

Puritan Report for Batch – 25-806-1WC FDNA Lot# 3197

Prepared by the University of Maine DNA Sequencing Facility/ Patty Singer,
September 4, 2012

Swabs were received for testing on August 22, 2012

Testing Scheme

DNA Test: 80 Test Swabs [27 from Beginning (1-27), 26 from Middle (28-53) and
27 from End (54-80)]

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

2 Genomic DNA Control Reactions (84-85)

1 No DNA Control (86)

DNase Test: 27 Test Swabs [9 Beg. (1-9), 9 Mid. (10-18) and 9 End (19-27)]

1 Positive Control

1 Negative Control

RNase Test: 27 Test Swabs [9 Beg. (1-9), 9 Mid. (10-18) and 9 End (19-27)]

1 Positive Control

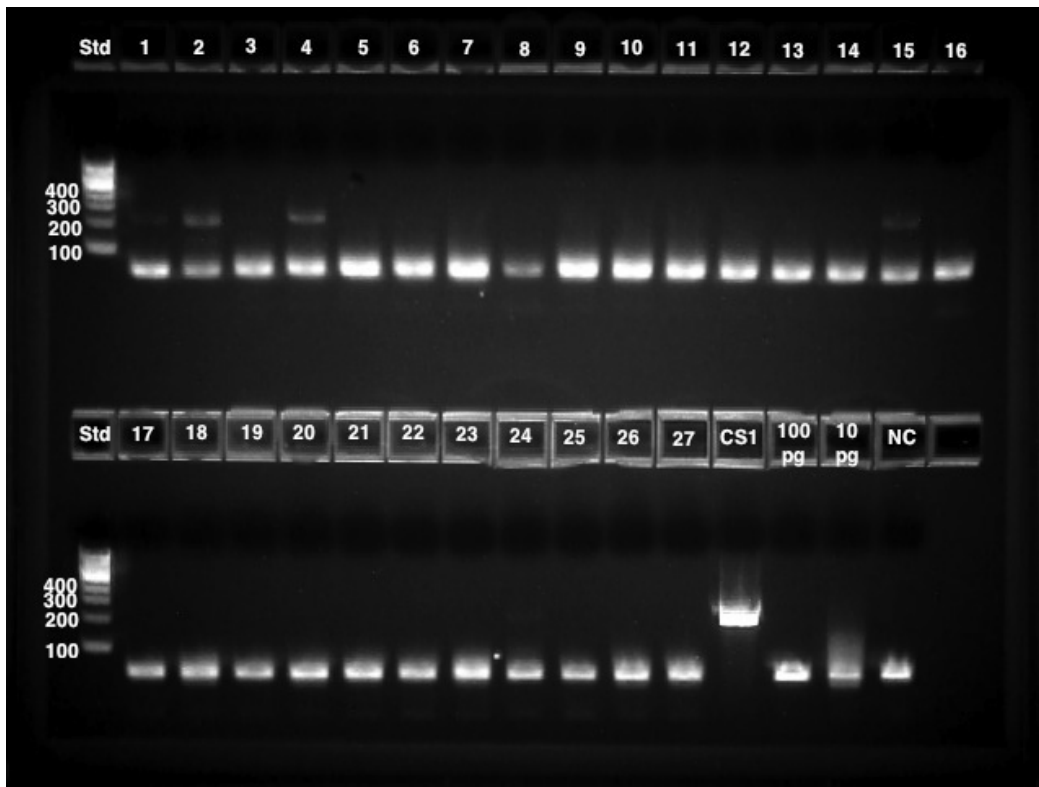
1 Negative Control

DNA Test

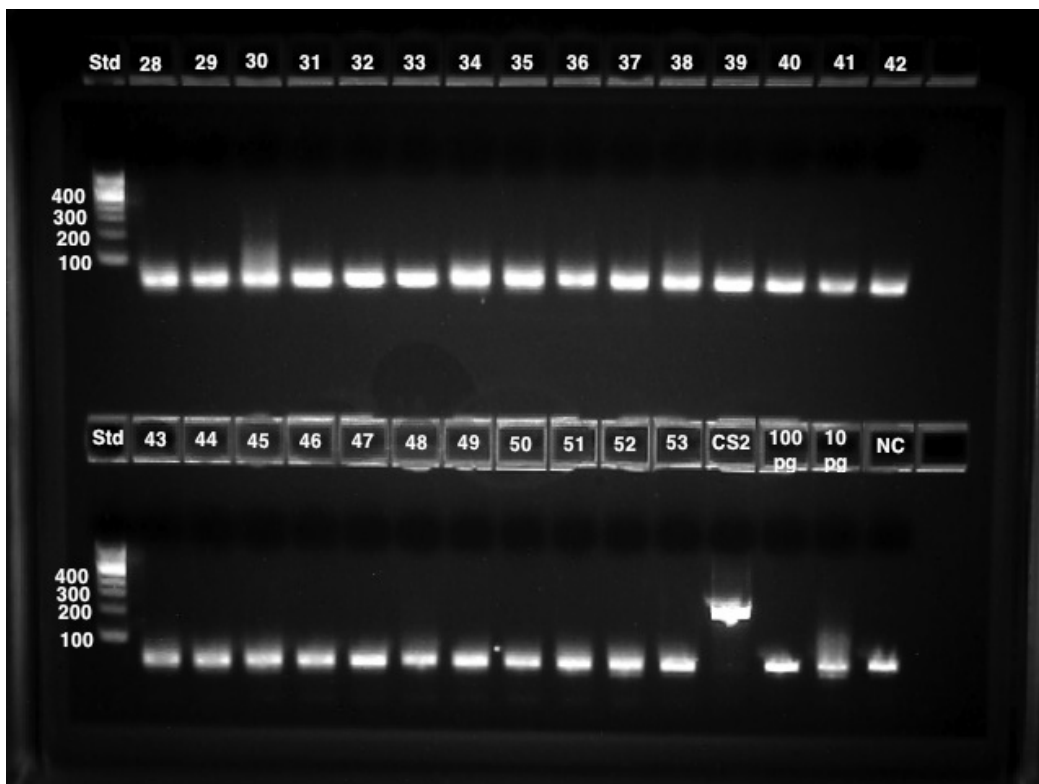
DNA was extracted from swabs using the Qiagen QIAamp DNA Blood Mini Kit in conjunction with the Qiagen QIAcube automated DNA prep instrument. In addition to the 80 sample swabs, DNA was also isolated from three positive control cheek swabs. PCR amplifications were performed on the DNA preps to determine 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 region tested (Beginning, Middle and End).

