Puritan Report for Batch – 25-806 2PC Lot# 3127, Blue Pink and Yellow

Prepared by the University of Maine DNA Sequencing Facility/ Patty Singer,
June 8, 2012

Swabs were received for testing on May 22, 2012

Testing Scheme

DNA Test: 80 Test Swabs (27 from Blue (1-27), 26 from Pink (28-53) and 27 from Yellow (54-80)). Blue (9-Beg, 9-Mid and 9-End), Pink (9-Beg, 9-Mid and 8-End), Yellow (9-Beg, 9-Mid and 9-End)

3 Positive Control Cheek Swabs CS1-Blue, CS2-Pink and CS3-Yellow (81-83)

2 Genomic DNA Control Reactions (84-85)

1 No DNA Control (86)

DNase Test: 27 Test Swabs (9 Blue (1-9), 9 Pink (10-18) and 9 Yellow (19-27)). Take 3-Beg, 3-Mid and 3-End from each color of swab.

1 Positive Control

1 Negative Control

RNase Test: 27 Test Swabs (9 Blue (1-9), 9 Pink (10-18) and 9 Yellow (19-27)). Take 3-Beg, 3-Mid and 3-End from each color of swab.

1 Positive Control

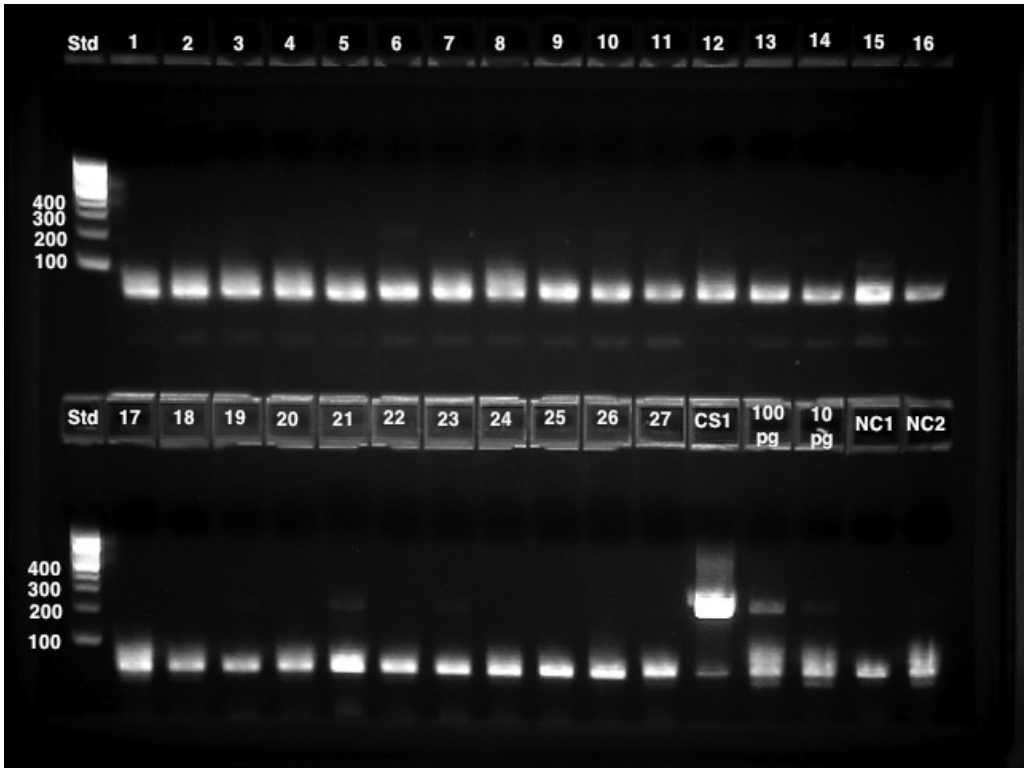
