

# Fetal Microchimerism and Cancer in Golden Retrievers



Jessica Mautone, Sarah Hansen, Senthil Kumar, Jeffrey Bryan

Comparative Oncology and Epigenetics Laboratory, College of Veterinary Medicine at the University of Missouri

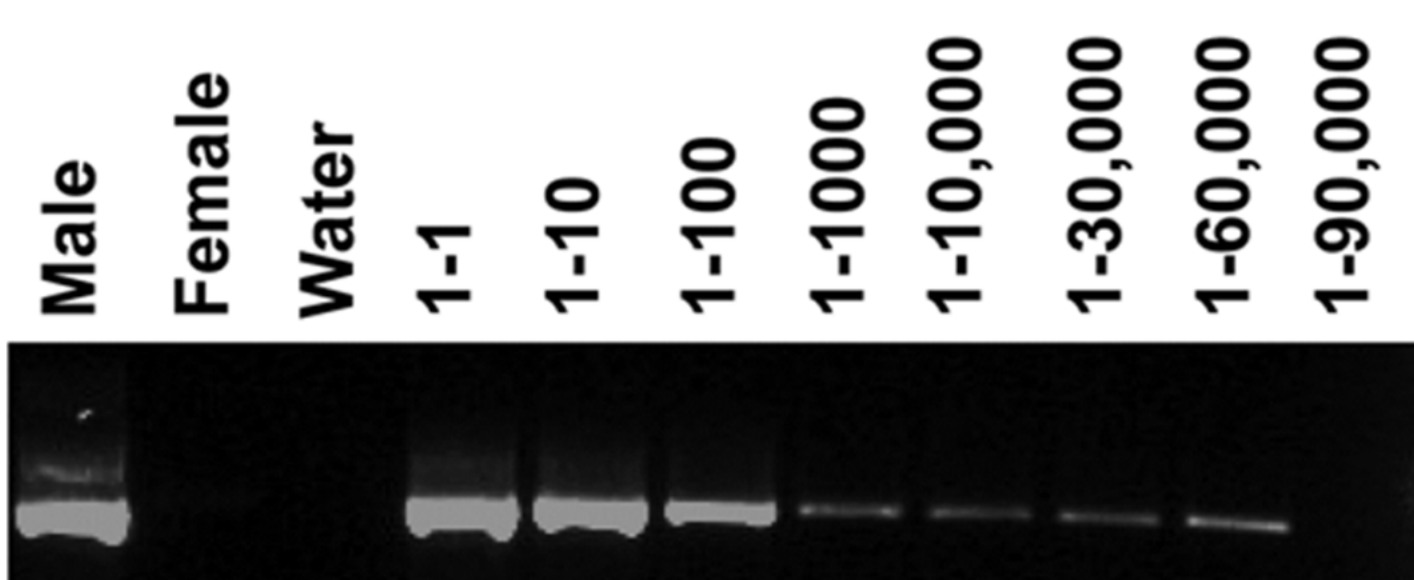
## Background

Fetal Microchimerism (FMC) occurs when cells from a fetus migrate across the placenta to the mother and persist for her lifetime. Studies in humans suggest that these fetal cells can influence cancer rates. FMC is associated with lower risk of developing breast cancer and higher risk of developing colon cancer. FMC is also associated with an increased risk of developing autoimmune diseases in humans such as Grave's disease and autoimmune thyroid disorders. Humans with FMC are less likely to reject organs from a sibling or parent following transplant. Our laboratory has recently described this phenomenon in parous dogs (also reported in cattle, rats, and mice). The objective of this study is to determine the FMC frequency in nulliparous dogs and dogs with mammary cancer or lymphoma. We hypothesize that both the nulliparous dogs and dogs with mammary cancer or lymphoma will have lower rates of FMC than the parous dogs previously described.

## Challenges of PCR

A nested PCR assay provides increased sensitivity as well as sequence specificity for the desired target sequence. However, frequent amplification also increases the risk of amplicon contamination.

