Electronic Supplementary Material (ESI) for Lab on a Chip.

Pyrosequencing on a glass surface, Supplementary information

In this supporting document we would like to describe washing efficiency , active mixing, as well as effect of DNA concentration used with SPP and finally also experiment with homopolymeric stretches sequencing. We feel these experiments are too detailed to be in the main manuscript.

1. Washing efficiency

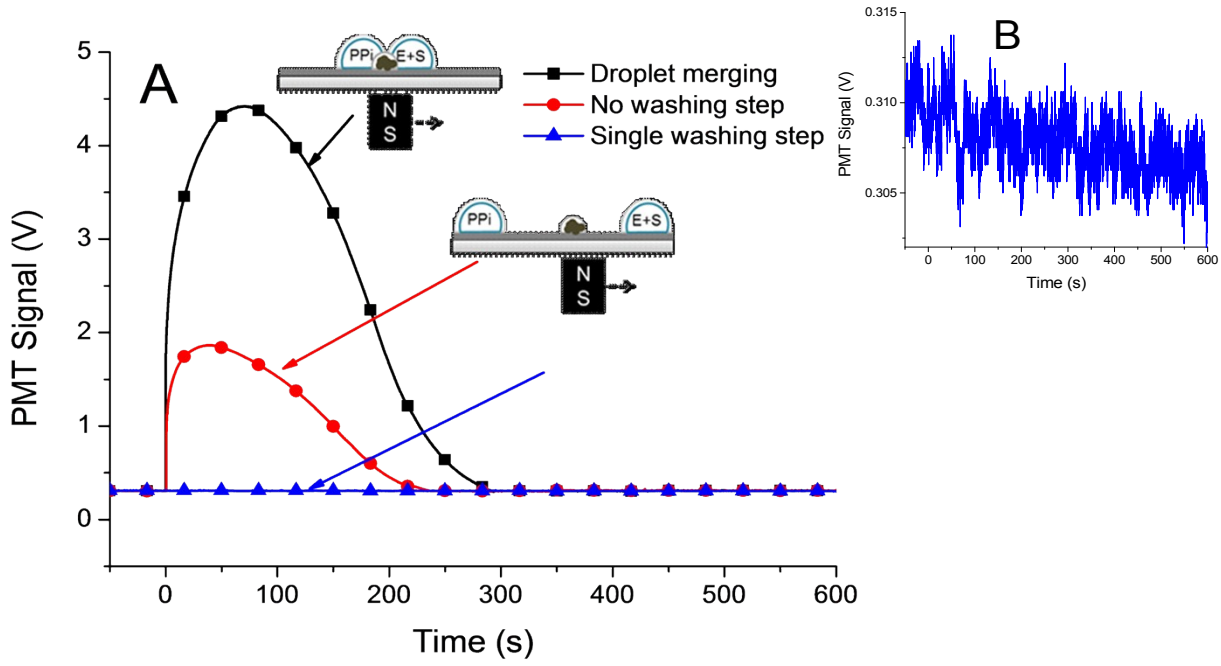

Figure 1: (A) We moved SPP between droplets containing inorganic pyrophosphate (PPi), enzymes and substrates used for pyrosequencing while the resulting luminescence was continuously monitored by PMT and recorded by an oscilloscope. We have merged both (black squares), extracted the SSP from one droplet to the next (red circles) and included a washing step between (blue triangles). Results demonstrated that efficiency of washing step implementation effectively suppressed the signal background. Peak amplitude (minus baseline subtraction) dropped from ≈4.12 V (black squares) to ≈1.56V (red circles) and to practically ≈0 V (blue upward triangles) once the washing step was included. (B) The resulting PMT signal difference was ≈10 mV unrelated to the reaction. Amplitude of the area under curve (AUC) dropped from ≈0.70 mC to ≈0.21 mC (no washing) and eventualy to ≈0 mC (washing).

