Application of a Simple SNP Detection Method for Mapping and Association Studies

We demonstrate the application of agarose-based TILLING (Targeting Induced Local Lesions IN Genomes) for mapping and association studies. Using the agarose-based TILLING procedure we were able to detect SNPs in pools of 8 DNA templates and expand the window for SNP detection by analyzing large amplicons (~3 kb). Using mapping populations and pedigrees in breeding programs, we evaluated SNP polymorphism of candidate genes showing differential expression patterns during host-pathogen interaction. We applied this technique for mapping oxalate oxidase, a candidate defense gene in RILs obtained from parental lines Sanhuangzhan 2 (SHZ-2) (*indica*) and Lijiangxin-tuan-heigu (LTH) (*japonica*). Results suggested that mismatch detection offers a simple way to track specific genes in breeding pedigrees and segregating populations without upfront investment in sequencing.

POOLING CAPACITY

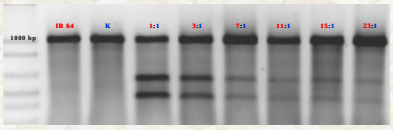


Figure 1. *Evaluation of pool capacity. DNA from the genotypes IR64 and Kun Min Tsieh Hunan (K) were combined in ratios indicated above the lanes (IR64: H). The TPP gene (Table 3) was PCR amplified, subjected to heteroduplex formation followed by CEL I digestion.*

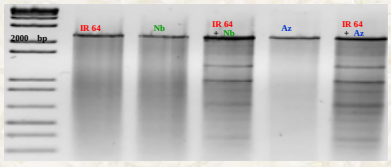
DNA pooling:

Pool capacity depends on
1. **efficiency of PCR**
2. **number of expected SNPs between samples**

TILLING – a pool consists of DNA from 8 plants. Currently we use 8-fold pools to scan our EMS mutant population (2,400 M2 lines)

EcoTILLING – DNA of two genotypes are contrasted in a 1:1 ratio. In rice we contrast the germplasm varieties to IR64 (*indica*) and Nipponbare (*japonica*).

AMPLICON SIZE



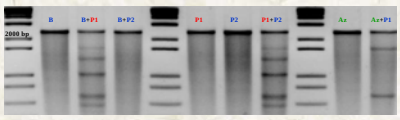
Amplicon size:

Agarose - amplicon size could be as large as 3 kb; run-time within 2 hours

LiCor genotyper – recommended amplicon size 1.5-2 kb; run-time 5 hours

Figure 2. *EcoTILLING of the MYB1 gene (Table 2) between IR64, Nipponbare (Nb) and Azucena (Az).*

APPLICATION



This low-cost approach of mismatch detection offers a simple way to track specific genes in breeding pedigrees and segregating populations without upfront investment in sequencing.

Figure 3. *Detecting the parental allele carried by a BC3F3 line for the MYB1 gene (Table 2). P1-Sanhuangzhan 2 (SHZ-2) (indica); P2-Texianzhan-13 (TXZ-13) (indica), B-BC3F3 line and Az-Azucena. SNPs were detected between the BC10 and SHZ2 (indicating the allele carried by BC10 for MYB1 was of TXZ-13 type); SHZ-2 and TXZ – 13; and also SHZ-2 and Az. The full length product is 2.3 kb.*

Table 1. Comparison between agarose and LiCor-based EcoTILLING.

Parameter	SNP detection on agarose gel	SNP detection using the LiCor Genotyper
DNA Pooling	2 per pool for EcoTILLING; 8 per pool for TILLING	2 per pool for EcoTILLING; 8 per pool for TILLING
PCR primer	Unlabeled	Labeled (IRD 700 and IRD 800)
Amplicon size	2- 3 kb	1.2-1.5 kb
Post-CEL cleavage clean up	None	Removal of excess salt and concentrating the sample required
Gel Analysis	Agarose / ethidium bromide	Polyacrylamide gel / LiCor genotyper
Overall time for electrophoresis	2 hours	5 hours
Resolution of closely spaced SNPs	May not be distinguishable; hence having single amplicon is critical	Distinguishable, less critical to have single amplicon
Relative cost	Low	High

