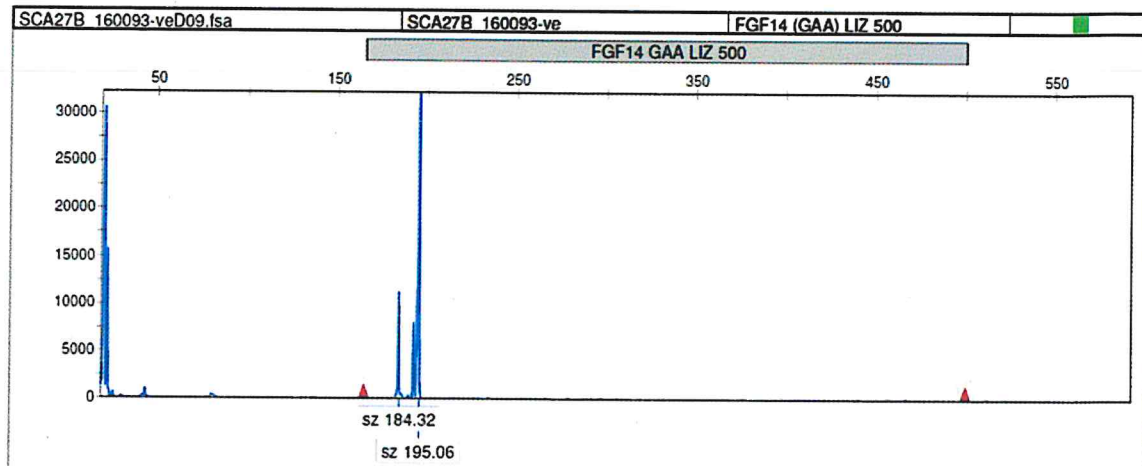
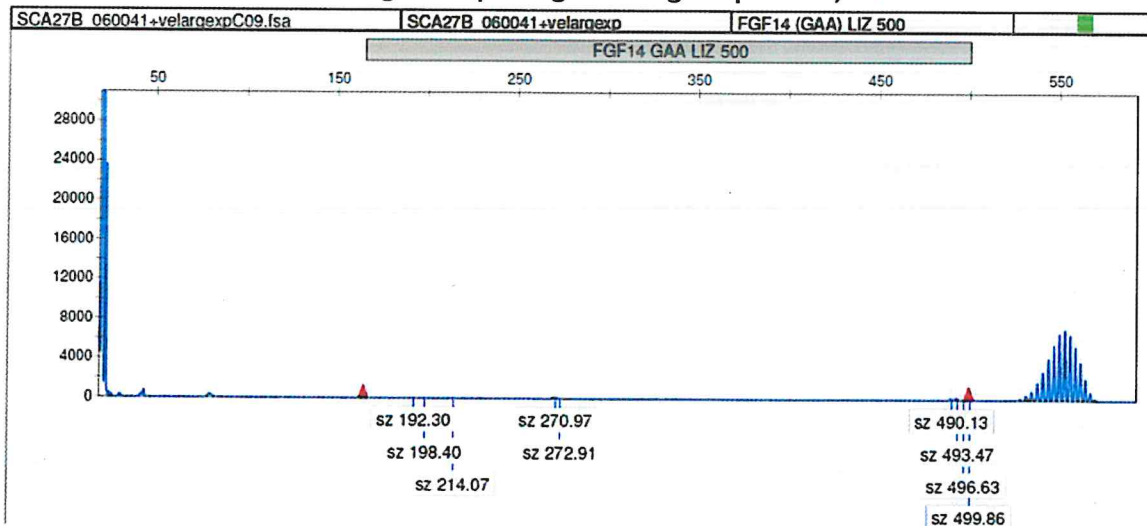


SCA27B analyses results 19-11-24 CV**Test sample: 240422****1. LR-PCR product analysis by capillary electrophoresis****Negative control 160093:****SCA27B calculation algorithm for fragments <243 bp, (8-25 GAA repeats):**Fragment Size - 165 = $X/3.0 = Y - 1$ = repeats # (correction made based on sequenced controls)Expected GAA repeat sizes: 9/9Detected alleles: 195bpPCR product size without GAA repeat alleles: 165bpCalculated CAG repeat sizes: 1. $195 - 165 = 30 \text{ bp} / 3 = 10 - 1 = 9 \text{ GAA repeats}$ **Non-affected control with large non-pathogenic range expansion, 060041:****SCA27B calculation algorithm for fragments 500-600 bp, (~125-160 GAA repeats):**Fragment Size - 165 = $X/2.9 = Y + 9$ = repeats # (correction made based on sequenced controls) ± 5 Expected GAA repeat sizes: 141/141Detected alleles: 549bp