2. Active mixing and reaction time

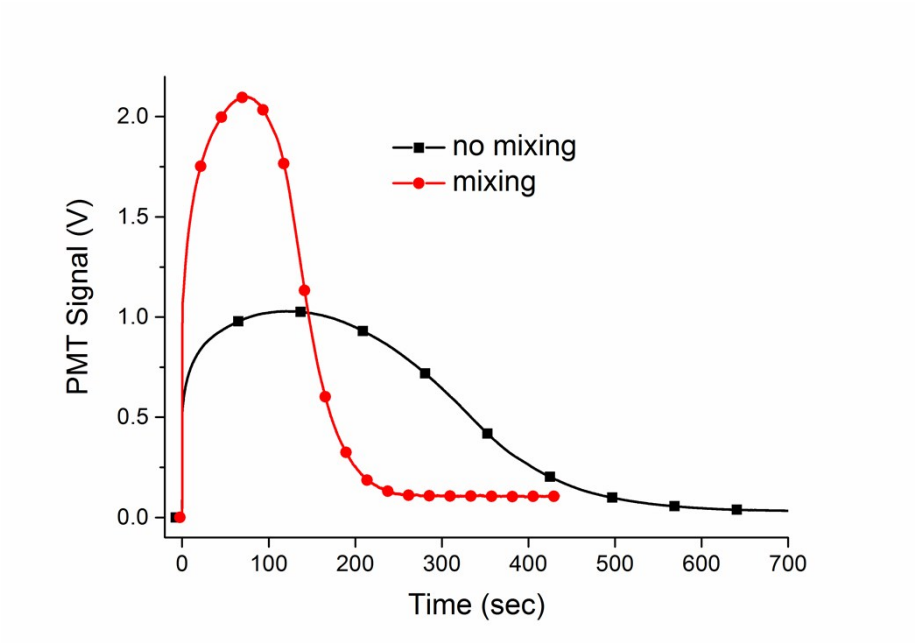

Figure 2: We have moved SPP bound to ssDNA between droplets containing enzymes, substrates and dNTP while the resulting luminescence was continuously monitored by PMT and recorded by an oscilloscope. In one case we recorded the ongoing reactions while having the magnet fixed in position (black squares). In the other case, the magnet was moved across the reaction droplet with spedd of ≈2 mm/s while the pyrosequencing reactions were ongoing (red circles).

3. Effect of DNA concentration

The sequence B-5‘-ATGGGGGGGGGGATGGGGGGGGGATGGGGGGGGA TGG GGGGGATTGGGGGGTTGG GGGATGGGGTCTGGTATGGATGTAAACGCCTGG TATCTTTATAGTCCA-3‘ was determined using the sequencing-primer 5‘-GGACTATAAAGATACCAGGCGTT-3’ with different concentrations of DNA, such as ≈0.315 pmol, ≈1.25 pmol, ≈5,pmol, ≈15 pmol and ≈20 pmol.

