

Sex Determination Protocol:

30 μ l Reaction--300ng of DNA

<u>Reagent</u>	<u>Amount μl</u>	<u>Final Conc.</u>
10x PCR Buffer	3.0	1X
12.5 mM dNTP	0.5	200 μ M
25mM MgCl ₂	1.8	1.5mM
Amplitaq Gold	0.4	2 units
Sry Primer Set (10 μ M)	2	0.33 μ M each
Actin Primer Set (10 μ M)	2	0.33 μ M each
Genomic DNA (60 ng/ μ l)	5	300ng
Water	15.3	Total 30 μ l

Conditions:

95°C 10 minutes (Hot Start)

35 cycles

94°C 15 sec.

60°C 30 sec.

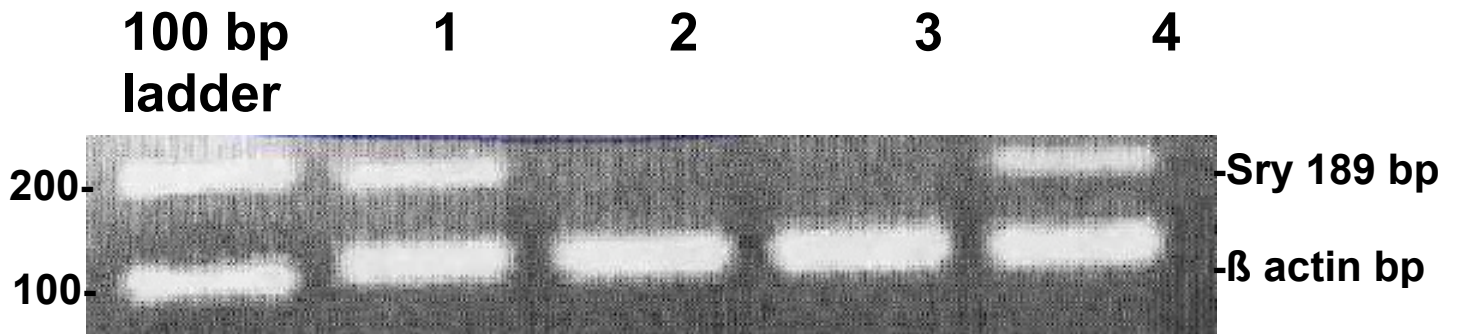
72°C 30 sec.

72°C 7 minutes

Load 25 μ l in 2 μ l of 6X loading dye on a 2% Agarose gel.

Sry PCR Product---- 189 bp

Actin PCR Product-- 106 bp



- 1) Male Genomic DNA (Blood)
- 2) Female Genomic DNA (Blood)
- 3) Fetus # 1 (Genomic DNA)
- 4) Fetus # 2 (Genomic DNA)