Internal validation of a commercial 17-locus Y-STR system (AmpFISTR® Yfiler™, Applied Biosystems) has been performed on the ABI PRISM® 3130 Genetic Analyzer for use in forensic cases. The Yfiler™ kit was validated according to SWGDAM guidelines. Our results show that it is possible to obtain full profiles with as little as 40 pg of male DNA even in the presence of 10,000-fold amounts of female DNA. Reaction volume was optimized for 10 µl. Male-male mixtures yielded full profiles of the minor contributor with 10-fold excess of the major contributor. Stutter values for each locus were determined from data generated for the population study which included Y-STR profiles from 156 caucasian males from the Montreal and Lac St-Jean areas of Québec, Canada. The study recorded 141 different haplotypes of which 131 were unique with a haplotype diversity of 0.9965. A number of non-probative forensic samples from rape kit epithelial fractions and fingernail scrapings were also successfully tested.