Novel approach for sex identification in equine forensic samples using PCR amplification

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Introduction & Background

- Importance of sex determination
  - Determine sex of preimplantation stage embryo biopsies in IVF/embryo transfer
  - Prenatal sex identification and fetal tissue biopsies of the developing fetus[1]
  - Study developmental differences between individual embryos[2]
  - "Correct allocation of export refunds"[3] and slaughter of correct gender for religious beliefs[3]
  - Population ecological studies, lab sample controls, archeology, and various forensic uses[4]
- DDX3 mammalian gene
  - ATP-dependent RNA helicase: involved in altering structure of RNA[5], functions as transcriptional regulator[5], involved in nuclear export of retroviral RNA[5]
  - Ubiquitous in all mammalian species, found on X- and Y-chromosomes
  - Y-chromosome homolog contains unique deletion and insertion regions

Purpose, Objectives, and Process

- Purpose: design a primer pair with build in PCR control, verify with equine DNA samples and PCR assay
- Objectives:
  - Create alternative means of detecting sex in equine embryos and forensic samples
  - Design a primer pair that contains a built-in control for PCR amplification
  - Contribute to genetic understanding of X- and Y-chromosomes
- Involved process:
  1. Design primer pair
  2. Collect equine blood samples and extract DNA
  3. Conduct PCR assay
  4. Conduct gel electrophoresis for qualitative evaluation

Human-Equine Alignment

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CLUSTAL 2.1 multiple sequence alignment


ref|NC_000024.10|_12914495-129 H-Y    TCAAGGGCATTTAGAACCCTTTGTCATCTGTTAATATTCAGAAATGATAA
gb|KP686224.1|_1598-1996       E-DDX3Y----------------------------------------------------
ref|NC_000023.11|_41343690-413 H-X    ----------------------TCTGTTTAAAAGTAATGAGCAGGATTTG
ref|NC_009175.3|_34841590-3484 E-X    ----------------------GTAGATTTTTCTGTGGTTGAGTAAATATG


ref|NC_000024.10|_12914495-129       GCCAGTGTTTTGTTTTCAGGATCTGGGAAAACTGCAGCATTTCTTTTACC
gb|KP686224.1|_1598-1996             ---------------------------AAAACTGCGGCATTTCTTTTGCC
ref|NC_000023.11|_41343690-413       TTTGTTTGTTTTTGAACAGGGTCTGGAAAAACTGCAGCATTTCTGTTGCC
ref|NC_009175.3|_34841590-3484       CTT---TCTCTTAAAACAGGGTCTGGAAAAACTGCAGCATTTCTCTTGCC
                                                      ******** ******** ** **


ref|NC_000024.10|_12914495-129       CATACTGAGTCAGATATATACAGATGGTCCAGGAGAAGCTTTGAAGGCTG
gb|KP686224.1|_1598-1996             CATCTTGAGTCAGATTTATACAGATGGTCCAGGCGAGGCTTTGAAGGCTG
ref|NC_000023.11|_41343690-413       CATCTTGAGTCAGATTTATTCAGATGGTCCAGGCGAGGCTTTGAGGGCCA
ref|NC_009175.3|_34841590-3484       CATCTTGAGTCAGATTTATTCAGATGGTCCAGGCGAGGCTTTGAGGGCCA
                                     ***  ********** *** ************** ** ******* ***