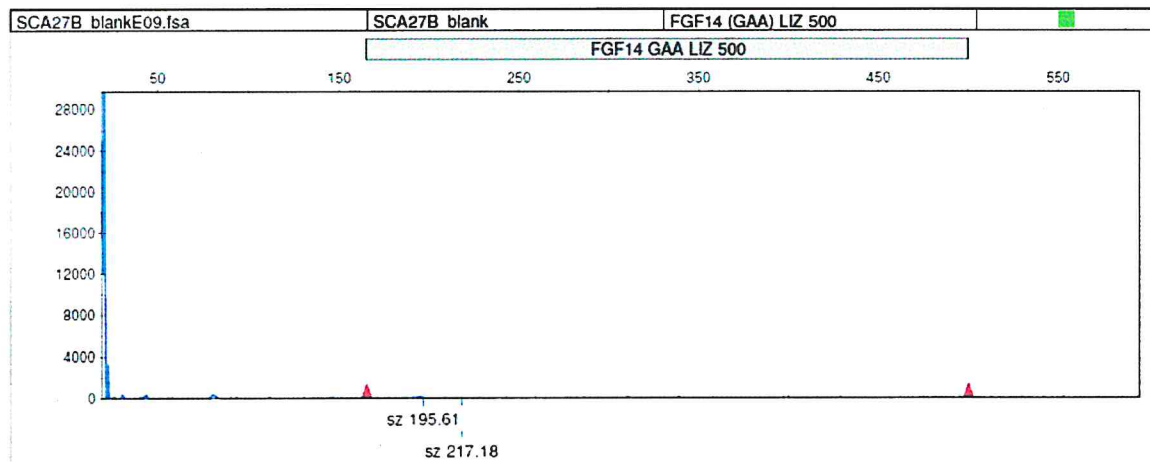
✓

46
22-11-2024

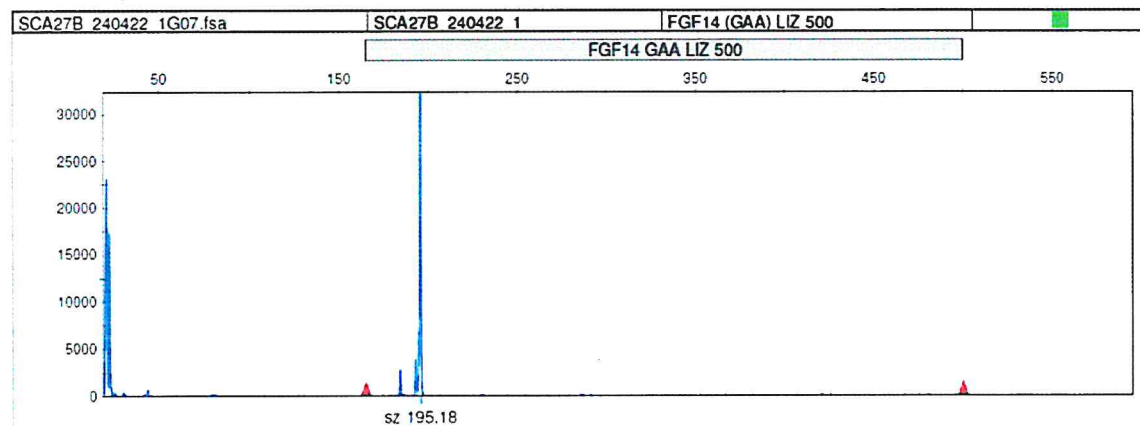
PCR product size without GAA repeat alleles: 165bp

Calculated GAA repeat sizes: 1. $549 - 165 = 384\text{bp} / 2.9 = 132 + 9 = 141$ GAA repeats ± 5

