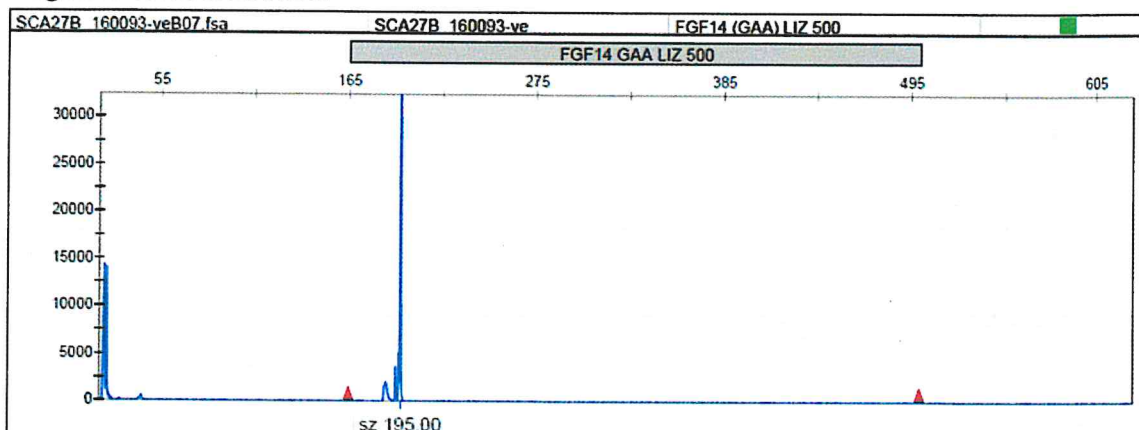
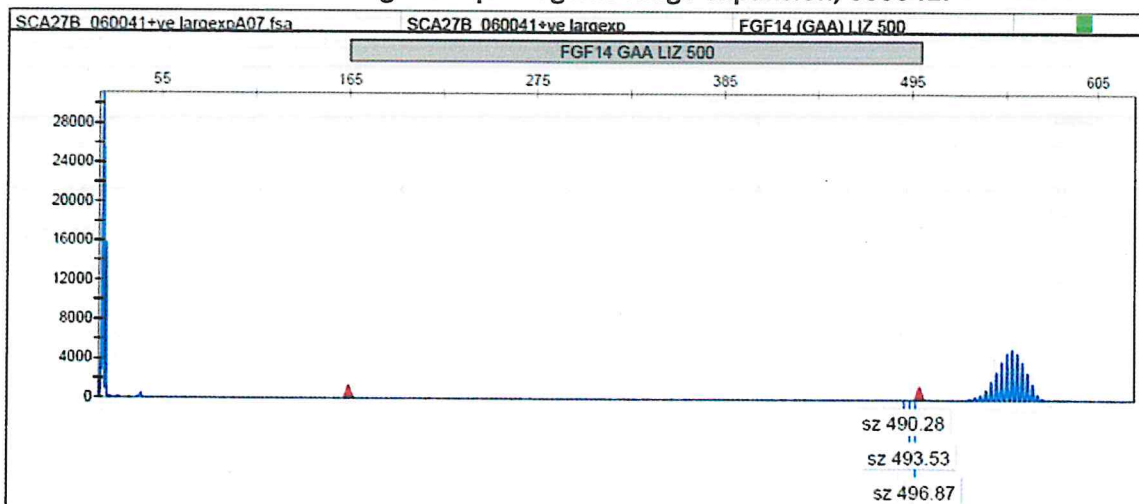
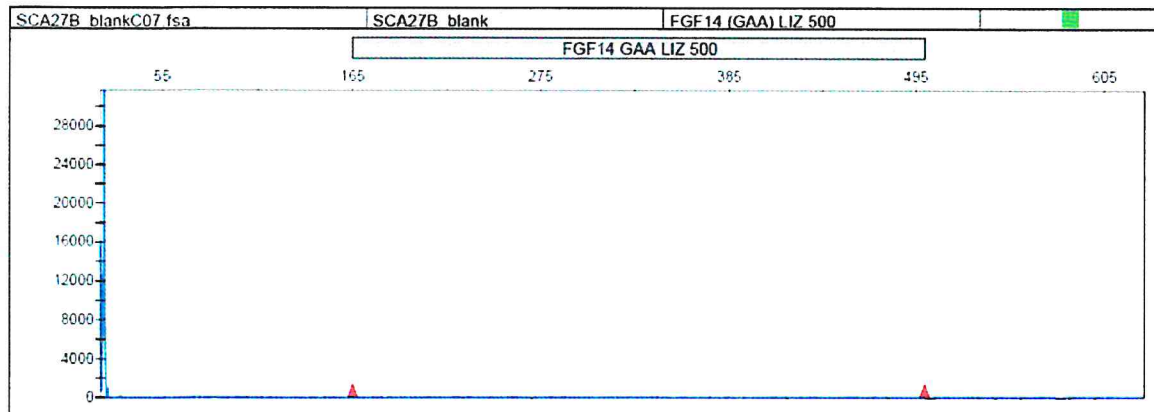
