Puritan Report for Batch – 25-3306-U TT FDNA Lot# 3694

Prepared by the University of Maine DNA Sequencing Facility/ Patty Singer,
January 22, 2014

Swabs were received for testing on January 13, 2014

Testing Scheme

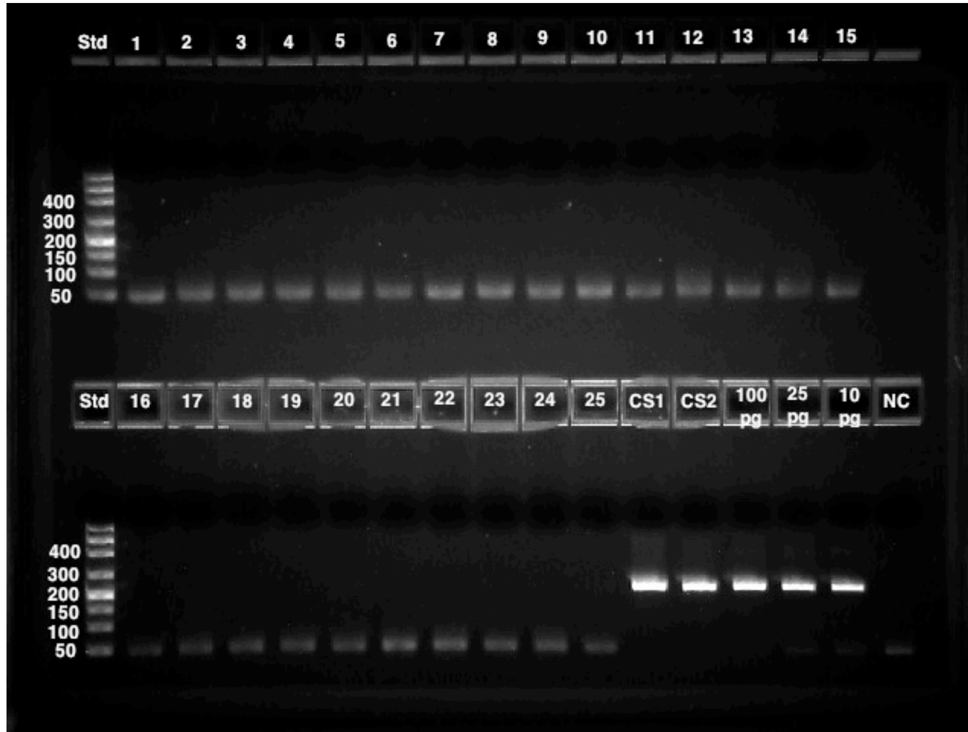
DNA Test:	50 Test Swabs [17 from Beginning (1-17), 16 from Middle (18-33) and 17 from End (34-50)]
	3 Positive Control Cheek Swabs CS1, CS2 and CS3 (51-53)
	3 Genomic DNA Control Reactions (54-56)
	1 No DNA Control (57)
DNase Test:	27 Test Swabs [9 Beg. (1-9), 9 Mid. (10-18) and 9 End (19-27)]
	1 Positive 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

