INTERNAL VALIDATION OF THE PROMEGA POWERPLEX® 16-LOCUS FLUORESCENT STR MULTIPLEX SYSTEM

Jim Sloots[1], Joel McGrory[2], Dave Hunt[1], Rebecca Ford[1], Lindsay Cain[3], Roger Frappier[2], and Jonathan Newman[2]

[1]Biology Section, Centre of Forensic Sciences, Sault Ste Marie, ON
[2]Biology Section, Centre of Forensic Sciences, Toronto, ON
[3]University of Strathclyde, Glasgow, Scotland

The Centre of Forensic Sciences, in both its Toronto and Sault Ste. Marie locations, has completed internal validation of the PowerPlex®16 DNA typing system that satisfies both the current and revised (effective Jan. 2004) SWGDAM validation guidelines. The Promega PowerPlex®16 DNA typing system amplifies the 13 CODIS loci along with Penta D, Penta E and Amelogenin in a single reaction affording the laboratory greater efficiencies, discrimination power and throughput.

Organically extracted DNA was quantitated using the Quantiblot® detection kit and was amplified in a 9600 thermocycler (PE/ABI). The amplified fragments were detected and analyzed using a 5% Long Ranger® Singel® polyacrylamide gel on a 377 Automated Sequencer (ABI) using GeneScan® Collection (v. 2.6) and Analysis Software (v. 3.1.2) followed by allele calling with Genotyper® Software (v 2.5).

Initial experimentation with template, amplification volume and cycling parameters indicated that robust performance of the kit was obtained using 1ng of target in a 15µL reaction (as per all other STR analysis protocols at CFS) amplified for a total of 30 cycles. With these conditions, 0.5µL of amplified product gave approximately 2000 rfu/locus.

Sensitivity studies demonstrated that complete profiles were obtainable with 250pg of template DNA, although increased peak height discordance and a number of peaks with rfu's below 50 were noted. At 125pg, less than 50% of the samples gave complete profiles, and at 63pg no complete profiles were observed.

When 1ng of template DNA was amplified, stutter averaged below 9% at all loci while heterozygote peak height concordance averaged above 80% for all loci. Other artifacts including the observation of n-1 peaks will be presented.

Sizing precision using the Long Ranger® Singel® gel system with ILS600 and the Promega Allelic ladder gave an average standard deviation for all alleles in all loci of less than 0.07 nucleotides.

Alleles from the minor source in mixtures of DNA from two persons could be detected at almost all loci when the minor component was present at as little as 10%; and on the basis of at least one locus, mixtures could still be detected when the minor component was present at 5%.

Profiles generated using NIST traceable reference materials, other non-probative forensic samples, compromised samples or various samples from the same individual were consistent with observations noted in other typing systems.

Overall, our validation study has demonstrated that the Promega PowerPlex®16 multiplex system provides consistent and robust amplification with a variety of different template challenges, and is suitable for casework implementation in our laboratory.