1 Negative Control

DNA Test

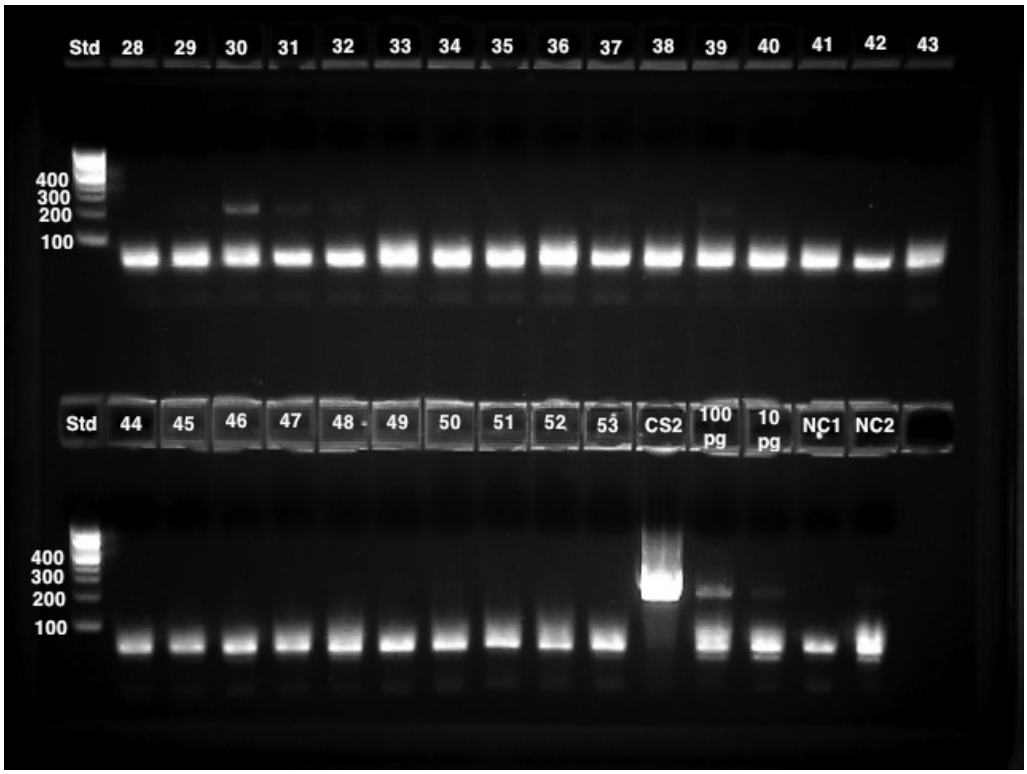
DNA was extracted from swabs using the Qiagen QIAamp DNA Blood Mini Kit in conjunction with the Qiagen QIAcube automated DNA prep instrument. In addition to the 80 sample swabs, DNA was also isolated from three positive control cheek swabs. PCR amplifications were then performed on the DNA preps to determine whether DNA is present on the sample swabs. In addition to the 83 DNA preps, amplifications were also done on two control genomic DNA amounts (100 pg and 10 pg) as well as a no DNA control for a total of 86 PCR amplifications. The primers used for the amplifications are the human DNA repeat region AluYb8 (225bp).

After amplification an aliquot of each reaction was run on a 2.2% double tier Lonza flash gel. A DNA ladder was also loaded as a size standard. One gel was run for each swab color tested (Blue, Pink and Yellow).

