Puritan Batch 6 Report
May 26, 2011
Prepared by:
Christina Newman, Technical Head – Genomics Core
Maine Institute for Human Genetics and Health

1. Swab Preparation

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

Type: 25-806 1WC FDNA, Lot# 2819 Exp: 4/2016
40 swabs from beginning, middle and end of batch production (120 total)

The swabs were used for analysis, as described below:

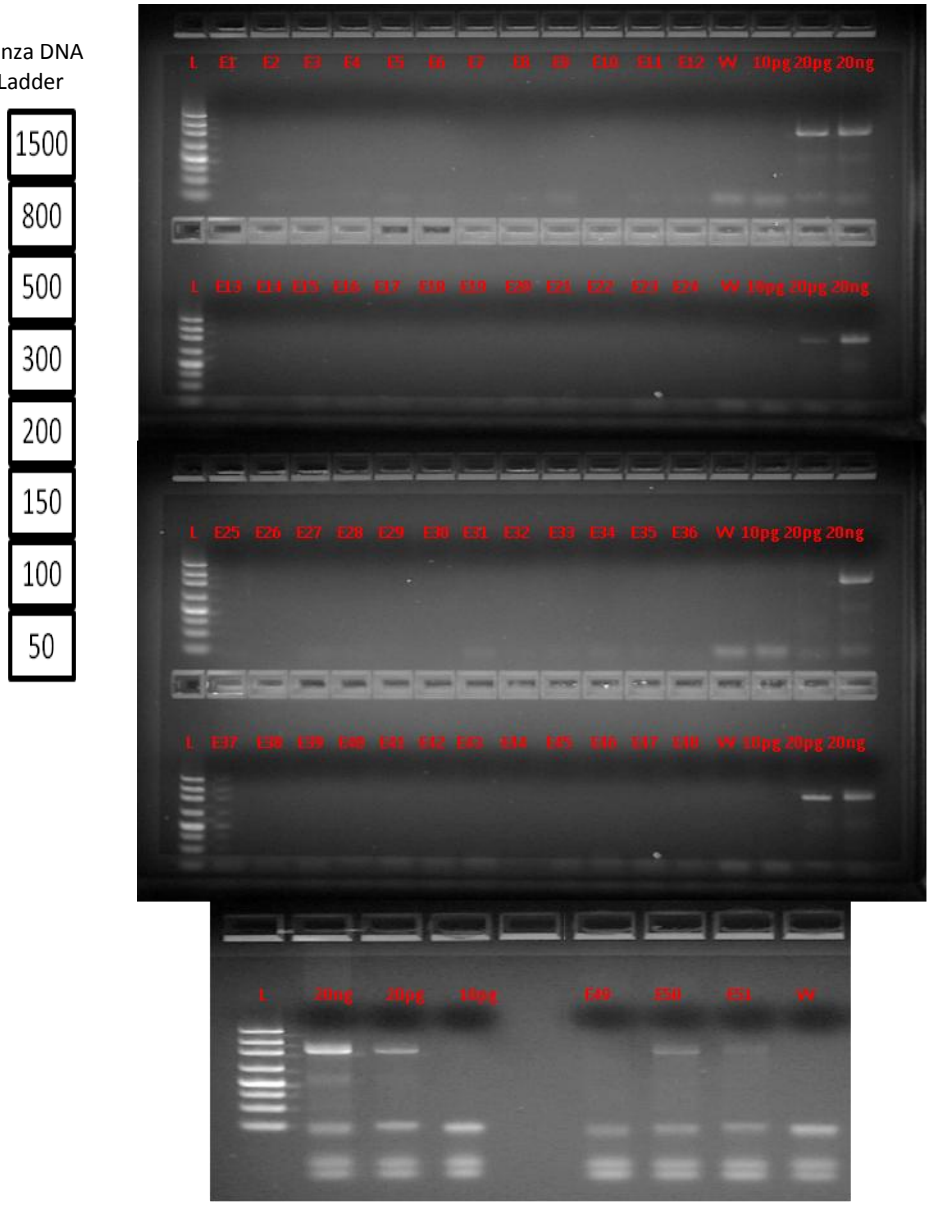
51 swabs:	DNA test
	48 test swabs (E1 – E48)
	3 positive control swabs (E49 beginning, E50 middle, E51 end)
28 swabs:	DNAse test
	27 test swabs (E52 – E78)
	1 positive control swab (E79)
	1 negative control (no swab)
28 swabs:	RNAse test
	27 test swabs (E80 – E106)
	1 positive control swab (E107)
	1 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.



