Puritan Batch 3 Report
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1. Swab Preparation

For simplicity, the swabs were coded as "B".

Type: 25-806 1WC FDNA, Lot# 2812 Exp: 3/2016
40 swabs from beginning, middle and end of batch production (120 total)

The swabs were used for analysis, as described below:

51 swabs:	DNA test
	48 test swabs (B1 – B48)
	3 positive control swabs (B49 beginning, B50 middle, B51 end)
28 swabs:	DNAse test
	27 test swabs (B52 – B78)
	1 positive control swab (B79)
	1 negative control (no swab)
28 swabs:	RNAse test
	27 test swabs (B80 – B106)
	1 positive control swab (B107)
	1 negative control (no swab)

2. Test for DNA contamination

DNA was extracted from each swab using the QIAmp DNA Mini extraction kit and protocol for buccal swabs – the elution volume was 150 ul. For the three positive control swabs, a buccal sample was collected, allowed to dry overnight and then used for DNA extraction. To avoid contamination, DNA was extracted from the positive control swabs separately from the test swabs. Additional positive amplification controls (pac) of 10pg, 20pg and 20ng were added to the PCR.

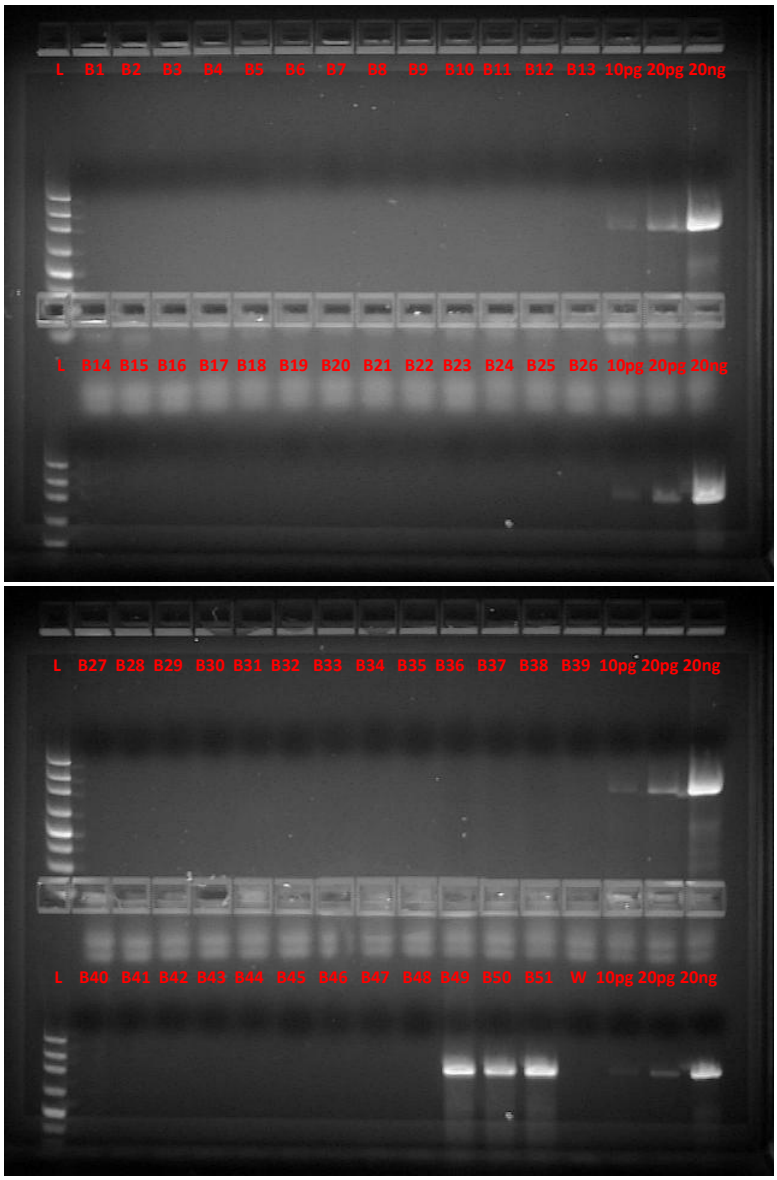
5 ul from each sample was amplified for 40 cycles in a PCR reaction using primers specific for Alu. The total reaction volume was 20 ul. After PCR, 5 ul of each reaction was combined with 1 ul of 5x loading dye and run on a Lonza DNA flashgel. Images of the gels are shown below.

Figure 1.

