

**DETERMINING RESTRICTION DIGEST EFFICIENCY USING THE SNAPSHOT SINGLE BASE EXTENSION METHOD**

**Roland van Oorschot, Kaye Ballantyne, Manfred Kayser and John Mitchell**

*Biology Division, Victoria Police Forensic Services Department, 31 Forensic Drive, Macleod, VIC 3085, Australia*

---

Incomplete restriction endonuclease digestion may cause complications during particular applications, where any undigested product may interfere with genotyping accuracy, cloning efficiency or PCR amplification. In some instances it may be useful to measure the amount of undigested DNA remaining in the sample. A single-base extension SNP genotyping method (SNaPshot, Applied Biosystems) was adapted to interrogate restriction sites and their digestibility. The digested DNA was amplified by nested PCR to specifically amplify sections containing the restriction sites of interest. Single interrogation primers terminating immediately 5' of the cleavage site were used, in conjunction with the SNaPshot multiplex system and capillary electrophoresis, to detect intact, amplifiable product remaining in the digest. This method detected as little as 10 pg of intact DNA, within a larger proportion of digested DNA. Across two different restriction enzymes the amount remaining undigested was found to be dependant on the amount of DNA, ranging from 0.08% with 200 ng to 1.25% with 600 ng. This method may be useful for applications such as cloning or Southern Blotting, where complete digestion is required. For our application, we were able to determine that whole genome amplification can amplify <10 intact copies of STR alleles to reportable levels, even in the presence of over 10 ng of digested DNA.