We developed an open source software Kongoh for DNA mixture interpretation based on a continuous model. In Kongoh, the likelihood ratio (LR) is calculated on the basis of expected peak heights determined by five biological parameters: the mixture ratio, DNA degradation, locus-specific amplification efficiency, heterozygote balance, and stutter ratio. Other continuous-model software have been developed, but the peak height and biological parameters are slightly different in each software. In this study, we investigated the difference between Kongoh and two open source software (i.e., EuroForMix and likeLTD) by comparing the LR values and estimated number of contributors calculated by each software.

We experimentally prepared 27 two-person mixtures, 27 three-person mixtures, and 18 four-person mixtures in various mixture ratios. These samples were amplified using the Identifiler Plus Kit, and PCR products were analyzed using the 3130xl Genetic Analyzer with an analytical threshold of 30 relative fluorescence units. We then calculated the likelihood of both the prosecutor hypothesis (i.e., a person of interest (POI) is a contributor) and defense hypothesis (i.e., an unknown person is a contributor) for each mixture. The number of contributors were changed (1-4 contributor(s) in Kongoh and EuroForMix and 1-3 contributor(s) in likeLTD), and LR values were calculated from the ratio of maximum likelihood of each hypothesis. We also estimated the number of contributors based on the maximum likelihood.

The LR values of each software tended to be strongly supportive (i.e., LR > 10,000) of the prosecutor hypothesis when the POI is a true contributor. The values calculated by the three software tended to be similar even when the amount of DNA of the POI was small. Accuracy of the estimated number of contributors was the highest in Kongoh (e.g., accuracy in two-person mixtures: 85.2% in Kongoh, 77.8% in EuroForMix, and 51.9% in likeLTD), because Kongoh incorporates allele- or locus-specific effects using experimental data. Conversely, EuroForMix and likeLTD provide a versatile method by modelling the expected peak heights broadly, regardless of allele- or locus-specific effects. Therefore, the likelihood values estimated by Kongoh are expected to be more rigorous than those by any other software.