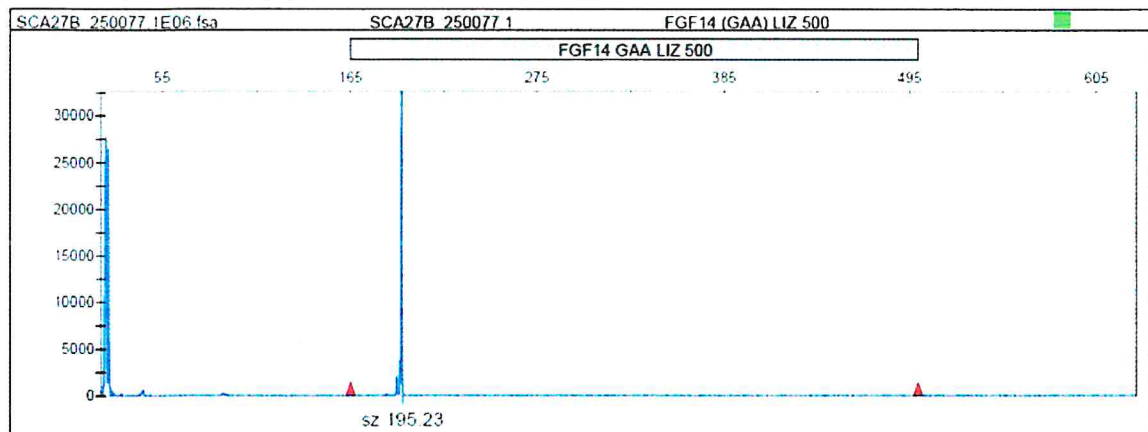
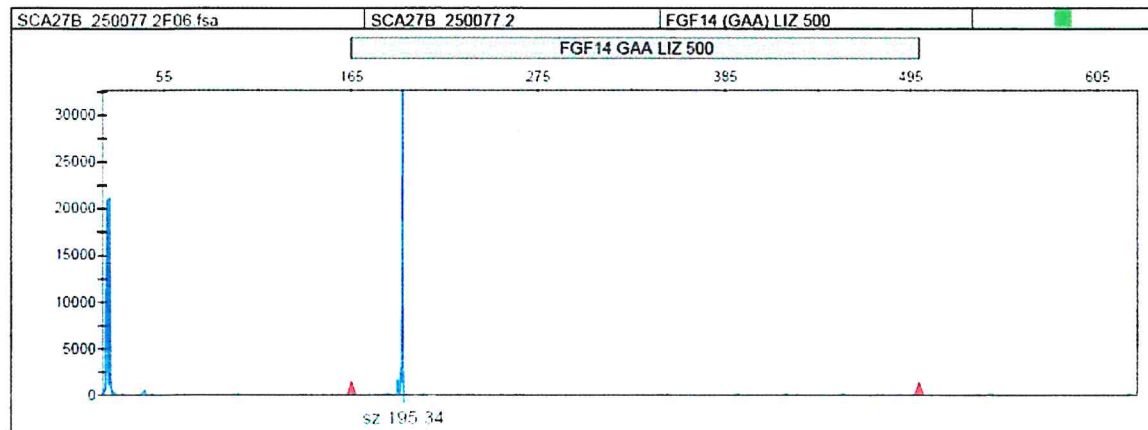