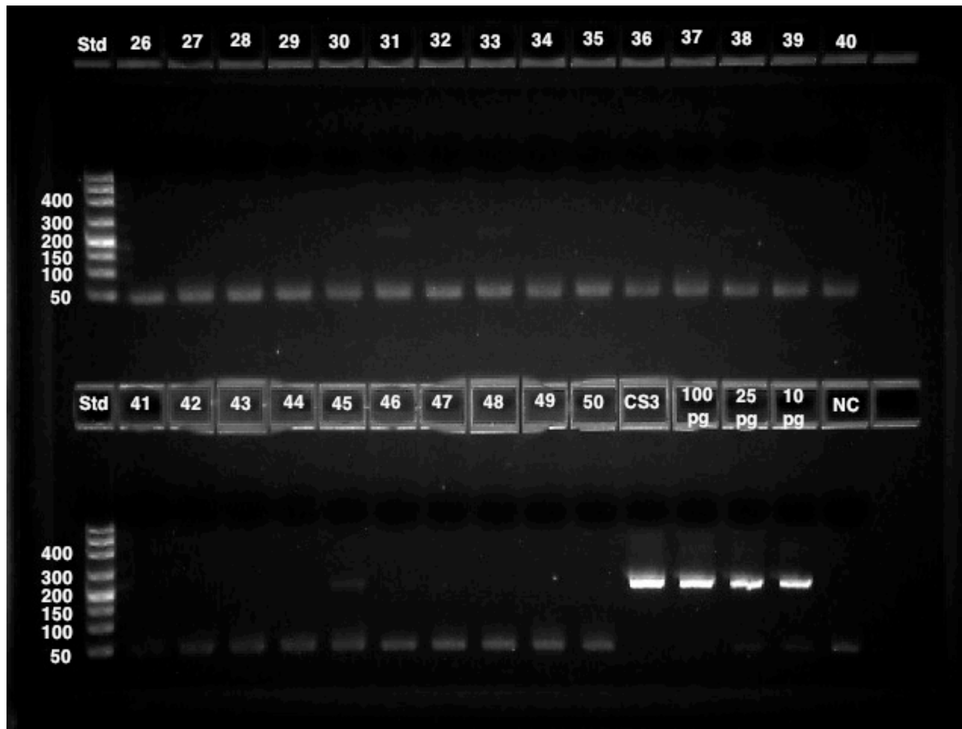
DNA was extracted from swabs using the Qiagen QIAamp DNA Blood Mini Kit in conjunction with the Qiagen QIAcube automated DNA prep instrument. In addition to the 50 sample swabs, DNA was also isolated from three positive control cheek swabs. PCR amplifications were performed on the DNA preps to determine 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 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).

