Puritan Report for Batch – 25-3306-U TT FDNA Lot# 3740

Prepared by the University of Maine DNA Sequencing Facility/ Patty Singer,
April 4, 2014

Swabs were received for testing on March 17, 2014

Testing Scheme

DNA Test: 50 Test Swabs [17 from Beginning (1-17), 16 from Middle (18-33) and 17 from End (34-50)]

3 Positive Control Cheek Swabs CS1, CS2 and CS3 (51-53)

3 Genomic DNA Control Reactions (54-56)

1 No DNA Control (57)

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

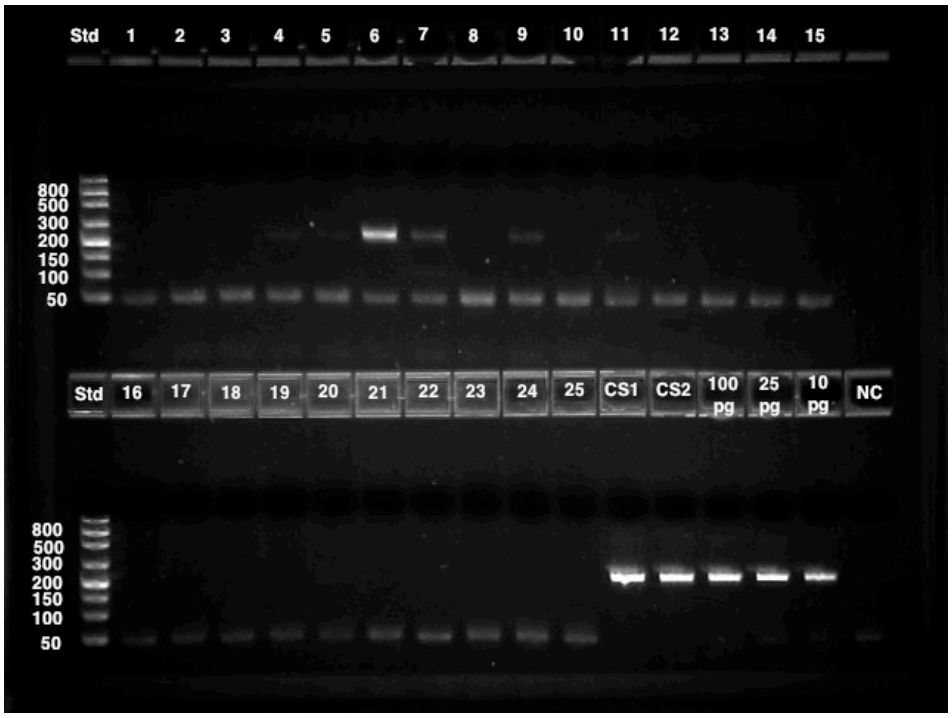
1 Negative Control

DNA Test

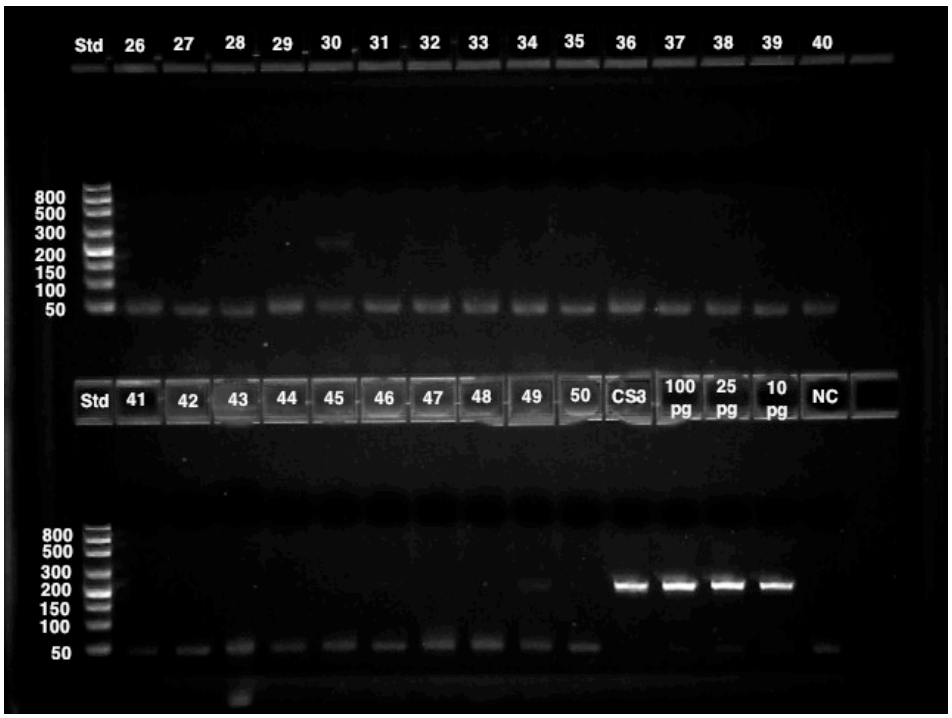
DNA was extracted from swabs using the Qiagen QIAamp DNA Blood Mini Kit in conjunction with the Qiagen QIAcube automated DNA prep instrument. In addition to the 50 sample swabs, DNA was also isolated from three positive control cheek swabs. PCR amplifications were performed on the DNA preps to determine whether DNA is present on the sample swabs. In addition to the 53 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 57 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 2.2% double tier Lonza flash gels. A DNA ladder was also loaded as a size standard.