DNA Test

Lonza DNA Ladder

1500
800
500
300
200
150
100
50



FlashGel DNA 2.2% 16+1 Double Tier Lot: 230163 Exp: 8-28-2011

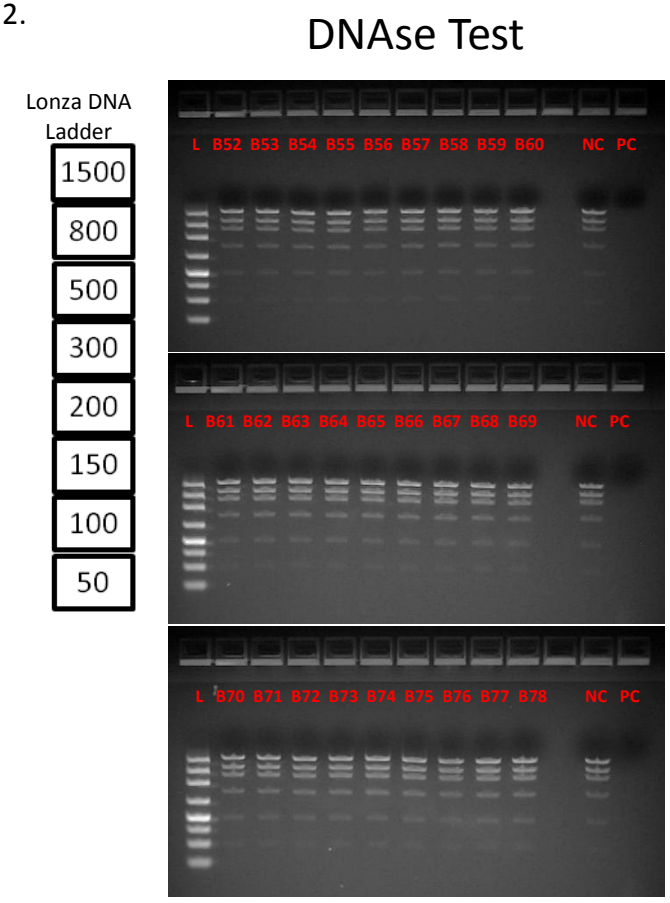
Samples B1 – B16 = Beginning, B17 – B32 = Middle, B33 – B48 = End, B49 = Positive Extraction Control – Beginning, B50 = Positive Extraction Control – Middle, B51 = Positive Extraction Control – End. 10pg, 20pg, 20ng = Positive PCR Controls.

Result: No DNA was detected - within the limits of detection - on the test swabs, as evidenced by the lack of a band on the gels. The positive controls (B49, B50 and B51) were selected from each position within the batch (beginning, middle and end, respectively). These swabs performed as expected, revealing the presence of nucleic acid from a buccal swab preparation. **Lot 2812 (25-806 1WC FDNA) is DNA-free.**

3. Test for presence of DNAse contamination

Swabs from each of the 3 shift periods (beginning, middle and end) were labeled B52 through B79 and were tested for DNAse contamination. One swab was treated with DNAse and used as a positive control. In this test, the swabs were saturated with 200 ul 1X PCR buffer containing 200ul of a Low Mass DNA ladder; the positive control swab was also incubated with DNAse. A negative control with just buffer and ladder (no swab) was also prepared. All the samples were incubated at 37^{o}C for 1 hour. 5 ul of each sample was combined with 1 ul 5x loading dye and loaded on a Lonza DNA flashgel. The image of the gel is shown below.

Figure 2.



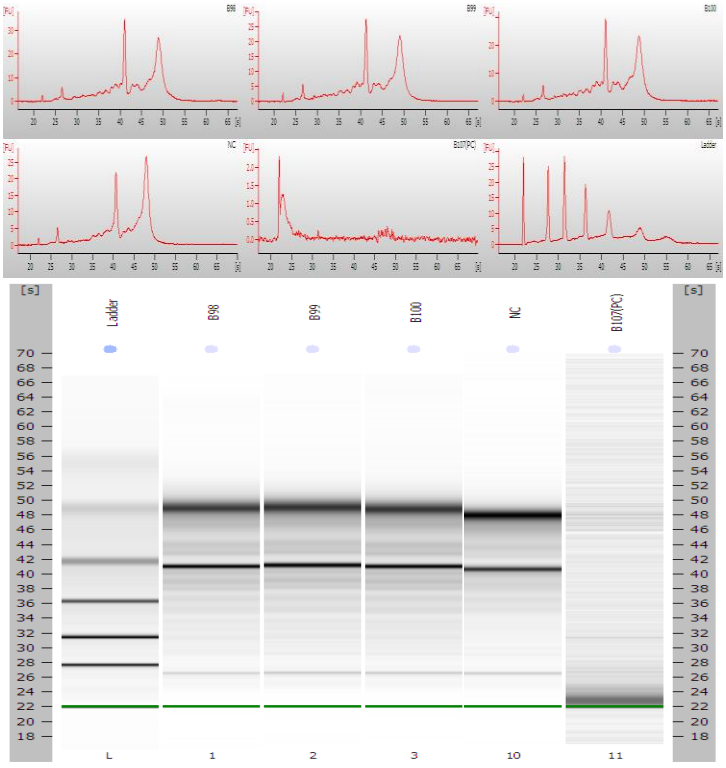
FlashGel DNA 2.2% 12+1 Single Tier Lot: 217970
Exp: 6-17-2011
Samples B52 – B60 = Beginning, B61 – B69 = Middle, B70 – B78 = End, NC, PC

Result: No DNAse was detected on the test swabs, as evidenced by the presence of a ladder in each lane on the gel. The ladder is completely degraded in the positive control lane, as expected. **Lot 2812 (25-806 1WC FDNA) is DNAse-free.**

4. Test for the presence of RNAse contamination

Swabs from each of the 3 shift periods (beginning, middle and end) were labeled B80 through B107 and were tested for RNAse contamination. One swab was treated with RNAse and used as a positive control. In this test, the swabs were saturated with 200 ul 1X PCR buffer containing 300ul of a high quality RNA at 510ng/ul concentration; the positive control swab was also incubated with RNAse A. A negative control with just buffer and RNA (no swab) was also prepared. All the samples were incubated at 37^{o}C for 30 minutes. Each sample was analyzed using the Agilent Bioanalyzer RNA Pico kit, following the manufacturer's instructions. One of the electropherograms is shown below.

Figure 3.



Result: No RNAse was detected on the swabs tested,as evidenced by the presence of two RNA peaks in each lane on the gel. The 28S:18S ratio for these samples was between 1.7 and 3.0, while the RIN values were ≥ 6.0. The negative control demonstrates these same peaks and the positive control is completely degraded, as expected. **Lot 2812 (25-806 1WC FDNA) is RNAse-free.**