Table 2. Genes and primer sequences used in the study. (O.O –oxalate oxidase)

Gene Name	TIGR Locus ID	Primer (F-forward; R-reverse)
MYB1	Os12g41920	F - ATAATATgggTgCCCCgAAgCAgAAAT
		R - TCCTACAgTTCTCCACTTCCCCATTCC
TPP	Os02g44230	F - ggCACACTgTCgCCTATTgTggATg
		R - gTTTACgAgCCgTgCgACCAgTTTC
O.O	Os080g09000	F - AgTTAgCTTCCCATCAgAgAAAgA
		R- ggTTgTTTgCAAgCACACATAAT
O.O	Os080g09020	F- gCTTAATTgACTTgTTCTCCATCA
		R – AgTCCgTAAgCggACAATATTTAg

Mapping Procedure

- **Candidate gene (CG) is amplified from mixed genomic DNA of the parental lines**
- **The PCR product is subjected to heteroduplex formation and digested with CEL I endonuclease.**
- **The presence of cleaved products indicates the presence of SNPs between the parental lines.**
- **Genomic DNA of individual lines of the corresponding mapping population is combined with that of either parents and used as template for TILLING of the CG.**
- **The absence of cleaved products indicates that the line carries the same allele as that of the parent that it was combined with. The presence of cleaved products indicates the alternative allele.**

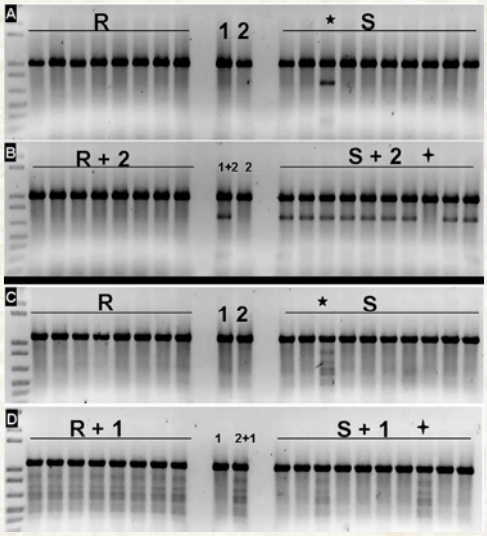


Figure 4. *Detection of parental allele carried by the 18 RILs that exhibited extreme phenotypes. R – resistant; S – Susceptible; 1 – LTH; 2 – SHZ-2.*
★ *indicates a susceptible RIL that is heterozygous for both loci (A, C) and*
✝ *indicates a putative RIL (B, D). A and B – Os08g09000; C and D – 08g09020 (Table 2)*

The CEL I assay was performed on the entire RIL population (n=215) for two oxalate oxidase genes. SNPs were detected for both genes. Single marker analysis was subsequently performed using the SNP marker and trait data. R^2 values were consistent with the previous QTL mapping results.

Table 3. Single marker analysis for candidate genes encoding members of the oxalate oxidase family

		LD PO6	LS PO6	DLA (IRRIGH)	DLA (GDGH)
Os08g09000	R^2 (%)	5.7	6.7	21.3	19.6
	p-value	0.007	0.004	< 0.001	< 0.001
Os08g09020	R^2 (%)	4.5	6.7	19.1	25.4
	p-value	0.016	0.004	< 0.001	< 0.001

LD: Lesion density
LS: Lesion size
DLA: Diseased leaf area

PO6 blast isolate
IRRIGH: IRRI glass house
GDGH: Guangdong glass house

Summary & Future work

- **Developed a simplified procedure of detecting CEL-1 cleaved products on conventional agarose gels**
- **This cost-effective method enables laboratories from developing countries to participate more easily in SNP and gene function discovery**
- **Applications in mapping**
  - **Useful tool in cases where levels of polymorphisms between parental lines are low eg. within *japonica* lines or other crops such as wheat**
  - **Target gene selection**
  - **Fine mapping**
  - **Bulk-segregant analysis and QTL mapping**
- **Due to the abundance of SNPs most candidate genes identified through gene expression studies can be used for mapping and pedigree analysis**
- **The use of SNP detection may provide a useful bridge to evaluate orthologous markers across species**

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