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## High Throughput Screening of Single Nucleotide Polymorphisms (SNPs) Using the BESS-T&G™ Base Reader Kit and the ABI PRISM® 3700 DNA Sequencer

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### Introduction

With the near completion of the human genome sequencing project, there is increasing interest in identifying single nucleotide polymorphisms (SNPs) that can be linked to phenotypes of clinical significance. The ability to screen large numbers of samples is essential both for SNP discovery and for screening to establish linkage relationships.

In the past, screening for mutations involved the use of rudimentary techniques, such as single strand

conformation polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE) and other inefficient processes. Such inaccurate and labor-intensive techniques are not viable for handling large numbers of samples due to reproducibility, sensitivity and data management issues. Thus, a simple, accurate, and robust SNP detection technique, coupled with a rapid analysis system, is needed for discovery and scoring of specific SNPs in a high-throughput screening environment.

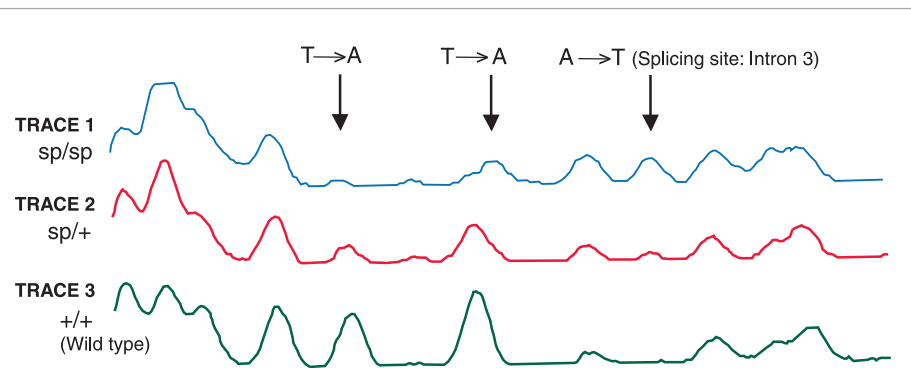
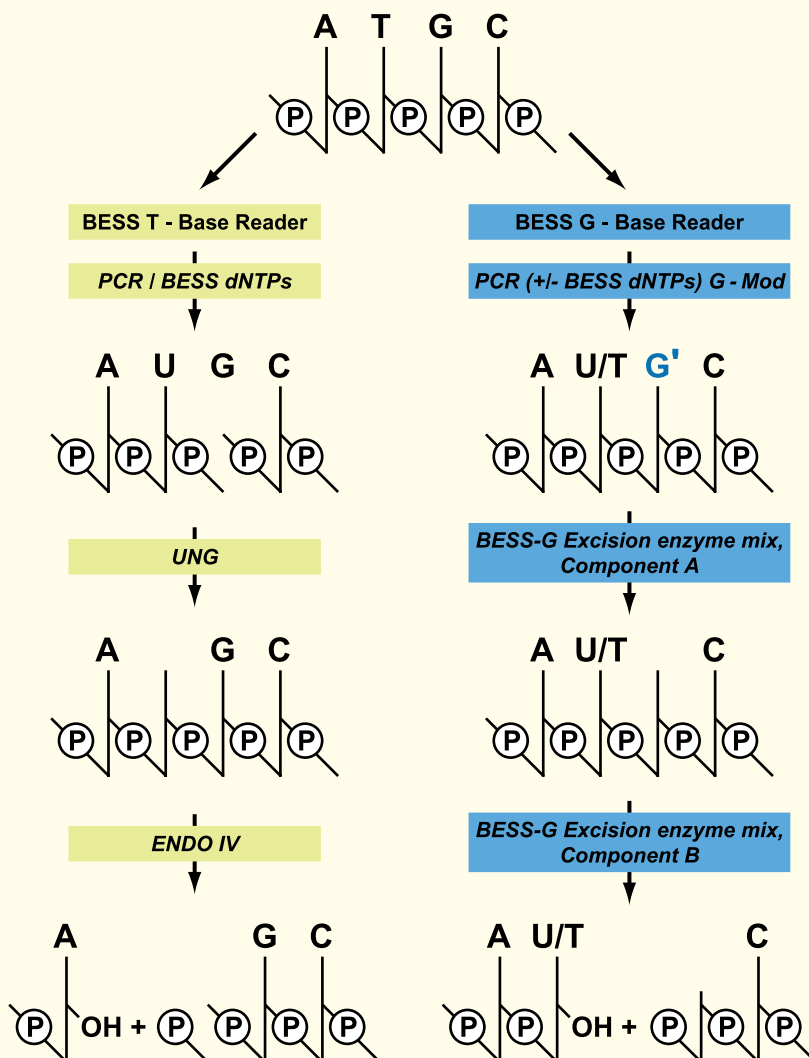


