

Puritan Report for Batch – 25-806 1 WC FDNA Lot# 3636

Prepared by the University of Maine DNA Sequencing Facility/ Patty Singer,
November 21, 2013

Swabs were received for testing on November 7, 2013

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)

3 Genomic DNA Control Reactions (84-86)

1 No DNA Control (87)

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

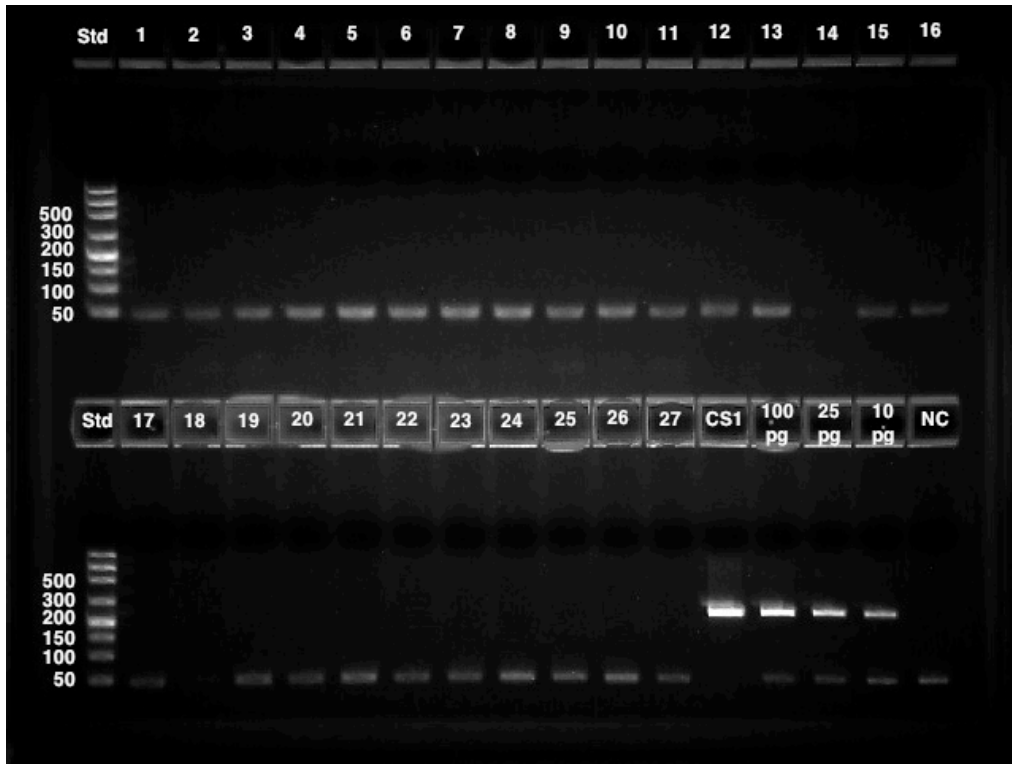
1 Negative Control

DNA Test

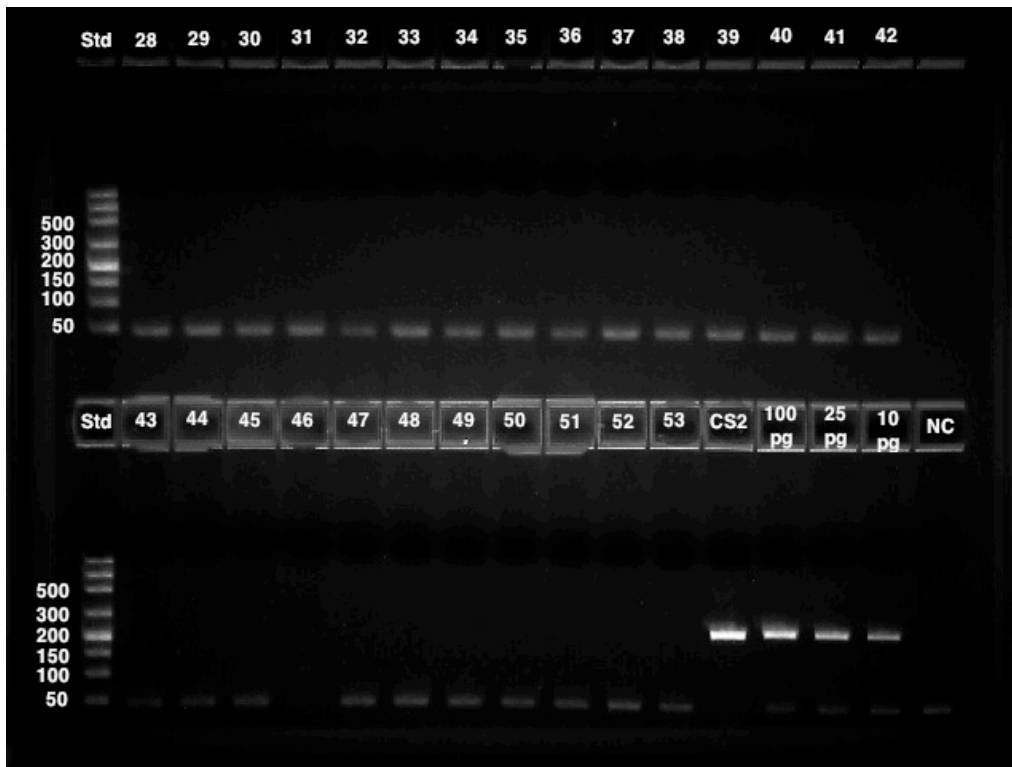
DNA was extracted from swabs using the Qiagen QIAamp DNA Blood Mini Kit in conjunction with the Qiagen QIAcube automated DNA prep instrument. In addition to the 80 sample swabs, DNA was also isolated from three positive control cheek swabs. PCR amplifications were performed on the DNA preps to determine whether DNA is present on the sample swabs. In addition to the 83 DNA preps, amplifications were also done on three control genomic DNA amounts (100 pg, 25 pg and 10 pg) as well as a no DNA control for a total of 87 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).