PCR Swab Gel 1



PCR Swab Gel 2

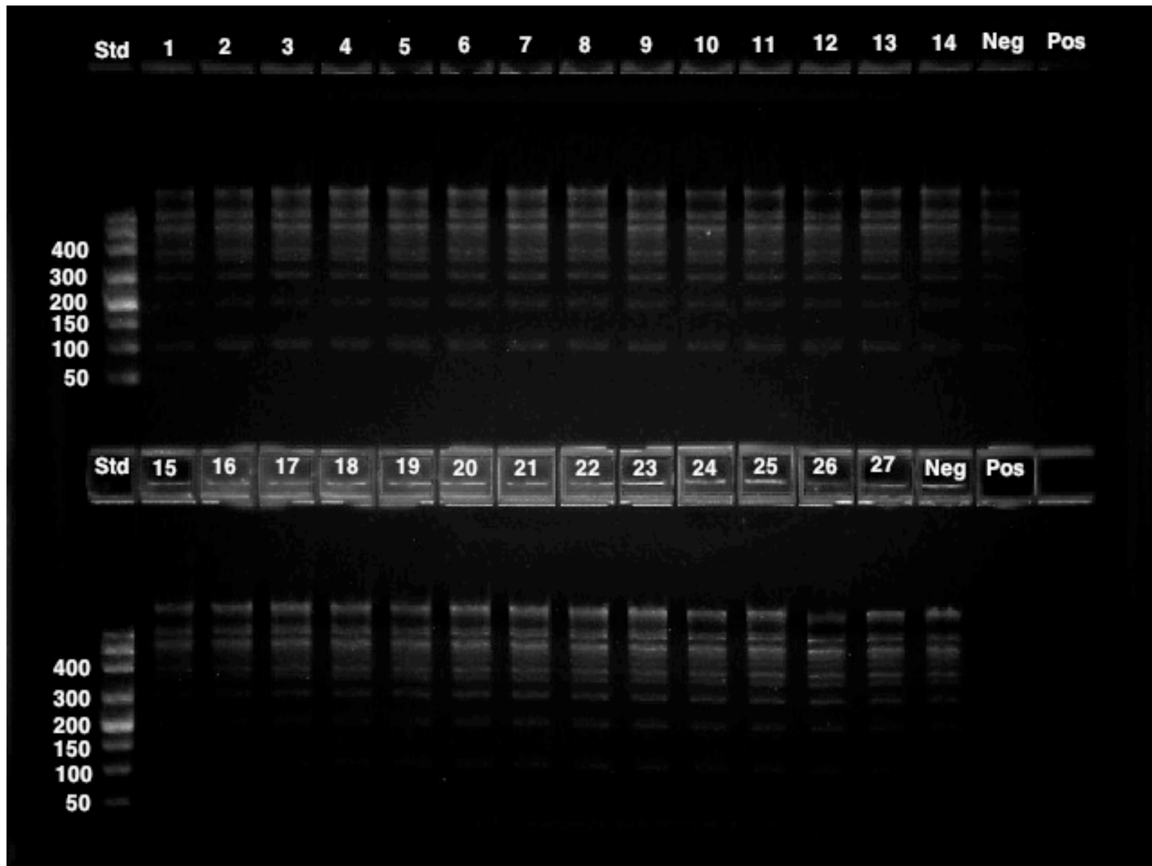


These results show that there is no DNA present on the sample swabs tested. Based on the above data these swabs are considered to be DNA-free and PASS.

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

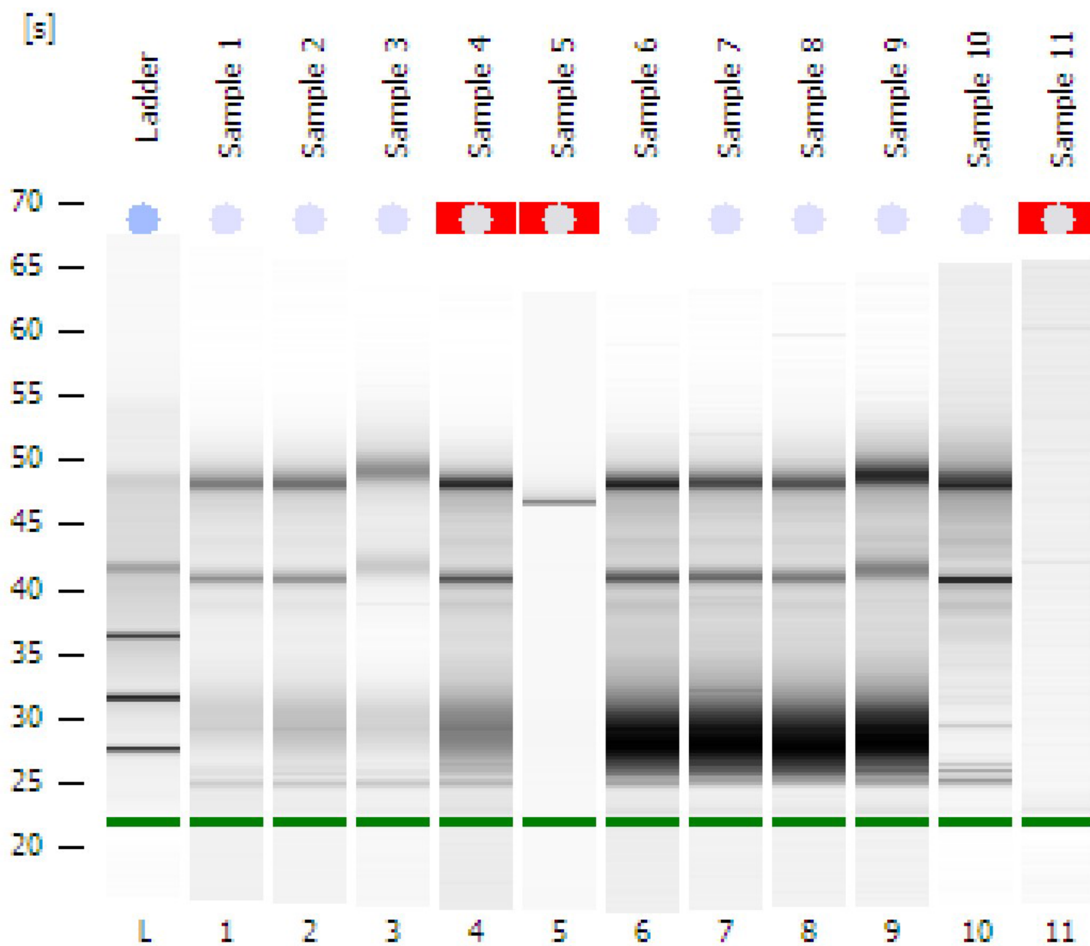


There is no degradation of substrate DNA after incubation with the swabs so these swabs are considered to be DNase-free and PASS.

