

# An Evaluation of the PowerSeq™ Auto system: A Multiplex Short Tandem Repeat Marker Kit Compatible with Massively Parallel Sequencing

Xiangpei Zeng <sup>1</sup>, Jonathan King <sup>1</sup>, Spencer Hermanson <sup>2</sup>, Jaynish Patel <sup>2</sup>, Douglas R. Storts <sup>2</sup>,  
Bruce Budowle <sup>1,3</sup>

<sup>1</sup> Institute of Applied Genetics, Department of Molecular and Medical Genetics, University of North Texas Health Science Center, 3500 Camp Bowie Blvd., Fort Worth, Texas 76107

<sup>2</sup> Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711

<sup>3</sup> Center of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University, Jeddah, Saudi Arabia

## Introduction:

Multiplex amplification with fluorescent detection during capillary electrophoresis (CE) has been used routinely for STR typing in forensic genetics. However, CE-based method has some limitations, i.e., the number of markers that can be multiplexed is rather limited, and STR polymorphisms are based on length, which in turn does not enable elucidation of the primary sequence within a STR allele. Massively parallel sequencing (MPS) technology, with its high throughput, can provide direct sequence of far more markers and samples in one analysis than Sanger sequencing. In addition, MPS technology allows STR amplicons to be designed with more similar and shorter in length. The STR allelic data are both sequence and length based. Thus, STR polymorphisms generated by MPS are backwards compatible with current STR data with added value of increased discrimination power. The performance of the PowerSeq™ Auto system (Promega), a new prototype multiplex STR kit that is designed for the Illumina MiSeq platform, was evaluated.

## Methods:

- A PCR sensitivity study was conducted using three DNA samples at a range of different amounts of input DNA (16-500 pg).
- A reproducibility study was performed using six individuals to determine allele coverage ratio (ACR) variation, interlocus coverage and balance.
- A mixture study was conducted by mixing two DNA samples (one male and one female) at different ratios (total amount 500 pg).
- A mock forensic casework study was performed using forensic casework-like single source and mixture samples.
- A concordant study was conducted using PowerSeq Auto system (MiSeq) and PowerPlex™ Fusion kit (CE) for single source and mixture samples.

## Results:

- The results indicated that depth of coverage (DoC) was high and ACRs were relatively balanced at 500 pg of input DNA. Full profiles could be generated using as little as 62 pg of input DNA.
- The profiles generated were reproducible and consistent among replicates for a given sample.
- PowerSeq Auto system could detect partial STR profiles of the minor contributor down to a 19:1 mixture.
- Full or partial profiles could be obtained from different types of single source samples and mixture samples.
- This MPS-based system was able to enhance mixture interpretation.
- Data were consistent between the PowerSeq Auto system and PowerPlex™ Fusion kit.

**Discussion:**

These results indicate that the PowerSeq™ Auto system and the MiSeq system can generate reliable and consistent results compared with current CE-based methods. In addition, this MPS-based system is able to improve mixture analysis with the detection of intra-repeat variations within STR alleles.

**References:**

1. X. Zeng, J. King, S. Hermanson, J. Patel, D.R. Storts, B. Budowle, An evaluation of the PowerSeq™ Auto System: A multiplex short tandem repeat marker kit compatible with massively parallel sequencing, *Forensic Sci. Int. Genet.* 19 (2015) 172-179.