Objective: To develop a useful tool for forensic DNA analysis of sexual assault evidence, we tried to apply cell sorting methods to isolate target sperm cells from forensic mixtures for individual identification.

Methods: Fluorescence- and magnetic-activated cell sorting (FACS and MACS) are two proven strategies for the separation of specific cells in mixtures.

1. MACS cell sorting strategy for isolating sperm cells from forensic mixture samples using sperm specific antibodies (AKAP3) for STR profiling.

2. FACS cell sorting strategy for isolating target individual sperm cells involving two or three donors using FITC-labeled blood group A/B antigen antibody for STR profiling.

3. Mock casework: To prepare mock case sample, 5 μL of semen from 2 donors with different blood type was thoroughly mixed and spotted onto cotton cloth (2.5 cm in diameter) and let it dry at room temperature. The semen stain was cut into 1×1 cm² squares and soaked in PBS to prepare sperm cell suspension.