RNase Test

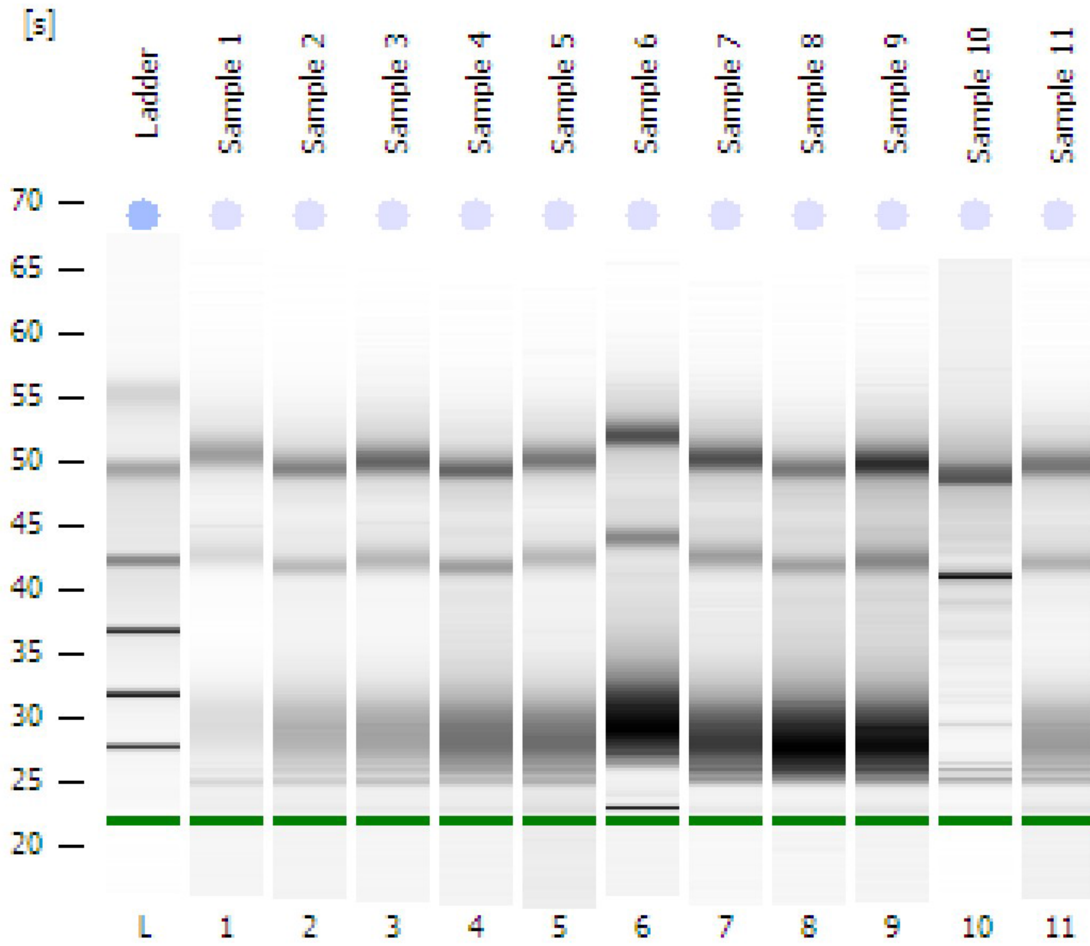
Twenty-seven swabs were tested for the presence of RNase activity. Two controls, one positive and one negative, were also tested. The swabs and controls were incubated with total RNA added as the substrate. The controls contained no swabs; the positive control had the addition of RNase A while the negative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



