**PCR Genotyping of *Sox9 Asp272del NEW PROTOCOL***

**PCR primers**

Sox9 del wt only-F ATCGACTTCCGCGACG

Sox9 del GT-R TGGCAAGTATTGGTCAAACTCA

Sox9 del mutant only-F CCATCGACTTCCGCGTGG

Sox9 del GT-R TGGCAAGTATTGGTCAAACTCA

Note: Reverse primers are the same. It is also the same reversed primer used for the OLD PROTOCOL.

**Product Sizes** Mutant band: 156 bp; Wild type band: 156 bp

Need to run two separate PCRs for wt and mutant primers. Wild type animals will only show a 156bp band in wt reaction. Heterozygous will show two 156bp bands in both reactions. Mutant animals will only show a 156bp band in mutant reaction.

**PCR Reaction Mix**

10 µl Green Master Mix (Promega M7122)

7 µl di-H2O

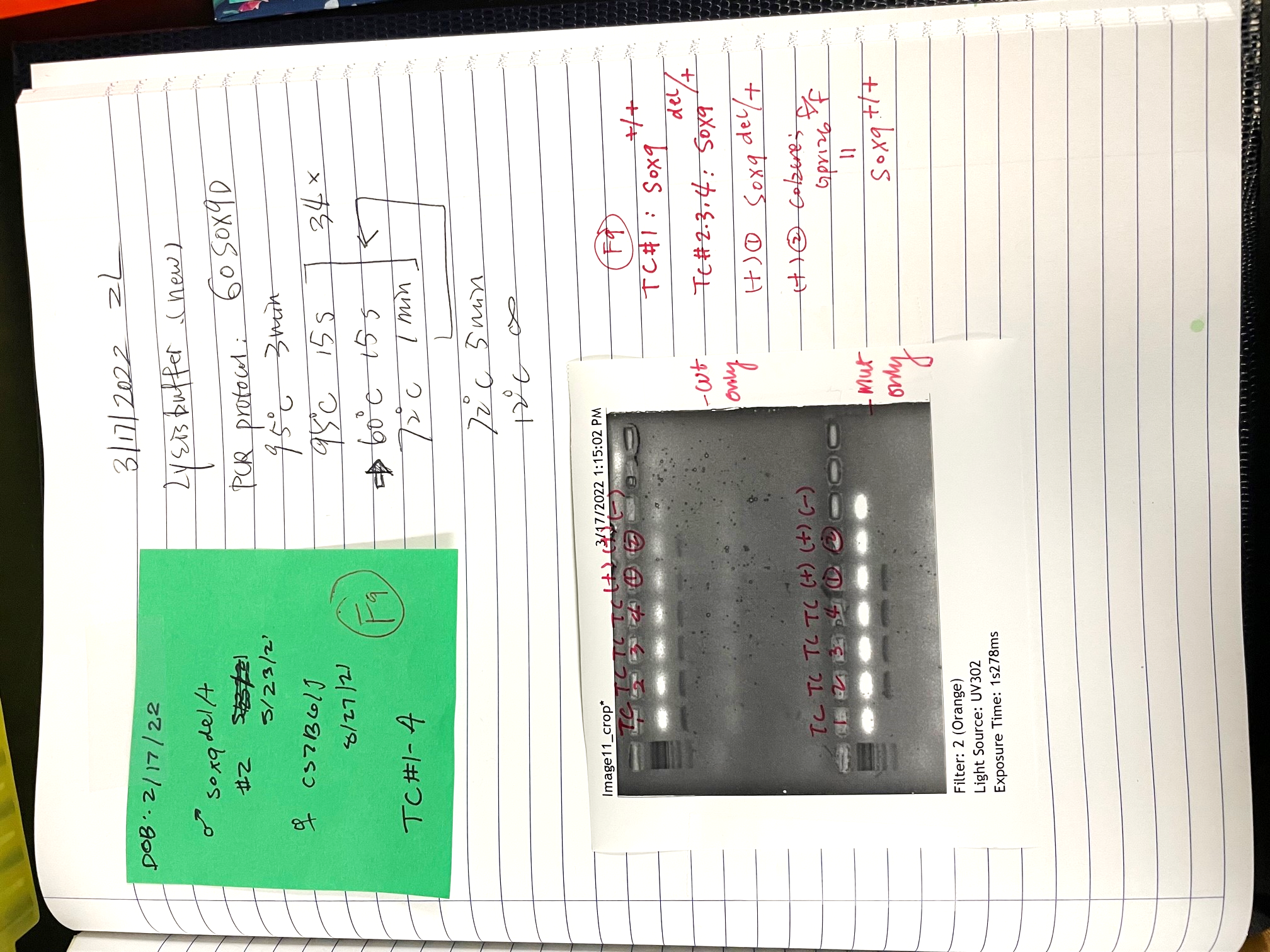
1 µl Forward/Reverse primer mix \*

2 µl gDNA

20 µl

\*to make primer mix, add 10 µl of forward primer (100 µM stock) and 10 µl of reversed primer (100 µM stock) to 80 µl of di-H2O.

**PCR Program (SOX960D)**



1. 95 °C 3min
2. 95 °C 15sec
3. 60 °C 15sec
4. 72 °C 1min