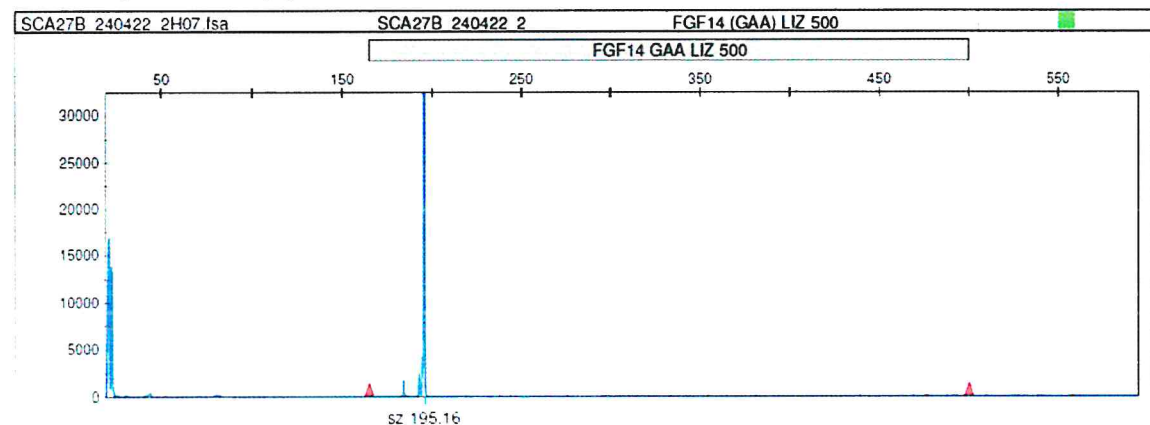
Blank:



Test sample 240422(1):



Test sample 240422(2):



Detected alleles: 195bp

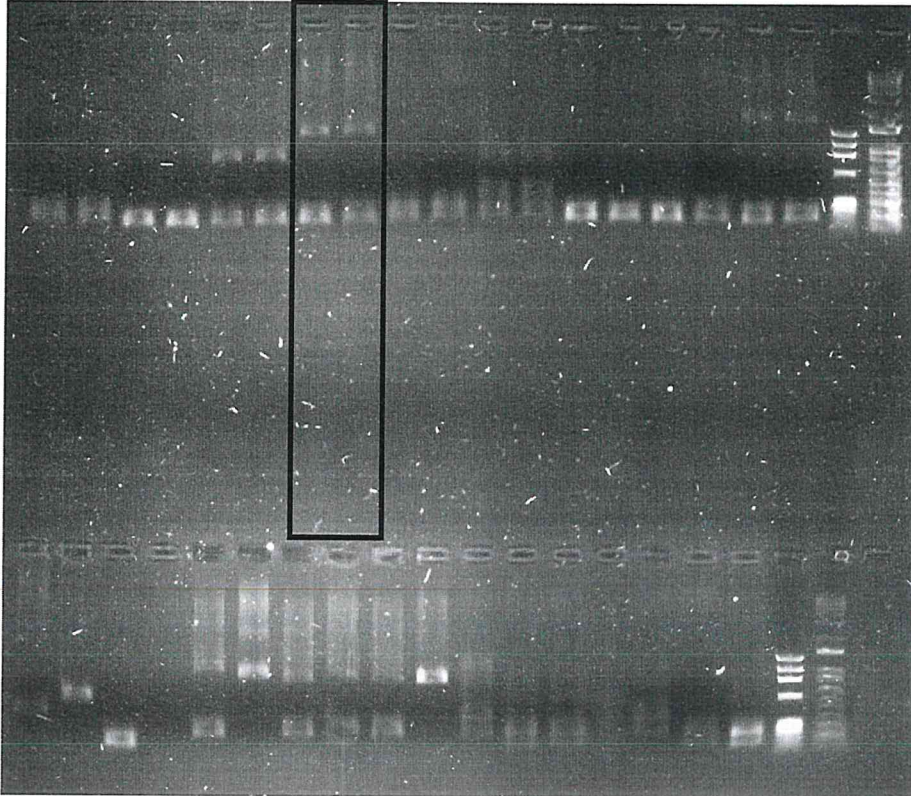
SCA27B calculation algorithm for fragments <243 bp, (8-25 GAA repeats):

Fragment Size - 165 = $X/3.0 = Y - 1$ = repeats # (correction made based on sequenced controls)