### Test Sensitivity

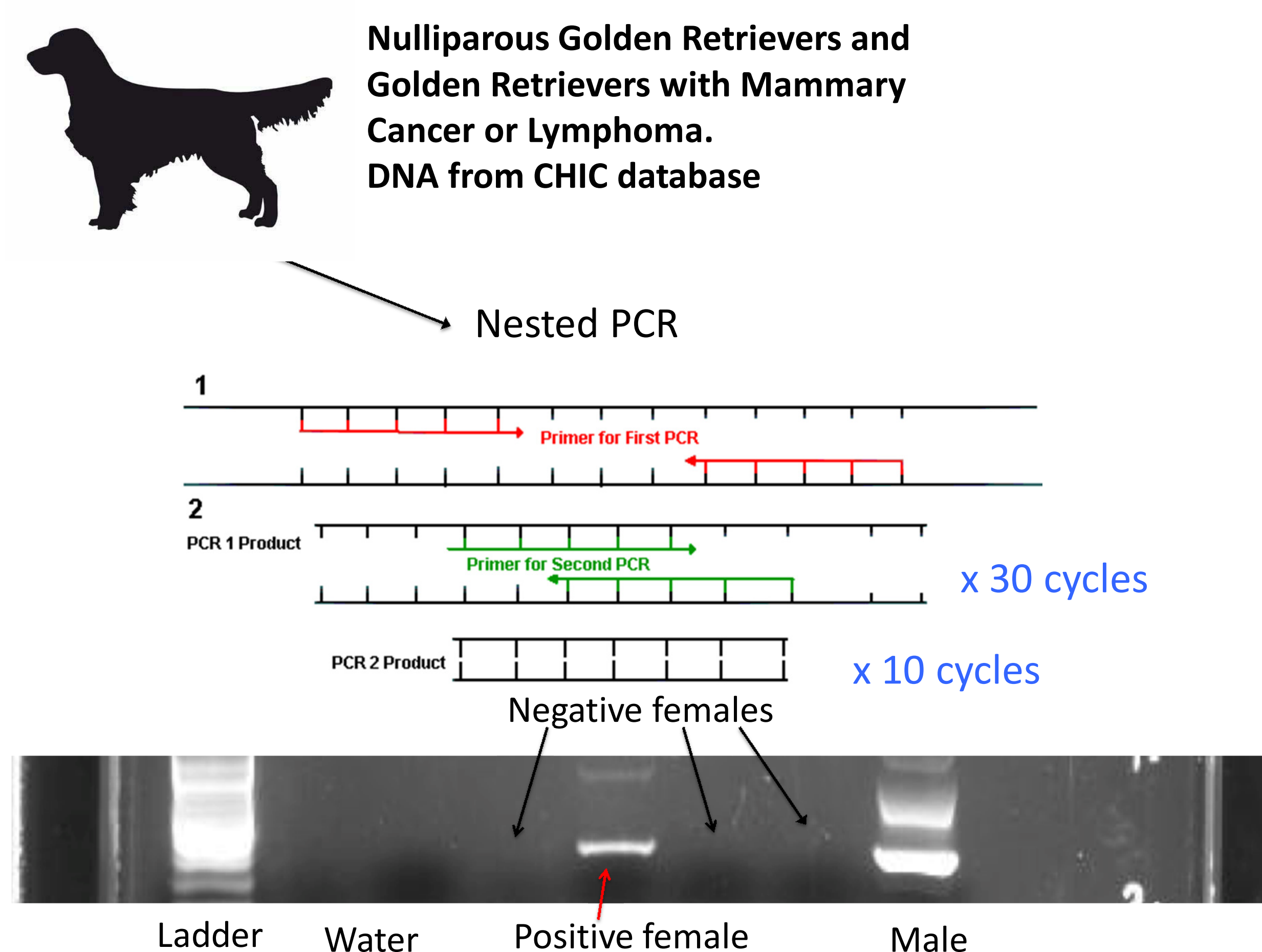


Contamination in the lab has presented a huge challenge that we are working to overcome. Changes have been made to try to reduce potential for contamination such as moving all preparation of PCR products to a different room under a UV hood, using filtered pipette tips, wearing protective clothing, and using new pipettors.