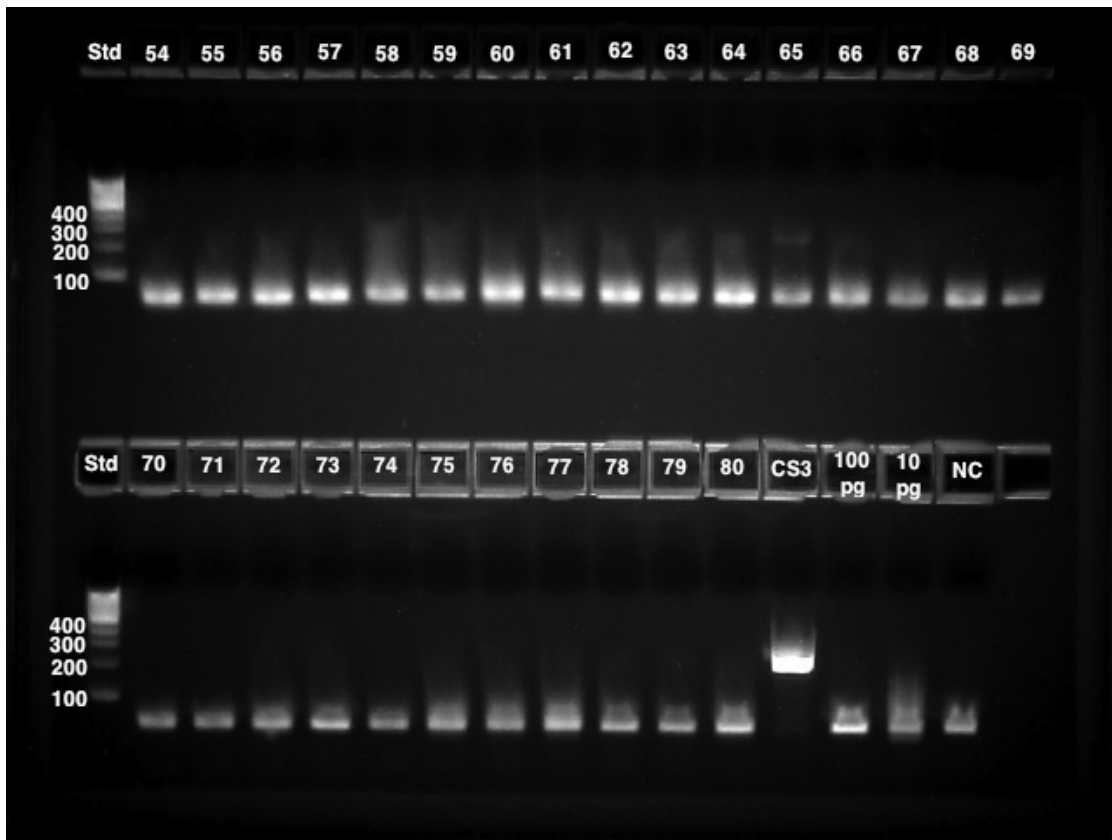
Beginning Swab Gel



Middle Swab Gel

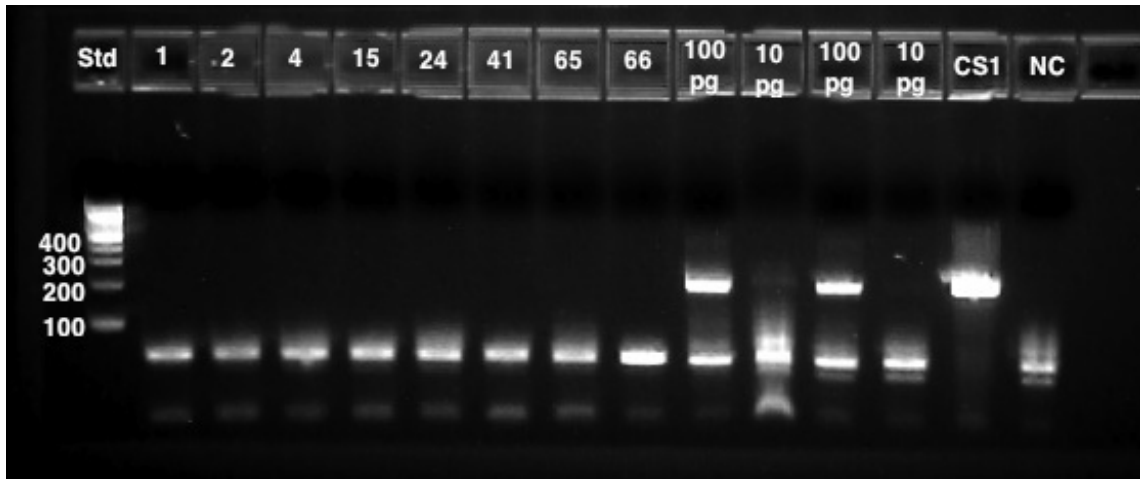


End Swab Gel



These results show that there is no DNA contamination in the swabs tested. However, two new genomic DNA dilutions were used as controls and they did not amplify as they should. New dilutions were made and new amplifications were performed as well as re-amplifications of those few samples that showed very minor amplification products.