SCA27B analyses results 24-02-25 CV**Test sample: 250077****1. LR-PCR product analysis by capillary electrophoresis****Negative control 160093:****SCA27B calculation algorithm for fragments <243 bp, (8-25 GAA repeats):**Fragment Size - 165 = $X/3.0 = Y - 1$ = repeats # (correction made based on sequenced 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: 549bpPCR product size without GAA repeat alleles: 165bpCalculated GAA repeat sizes: 1. $549 - 165 = 384 \text{ bp} / 2.9 = 132 + 9 = 141 \text{ GAA repeats} \pm 5$

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Blank:**Test sample 250077(1):****Test sample 250077(2):**

Detected alleles: 195 bp

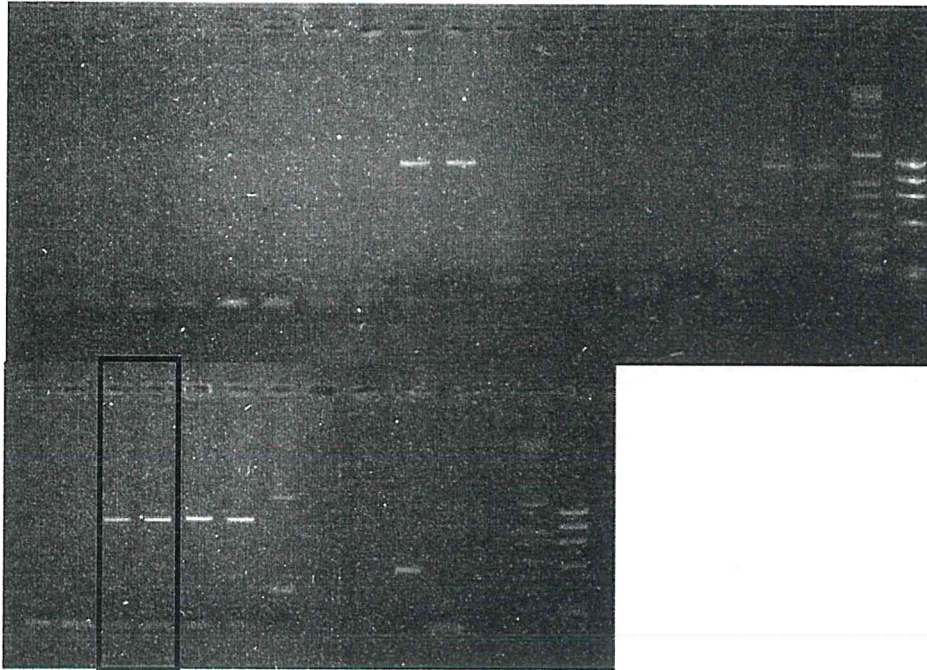
SCA27B calculation algorithm for fragments <243 bp, (8-25 GAA repeats):

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 = 30bp / 3 = 10 - 1 = 9 GAA repeats

2. LR-PCR product analysis by agarose gel electrophoresis



✓

Result: A large size product was detected in addition to the small one for sample 250077.