We thank Zoetis and the Scott Endowed Program in Veterinary Oncology for funding necessary to complete this project

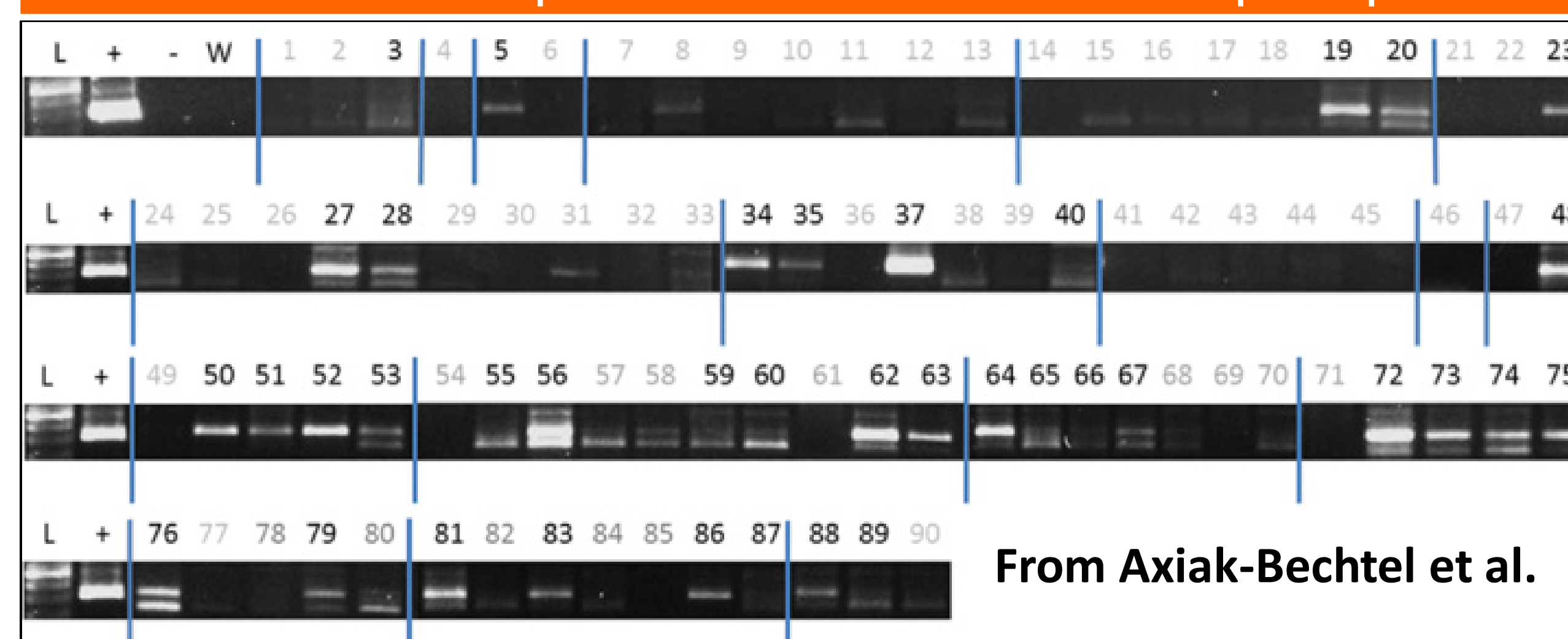
## Materials and Methods

Polymerase chain reaction (PCR) is used to amplify the Y chromosome (if present) in female whole-blood DNA extracts. Two amplifications by PCR are run to give a final nested PCR product which is then visualized by electrophoresis on an agarose gel. Two sets of primers are used in this PCR. A 650bp fragment of the male Y chromosome for the first reaction and a second 320bp fragment (within the 650bp) to be used in the nested reaction. In each gel, a 100bp ladder is run, at least one water lane to check for contamination, and a male positive control is used. Gels were analyzed using ImageJ at the expected 320bp region and the 1900 bp region for background. Relative intensities greater than the mean plus two standard deviations of negative lanes were considered to be positive.