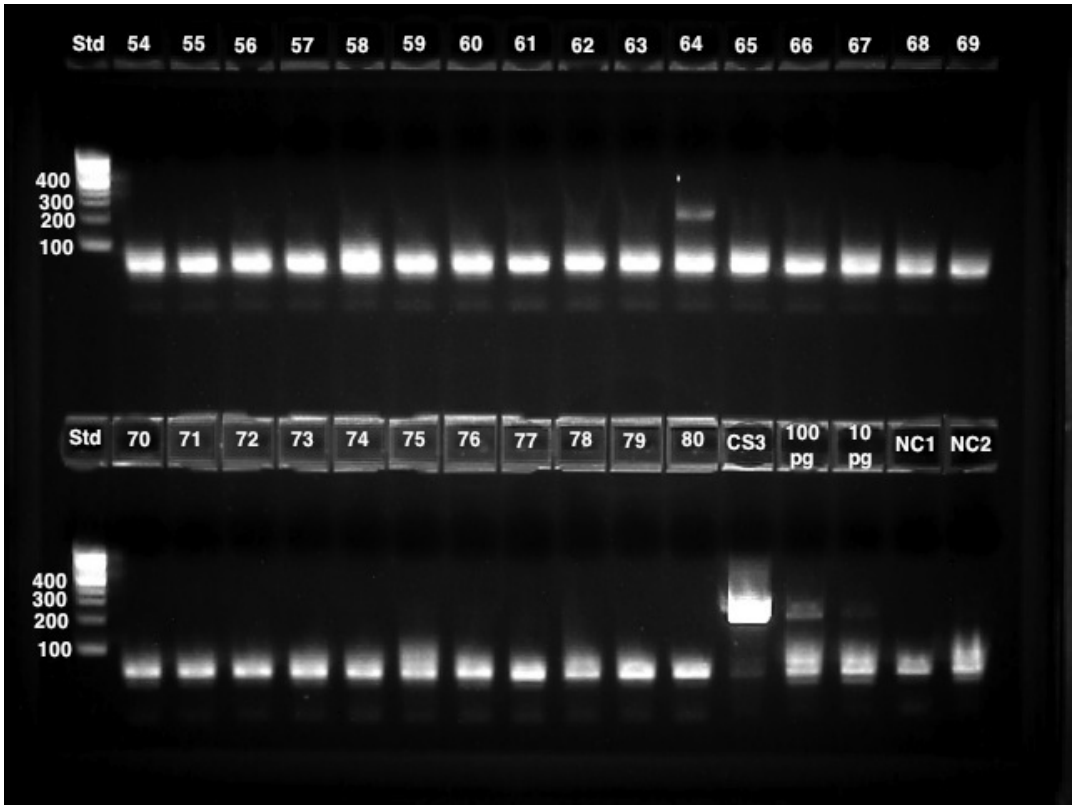
Blue Swab Gel



Pink Swab Gel

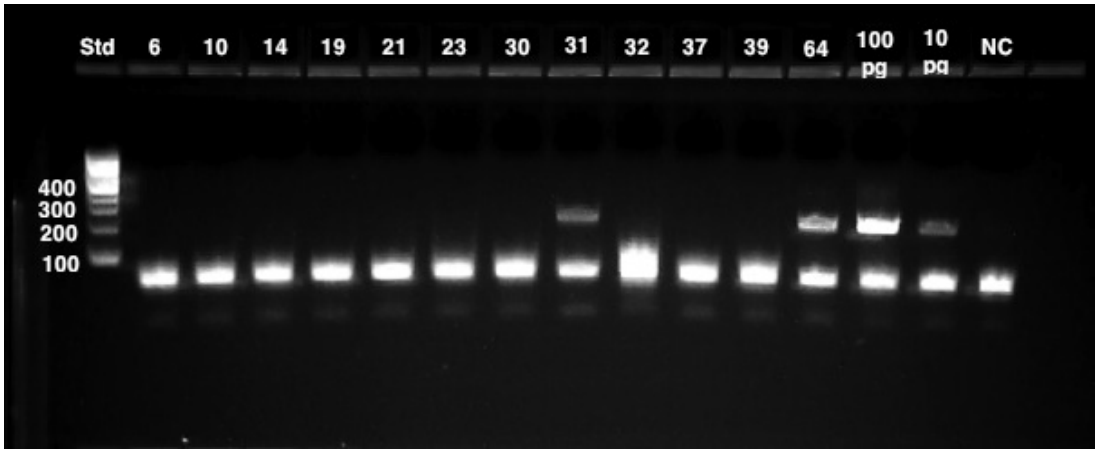


Yellow Swab Gel



The results show that, with a few exceptions, there were no detectible amplification products for the test swabs. The exceptions are likely a result of minor contamination issues during the amplification procedure. To test this, all potential positive test swabs, were re-amplified to confirm or dispute original amplifications.