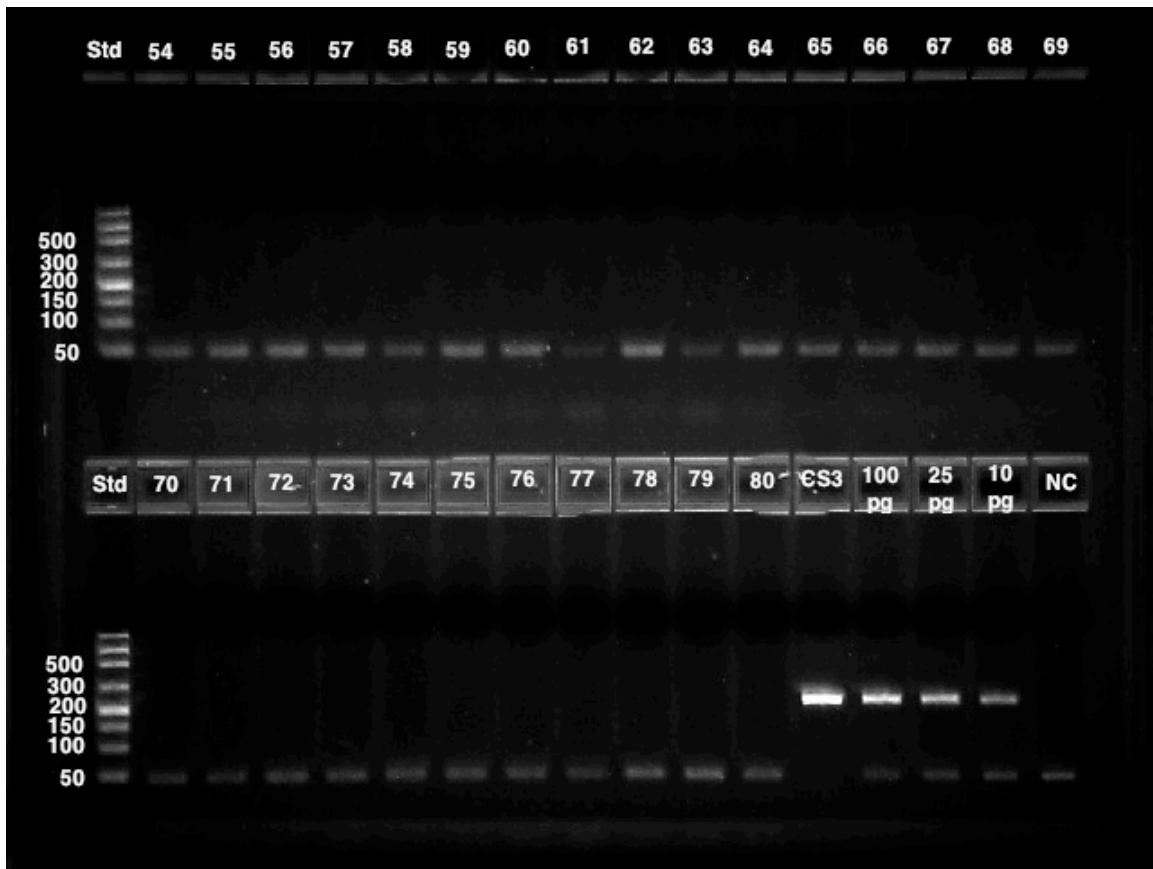
Beginning Swab Gel



Middle Swab Gel



End Swab Gel

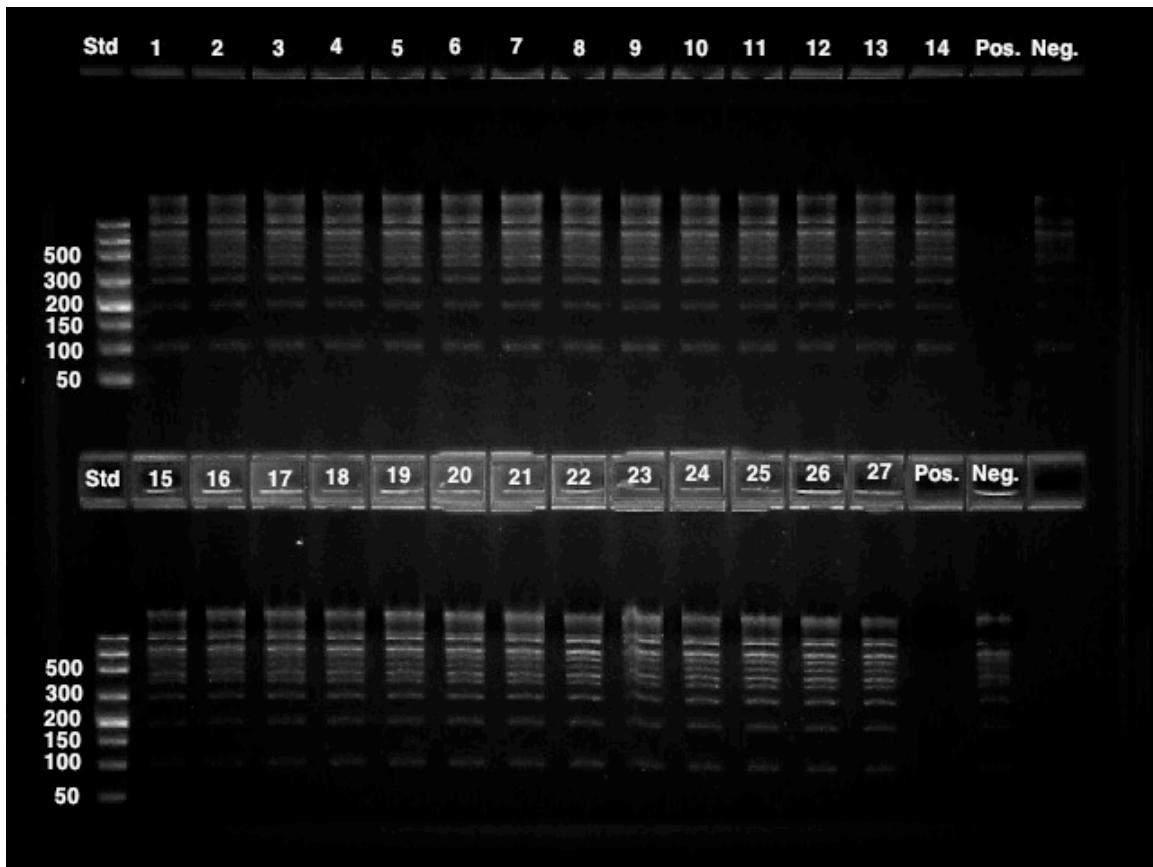


These results show that there is no