ref|NC_000024.10|_12914495-129       TGAAGGTAAAGGTTTTGTTATAAAATCAGACATTTTTGTTTTAAAAAGCT
gb|KP686224.1|_1598-1996             TGAAGGTAAAGATTTCTTTATAAAATAAGT--TTTTCCTTTGAAAAAGTT
ref|NC_000023.11|_41343690-413       TGAAGGTAGATGTTTCTTTATAAAATGGGAAATT---GT-----AGAACT
ref|NC_009175.3|_34841590-3484       TGAAGGTAAATATTTCTTTAAAAAGTGGGAAATTATTGT-----AGAGCT
                                     ******** *  ***  *** *** *  *   **    *     * *  *
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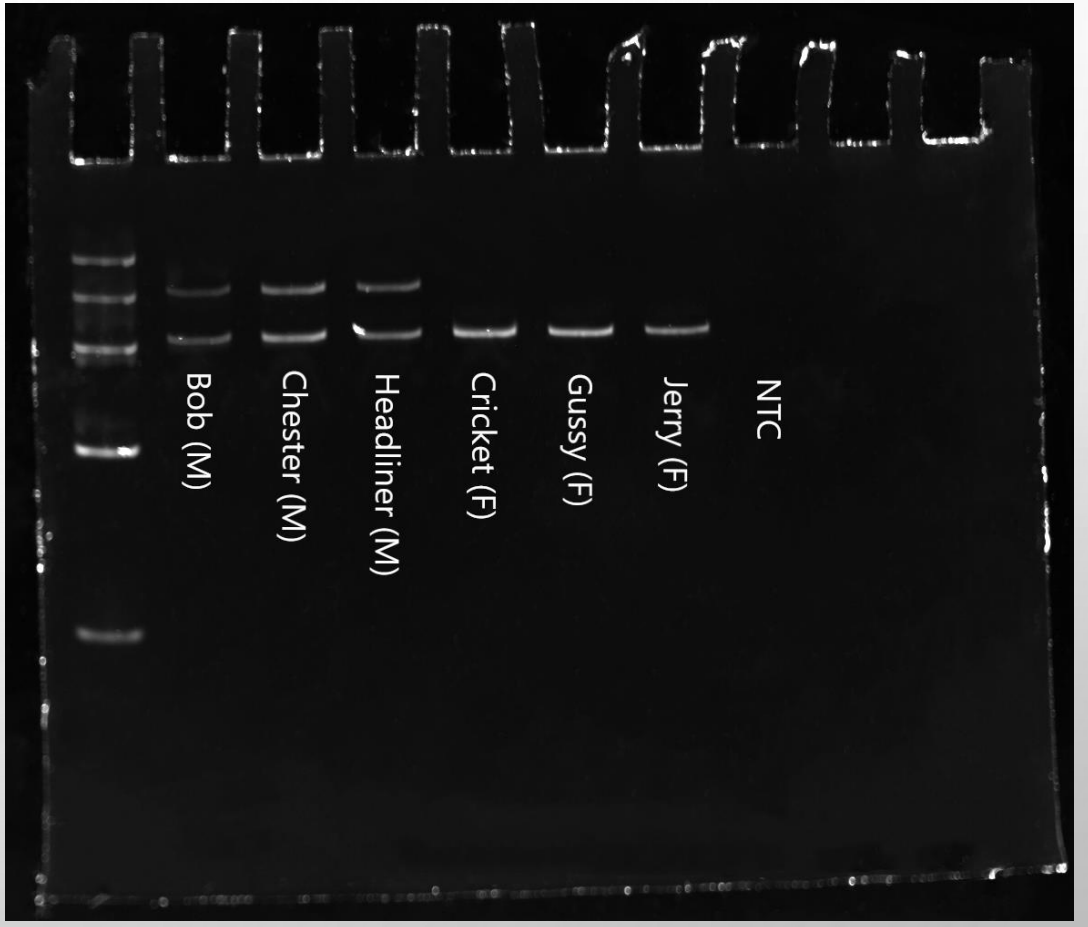
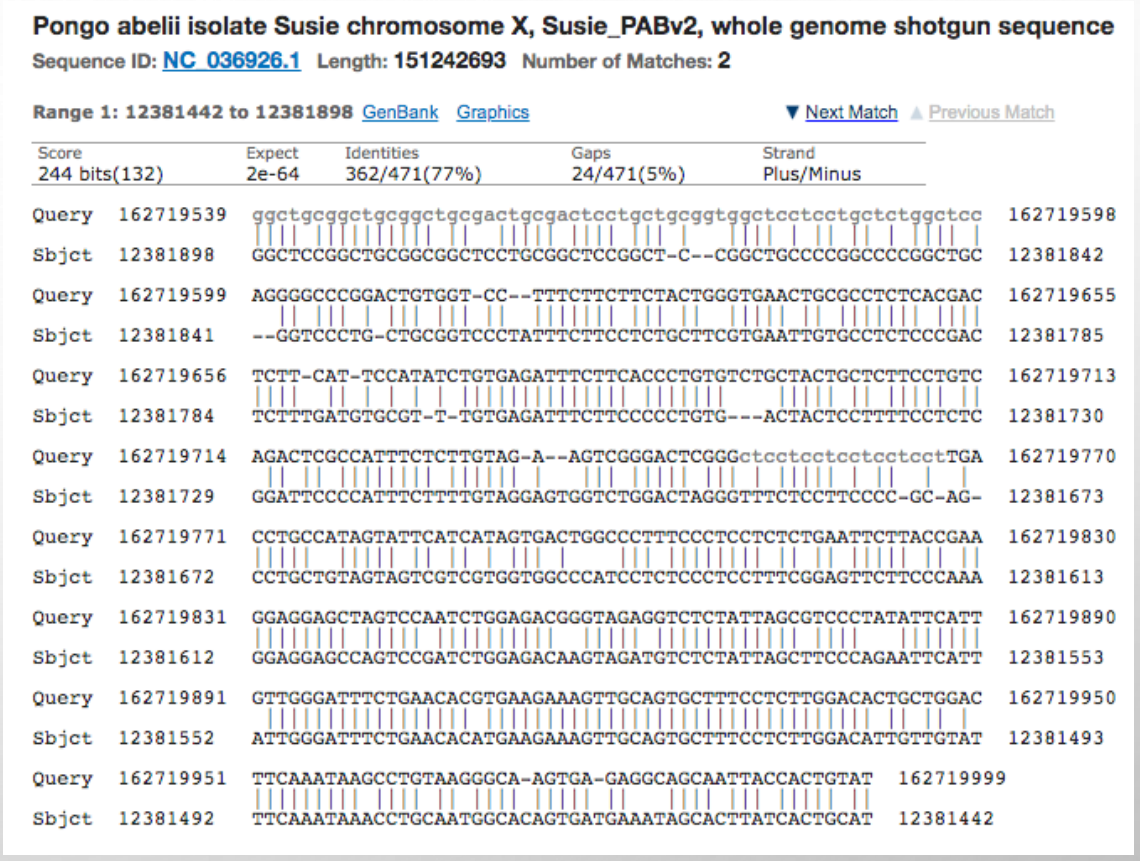
Results & Discussion



