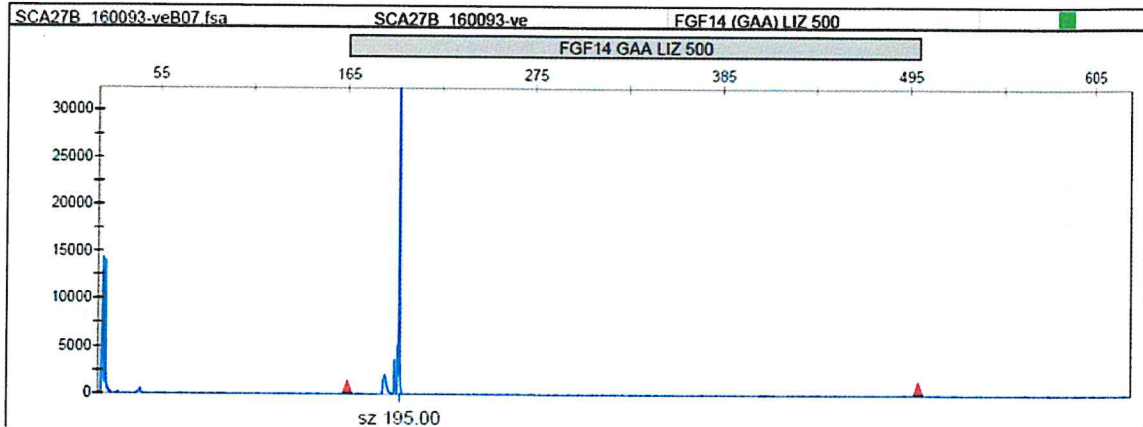
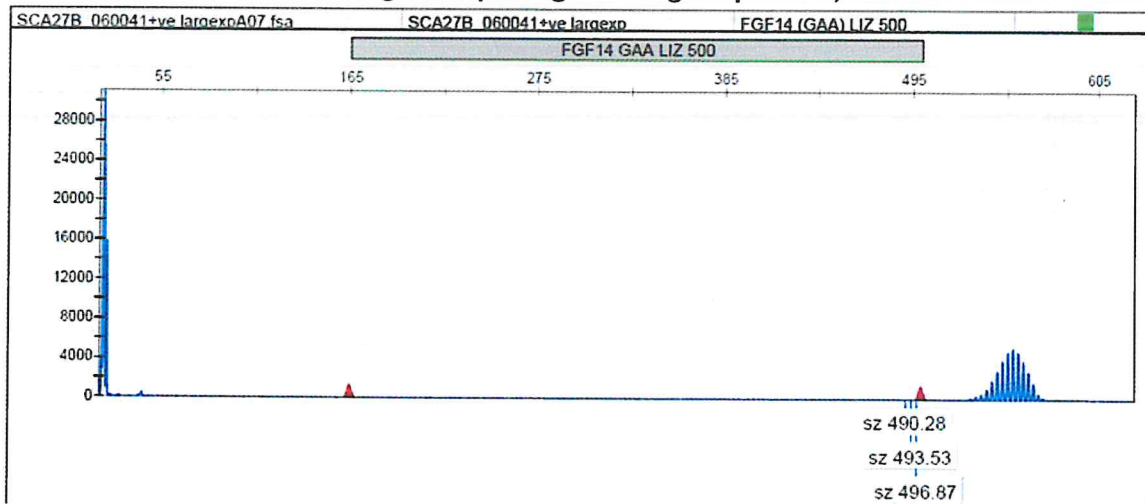
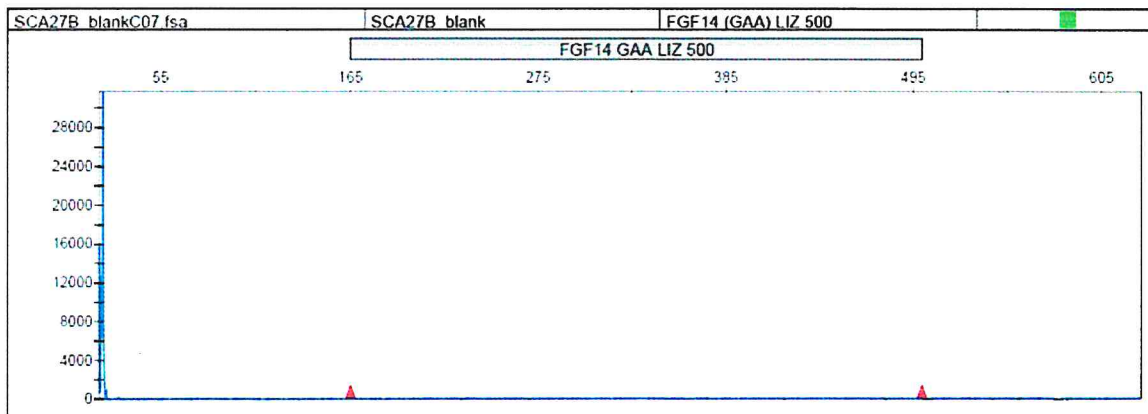
