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To maintain ecological balance, monitoring species populations is essential, and identifying gender is one of the key factors influencing population trends. Shrews are the smallest mammals on Earth and show little visible sexual dimorphism, which makes it difficult to determine sex. Dissection is the most common method used; however, it is invasive and not reliable on live samples. With fetal or immature specimens, it is still a challenge, even with the aid of a microscope. To address the limitations, genetics provides a more accurate and less invasive approach. This study focused on developing a non-invasive assay of sex identification for shrews using external tissues, such as feet and toes. DNA was extracted from the shrew samples with Chelex 100 solution. PCR samples were prepared by mixing the SRY and ZFY primers, Taq DNA polymerase, nuclease-free water, and extracted DNA. A sample without extracted DNA was used as a negative control. Amplified products were visualized by gel electrophoresis. The male samples show two distinct bands corresponding to both the SRY and ZFY primers, while female samples only produce the ZFY band. This research provides a non-invasive alternative for the shrew population studies and reduces specimen damage.