5. Go to 2, repeat 34x
6. 72 °C 5min
7. Keep at 12 °C

**PCR Genotyping of *Sox9 Asp272del OLD PROTOCOL***

**PCR primers**

Sox9 del GT-F GTCTTTCTCTTTTATGGCCTGC

Sox9 del GT-R TGGCAAGTATTGGTCAAACTCA

**Product Sizes** Mutant band: 233 bp; Wild type band: 236 bp

Note: Use 4% MetaPhor high resolution gel to separate bands. Run a longer gel (>50min). 90min gives better results.

**PCR Reaction Mix**

10 µl Green Master Mix (Promega M7122)

7 µl di-H2O

1 µl Forward/Reverse primer mix \*

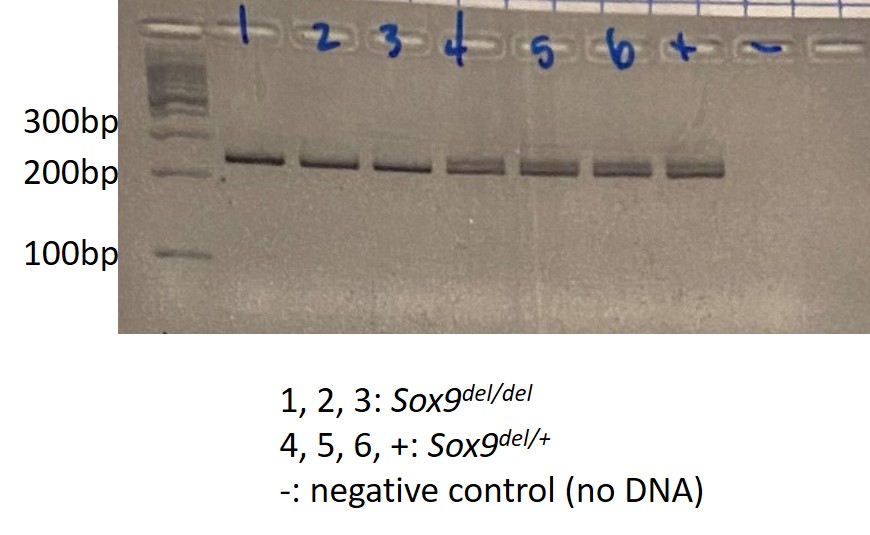
2 µl gDNA

20 µl

\*to make primer mix, add 10 µl of forward primer (100 µM stock) and 10 µl of reversed primer (100 µM stock) to 80 µl of di-H2O.

**PCR Program (55C)**

1. 94 °C 3min
2. 94 °C 30sec



1. 55 °C 30sec
2. 72 °C 1min
3. Go to 2, repeat 34x
4. 72 °C 5min
5. Keep at 12 °C

(Note: heterozygous *Sox9del/+* will show two bands. Wild type (*Sox9+/+*) and *Sox9del/del* will show only one band. This protocol **cannot** distinguish wild type and *Sox9del/del.* Sequencing is required!!)