

Simultaneous SNP Identification in p53 and HPV Viral Typing with Multiplex Base Excision Sequence Scanning (BESS)

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Introduction

Base Excision Sequence Scanning (BESS) has been shown to be a highly sensitive method both for determining the existence of and exact sequence changes involved in single nucleotide polymorphisms (SNPs),¹ and for viral and bacterial typing.^{2,3} This article demonstrates that BESS can be used to simultaneously analyze two different targets by using PCR primers with different fluorescent labels and multiplex PCR techniques. In this work, we use multiplex-BESS (M-BESS) to examine the correlation between a SNP in the p53 tumor suppressor gene with human papilloma viral (HPV) type in cervical scrape DNA.

Methods

Purified DNA samples were kindly provided by Dr. Tsilya Gerasimova and Dr. Lucia Pirisi-Creek of the Molecular Biology Laboratory, in the Department of Pathology, at the University of South Carolina School of Medicine.

Multiplex PCR amplification of Exon 4 of the p53 gene and the HPV L1 gene was optimized for BESS-T & G™ Base Reader Analysis using the BESS PCR Optimization Kit. DNA amplification was performed using approximately 100 ng of sample DNA, 10 pmol of each primer in BESS Optimization PreMix E and 2.5 units of MasterAmp™ Taq DNA polymerase in 50-µl reactions. The primers used to amplify the HPV L1 ORF were: MY09, and 6-FAM-labeled MY11.¹ The primers used to detect the mutation in codon 72 of the p53 gene were: TET-labeled, 5' TCC CCC TTG CCG TCC CAA 3' and HEX-labeled, 5' TGA AGT CTC ATG GAA GCC AGC 3'. Amplifications were performed with either set of primers independently, and with both sets simultaneously (multiplex PCR). The BESS-G™ modification reaction was performed on 5 µl of PCR product and these modified products were treated with the BESS-G™ Excision Enzyme Mix for 30 minutes. BESS-T™ analysis required only incubation with the BESS-T Excision Enzyme Mix. Two microliters of each BESS Excision reaction were diluted in formamide and run on an ABI PRISM™ 310 Genetic Analyzer using GeneScan® 500 (TAMRA) Size Standards and GeneScan® Analysis software.

Results

Recently it has been noted that an over-representation of a specific polymorphism in p53 (arginine-72) is found in patients with HPV-associated tumors.⁴ Primers to exon 4 of the p53 gene were used to monitor the p53 SNP resulting in arginine-72 (CGC). The multiplex PCR also contained primers to the L1 open reading frame of HPV to analyze the HPV type of the cervical scrape DNA samples. A sample of M-BESS results obtained using differentially labeled primers and analyzed on an automated DNA sequencer is shown in Figure 1.

Fourteen samples with prominent HPV PCR products were typed by BESS-T analysis. Of the 14 samples, clearly positive for HPV, 10 were quickly typed using the published sequences of the L1 ORF (Table 1). The remaining 4 samples were clearly HPV positive, but were listed as “not determined” because the T ladder produced by BESS-T Analysis did not match any of the 33 genotypes examined. Sample electropherograms of the HPV typing data are shown in Figure 1. Two of the samples, which were positive

for HPV but of undetermined type, had complex T patterns (samples 8 and 14), indicating a possible co-infection by at least two types of HPV (data not shown).

BESS-G analysis of p53 defined three HPV-positive samples that had a G present in the second position of codon 72 (arginine-72). Sample BESS-G electropherograms of the p53 tumor suppressor gene are shown in Figure 1. The absence of the G in codon 72 (proline-72) was only detected in 3 samples, all of which carried “not determined” HPV genotypes (samples 8, 11, and 14 in Table 1). Interestingly these were the three samples not identified by comparison to any of the 33 most common HPV genotypes, and included the two samples that contained at least two HPV types.

Discussion

We have demonstrated the utility of using Multiplex Base Excision Sequence Scanning (M-BESS) in simultaneous HPV typing and specific mutation analysis, adding a tool for researchers performing epidemiological studies requiring the analysis of multiple PCR products.

Table 1. Multiplex-BESS determination of HPV Type and p53 codon 72 polymorphism

Sample	HPV Status	HPV Type (BESS)	p53 Codon
1	Positive	undetermined	Arg
2	Positive	58	Arg
3	Positive	16	Arg
4	Positive	16	Arg
5	Positive	6b	Arg
6	Positive	58	Arg
7	Positive	16	Arg
8	Positive	undetermined#	Pro
9	Positive	66	Arg
10	Positive	61	Arg
11	Positive	undetermined	Pro
12	Positive	6b	Arg
13	Positive	52	Arg
14	Positive	undetermined#	Pro

Fourteen clinical samples were HPV-typed by BESS™ Base Reader Analysis. The undetermined samples did not match the sequence of any of the 33 HPV genotypes examined.

These samples had at least two HPV strains present.