Conclusion & Future Research

- Gel electrophoresis showed confirmation of hypothesis – novel primers can accurately be used to identify sex in equine samples
- Future research:
  - qRT-PCR assay and quantitative melting curve analysis of novel primers
  - Compare and align DNA sequences of multiple species – study conservation of gene sequence



Pongo abelii isolate Susie chromosome X, Susie_PABv2, whole genome shotgun sequence

Sequence ID: NC_036926.1 Length: 151242693 Number of Matches: 2

Range 1: 12381442 to 12381898 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
244 bits(132)	2e-64	362/471(77%)	24/471(5%)	Plus/Minus

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Query  162719539  ggctgcggctgcggctgcgactgcgactcctgctgcggtggctcctcctgctctggctcc  162719598
Sbjct  12381898   GGCTCCGGCTGCGGCGGCTCCTGCGGCTCCGGCT-C--CGGCTGCCCCGGCCCCGGCTGC  12381842

Query  162719599  AGGGGCCCGGACTGTGGT-CC--TTTCTTCTTCTACTGGGTGAACTGCGCCTCTCACGAC  162719655
Sbjct  12381841   --GGTCCCTG-CTGCGGTCCCTATTTCTTCCTCTGCTTCGTGAATTGTGCCTCTCCCGAC  12381785

Query  162719656  TCTT-CAT-TCCATATCTGTGAGATTTCTTCACCCTGTGTCTGCTACTGCTCTTCCTGTC  162719713
Sbjct  12381784   TCTTTGATGTGCGT-T-TGTGAGATTTCTTCCCCCTGTG---ACTACTCCTTTTCCTCTC  12381730

Query  162719714  AGACTCGCCATTTCTCTTGTAG-A--AGTCGGGACTCGGGctcctcctcctcctcctTGA  162719770
Sbjct  12381729   GGATTCCCCATTTCTTTTGTAGGAGTGGTCTGGACTAGGGTTTCTCCTTCCCC-GC-AG-  12381673

Query  162719771  CCTGCCATAGTATTCATCATAGTGACTGGCCCTTTCCCTCCTCTCTGAATTCTTACCGAA  162719830
Sbjct  12381672   CCTGCTGTAGTAGTCGTCGTGGTGGCCCATCCTCTCCCTCCTTTCGGAGTTCTTCCCAAA  12381613

Query  162719831  GGAGGAGCTAGTCCAATCTGGAGACGGGTAGAGGTCTCTATTAGCGTCCCTATATTCATT  162719890
Sbjct  12381612   GGAGGAGCCAGTCCGATCTGGAGACAAGTAGATGTCTCTATTAGCTTCCCAGAATTCATT  12381553

Query  162719891  GTTGGGATTTCTGAACACGTGAAGAAAGTTGCAGTGCTTTCCTCTTGGACACTGCTGGAC  162719950
Sbjct  12381552   ATTGGGATTTCTGAACACATGAAGAAAGTTGCAGTGCTTTCCTCTTGGACATTGTTGTAT  12381493

Query  162719951  TTCAAATAAGCCTGTAAGGGCA-AGTGA-GAGGCAGCAATTACCACTGTAT  162719999
Sbjct  12381492   TTCAAATAAACCTGCAATGGCACAGTGATGAAATAGCACTTATCACTGCAT  12381442
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Example pairwise alignment: ZRSR2 gene, X-chromosome; *Pongo abelii* (Sumatran orangutan) and *Mus musculus* (common house mouse)

References

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