

Puritan Report for Batch – 25-806 2WC TT FDNA Lot# 3520

Prepared by the University of Maine DNA Sequencing Facility/ Patty Singer,
June 27, 2013

Swabs were received for testing on June 19, 2013

Testing Scheme

DNA Test: 32 Test Swabs [11 from Beginning (1-11), 10 from Middle (12-21) and
11 from End (22-32)]

3 Positive Control Cheek Swabs CS1, CS2 and CS3 (33-35)

3 Genomic DNA Control Reactions (36-38)

1 No DNA Control (39)

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 32 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 35 DNA preps, amplifications were also done on three control genomic DNA amounts (100 pg, 20 pg and 10 pg) as well as a no DNA control for a total of 39 PCR amplifications. The primers used for the amplifications are the human DNA repeat region AluYb8 (225bp).

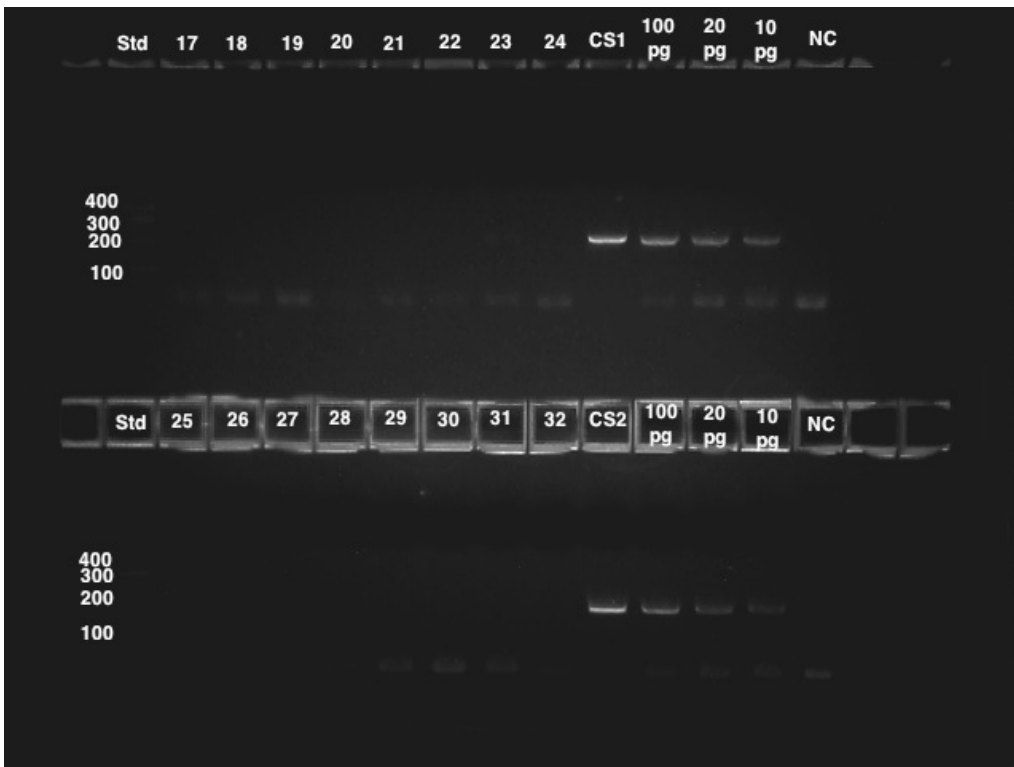
After amplification an aliquot of each reaction was run on a 2.2% double tier Lonza flash gel. A DNA ladder was also loaded as a size standard. One gel was run for each region tested (Beginning, Middle and End).

The results shown below on the gels show that there is no DNA contamination on the swabs tested. PCR amplifications were repeated on potentially positive samples 6, 11, 13 and 23 and there were no bands present.