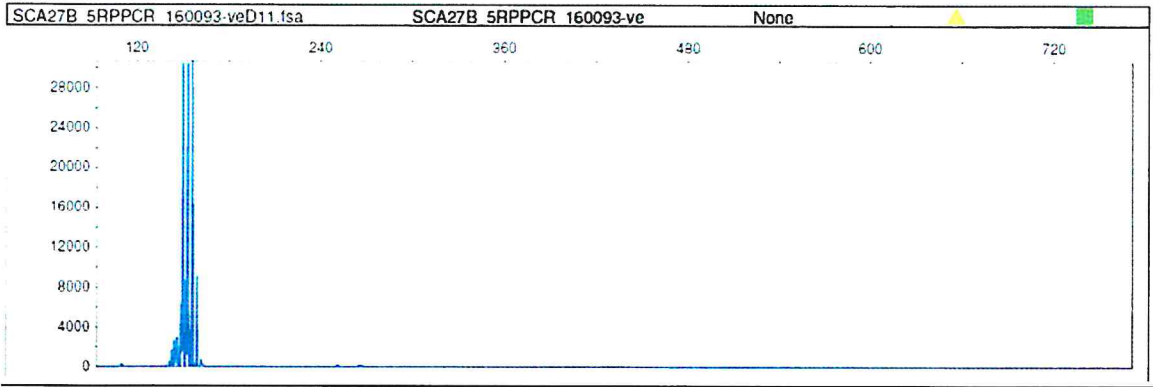
✓

KC

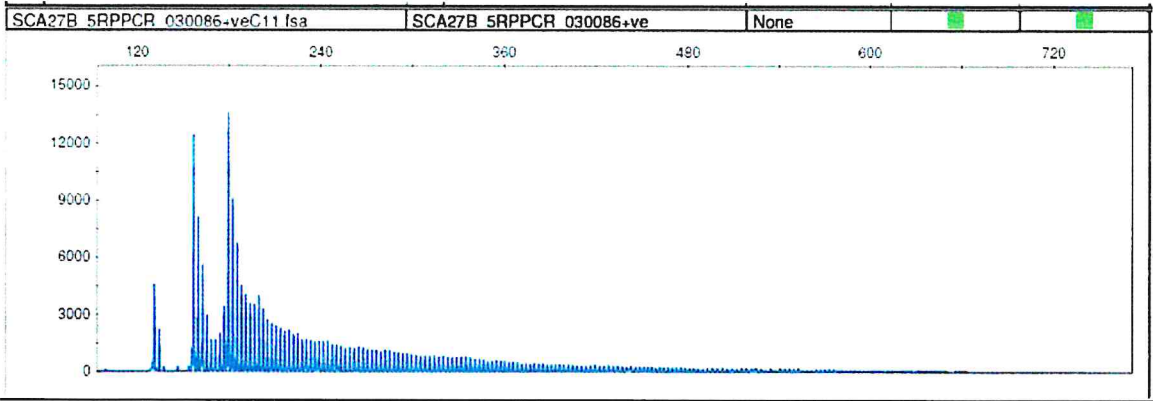
Upper zone
1.160078
2.160078
3.180405
4.180405
5.210189
6.210189
7.210438
8.210438
9.230004
10.230004
11.230019
12.230019
13.240408
14.240408
15.250003
16.250003
17.250054
18.250054
19.1kb plus ladder
20.PhiX174 ladder
Lower zone
1.250062
2.250062
3.250077 ←
4.250077 ←
5.250078
6.250078
7.030086+ve
8.empty
9.empty
10.060041>125
11.160093-ve
12.blank
13.1kb plus ladder
14.PhiX174 ladder