PCR Gel 1



PCR Gel 2

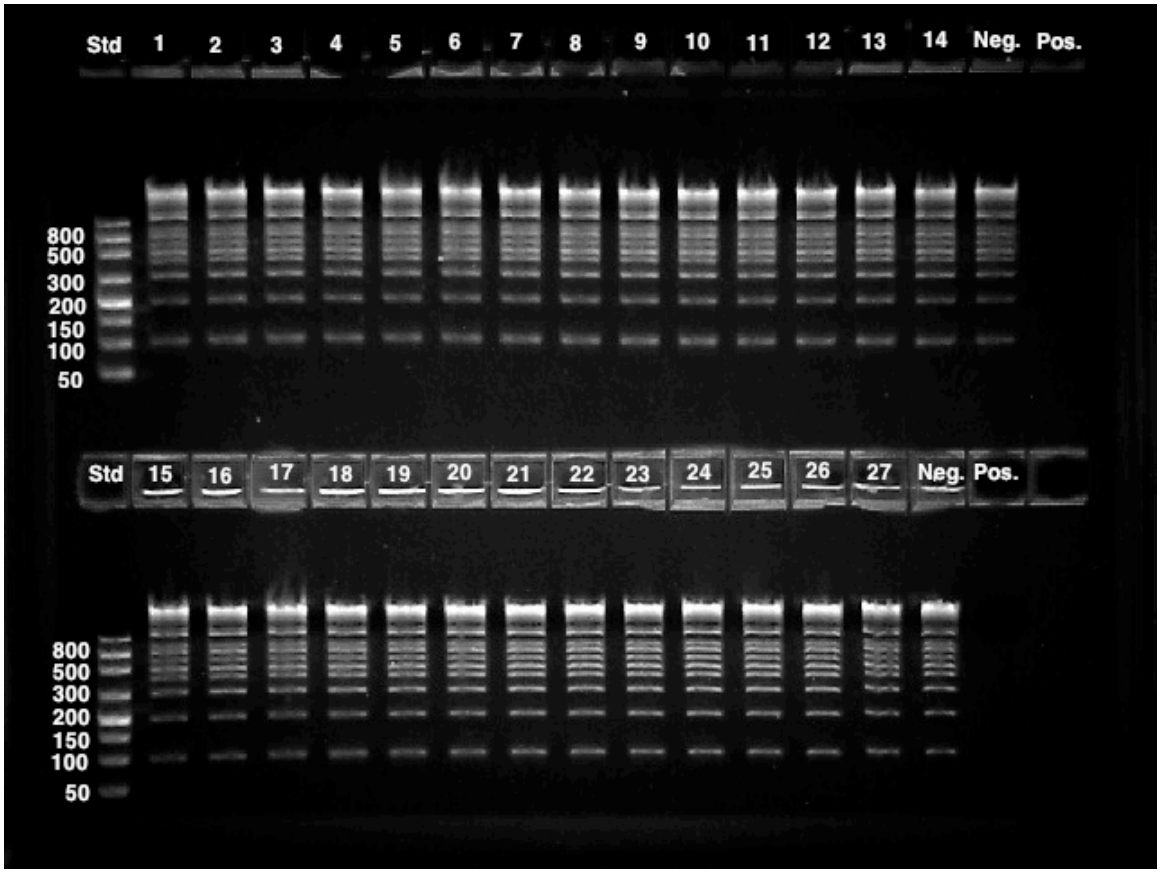


Based on the above data these swabs are considered to be DNA-free and PASS.

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