DNA present on the sample swabs tested. Based on the above data these swabs are considered to be DNA-free and PASS.

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 Gel

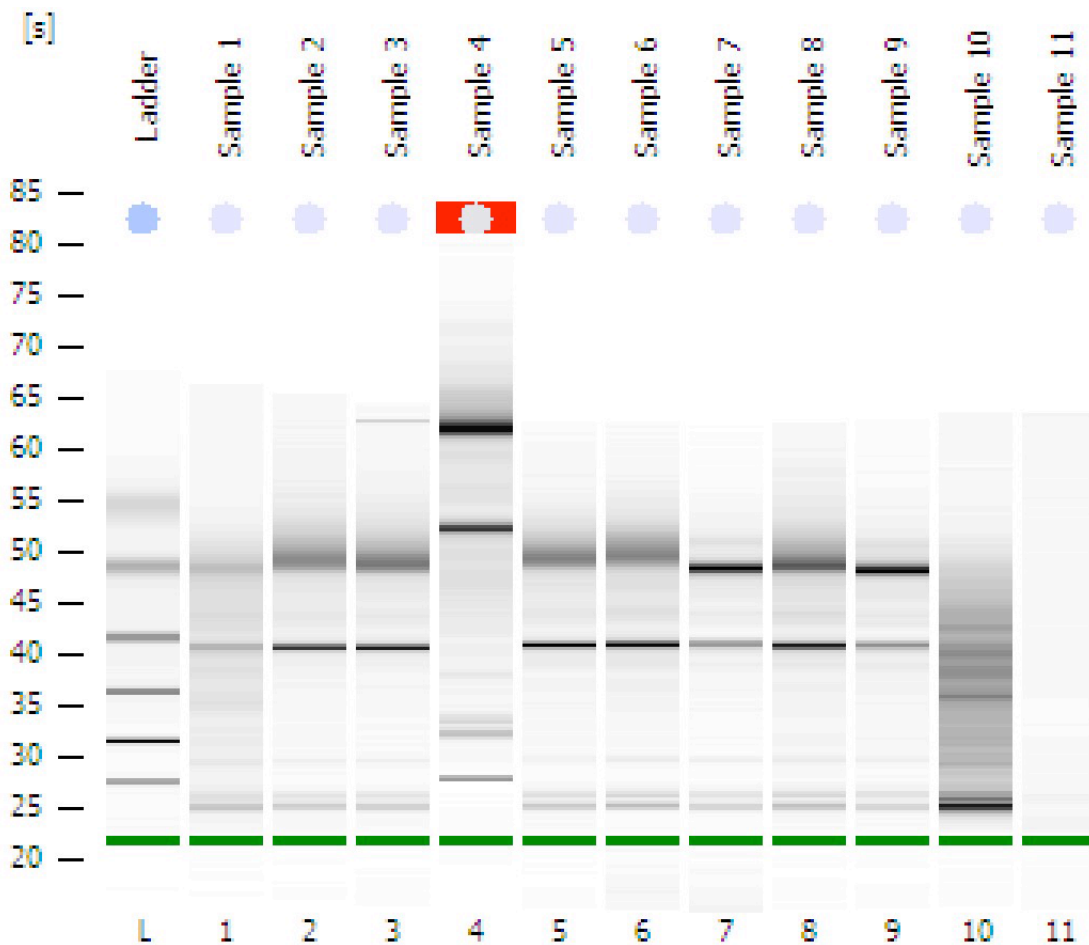


There is no degradation of substrate DNA after incubation with the swabs so these swabs are considered to be DNase-free and PASS.