FlashGel DNA 2.2% 16+1 Double Tier Lot: 230163 Exp: 8-28-2011
FlashGel DNA 2.2% 12+1 Single Tier Lot: 217970 Exp: 6-17-2011
Samples E1 – E16 = Beginning, E17 – E32 = Middle, E33 – E48 = End, E49 = Positive Extraction Control – Beginning, E50 = Positive Extraction Control – Middle, E51 = Positive Extraction Control – End. 10pg, 20pg, 20ng = Positive PCR Controls.

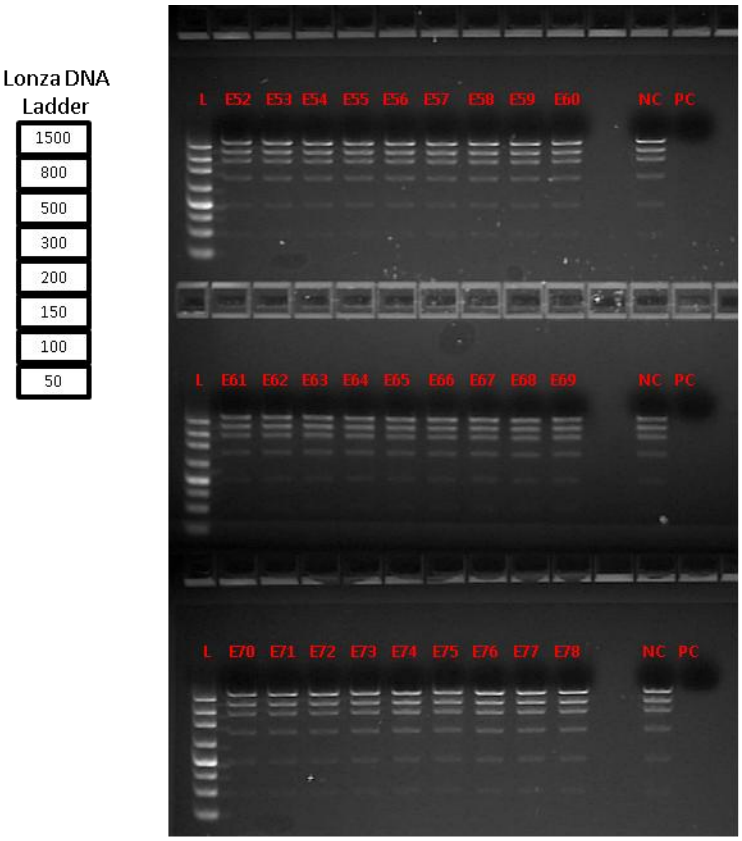
Result: No DNA was detected - within the limits of detection - on the test swabs, as evidenced by the lack of a band on the gels. The positive controls (E49, E50 and E51) were selected from each position within the batch (beginning, middle and end, respectively). The positive control swabs were collected from one individual and therefore each swab collected contained less DNA than the previous collection.

Although a clear band is not as evident in E49, these swabs gave expected results. **Lot 2819 (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 E52 through E79 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 300 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 E52 – E60 = Beginning, E61 – E69 = Middle, E70 – E78 = 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 2819 (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 E80 through E107 and were tested for RNAse contamination. One swab was treated with RNAse and used as a positive control. In the test, the swabs were saturated with 300 ul 1X PCR buffer and 600 ul of high-quality human RNA at a concentration of 500 ng/ul; the positive control swab was also incubated with RNAse. A negative control with just buffer and RNA (no swab) was also prepared. All the samples were incubated at 37^{o}C for 1 hour. Each sample was analyzed using the Agilent Bioanalyzer RNA Nano kit, following the manufacturer's instructions.

Results

The positive control lane showed RNA degradation and the negative control lane showed the presence of intact, high quality RNA. For the 27 sample swabs the presence of RNA was intact. **Lot 2819 (25-806 1WC FDNA) is RNAse-free**.