Re-amplification Gel

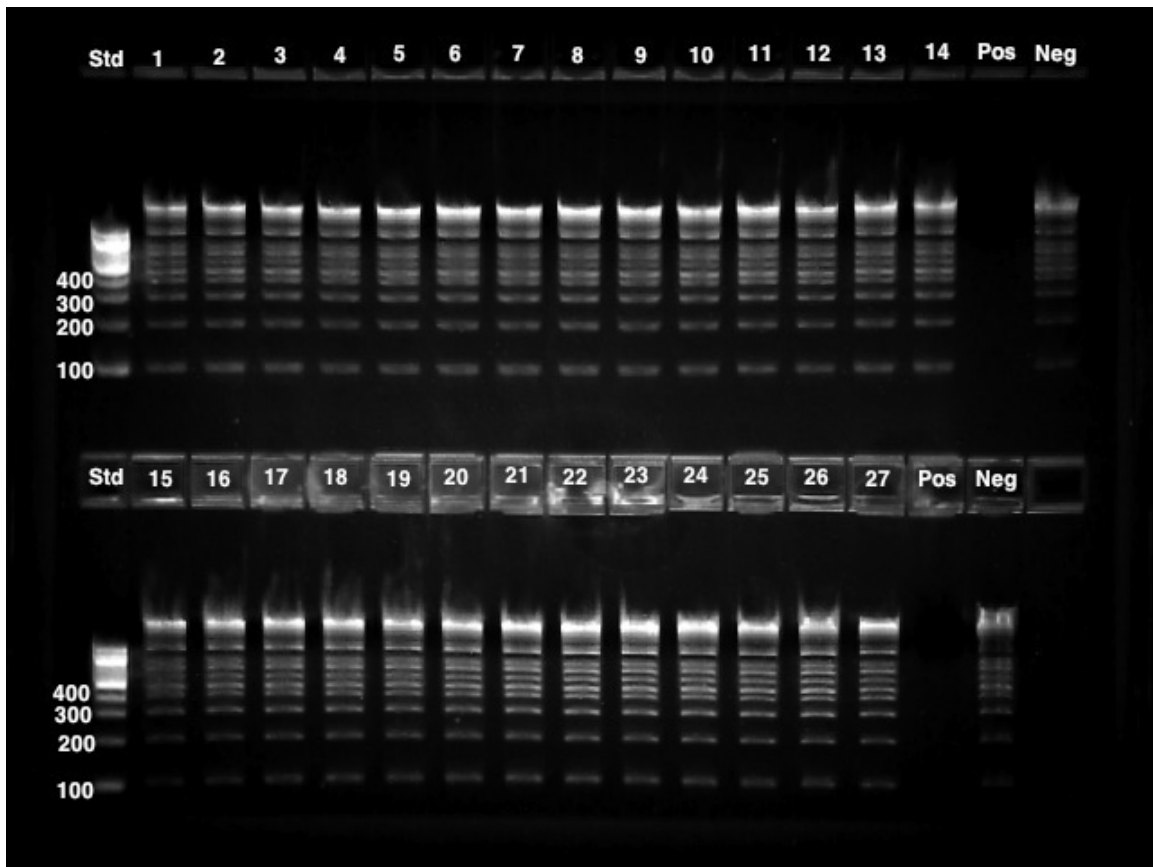


These results show that the minor bands seen in the swab sample initial amplifications were not real and there is no DNA contamination. The new standards worked as expected though the 10pg sample didn't amplify as well as it has in the past.

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 1 KB Plus DNA ladder added as the substrate. The controls contained no swabs; 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 there is DNase present on the swabs, then the 1 KB Plus DNA ladder from the test reactions should show degradation when compared to the negative control.