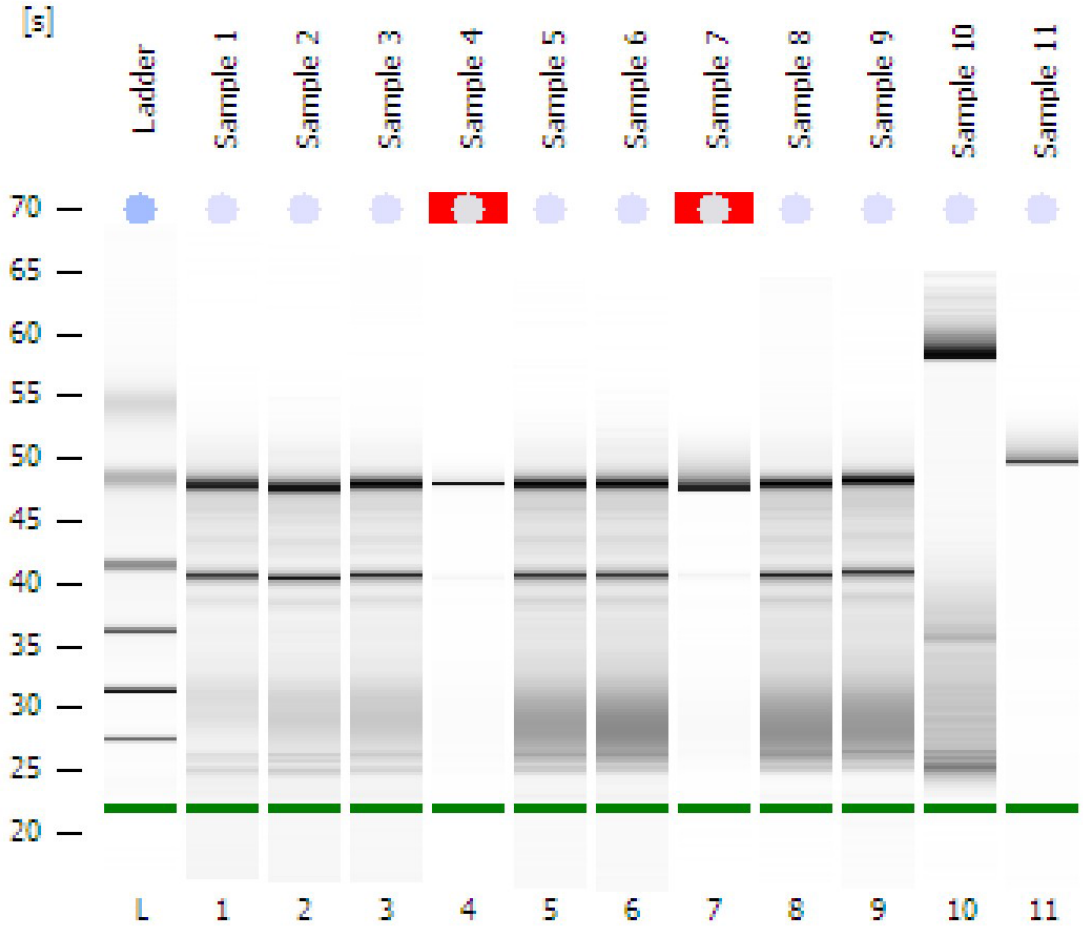


There is no degradation of substrate DNA after incubation with the swabs so these swabs are considered to be DNase-free and PASS.