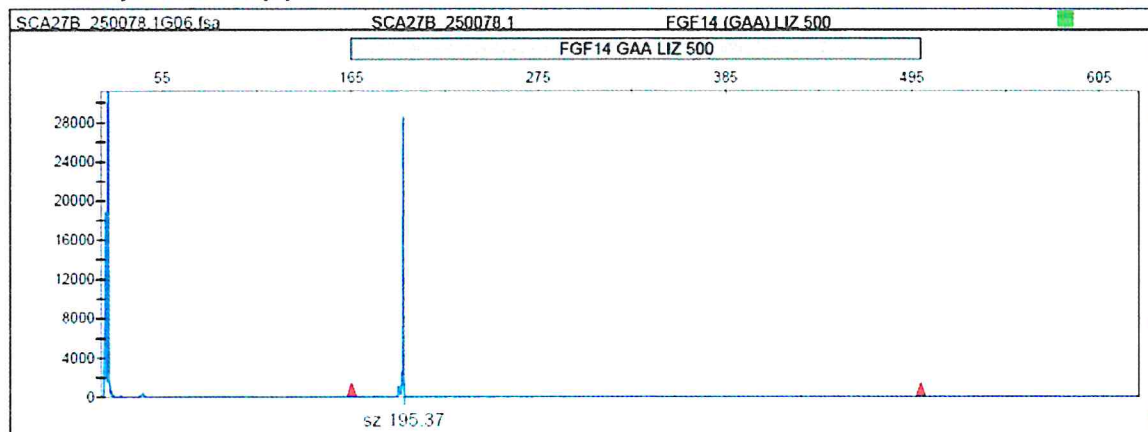
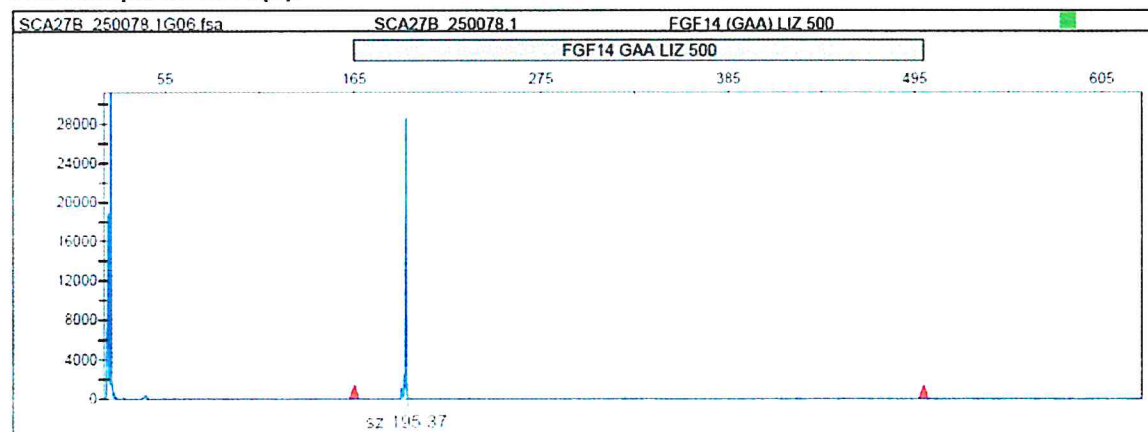