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

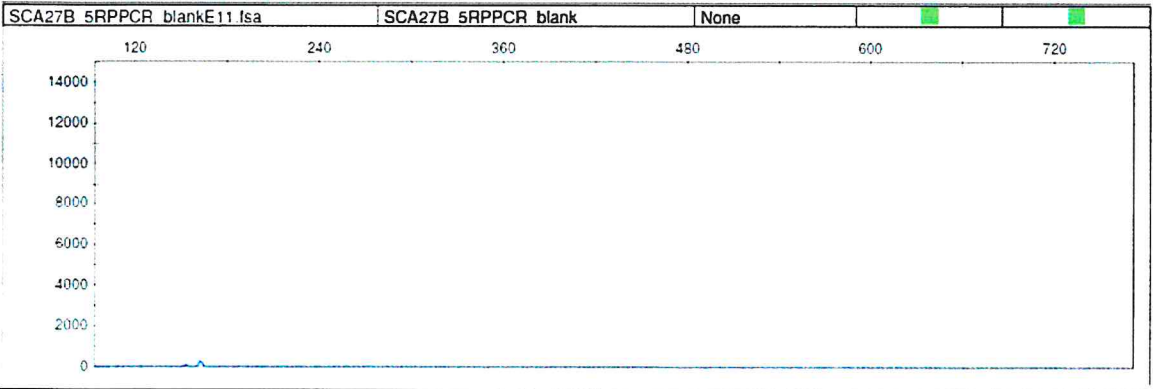
Negative control:



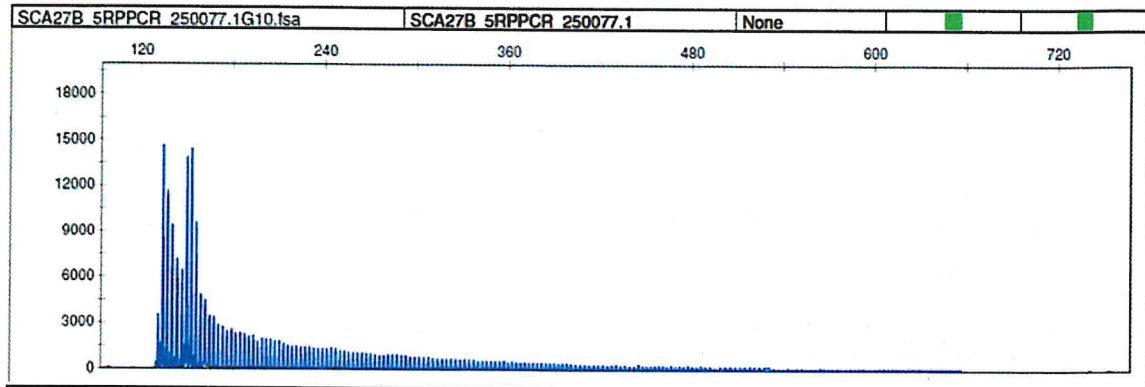
Positive Control:



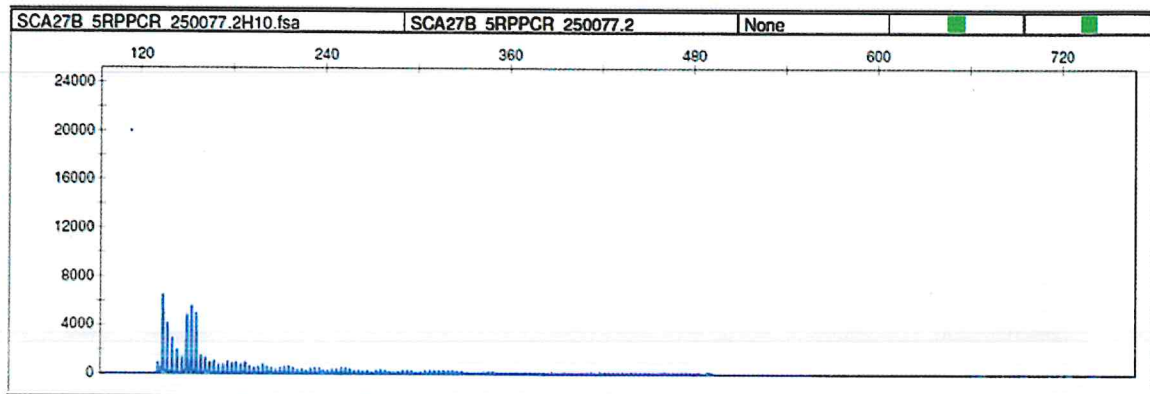
Blank:



Test sample 250077(1):

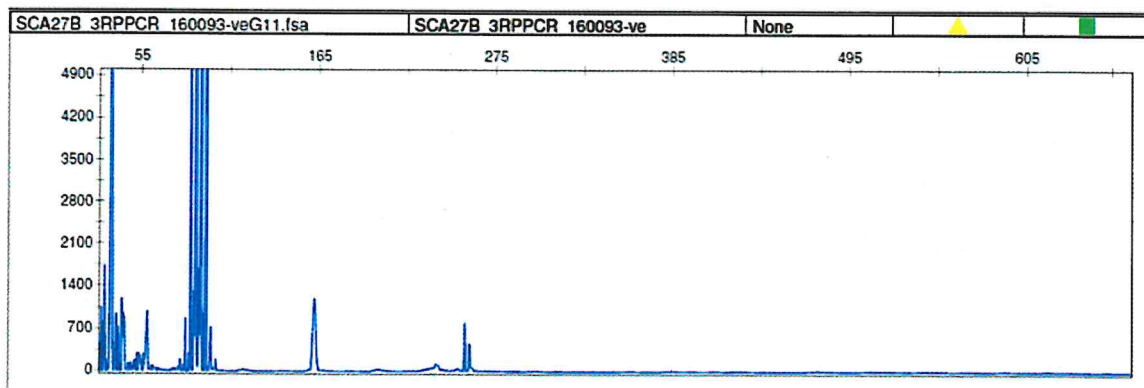


Test sample 250077(2):

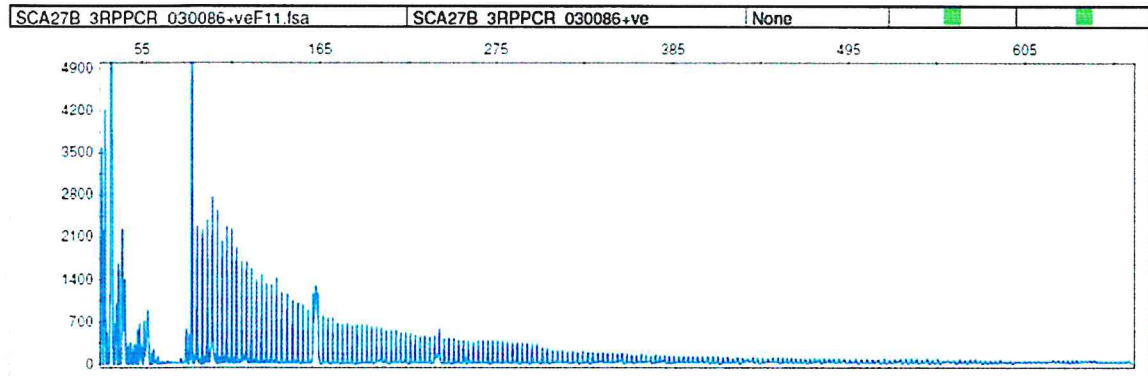


4. 3' RP-PCR analysis for GAA repeat allele

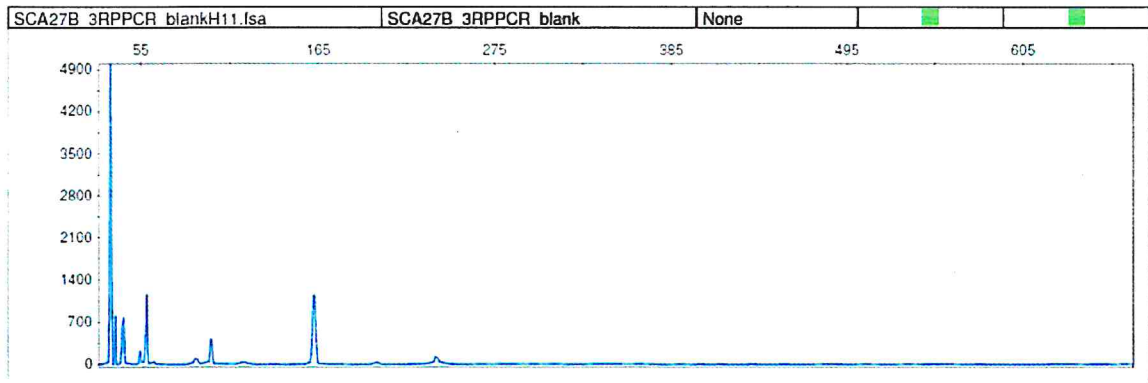
Negative control:



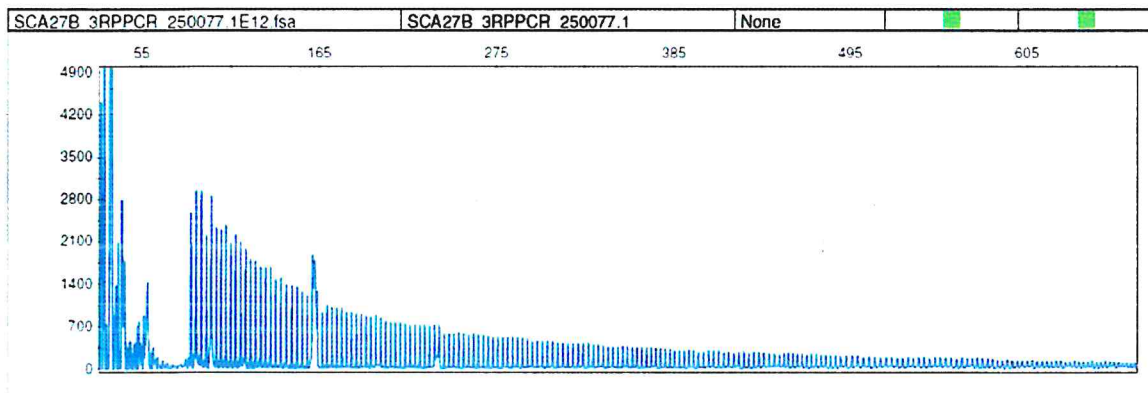
Positive Control:



Blank:



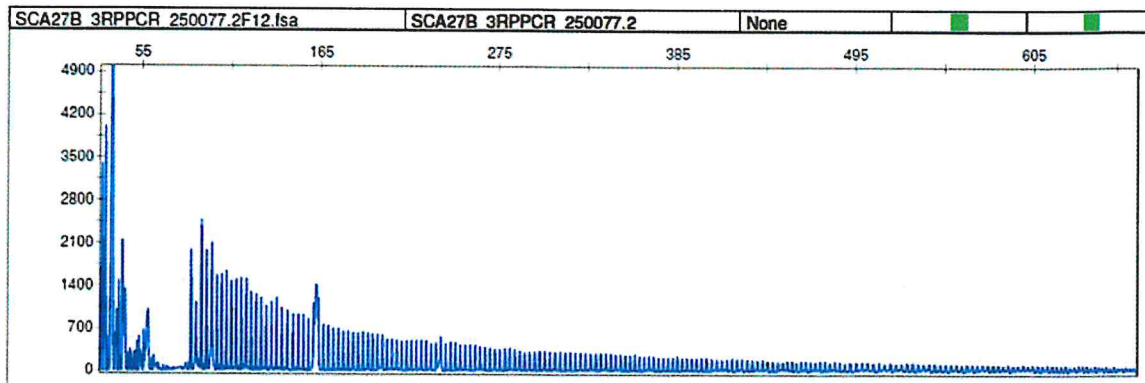
Test sample 250077(1):