Swab Gel One



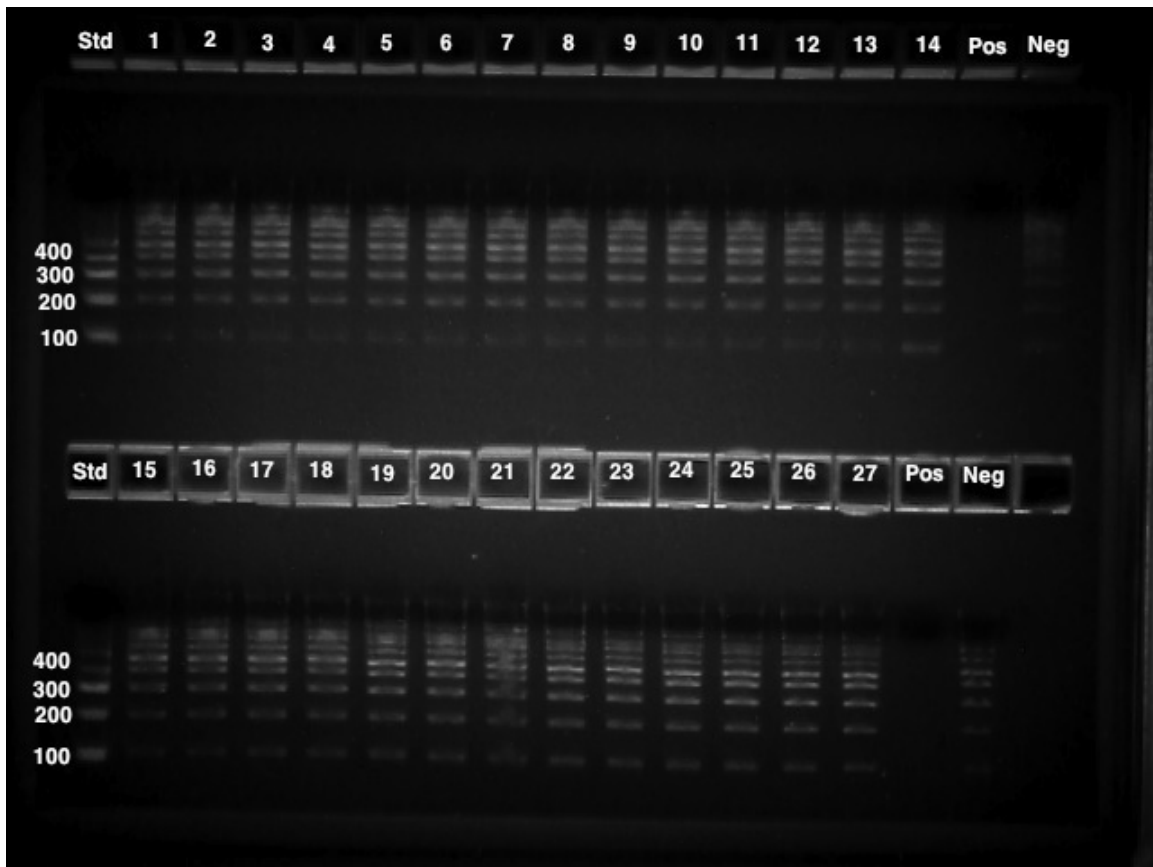
Swab Gel Two



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

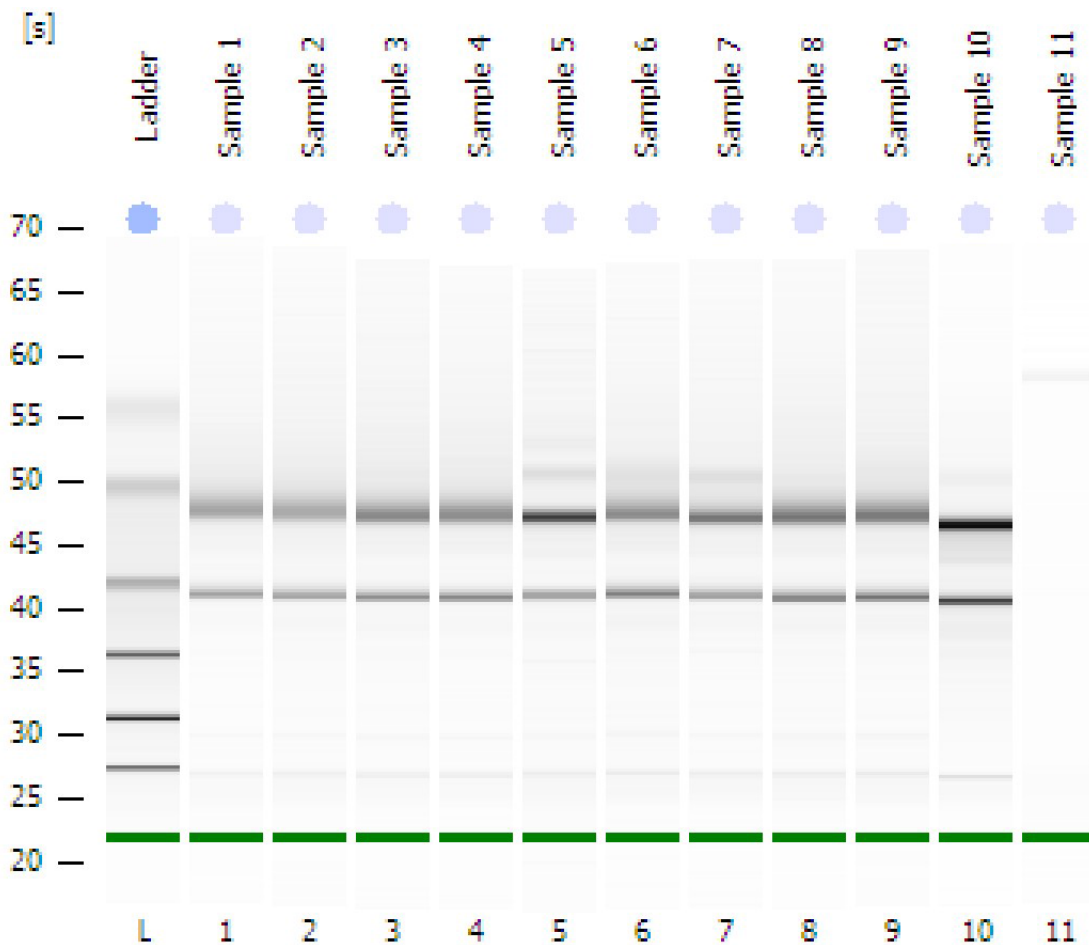


These results show that there is no degradation of the substrate DNA and thus no DNase present on the swabs tested.

RNase Test

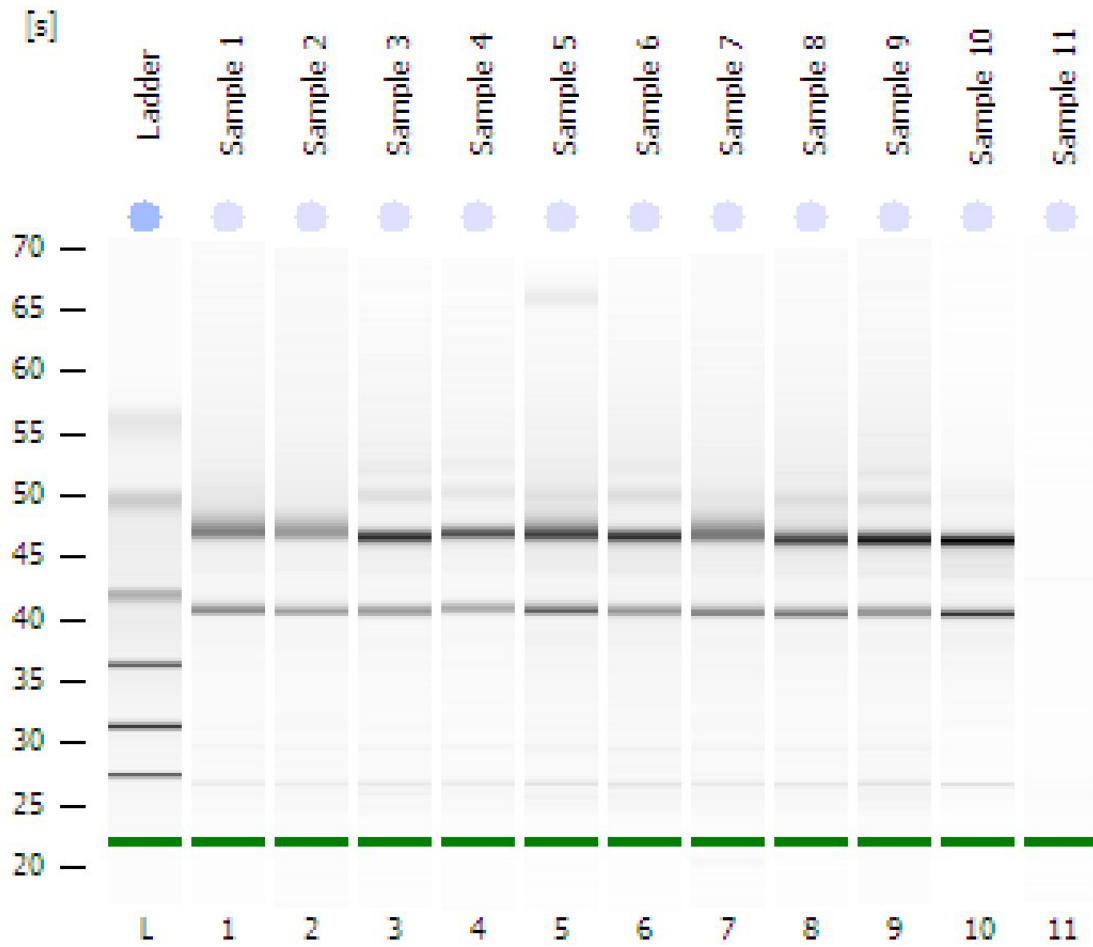
Twenty-seven swabs were tested for the presence of RNase activity. Two controls, one positive and one negative, were also tested. The swabs and controls were incubated with total RNA added as the substrate. The controls contained no swabs; the positive control had the addition of RNase A while the negative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