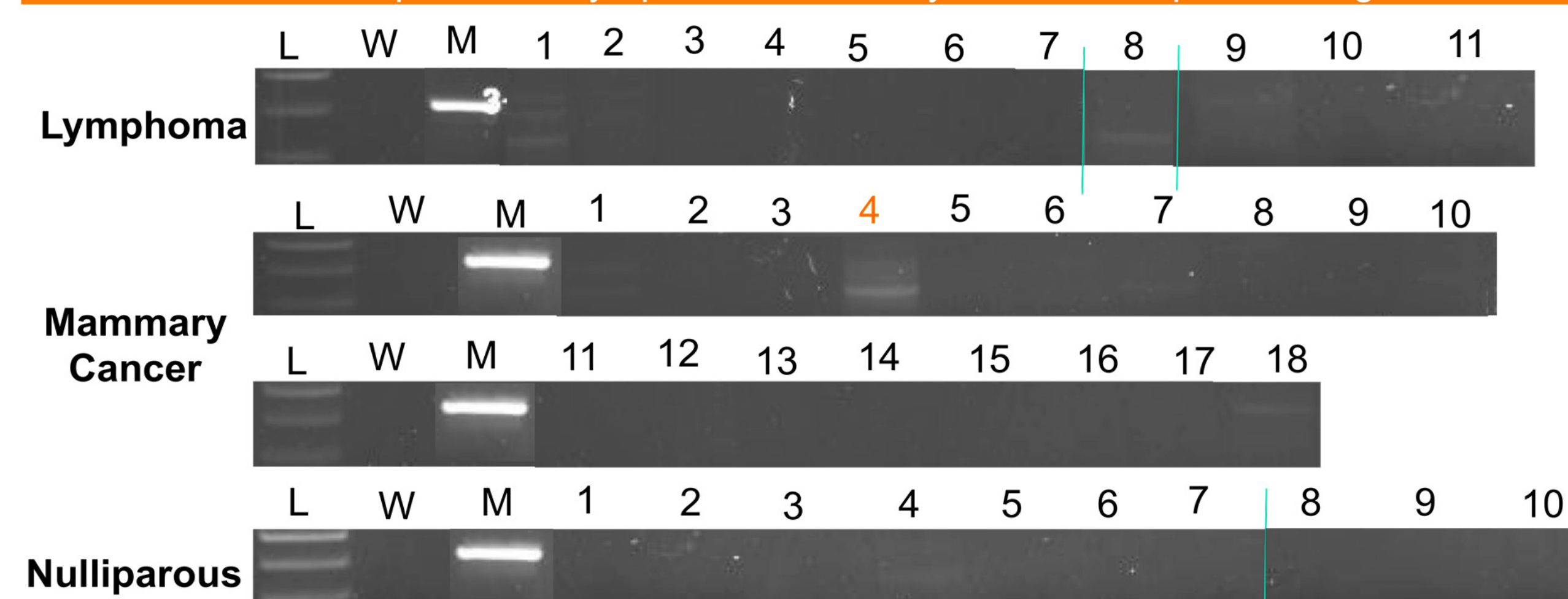
## Results

Banked DNA samples: as late as 96 months post-partum



From Axiak-Bechtel et al.

DNA Samples from Lymphoma/Mammary Cancer/Nulliparous Dogs



## Conclusions

Our previously reported studies demonstrated that fetal microchimerism can be identified in female parous dogs. The sensitivity of that assay, in which the Y chromosome can be detected, was shown to be between 1 male cell in 60,000 to 90,000 female cells (Axiak-Bechtel et al.)

Evaluating nulliparous dogs with the same assay, no bands in the appropriate size range were found. We will continue to run more samples, as we suspect that microchimerism is a low-frequency event in nulliparous dogs.

Dogs with lymphoma demonstrated no evidence of FMC

Dogs with mammary cancer demonstrated a 5% rate of positivity.

Both are lower than the previously identified rate in parous dogs ( $P < 0.015$ ). In the nulliparous dogs, none of the ten samples run so far appear to be microchimeric.

## Future Directions

1. Confirm all samples FMC status by running multiple PCR reactions.
2. Follow up with owners of the dogs to determine if the positive females had litters or were born with male siblings.



## Literature cited

1. Axiak-Bechtel, Sandra M., Senthil R. Kumar, Sarah A. Hansen, and Jeffery N. Bryan. "Y-chromosome DNA Is Present in the Blood of Female Dogs Suggesting the Presence of Fetal Microchimerism." *PLOS ONE* 8.7 (2013)