Molecular sexing technique methods

The down was removed from the lower part and the calamus and ~ 1mm of rachis dissected. Two dissected feather calamuses were submerged in 20 µl of Hot Sodium Hydroxide and tris (HotSHOT) lysing solution. Samples were heated at 95°C for 60 minutes and then neutralised with 20 µl of HotSHOT neutralising solution. 2 µl of the lysed cell solution was suspended in 8 µl of MasterMix (5.0 µl HSTaq [DNA Polymerase, PCR Buffer with 3mM MgCl2 and 400 µM of dNTPS], 1.60 µl RNAase free water, 1.0 µl Coral Red, 0.15 µl forward primer, 0.15 µl reverse primer, 0.1 µl Bovine serum albumin). The primers 2550F (5'-GTTACTGATTCGTCTACGAGA-3') and 2718R (5'-ATTGAAATGATCCAGTGCTTG-3') were used to amplify fragments of the *CHD* gene. For amplification an initial denaturing step of 95°C for 5 minutes was followed by a touchdown scheme with the annealing temperature lowered by 1°C per cycle, starting at 60°C until 50°C was reached. This was followed by an additional 30 cycles with an annealing temperature of 50°C. Denaturation occurred at 94°C for 30 seconds, annealing for 30 seconds and extension at 72°C for 30 seconds. This was followed with a final extension step of 72°C for 10 minutes. PCR products were run on a 1.5% agarose gel for 45 minutes at 120V in a standard TBE buffer and visualised by ethidium bromide staining.