PCR product size without GAA repeats: 165bp

Calculated GAA repeat sizes: 1. $195 - 165 = 30\text{bp} / 3 = 10 - 1 = 9$ GAA repeats

2. LR-PCR product analysis by agarose gel electrophoresis



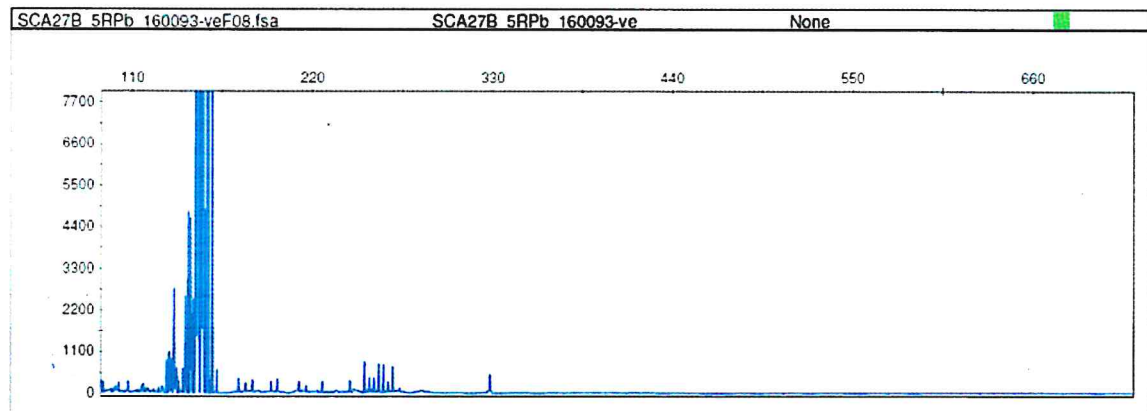
Result: A larger size product was detected in addition to the small one for sample 240422.

KC
N/E

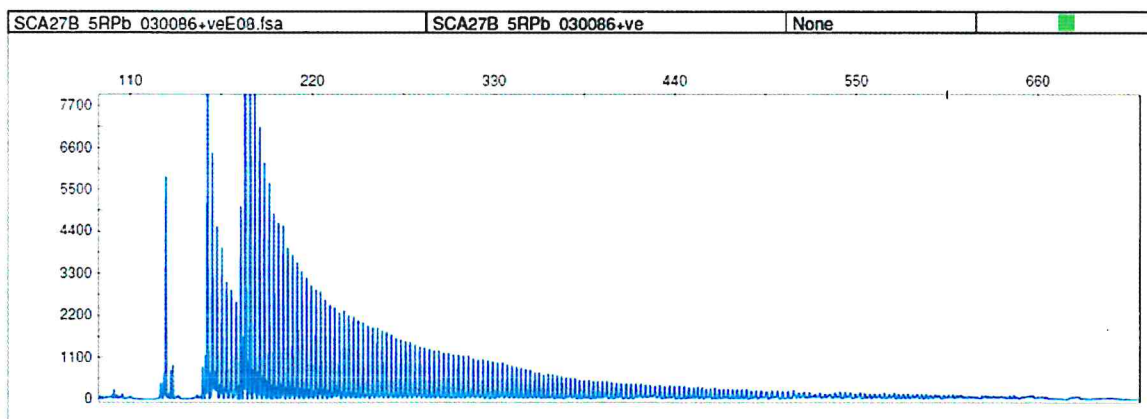
- 1.230245
- 2.230245
- 3.240090
- 4.240090
- 5.240179
- 6.240179
- 7.240422
- 8.240422
- 9.240472
- 10.240472
- 11.240473
- 12.240473
- 13.240474
- 14.240474
- 15.240507
- 16.240507
- 17.240515
- 18.240515
- 19.PhiX174
- 20.1kb plus ladder
- 21.030086+ve
- 22.060041>125
- 23.160093-ve
- 24.blank
- 25.240484
- 26.240485
- 27.240486
- 28.240487
- 29.240488
- 30.240489
- 31-37. other research
38. PhiX174
- 39.1kb plus ladder

