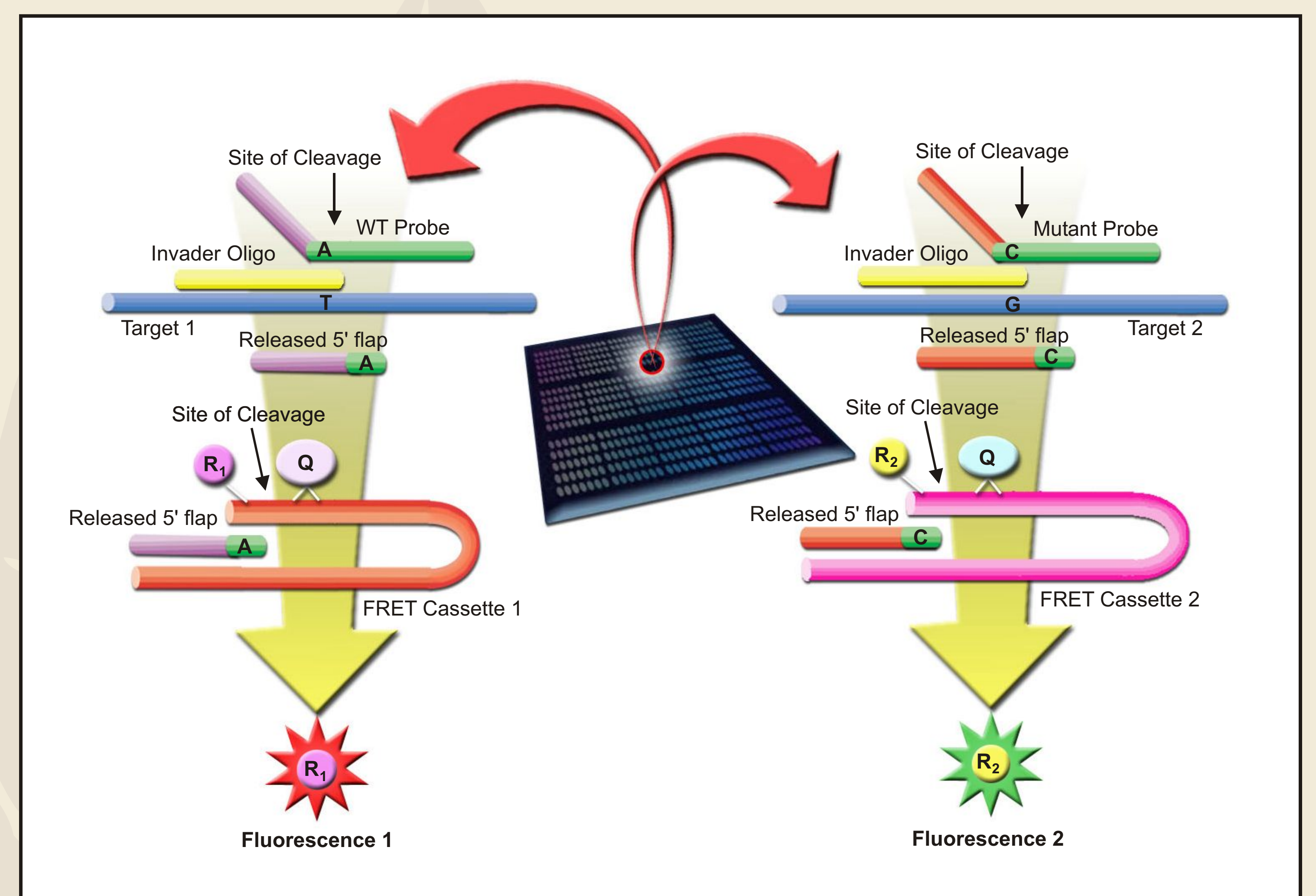


Figure 2. Both alleles of an SNP can be quantified from the fluorescence generated by each of the bplex Invader reactions.