a. Concentration of ≈0.315 pmol of DNA

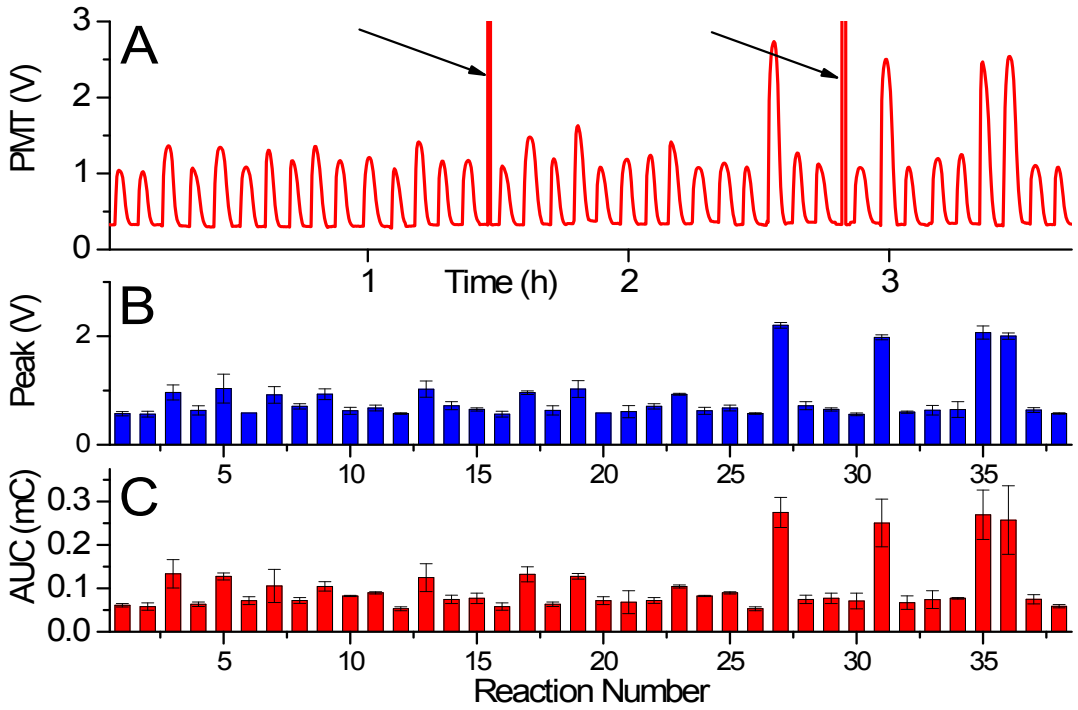

Figure 3 (A) A typical pyrogram consisting of 38 reactions performed on 3 glasses with two changes marked by black arrows. (B) Extracted peak voltages (mean value) with standard error as function of reaction number from three measurements. (C) Extracted AUC (mean value) with standard error as function of reaction number from three measurements. (D) Frequency count of mean peak values showing three distinguish groups according to number of incorporated nucleotides. (E) Frequency count of mean AUC shows again grouping according to number of incorporated nucleotides. Nevertheless the peak values provide more consistent data. (F) Peak amplitude (left) and AUC (right) as function of number of incorporated nucleotides. Even with this low DNA concentration the peaks can be distinguish from each other as the peak values are (0.628 ± 0.051) V, (0.974 ± 0.048) V and (2.063 ± 0.099) V (mean ± standard deviation) for 0, 1 and 2 incorporated nucleotidesm respectively. Value of AUCs are (0.071 ± 0.010) mC, (0.120 ± 0.013) mC and (0.263 ± 0.011) mC, all (mean ± standard deviation) for 0, 1 and 2 incorporated nucleotides. Nonetheless there is a nonlinearity with relatively large background signal.

