

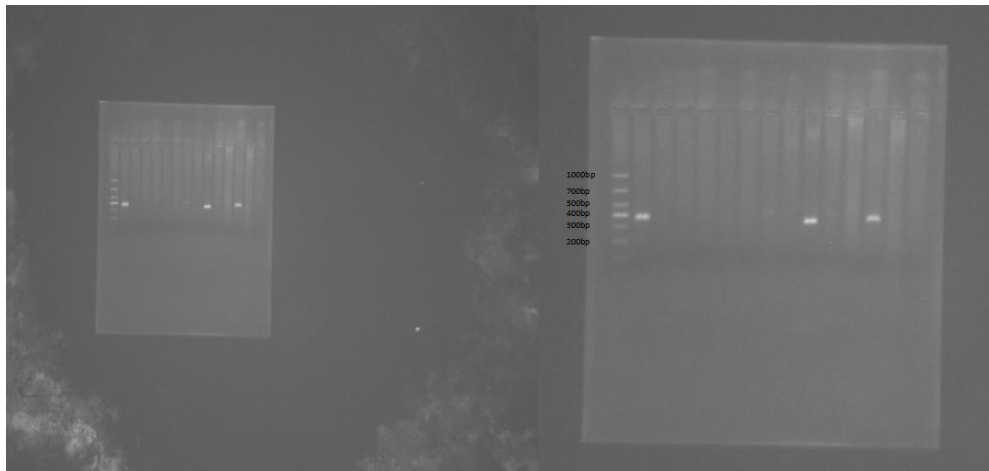


Figure 1 Gel image of the first-round amplified UP target band

DNA marker 1000bp

The left panel shows the original image; the right panel shows the image with size marker added.

primers F 5'TGCAATCTTTATATGTTTCGTT 3'

R 5'GATCTTTAAAGTTTCAATTCGT3'

Size of the amplified product was 350bp, Only three samples showed successful amplification of the target band.

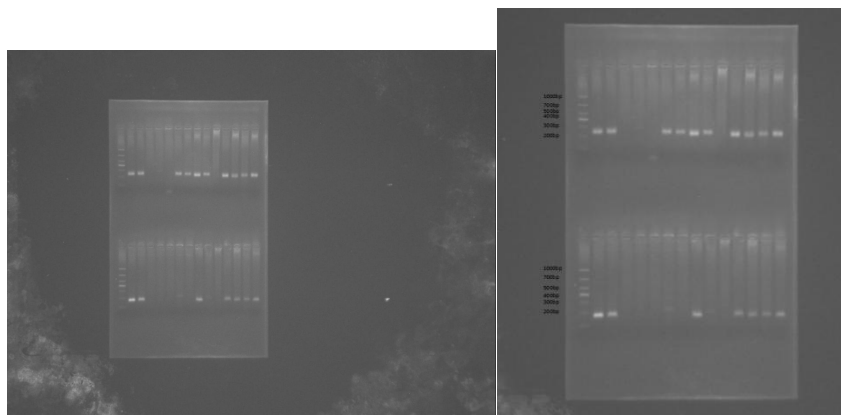


Figure 2 Gel image of the second-round amplified UP target band

DNA marker 1000bp

The left panel shows the original image; the right panel shows the image with size marker added.

primers F2 5'AATTCTGAGCTATGACATTAG 3'

R2 5'ATAGCAACTATTCCAGCTCCAAC3'

Given that DNA extracted from paraffin-embedded samples may be fragmented, we designed an additional pair of primers within the amplified region of the first primer set. By recombining the primers as F-R2 and F2-R, the target amplicon size was reduced to 200bp. This approach enabled successful amplification and subsequent sequencing of the target band in the second round of PCR for samples that failed to yield sequencing results in the first round of UP amplification. For each case, DNA extracted from different tissues was amplified to ensure the acquisition of sequencing samples.