Figure 1. Sample electropherograms showing the presence of mutations in the Pax-3 gene in mice obtained using the BESS-T & G™ Base Reader Kit and the ABI Prism® 3700 DNA Sequencer. Trace 1 shows BESS-T data for the homozygous mutant, with arrows indicating the locations of mutations of interest. Trace 2 is for the heterozygous condition, which shows a smaller T base peak at the splicing site for intron 3. Trace 3 shows the wild-type homozygous condition, with strong T peaks at arrows 1 and 2 and no detectable T peak at the intron 3 splice junction.

continued

## How Does BESS Work?

Base Excision Sequence Scanning (BESS)\* is the easiest, fastest, least expensive, most sensitive and most accurate method to generate T- and/or G-lane sequence data from either one or both strands of a PCR product made using labeled primers— without dideoxy sequencing. Unlike dideoxy sequencing, the sequence-determining nucleotides for BESS (dUMP for T or a modified dGMP for G) are not terminators, permitting the full length of PCR product to be generated. However, BESS generates fragments similar to those obtained in dideoxy sequencing reactions using a brief (15-30 minutes) enzymatic treatment of the PCR product. The BESS reaction products can be analyzed using a capillary or gel just like dideoxy sequencing reactions. The mechanisms of BESS reactions are presented below.



\* U.S. Patent No. 6,048,696 & other patents pending.

The ABI PRISM® 3700 DNA sequencer (PE Biosystems, Foster City, CA) is a new sequencing instrument that is capable of analyzing up to 96 samples simultaneously. This device uses capillary electrophoresis to resolve DNA fragments and uses Windows NT-based versions of the GeneScan™ and Genotyper™ fragment analysis software.

Base Excision Sequence Scanning (BESS) has been used previously to identify mutations that correlate to a number of medical conditions.<sup>1,2,3</sup> These reports demonstrated that BESS products were readily analyzed using ABI 310 and other sequencers<sup>2,3</sup> and thus, this technique was potentially adaptable to high-throughput mutation scanning applications.

In this report, we describe the application and optimization of BESS on the ABI 3700 sequencer. We selected the BESS-T&G™ Base Reader Kit for analyzing SNPs on this instrument because of its ability to locate and specifically identify all base sequence changes in a PCR-amplified DNA fragment and the ease of optimizing the BESS technique for use on a multiple-channel DNA sequence analyzer (Figure 1).

### Methods

To test the BESS-T&G Base Reader technology in a high throughput application, we looked at a specific A→T transversion in intron 3 of the Pax-3 gene of the Splotch mouse.<sup>4</sup> Genomic DNA was isolated from Splotch mouse cells and an approximately 244 bp fragment of the Pax-3 gene was amplified using the BESS PCR Optimization Kit (BESS 2X PreMix B, EPICENTRE), and 1.25 U of Taq Polymerase. Pax-3 amplification reactions were performed in a 25 µl reaction volume, using the following primers: UP primer: 5' FAM-TAG GGA GAG GGT TGA GTA CG-3';

DN primer: 5'-CTC GCT CAC TCA GGA TGC C-3'. Thermal cycling conditions were as follows: denaturation for 5 minutes at 95°C, then 35 cycles at 95°C for 1 minute, 52°C for 1 minute and 72°C for 1 minute. A final 10-minute extension step at 72°C completed the process. BESS-T&G Base Reader reactions were performed as described in the protocol. After completing the excision/cleavage reaction, 1 µl of the reaction product was added directly without further purification to 19 µl deionized formamide. Finally, 0.8 µl of GeneScan™ 400HD Internal Lane Size Standard (PE Biosystems) was added, and 20.8 µl of each sample was applied to the ABI PRISM® 3700 DNA Sequencer in 96-well plates. Resolution of BESS-T or BESS-G excision products was accomplished by creating a run module for each 96-well plate using a run-time of 6000 seconds, a sheath flow of 12000, run temperature 50°C, cuvette temperature of 40°C, full analysis range, peak threshold of 50, and Local southern size calling. The polymer used in the capillaries was Performance Optimized Polymer (POP) 6.