b. Concentration of ≈1.25 pmol of DNA

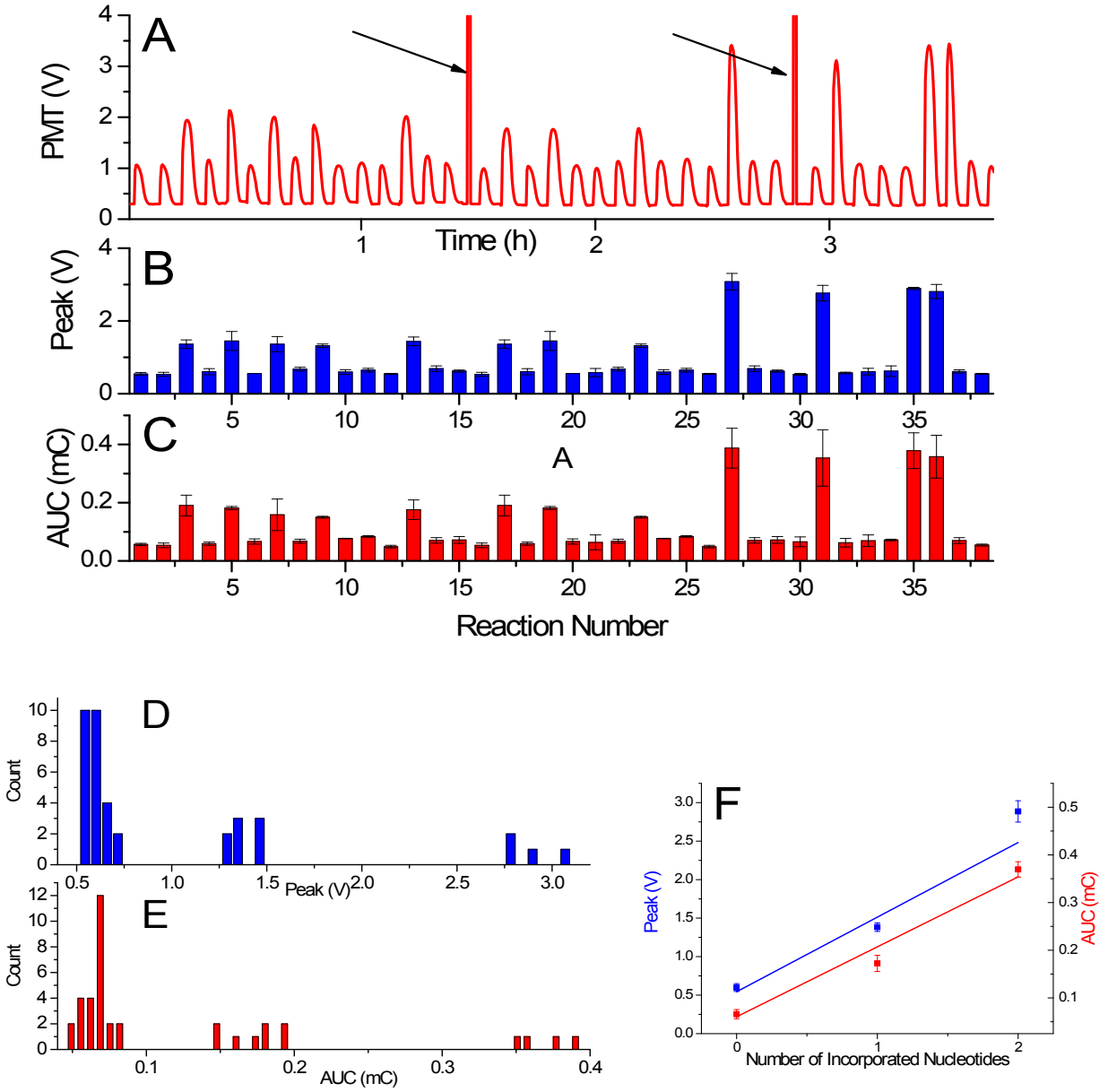

Figure 4 (A) A typical pyrogram consisting of 38 reactions performed on 3 glasses with two changes marked by black arrows. (B) Extracted peak voltages (mean value) with standard error as function of reaction number from three measurements. (C) Extracted AUC (mean value) with standard error as function of reaction number from three measurements. Frequency count of mean peak values (D) and AUCs (E) show three distinguish groups according to number of incorporated nucleotides. (F) Peak amplitude (left) and AUC (right) as function of number of incorporated nucleotides with slope of (0.969 ± 0.227) V and (0.147 ± 0.021) mC, both (mean ± standard deviation) for peak voltage and AUC, respectively. The nonlinearity is significantly smaller than the one for ≈0.315 pmol concentration of DNA with correlation coefficient of ≈0.959 and ≈0.896 for peak voltage and AUC, respectively.