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.

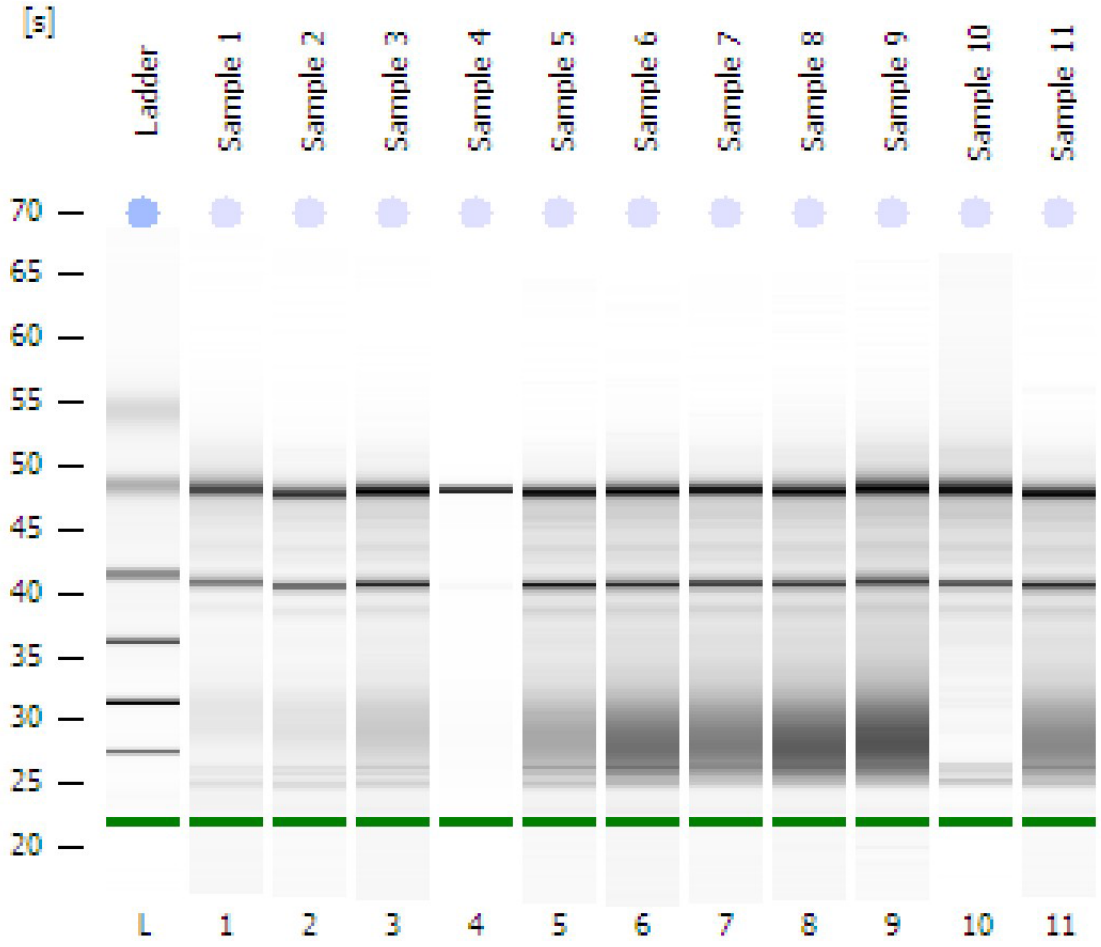
Beginning Swab Chip



Samples 1-9 are the nine beginning swabs tested. Sample 10 is the negative control and sample 11 is the positive control.

Sample 4, sample 7 and the negative control show an anomalous large peak that is obscuring the rRNA peaks because of its intensity. They will be rerun on a later chip.