RNase Test

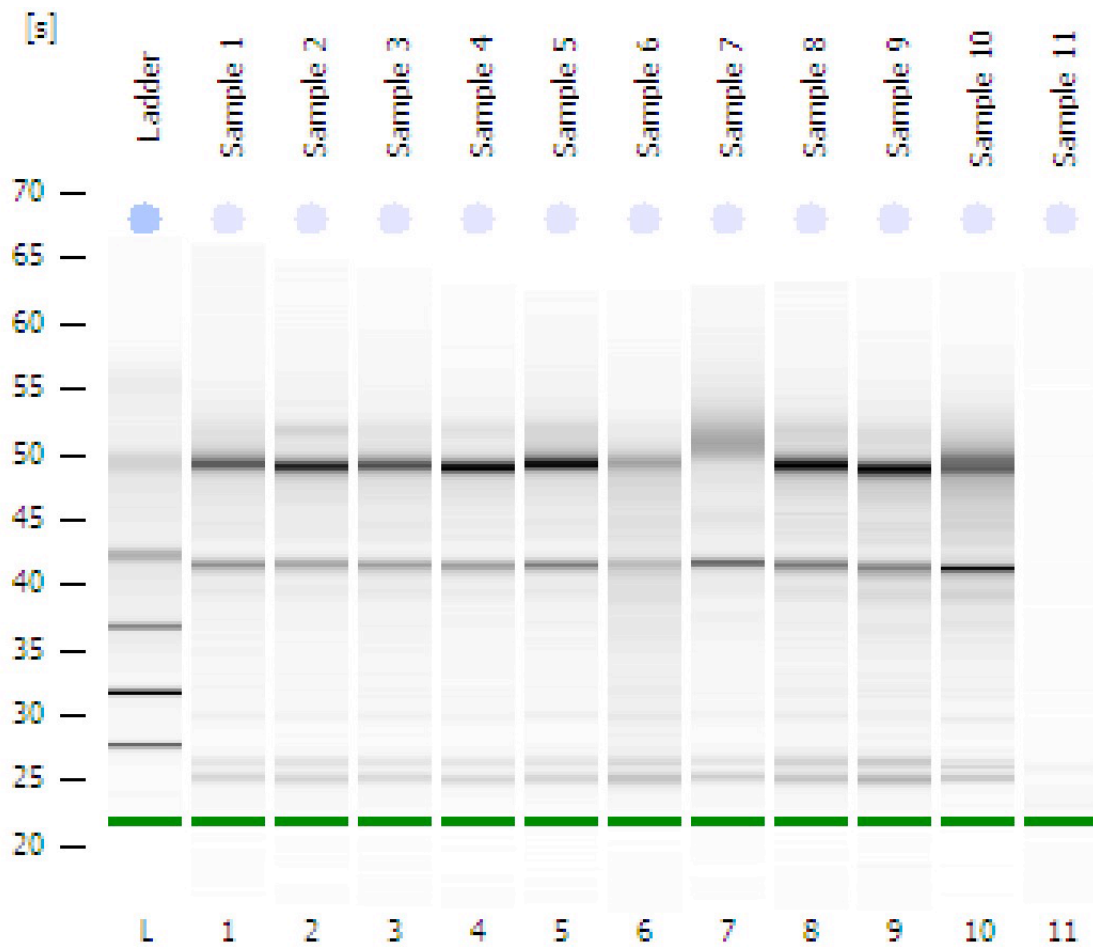
Twenty-seven swabs were tested for the presence of RNase activity. Two controls, one positive and one negative, were also tested. The swabs and controls were incubated with total RNA added as the substrate. The controls contained no swabs; the positive control had the addition of RNase A while the negative 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 Swab Chip