## Results and Discussion

The Splotch mouse contains a mutation in intron 3 of the Pax-3 gene that is a valuable model to study defects in neural tube closure.<sup>4</sup> Pax genes encode a series of DNA-binding transcription factors whose expression has been shown to occur in distinct regions of developing mouse embryos. Human syndromes associated with defects in Pax-3 are Waardenburg syndromes type 1 and 2, which include various defects, e.g., deafness, pigmentary deficiency or dystopia canthorum. The mutation in intron 3 results in aberrant splicing of the Pax-3 transcript, resulting in production of a non-functional protein. The BESS-T&G Base Reader readily located and

identified the mutation in intron 3 of the Pax-3 gene. In addition, heterozygotes were detected by observing a decrease in peak height of the affected base in the forward strand of the PCR product, which was verified by detecting a T→A transition opposite the mutated base on the reverse strand. A partial electropherogram of the A→T transversion from intron 3 of the Pax-3 gene is shown in Figure 1. While strong signal intensities were achieved in all 96 samples, the laser detection system showed signal variability depending on the capillary being analyzed. A comprehensive review of the data generated for the Pax-3 gene using the BESS-T&G Base Reader and ABI 3700 will be presented elsewhere.

The BESS system was previously demonstrated to work well with the ABI PRISM® 310 automated DNA sequencer, which is able to sequentially process up to 48 samples. The ABI 3700 is able to process 96 samples concurrently using GeneScan™ software in a Windows NT operating environment, thus enabling the use of BESS in a high-throughput mode.

## Conclusion

The BESS-T&G Base Reader Kit, used in conjunction with the ABI 3700 automated DNA sequencer, is an excellent tool for high throughput mutation discovery or scoring of SNPs in PCR-amplified DNA. The strong signal intensities generated using samples obtained from the simple BESS protocol permits generation of SNP or mutation data on PCR products of at least 600 bases.

## References

- Hawkins, GA, et al. (1999) *Electrophoresis* **20**, 1171.
- Brieger, A, et al. (1999) *Clin. Chem.* **45** (9), 1564.
- Brieger, J; et al. (1999) *Clin. Genet.* **56**, 210.
- Epstein, DJ, et al. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 532.

### BESS-T&G™ Base Reader Kit (also called BESS MutaScan™)

BTG8520-F73  
40 BESS-T™ Rxns & 40 BESS-G™ Rxns  
BTG85100-F73  
200 BESS-T™ Rxns & 200 BESS-G™ Rxns

*Includes BESS dNTP Mix, BESS-G™ Modification Reagent, BESS-G™ Excision Enzyme Mix, BESS-T™ Excision Enzyme Mix, BESS Excision Enzyme Buffer, Stop/Loading Buffer, Control Primer (forward), Control Primer (reverse), and Control Template.*

*Note: PCR enzyme not included*

### BESS-T™ Base Reader Kit (also called BESS T-Scan™)

BN712100-F73  
200 BESS-T™ Rxns

### BESS-G™ Base Reader Kit (also called BESS G-Tracker™)

GT85100-F73  
200 BESS-G™ Rxns

### BESS PCR Optimization Kit

BP8020-F73 20 Templates


*Contains 12 BESS 2X PCR PreMixes (A-L), 0.5 ml each.*

*Each BESS 2X PCR PreMix contains a buffered salt solution with all four dNTPs and a limiting amount of dUTP, a range of magnesium ions and MasterAmp™ PCR Enhancer (with betaine)\*.*

### MasterAmp™ Taq DNA Polymerase

Q82250N-F73 5 U/µl 250 U  
Enzyme only.

*\* Patents issued and pending.*

*For more information, please circle reader service number N731 on the reply card found in the center insert or visit our website at*  
 [www.epicentre.com/bess.htm](http://www.epicentre.com/bess.htm)