The Invader operating system requires no prior PCR amplification of genomic DNA for target sequence generation (Figure 2). All of the reagents, except for the target specific oligonucleotides, are dried down in a 96-well microplate. Sample DNAs were added and the plate was read by a fluorescence reader after 3 hours of incubation (Figure 1c). All 4 selected SNP sites for 5 different varieties corroborated the confirmed SNPs. The assays are quantitative and can determine the amount of genotype mixture in a seed bag. These results showed that the Invader assay could be used for plants having a hexaploid genome. The Invader operating system is reliable, quantitative, inexpensive and has a high throughput for SNP scoring.

Figure 1a

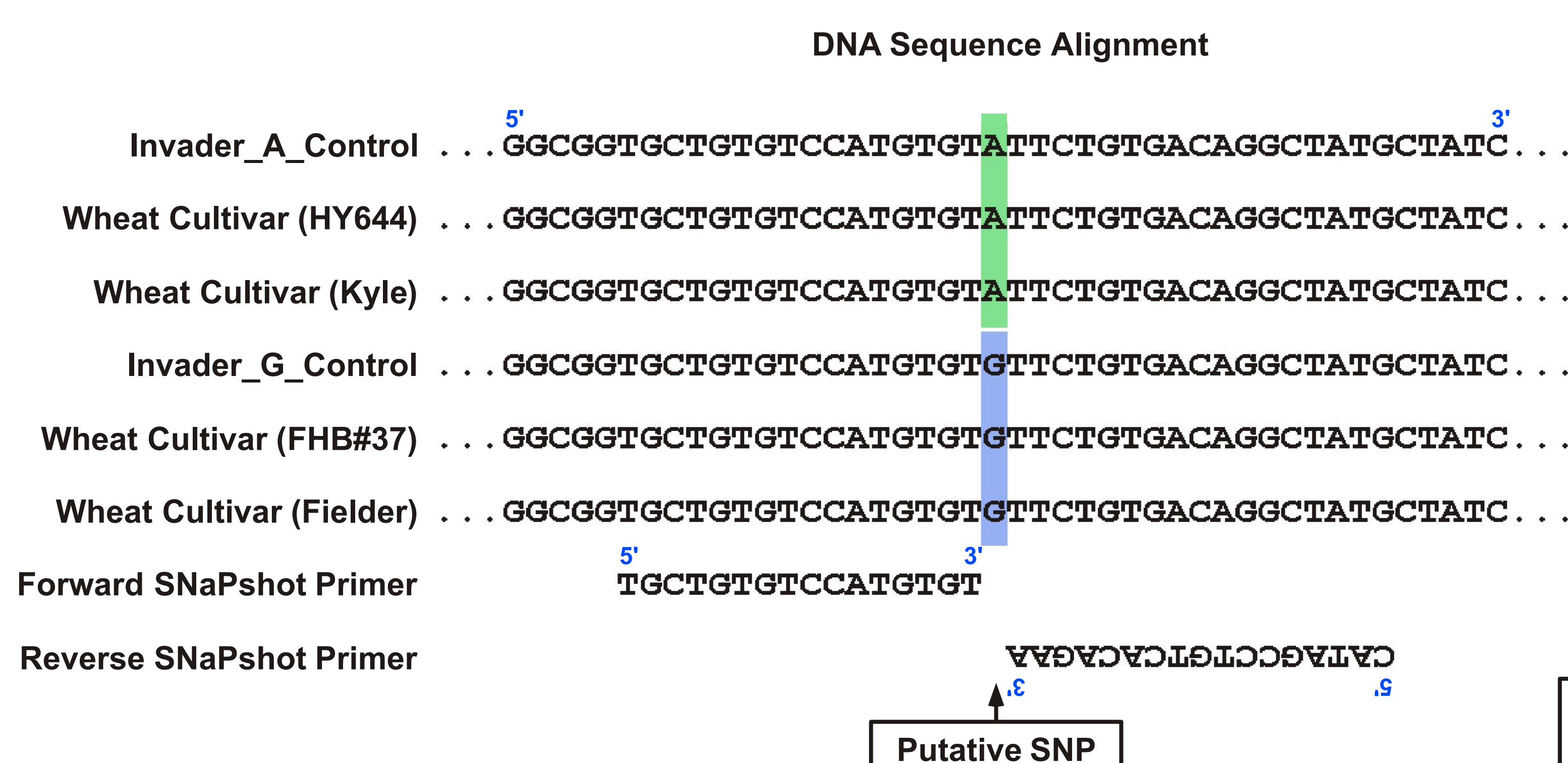


Figure 1b

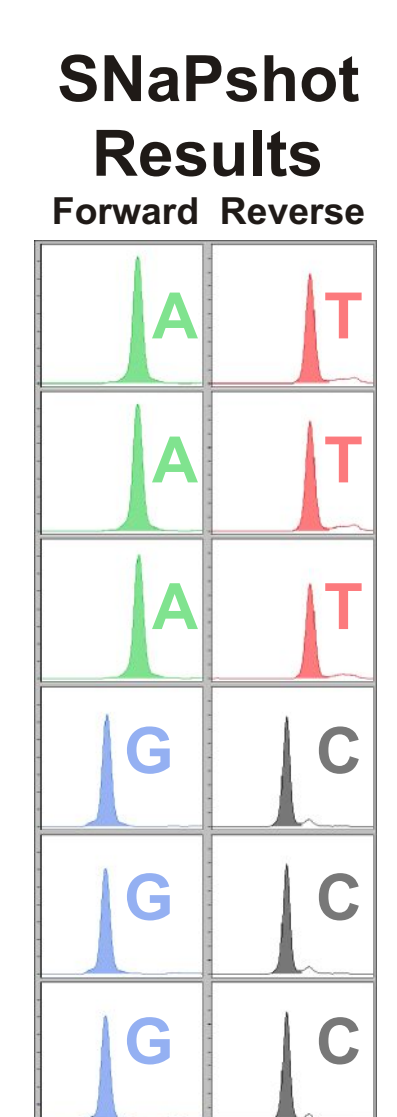
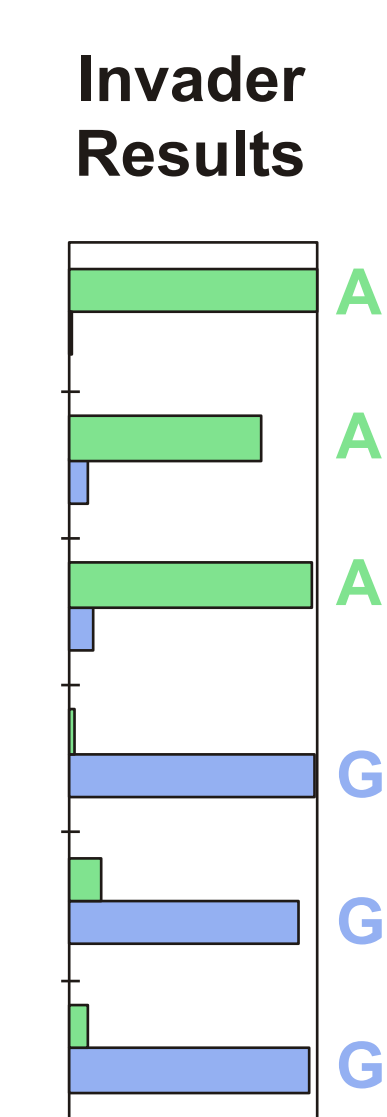


Figure 1c



SNaPshot Validated SNP

Invader Validated SNP

Figure 1a. DNA sequences of 4 wheat varieties are aligned to identify a putative SNP and to design SNaPshot primers and Invader probes.

Figure 1b. SNaPshot results of the SNP position from the forward & reverse SNaPshot primers. The SNaPshot results validate the SNP site.

Figure 1c. Invader results of the SNP position. The Invader results also validate the SNP site.

ACKNOWLEDGEMENTS

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