3. 5' RP-PCR analysis for GAA repeat allele

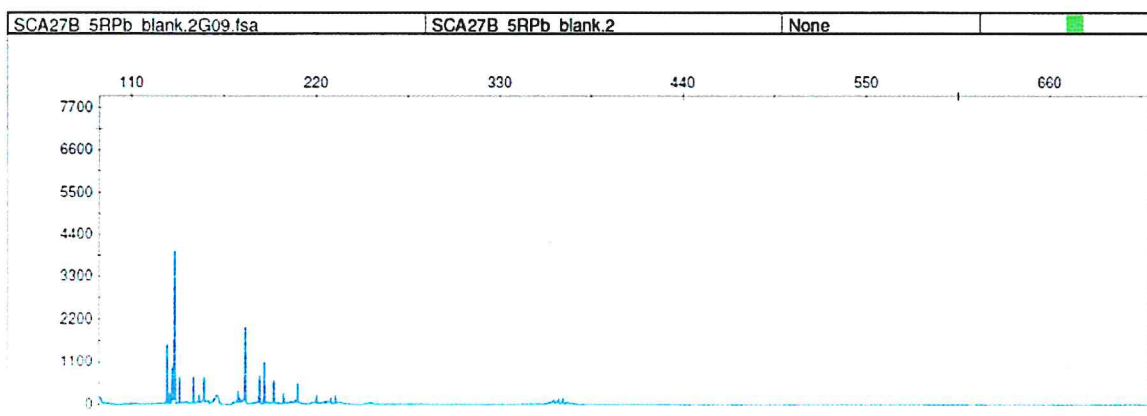
Negative control:



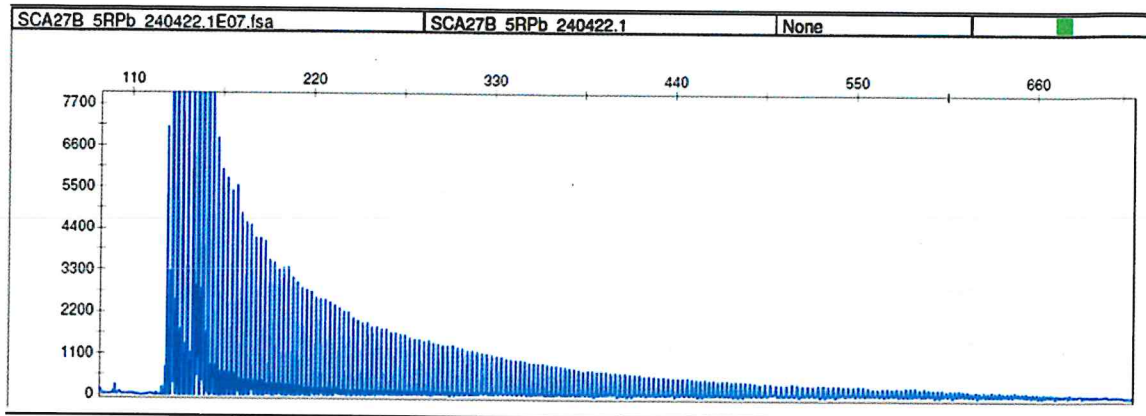
Positive Control:



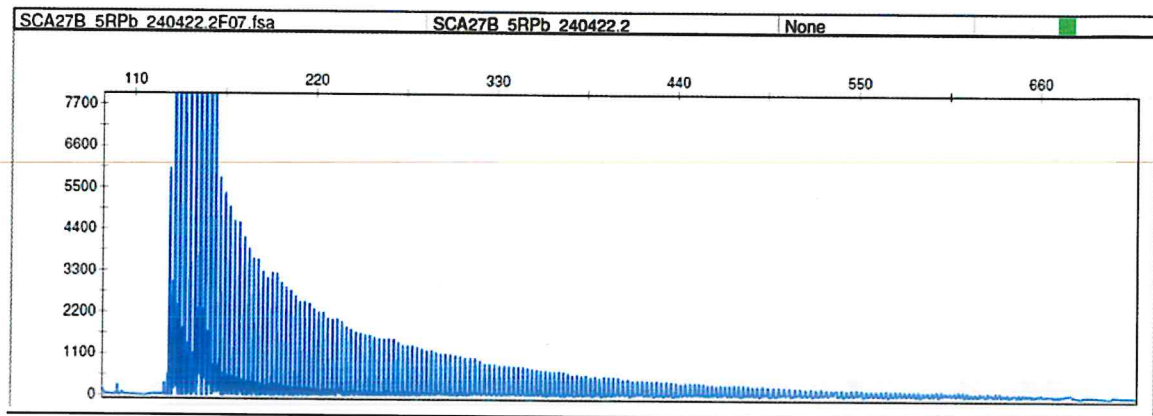
Blank:



Test sample 240422(1):

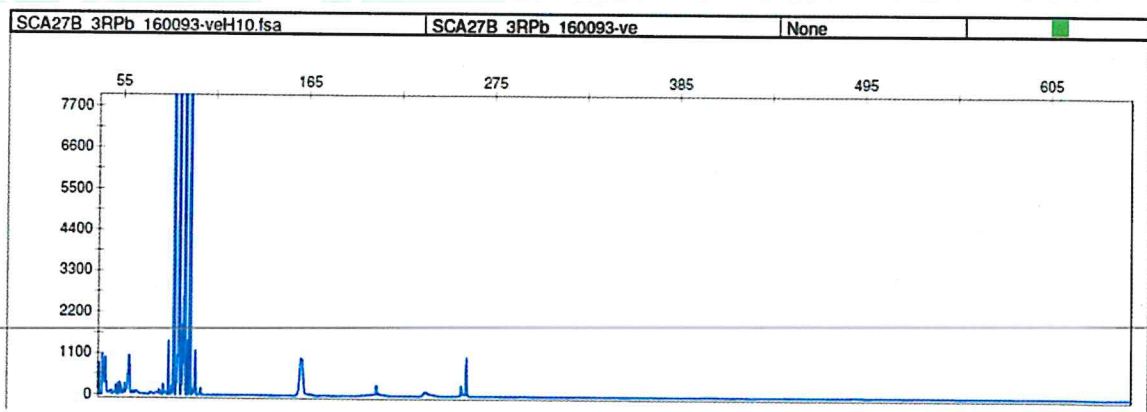


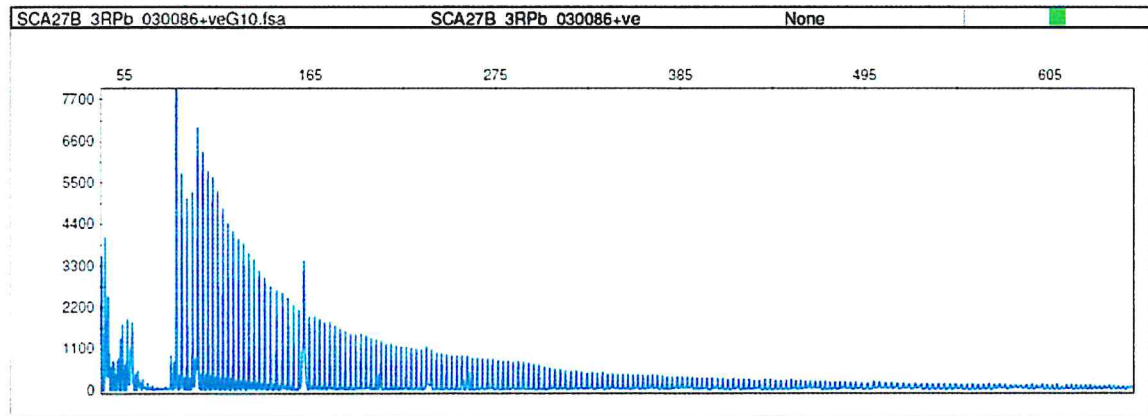
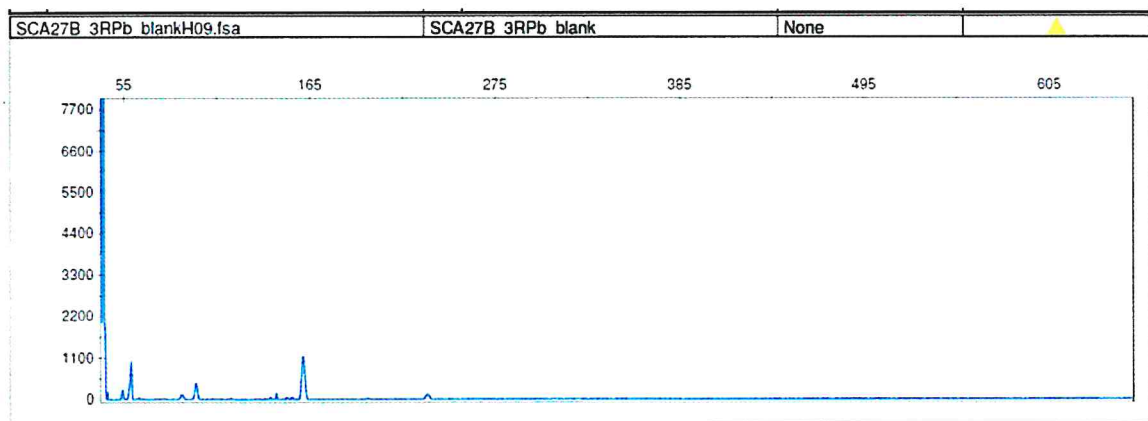
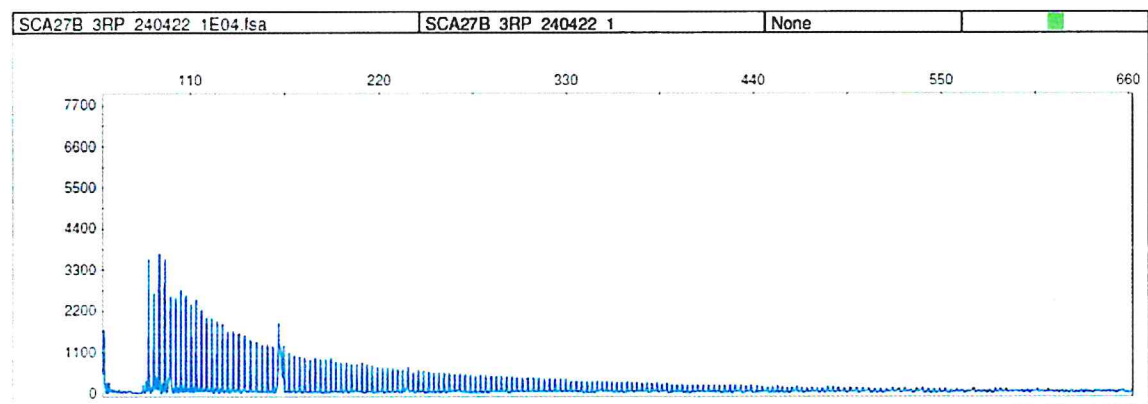
Test sample 240422(2):