Re-amplification Gel



Blue Swabs – 6, 10, 14, 19, 21 and 23

Pink Swabs – 30, 31, 32, 37 and 39

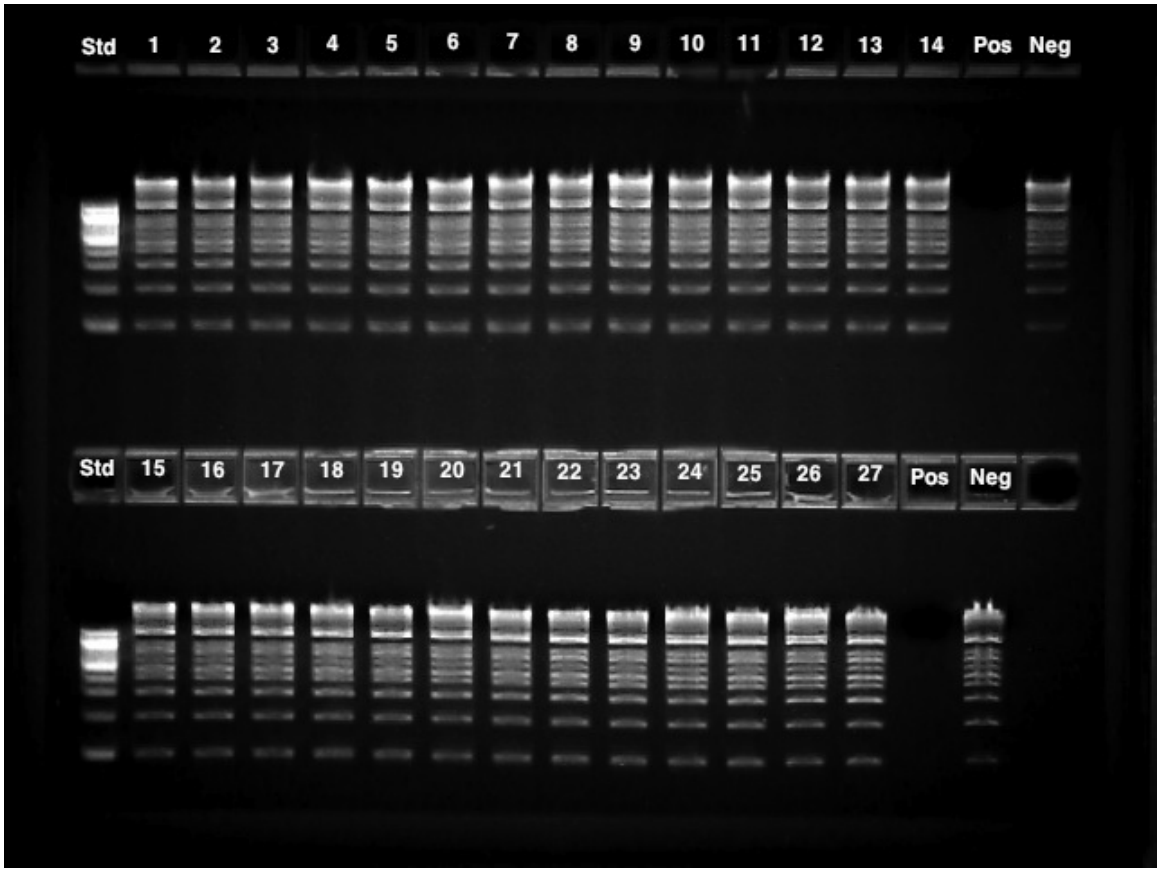
Yellow Swabs – 64

These results show that there are two DNA preps that show amplification of the AluYb8 sequence. The DNA present is likely due to contamination of those two samples during the preparation of the samples and not due to the presence of DNA on the swabs.