SCA27B analyses results 24-02-25 CV**Test sample: 250078****1. LR-PCR product analysis by capillary electrophoresis****Negative control 160093:****SCA27B calculation algorithm for fragments <243 bp, (8-25 GAA repeats):**Fragment Size - 165 = $X/3.0 = Y - 1$ = repeats # (correction made based on sequenced controls)Expected GAA repeat sizes: 9/9Detected alleles: 195bpPCR product size without GAA repeat alleles: 165bpCalculated CAG repeat sizes: 1. $195 - 165 = 30 \text{ bp} / 3 = 10 - 1 = 9$ 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. $549 - 165 = 384 \text{ bp} / 2.9 = 132 + 9 = 141$ GAA repeats ± 5

Blank:**Test sample 250078(1):****Test sample 250078(2):**

Detected alleles: 195 bp

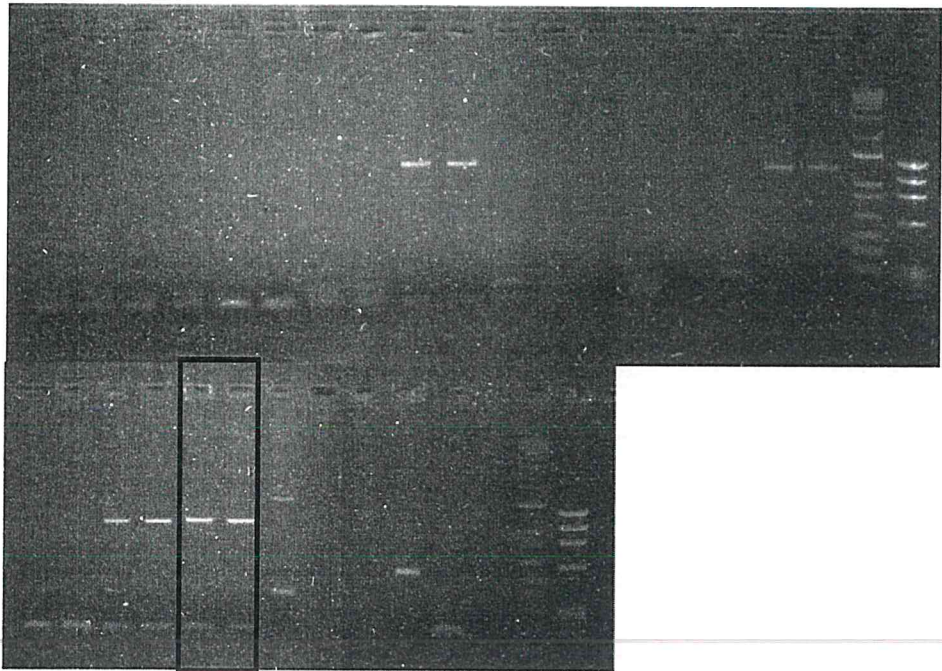
SCA27B calculation algorithm for fragments <243 bp, (8-25 GAA repeats):