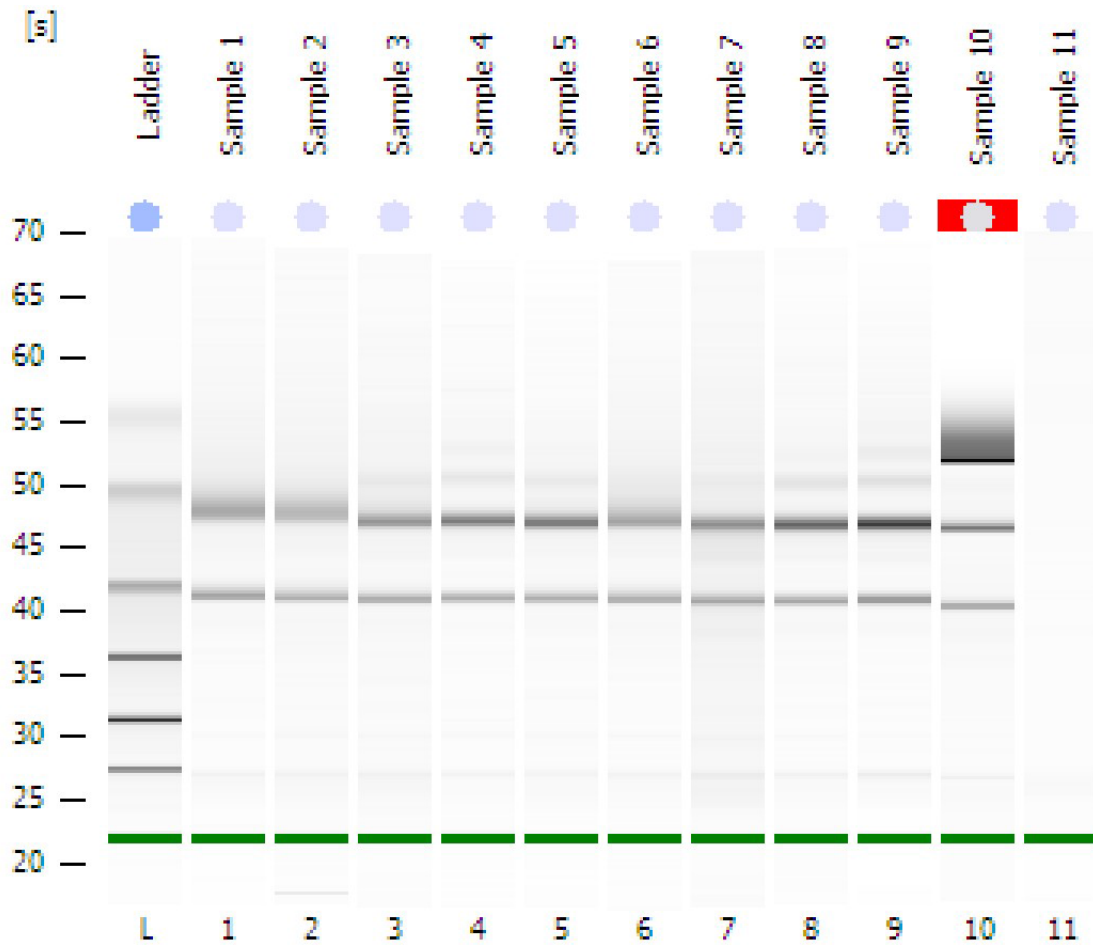
Samples 1-9 are the nine swab samples from the beginning box, sample 10 is the negative control and sample 11 is the positive control.

Middle Swabs Chip



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

End Swabs Chip



Samples 1-9 are the nine swab samples from the end box, sample 10 is the negative control and sample 11 is the positive control. The extra band in the negative control is due to the residual presence of RNaseZAP and not significant.

These results show that there is no degradation of the substrate RNA and thus no RNase present on the swabs tested.