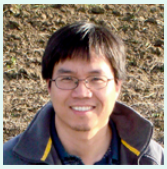


Gene Mapping via Bulked Segregant RNA-Seq (BSR-Seq)

Sanzhen Liu, Cheng-Ting Yeh, Ho Man Tang, Dan Nettleton, Patrick S. Schnable
Iowa State University, Ames, IA 50011-3650

Introduction

Bulked segregant analysis (BSA) is an efficient method to rapidly and efficiently map genes responsible for mutant phenotypes. We have developed a modification of BSA (BSR-Seq) that makes use of RNA-Seq reads to efficiently map genes even in populations for which no polymorphic markers have been previously identified.

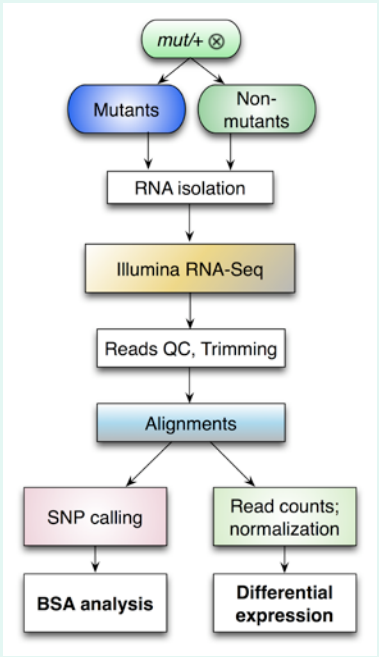
BSR-Seq

1. **Maps causal mutant gene to a small chromosomal interval**
2. **Provides genetic markers for fine-scale mapping**
3. **Defines differential expression in this chromosomal interval**
4. **Yields genome-wide transcription profiles**
5. **Works for any species with a sequenced genome**

BSR-Seq Experiment

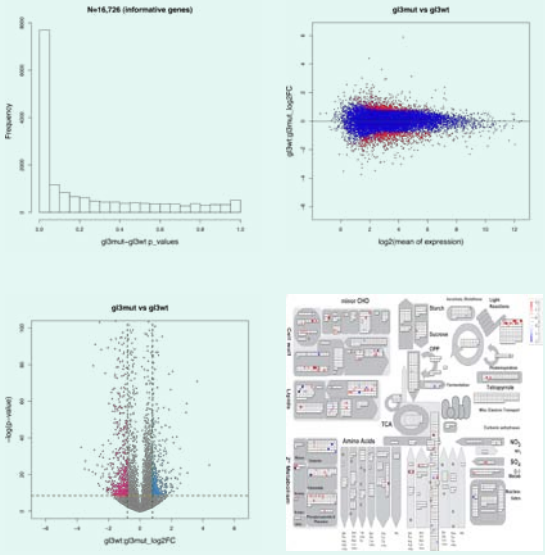

Mutants


Non-mutants



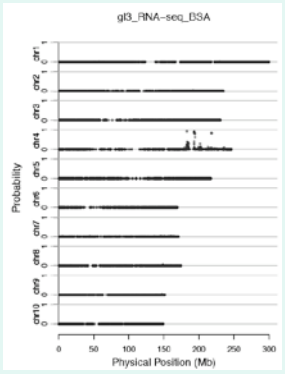
A recessive mutant, *gl3*, exhibits altered accumulation of epicuticular waxes on juvenile leaves. The mutant pool and the non-mutant pool were subjected to RNA-Seq.

Differential Expression



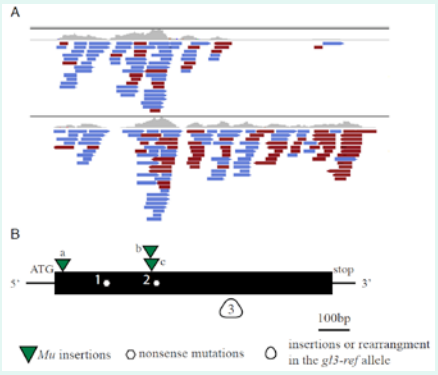
16,726 maize genes exhibit at least 40 uniquely mapped reads from the combined mutant and non-mutant pools. Of the 1,095 significantly differentially expressed genes, 446 were down-regulated and 633 were up-regulated in the *gl3* mutants as compared to their non-mutant siblings. MapMan was used to visualize the differential expression in various pathways.

Bulked Segregant Analysis



By subjecting the reference allele to BSR-Seq, we were able to map the *gl3* locus to a ~2 Mb interval by using a sophisticated empirical Bayesian approach.

Gene Cloning



The single gene located in the ~2 Mb mapping interval whose expression was down-regulated in the mutant pool was subsequently demonstrated to be the *gl3* gene via the analysis of multiple independently *Mu* transposon induced mutant alleles. The *gl3* gene encodes a putative *myb* transcription factor, which directly or indirectly affects the expression of a number of genes involved in the biosynthesis of very-long-chain fatty acids.

Acknowledgements

We thank Mr. Philip Stinard and Dr. Charles R. Dietrich for conducting the *gl3* tagging experiments and Ms. Li Fan and Ms. Lisa Coffey for maintaining the *gl3* mutant alleles. This research was supported in part by grants from the NSF (award number DBI-6344852).

SCHNABLE LAB
Plant Genomics

IOWA STATE UNIVERSITY