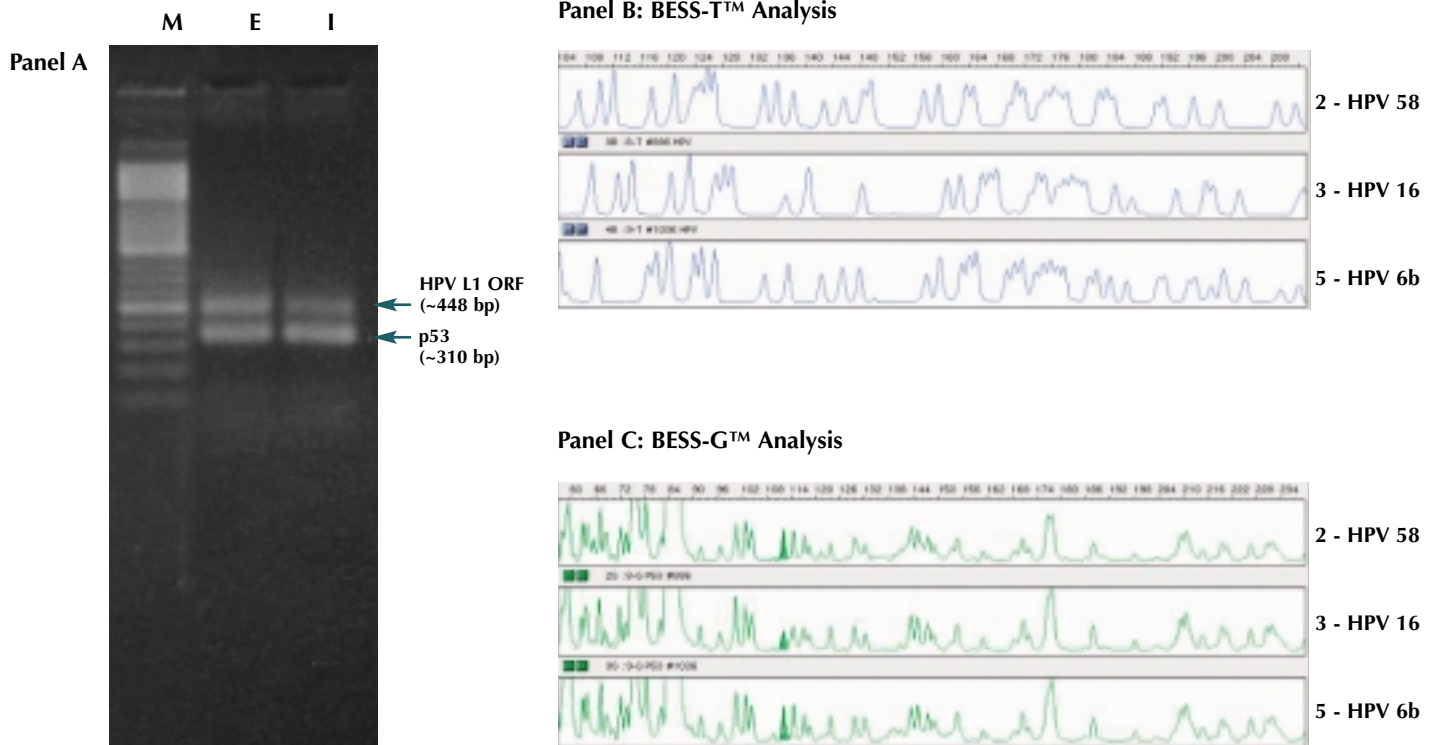


Figure 1. Simultaneous analysis of the HPV L1 ORF and the p53 gene by BESS™ Base Reader Analysis. *Panel A:* Multiplex PCR amplification of the HPV L1 ORF and p53 gene using the BESS™ PCR Optimization Kit PreMixes. The results using a “hot start” with PreMixes E and I are shown. M, DNA Ladder. *Panel B:* Rapid HPV typing by BESS-T™ analysis of the L1 ORF. The samples were analyzed using the HPV L1 ORF primers 6-FAM-labeled MY11 and unlabeled MY09. The three different HPV strains were easily determined by comparing the T peak patterns above with the known sequence of the viral strains. *Panel C:* Identification of the codon 72 polymorphism in the p53 gene by BESS-G™ analysis. The samples were amplified using a TET-labeled forward primer (shown) and a HEX-labeled reverse primer (data not shown). The data from the forward primer alone was sufficient to detect the polymorphism.

M-BESS allows the rapid generation of data at low cost per sample. M-BESS analysis can be performed on multiple loci simultaneously as long as different fluorescent-labeled primers are used for each locus of interest. In this preliminary study, we have shown that simultaneous BESS analysis of two DNA targets from a clinical sample can provide sufficient information for typing HPV and exactly identifying specific mutations in p53. The M-BESS technique is a useful tool for many other analyses where either multiple SNPs or multiple typing reactions, or both, are required.

The BESS Website (www.epicentre.com) contains information about the BESS technique, including application notes, a complete list and internet links to references from the scientific literature, product protocols, and articles from the Epicentre Forum.

References

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- Nelson, JH, et al. (2000) *J. Clin. Micro.* **38**:688-695.
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- Storey, A. et al. (1998) *Nature* **393**:229-234.

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

BTG8520 20/40 Rxns
BTG85100 100/200 Rxns

The BESS-T&G™ Base Reader Kits contain sufficient reagents to fully analyze 20 or 100 templates respectively, using PCR reactions with a single-labeled primer or 40 or 200 templates, respectively, using two labeled primers per reaction (T sense and antisense strands, and G sense and antisense strands).

Note: PCR enzyme is not included

Contents:

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)
Control Template

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

BN712100 200 Reactions

Contents:

BESS-T™ Excision Enzyme Mix
BESS Excision Enzyme Buffer
BESS dNTP Mix
Control Primer (forward)
Control Primer (reverse)
Control Template
Stop/Loading Buffer

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

GT85100 200 Reactions

Contents:

BESS-G™ Modification Reagent
BESS-G™ Excision Enzyme Mix
BESS Excision Enzyme Buffer
BESS dNTP Mix
Control Primer (forward)
Control Primer (reverse)
Control Template
Stop/Loading Buffer