Middle Swab Chip



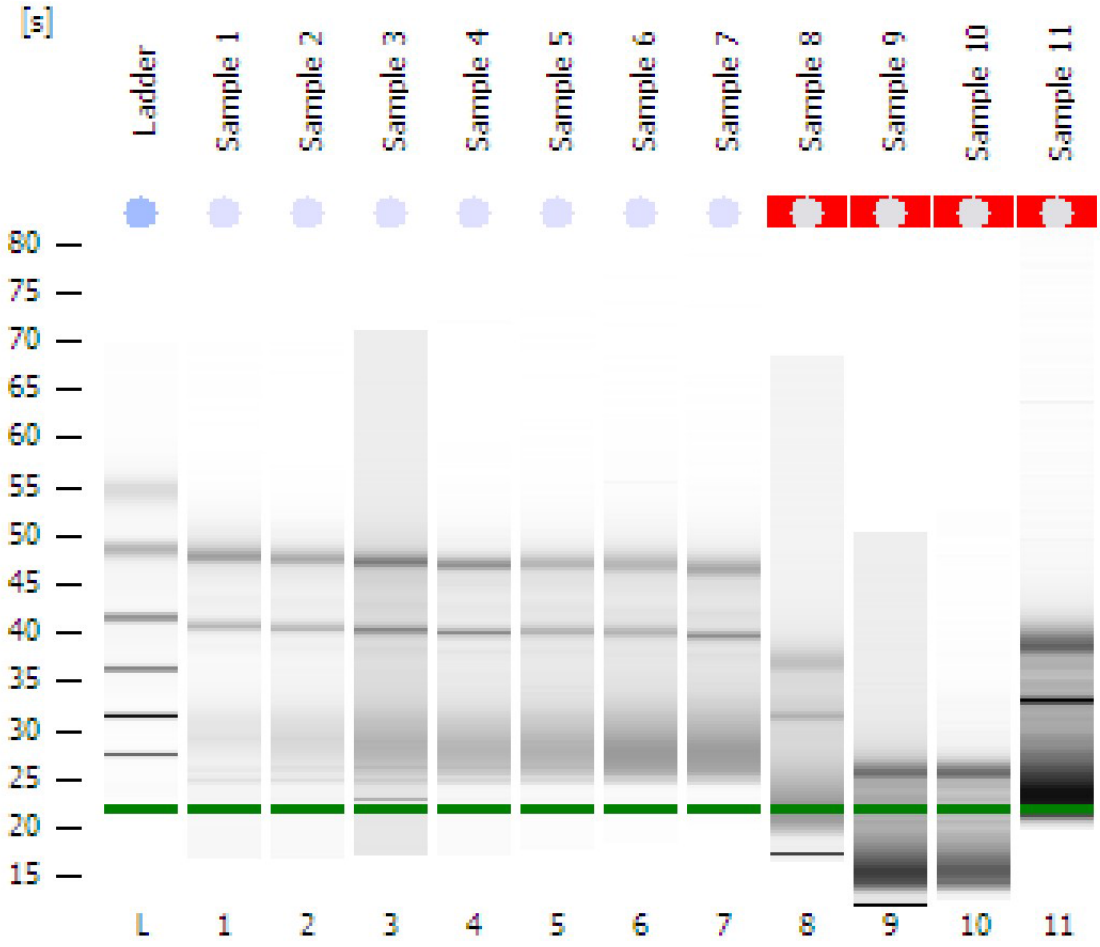
Samples 1-9 are the nine middle swabs tested.

Sample 10 is the negative control.

Sample 11 is the rerun of beginning swab 4

Sample 4 shows an anomalous peak that is obscuring the rRNA peaks.

End Swab Chip



Samples 1-9 are the nine end swabs tested

Sample 10 is a rerun of beginning swab 7.

Sample 11 is a rerun of middle swab 4.

Samples 8-11 are running much faster than they should and much faster than the rest of the samples. Because of this the dye marker and bands are not running in the correct place. Even though the samples aren't running properly the rRNA peaks are present.

Most of the RNAs are showing a broad peak prior to the 18S rRNA. This is not due to degradation but is something that is released from the pure flock swabs during incubation. We have seen this previously with the pure flock swabs and have determined that the "blob" is not degraded RNA.

These swabs should be considered to be RNase-free and PASS.