DNase Test

Twenty-seven sample swabs were tested for the presence of DNase activity. Two controls, one positive and one negative, were also tested. The swabs and controls were incubated with the 1Kb plus DNA ladder. The positive control had the addition of DNaseI while the negative control did not. Aliquots of each reaction were run on a 2.2% double tier Lonza flash gel. If the DNA ladder shows any degradation compared to the negative control then DNase is present.

DNase Gel

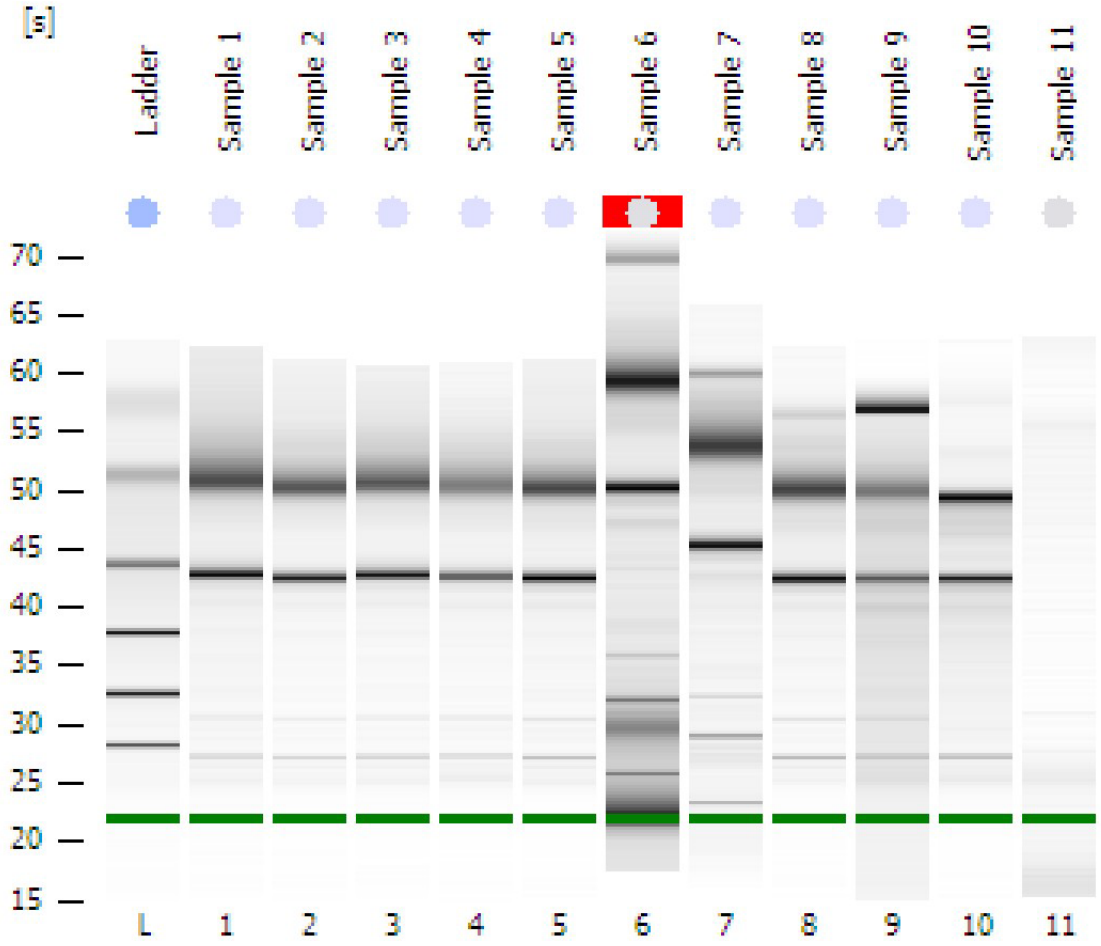


This gel demonstrates that there is no DNase present on any of the swabs tested.