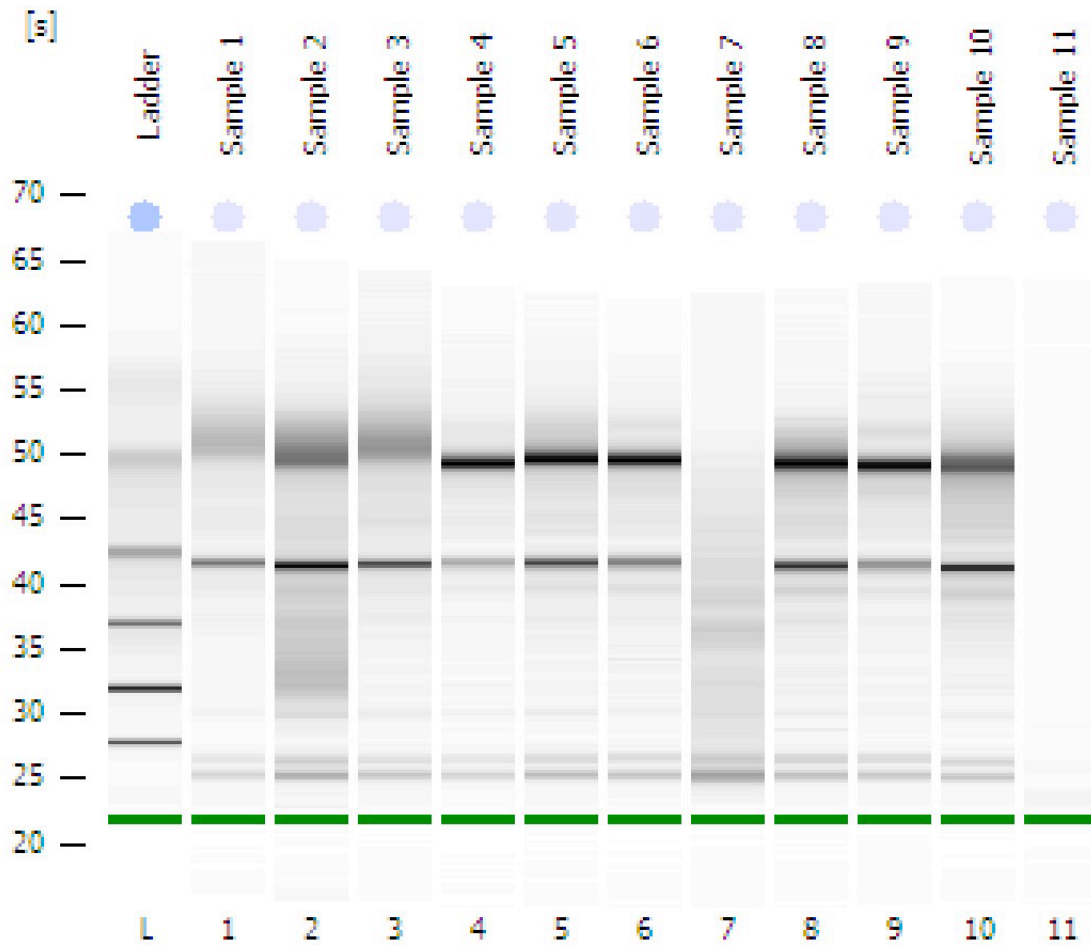
Samples 1-9 are the nine swab samples, sample 10 is the negative control and sample 11 is the positive control.

Middle Swab Chip



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

End Swab Chip



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

The above results show, overall, that there is no degradation of RNA due to the presence of the swabs. While there are a couple of lanes that do appear to show degradation I don't think it is real but due to instrument issues. Sample 11 on all three chips is the vary same sample and it appeared to be degraded on the first chip but dine on the subsequent two chips. Based on this data I would conclude that these swabs are RNA-free and PASS.