DNase Assay Gel

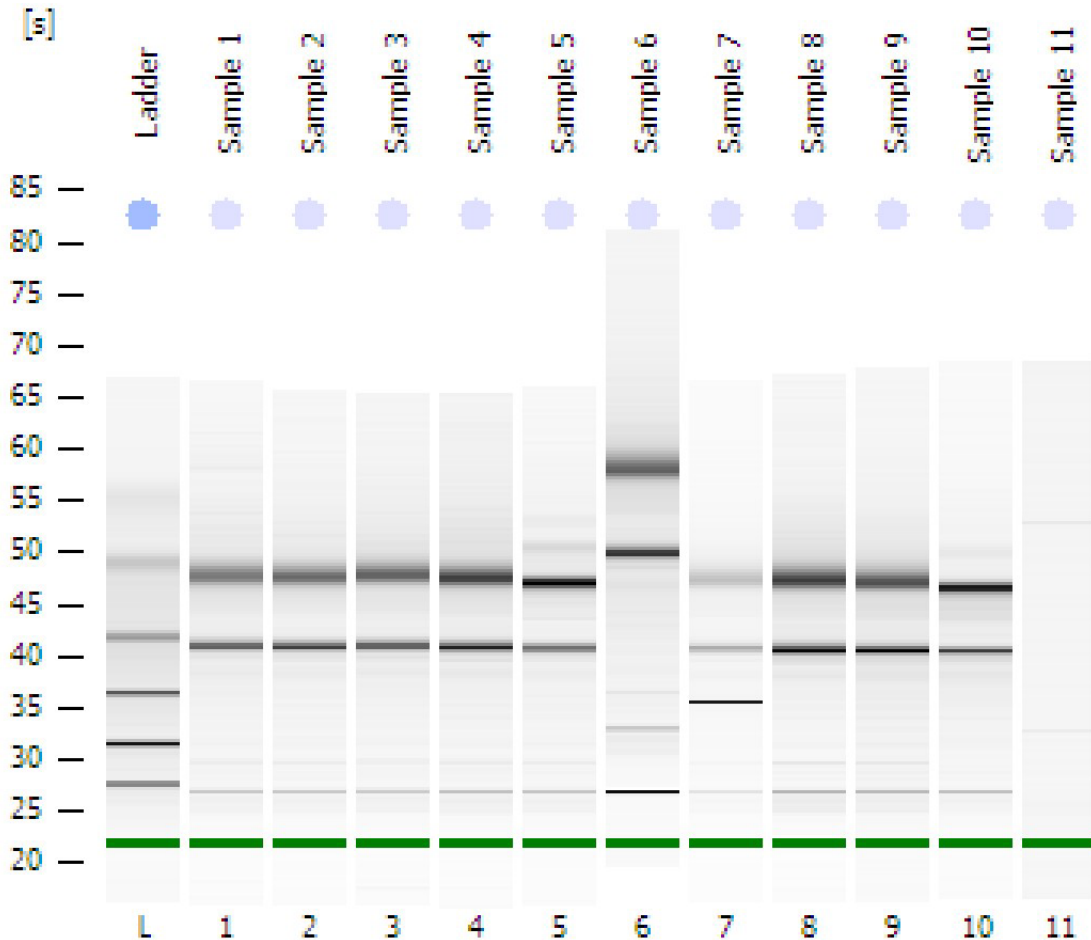


These results show that there is no DNase present on the swabs tested.

RNase Test

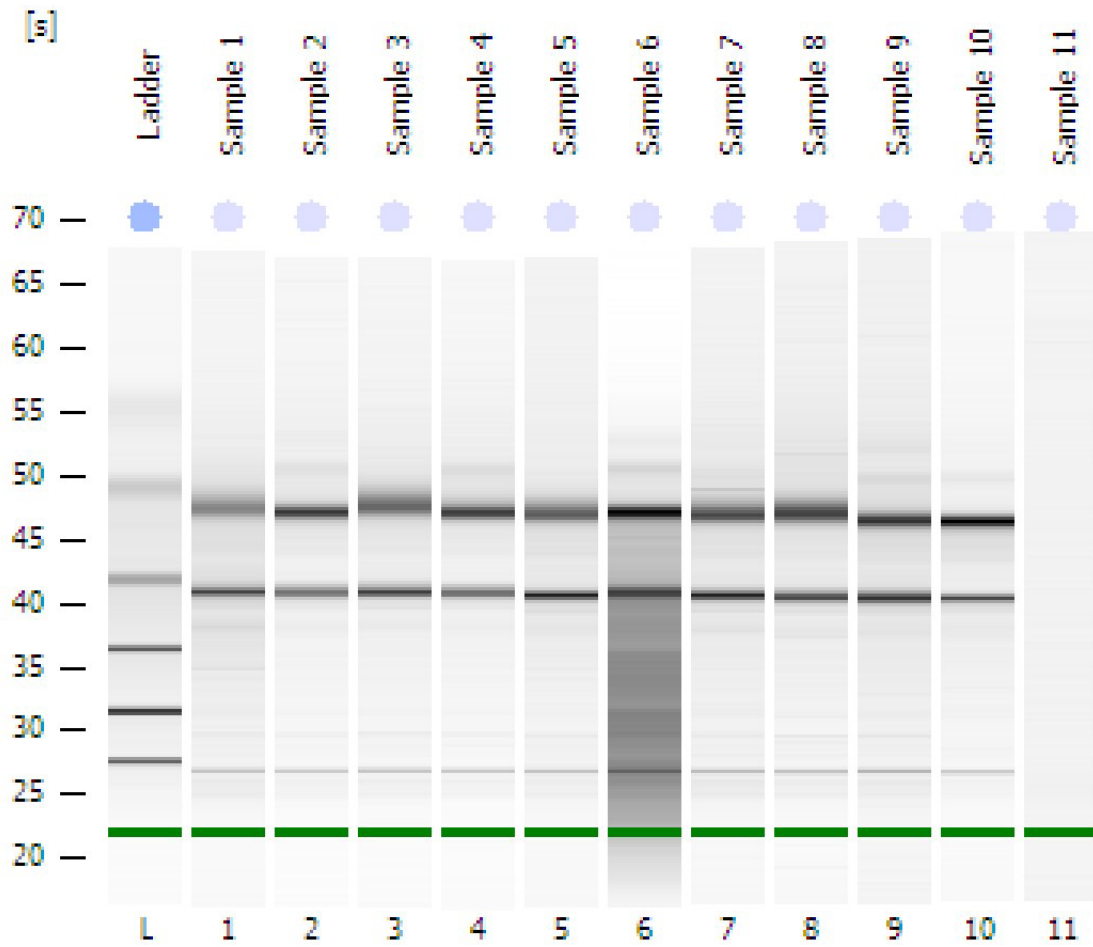
Twenty-seven swabs were tested for the presence of RNase activity. Two controls, one positive and one negative, were also tested. The swabs and controls were incubated with total RNA added as the substrate. The controls contained no swabs; the positive control had the addition of RNase A while the negative control did not. Aliquots of each reaction were run on the Agilent Bioanalyzer. If there is any RNase present on the swabs the ribosomal RNA bands should show degradation when compared to the negative control. One chip was run for each region tested, beginning, middle and end.