RNase Test

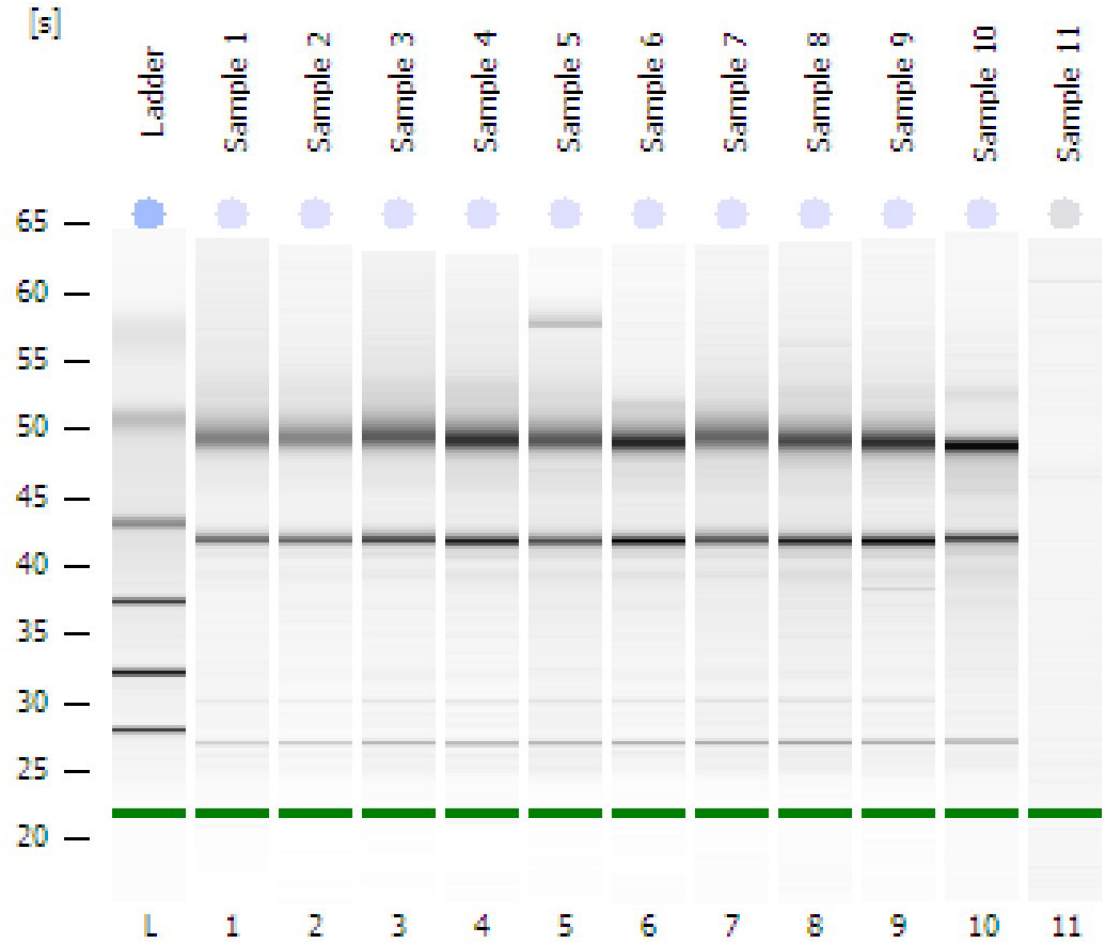
Twenty-seven swabs were tested for the presence of RNase activity. Two controls, one positive and one negative, were also tested. The swabs and controls were incubated with total RNA. The positive control had the addition of RNase A while the negative control did not. Aliquots of each reaction were run on the Agilent Bioanalyzer. If the ribosomal RNA bands show degradation compared to the negative control then RNAse is present. One chip was run for each swab color, blue, pink and yellow.