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

2. LR-PCR product analysis by agarose gel electrophoresis



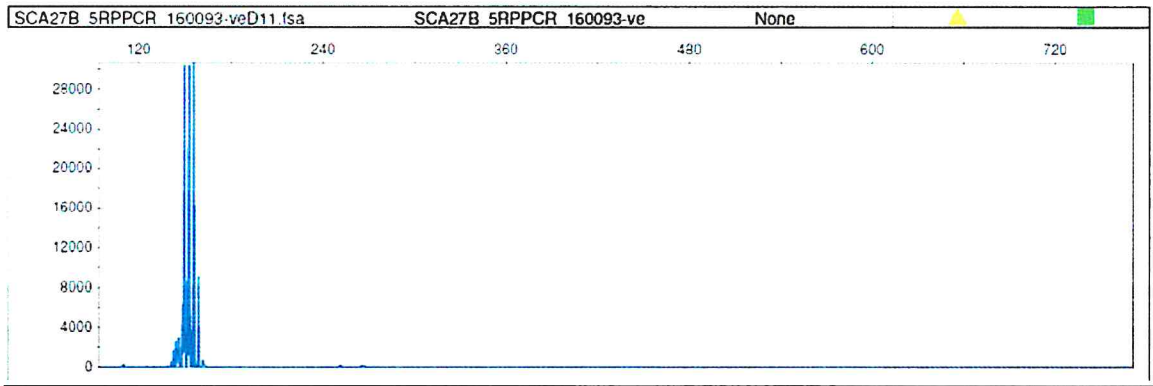
✓
Result: A large size product was detected in addition to the small one for sample 250078.

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

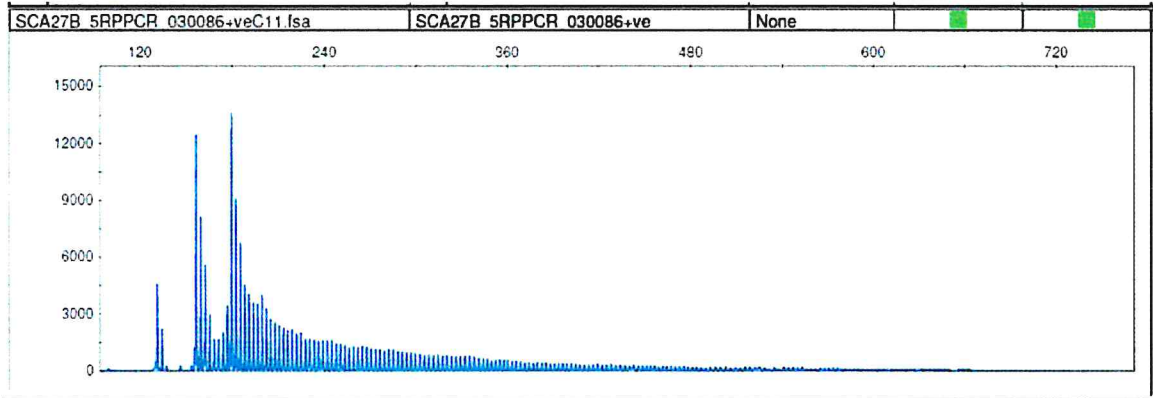
✓
KC

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

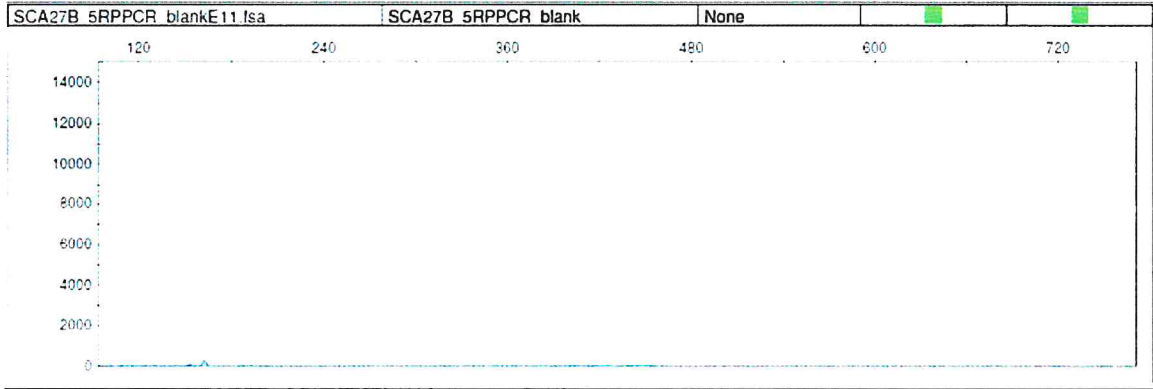
Negative control:



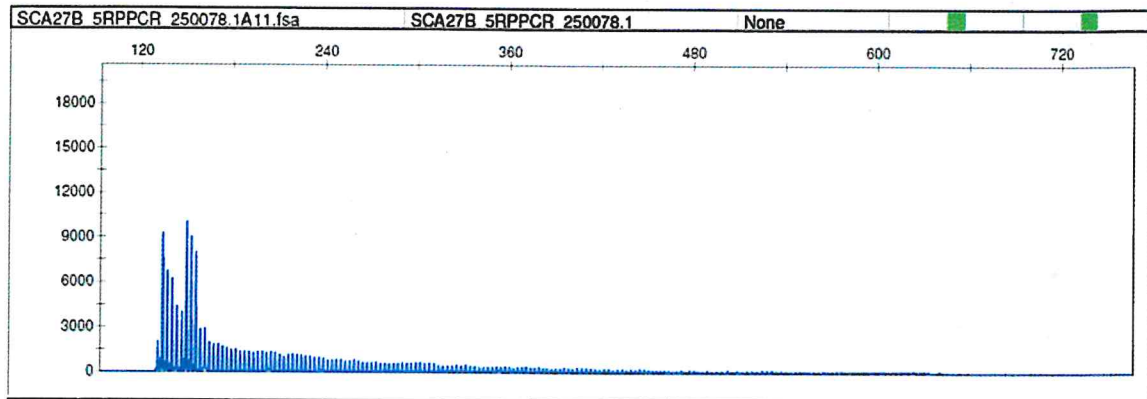
Positive Control:



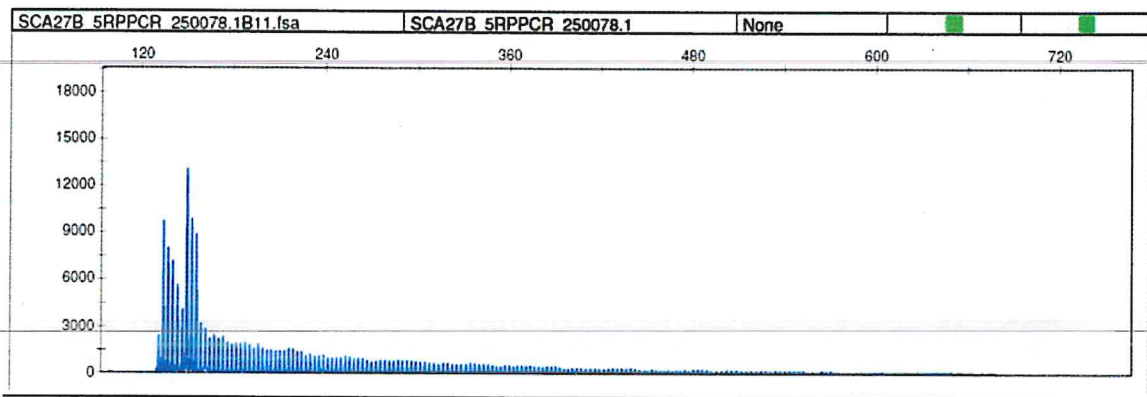
Blank:



Test sample 250078(1):