Beginning Swabs Chip



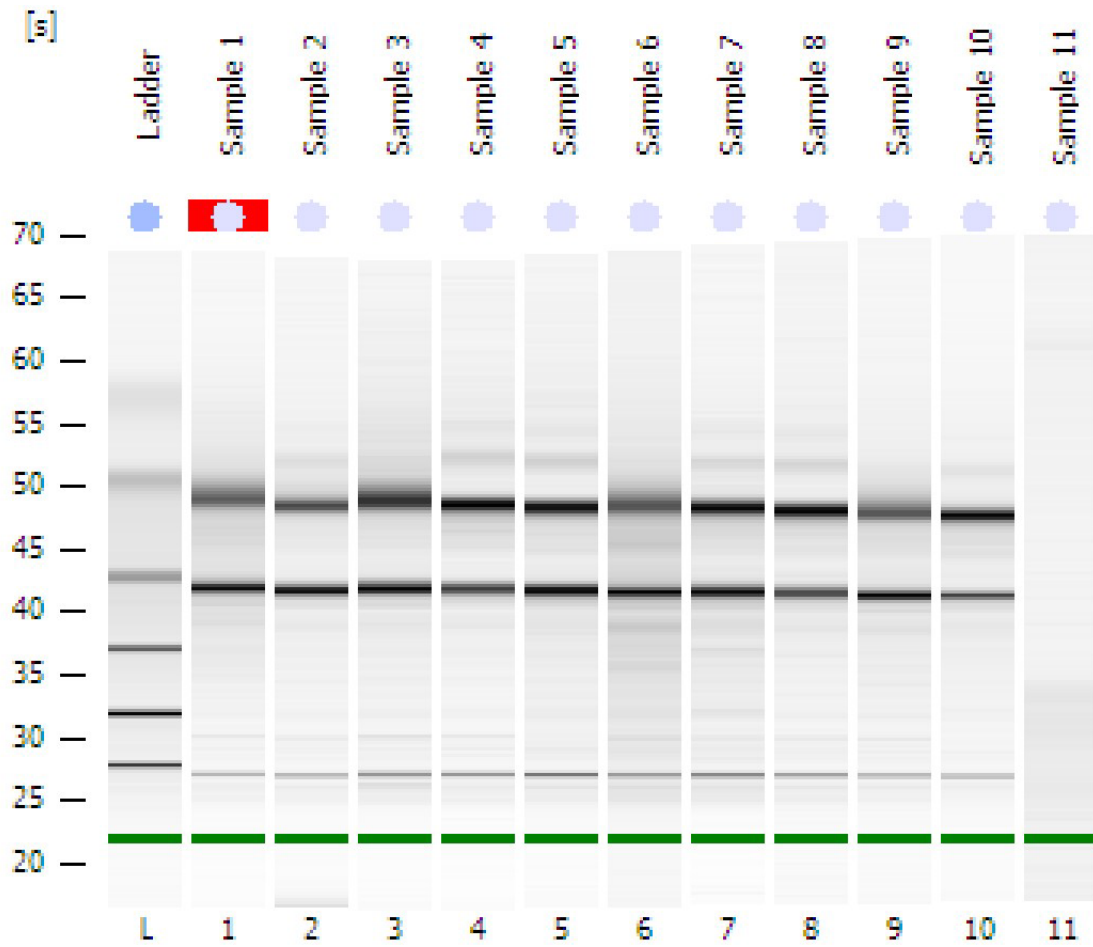
Samples 1-9 are the nine swab samples, sample 10 is the negative control and sample 11 is the positive control.

Middle Swabs Chip



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

End Swabs 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 contamination of the swabs tested.