Blue Swab Chip



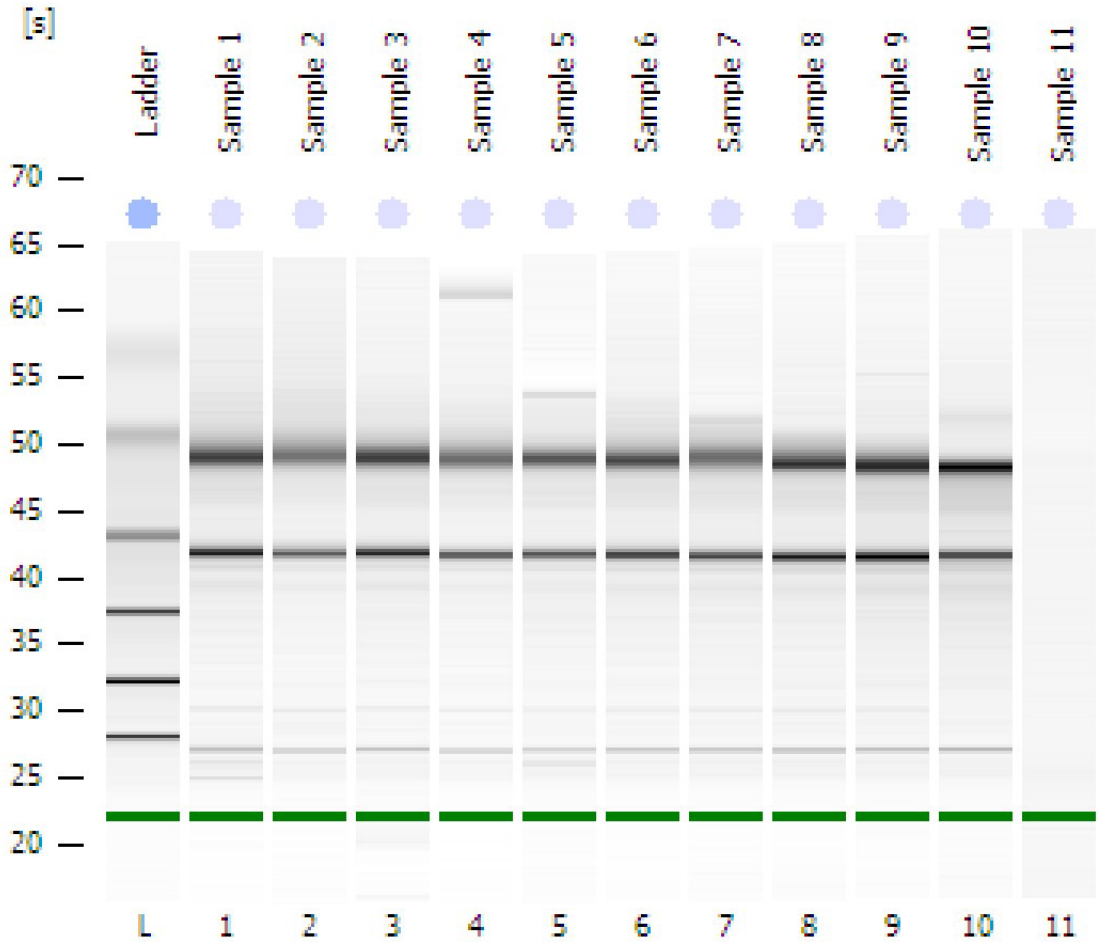
Samples 1-9 are the nine swab samples, sample 10 is the negative control and sample 11 is the positive control.

Pink Swab Chip



Samples 1-9 are the nine swab samples, sample 10 is the negative control and sample 11 is the positive control.

Yellow Swab Chip



Samples 1-9 are the nine swab samples, sample 10 is the negative control and sample 11 is the positive control.

These results show that there is no RNase present in the swabs tested.

Based on the tests performed on Batch 25-806 2PC lot# 3127 (blue, pink and yellow) the swabs can be considered to be DNA, DNase and RNase free.