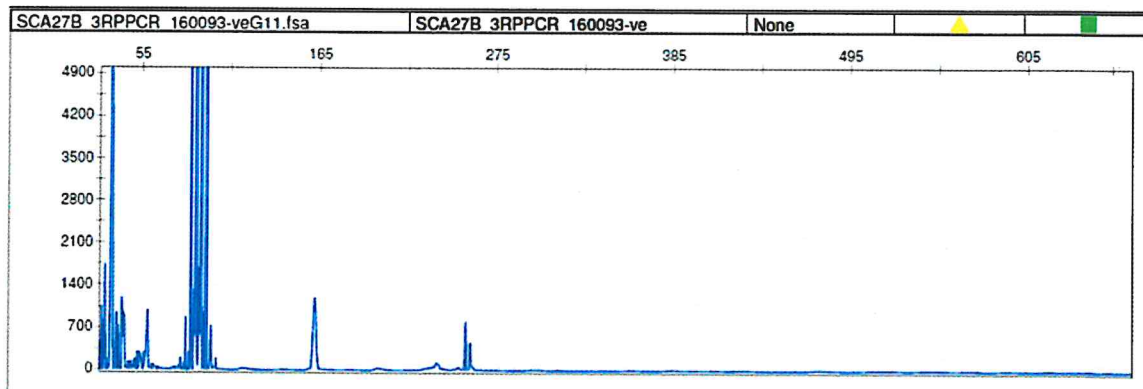


Test sample 250078(2):

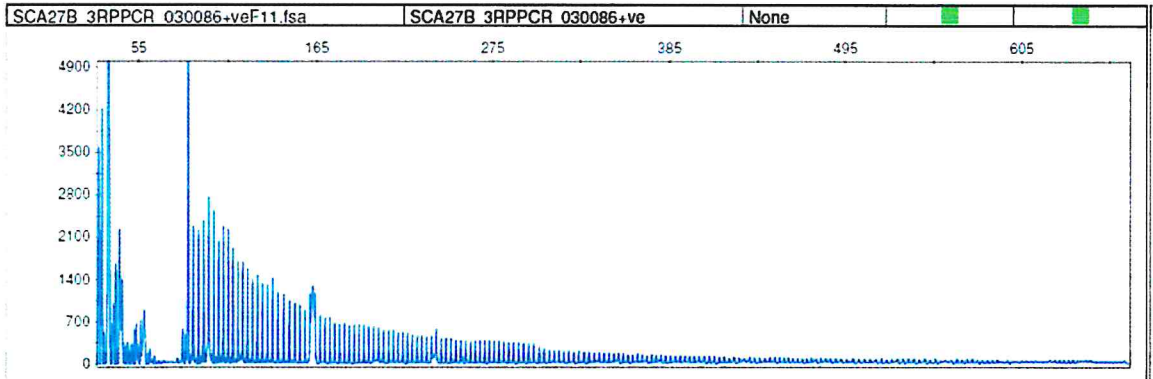


4. 3' RP-PCR analysis for GAA repeat allele

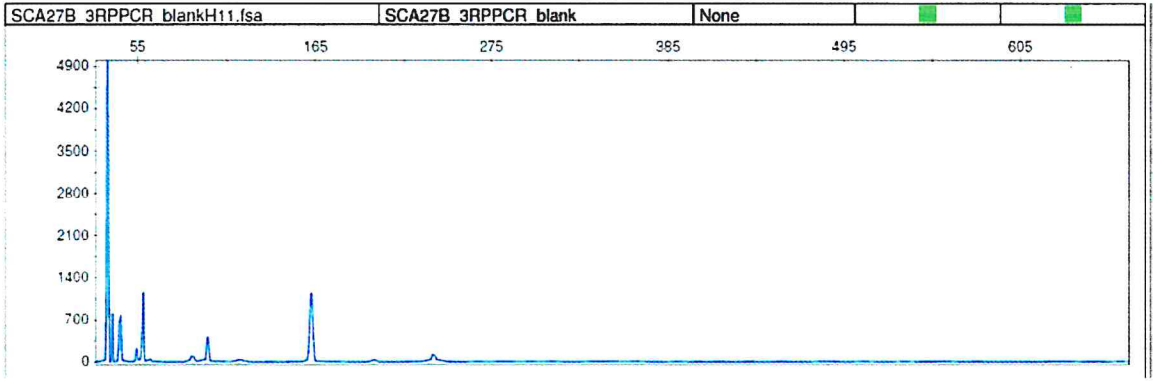
Negative control:



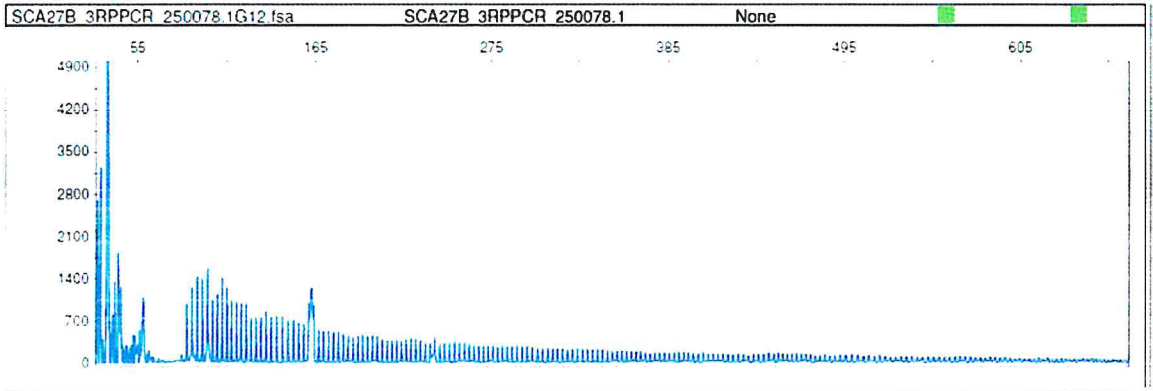
Positive Control:



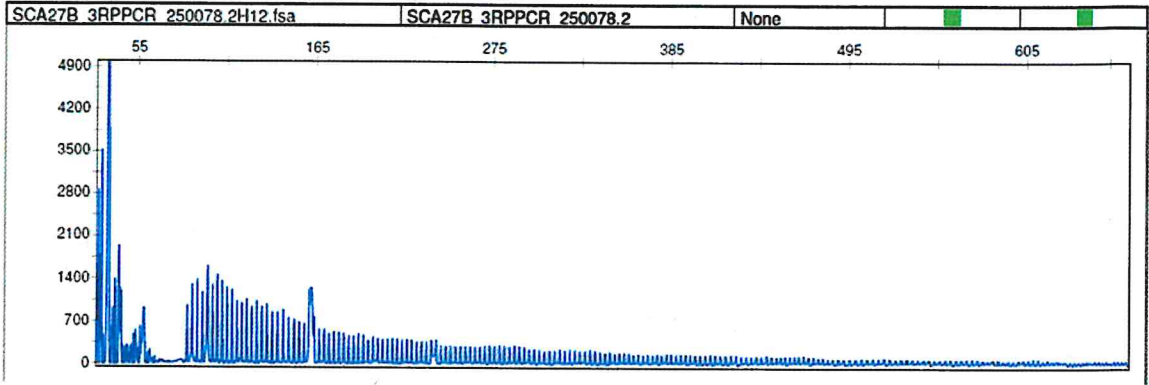
Blank:



Test sample 250078(1):