KC

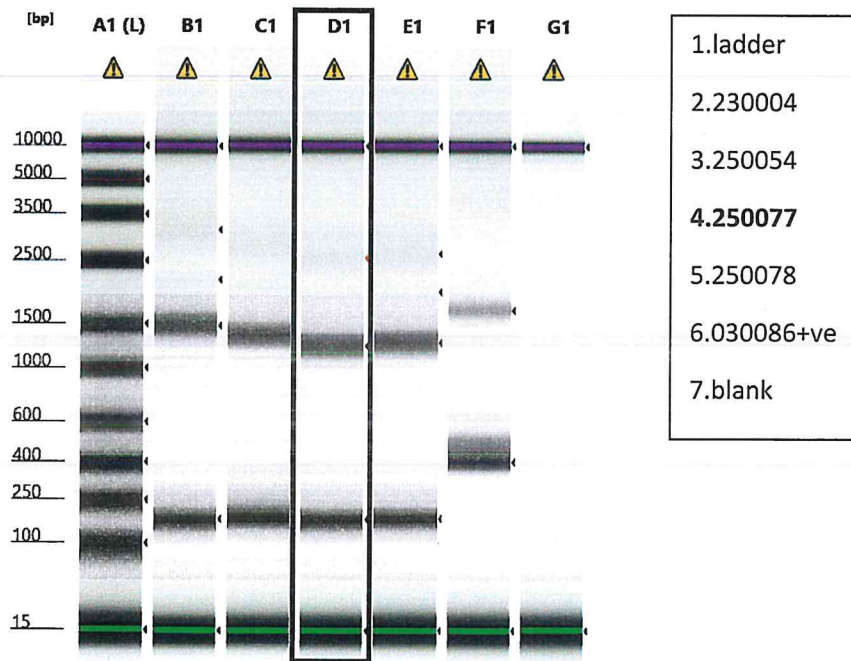
✓

Test sample 250077(2):



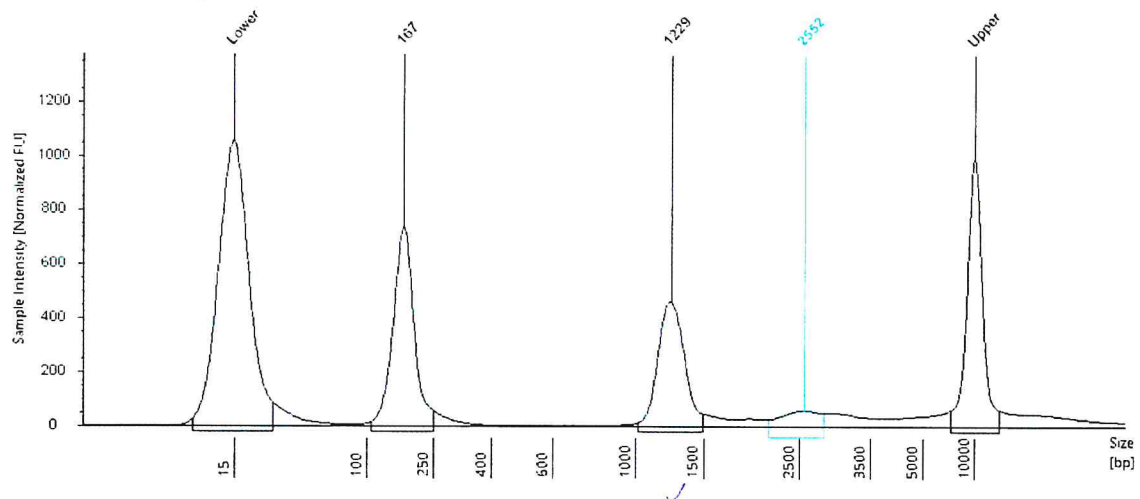
5. Tape Station 4150 automated electrophoresis using D5000 Screen Tape

Test sample 250077(1):



KC

D1: 250077 1/25



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: 1229 bp ✓

PCR product size without GAA repeats: 165bp

Calculated large allele GAA repeat sizes:

1. $1229 - 165 = 1064 \text{ bp} / 3 = 355 - 39.42 = 315 / 0.97 = 325 \text{ GAA repeats}$ ✓

9/325 GAA
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AC