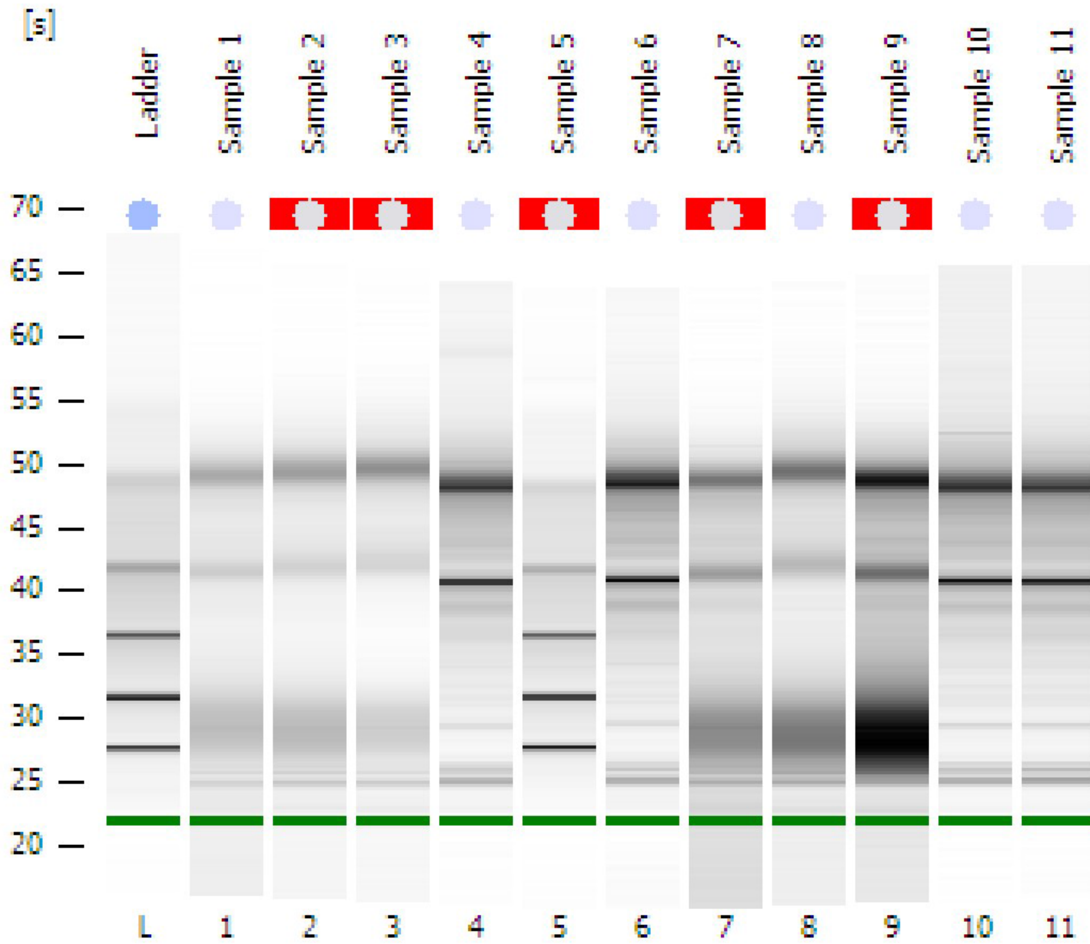
Samples 1-9 are the swab samples, sample 10 is the negative control and sample 11 is the positive control.

Middle Swab Chip



Samples 1-9 are the swab samples, sample 10 is the negative control and sample 11 is a rerun of sample 5 from the beginning swab chip.