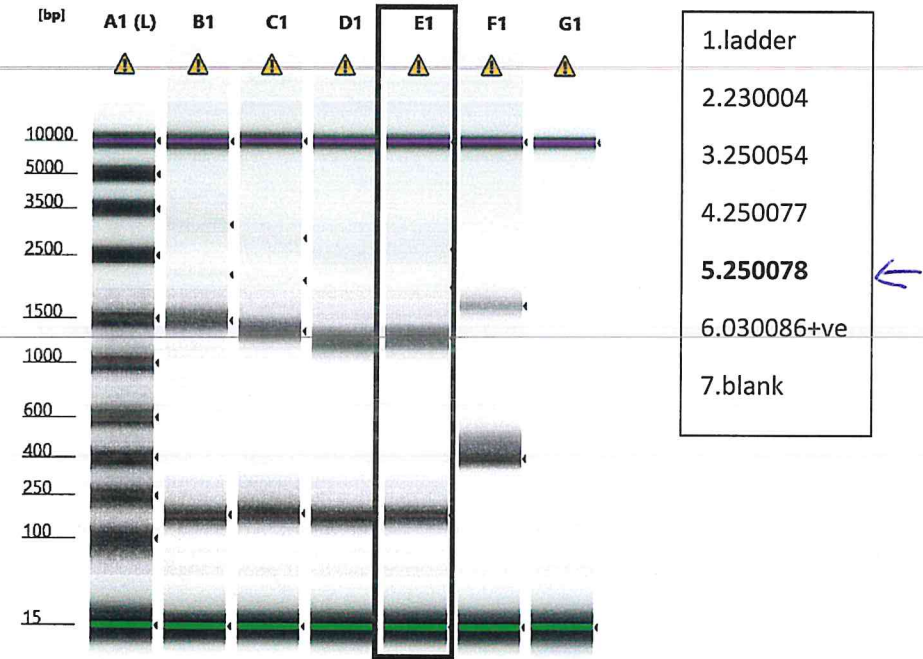


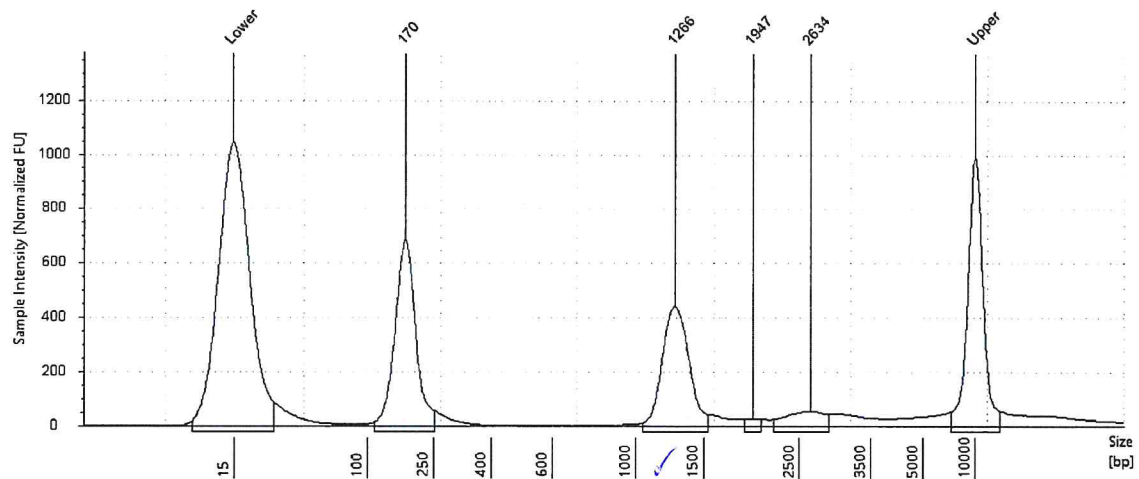
Test sample 250078(2):



5. Tape Station 4150 automated electrophoresis using D5000 Screen Tape

Test sample 250078(1):



E1: 250078 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: 1266 bp

PCR product size without GAA repeats: 165bp

Calculated large allele GAA repeat sizes:

1. $1266 - 165 = 1101 \text{ bp} / 3 = 367 - 39.42 = 328 / 0.97 = 338 \text{ GAA repeats}$

✓

9/338 GAA

AG 12-03-2025

KC