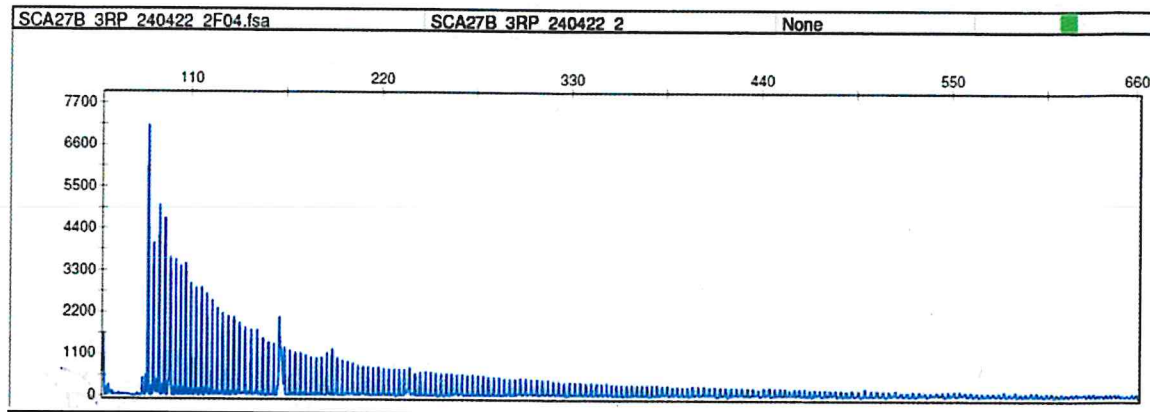
4. 3' RP-PCR analysis for GAA repeat allele

Negative control:



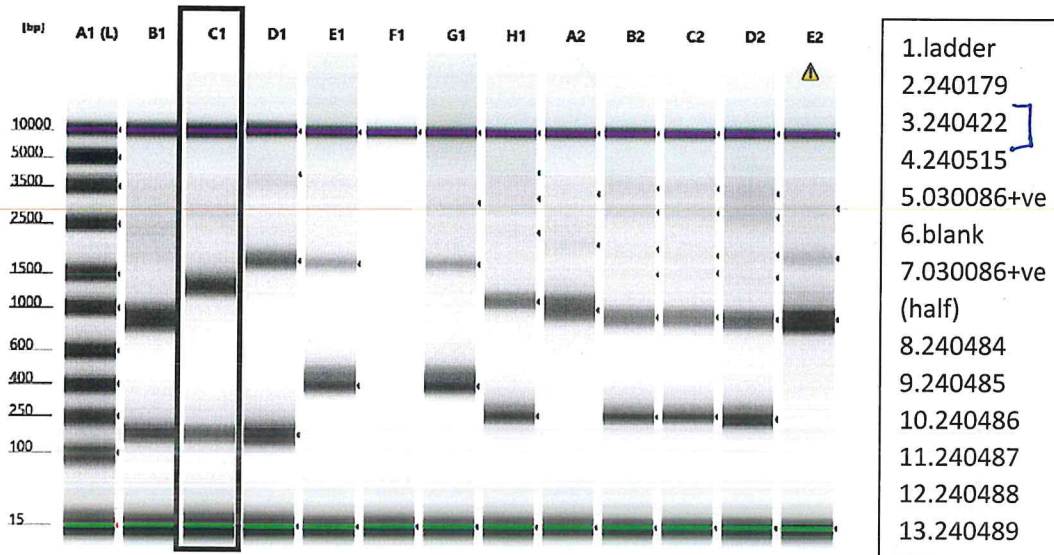
Positive Control:**Blank:****Test sample 240422(1):**

Test sample 240422(2):

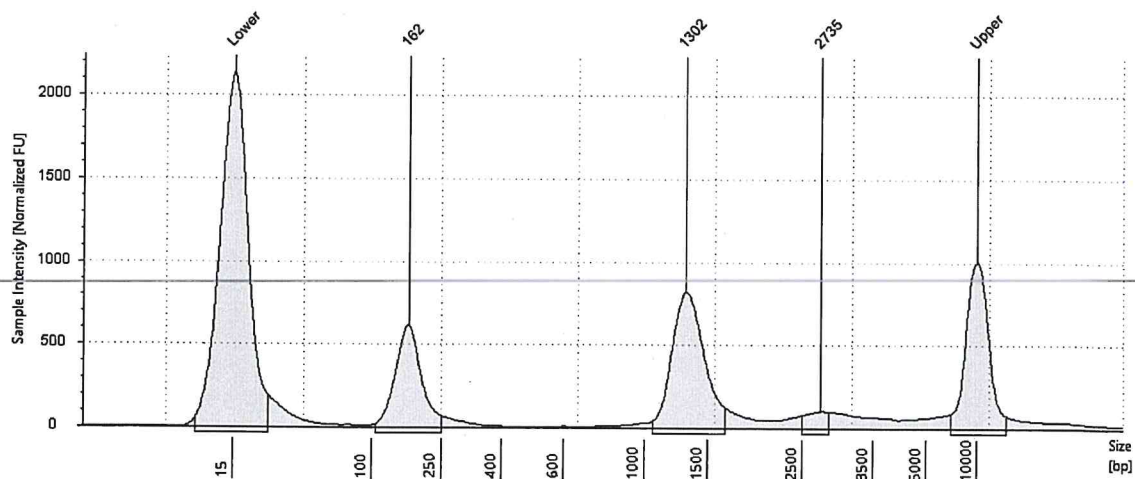


VC
E

5. Tape Station 4150 automated electrophoresis using D5000 Screen Tape



Test sample 240422(1):



SCA27B calculation algorithm for fragments >765 bp, (>200 GAA repeats):

Tape station adjustment

Fragment Size - 165 = X/3.0 = Y - 39.42 = Z/0.97 = repeats #

Detected alleles: 162bp/**1302bp**

PCR product size without GAA repeats: 165bp

Calculated large allele GAA repeat sizes: 1. $1302 - 165 = 1137\text{bp} / 3 = 379 - 39.42 =$

$339.58/0.97 = \mathbf{350 \text{ GAA repeats}}$

KC 350 GAA
repeats