End Swab Chip

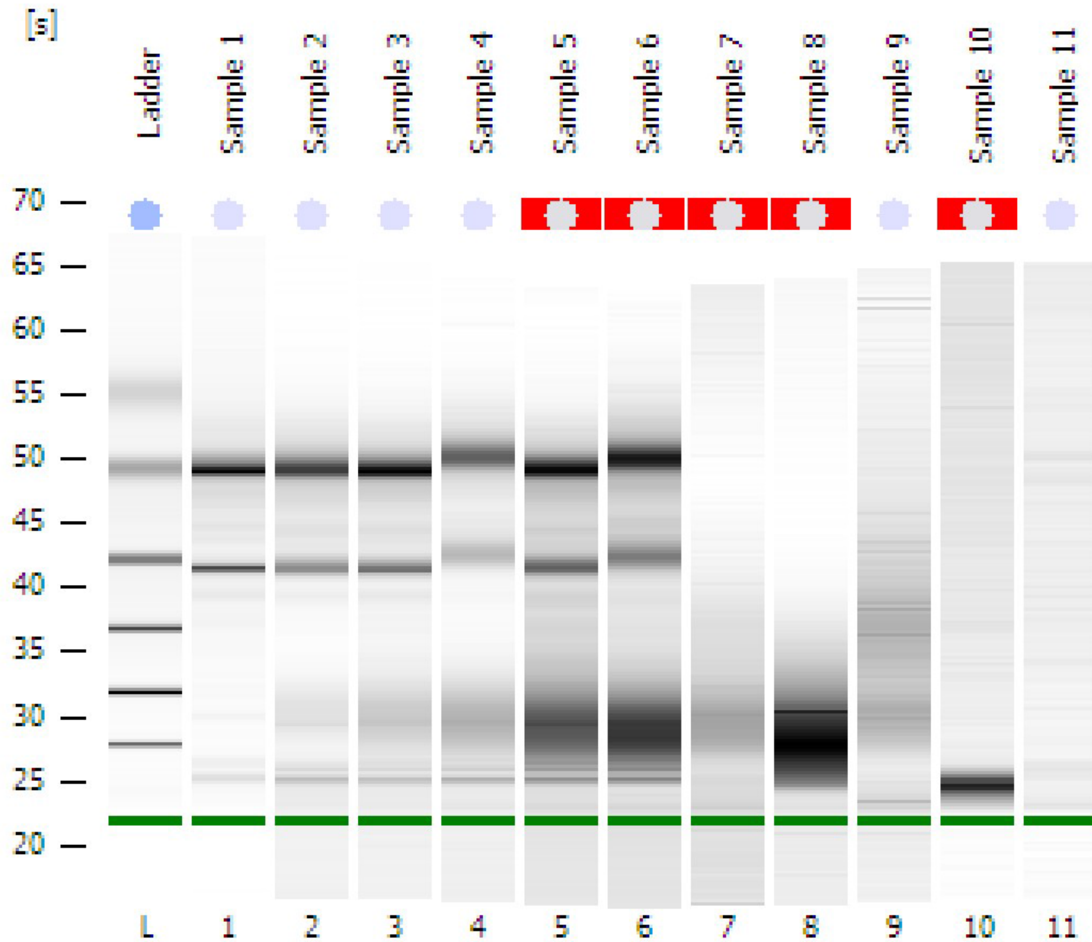


Samples 1-3 are swab samples, sample 4, 6, 10 and 11 are negative controls, sample 5 is the RNA ladder, samples 7-9 are swab samples.

The results above show that there seems to be something that is present on the swabs that is coming off the swab during incubation at 37°C. Although it looks like it could be degraded RNA it likely is not since the rRNA pecks are not disappearing.

Repeat the RNase assay to try and determine what may be happening. It may be the 5 minute centrifugation after the incubation is pulling something off the swabs

Repeat Assay Chip



Sample 1 – Total RNA with no incubation
Sample 2 – New swab with no spin after incubation
Sample 3 – New swab with a quick spin after incubation
Sample4 – New swab with the normal 5 minute spin after incubation
Sample 5 – New swab with a quick spin after incubation
Sample 6 – New swab with the normal 5 minute spin after incubation
Sample 7 – New swab with 5 min spin and then RNase treatment
Sample 8 – Sample 6 from the previous assay 2 days ago
Sample 9 – Sample 15 from before then treated with RNase
Sample 10 – Negative control
Sample 11 – Positive control

These results indicate to me that whatever is causing the low molecular weight band in the swab sample does not disappear after treatment with RNase so it is not degraded RNA. Therefore these samples should be considered to be RNase-free and PASS.