Mexican-mestizos are the most widely distributed population in both urban and rural regions throughout the country and constitute nearly 90% of the total population. They are the result of admixture of Spaniards, Amerindians, and Africans. Yucatan is a state with a high density of population with Mayan ascendancy at southeastern Mexico. Short tandem repeat (STR) polymorphisms are mainly used in forensic fields for paternity tests and personal identification at present. A sample of 120 unrelated mestizos from Yucatan was PCR-typed for 15 STR loci using the Power Plex 16 Promega kit (D3S1358, TH01, D21S11, D18S51, Penta E, D13S317, D7S820, D16S539, CSF1PO, Penta D, vWA, D8S1179, TPOX and FGA). Genotype distribution per each locus was in agreement with Hardy–Weinberg expectations for all 15 STRs. The most highly polymorphic loci were Penta E and D18S51, showing 17 and 15 alleles, respectively. Heterozygosity ranged for all 15 STRs from 0.6650 for D3S1358 to 0.8893 for Penta E. Number of alleles observed was ranged from 6 for TH01 and TPOX to 17 for Penta E. For each locus, allele frequencies were obtained, ranging from 0.0042 to: 0.4833 for D3S1358; 0.4208 for TH01, 0.2833 for D21S11, 0.2083 for D18S51, 0.2417 for penta E, 0.4750 for D5S818, 0.2583 for D13S317, 0.3125 for D7S820, 0.2833 for D16S539, 0.3166 for CSF1PO, 0.2667 for Penta D, 0.3625 for vWA, 0.3458 for D8S1179, 0.4625 for TPOX, 0.2542 for FGA. The most discriminating loci were Penta E (PD = 0. 0.972) and D18S51 (PD = 0. 0.962). The combined matching probability of these STRs showed 1 in 2.99 X1013 and the combined power of exclusion was 0.9999999. Our results suggest that STRs provide a powerful discrimination and show the importance of the generation of local databases for STRs when these markers are being currently used in forensic casework. Therefore, this database can be used as identity testing to estimate the frequency of a multiple PCR-based locus DNA profile in forensic cases as well as in paternity testing.