Scientific Protocol for Identification of Olive variety

https://npgsweb.ars-grin.gov/gringlobal/cropmarker.aspx?id=1563

Total DNA was isolated using either the CTAB method (Doyle and Doyle, 1987) or QIAGEN DNeasy Plant Mini-prep Kits (QIAGEN, Valencia, CA) and treated with RNase. Fourteen microsatellite markers were PCR amplified separately in a 10-´L reaction mixture containing 1X Standard Taq Buffer [New England BioLabs, Ipswich, MA (NEB)], 2 mM MgCl2, 0.375 mM each dNTP (ABI), 0.075 units/´l Taq DNA Polymerase (NEB), 0.05 pmol/´l each primer, and approximately 5 ng/´l DNA. PCR reactions were triplexed, i.e. made with three primer pairs combined in one reaction, each pair labeled with a different fluorescent dye. PCR was performed under the following conditions: 1 cycle of 94 C for 5 min, 30 cycles of 94 C for 30 sec, 55 C for 30 sec, and 72 C for 40 sec, and then a final elongation of 72 C for 7 min Amplified products were resolved using capillary electrophoresis on an ABI Prism 3100 Genetic Analyzer. One marker, IAS-pOe12, amplified two loci, and was separated for analysis as IAS-pOe12_A and IAS-pOe12_B, bringing the total number of loci for analysis to fifteen.