c. Concentration of ≈5 pmol of DNA

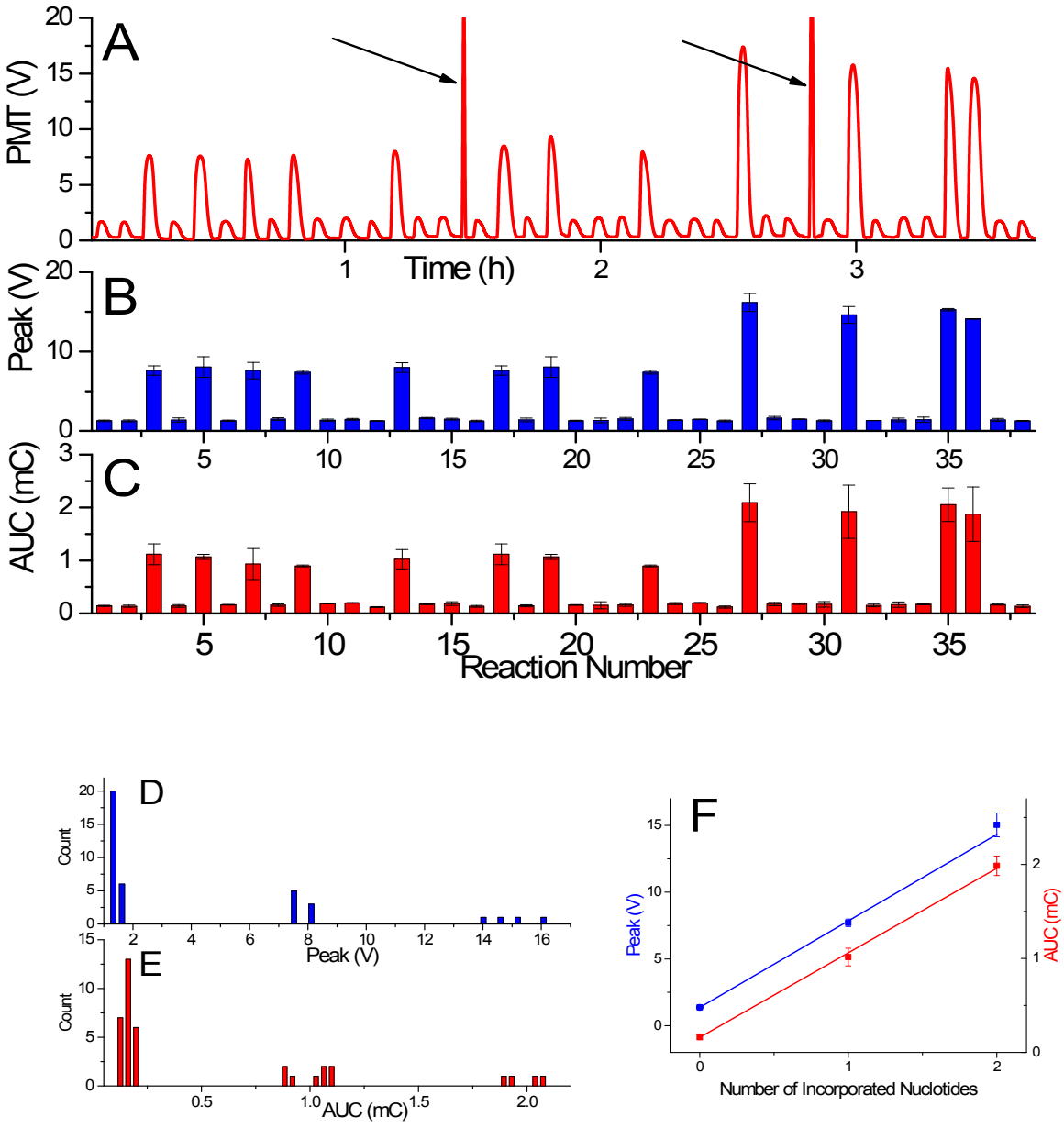

Figure 5 (A) A typical pyrogram consisting of 38 reactions performed on 3 glasses with two changes marked by black arrows. (B) Extracted peak voltages (mean value) with standard error as function of reaction number from three measurements. (C) Extracted AUC (mean value) with standard error as function of reaction number from three measurements. Frequency count of mean peak values (D) and AUCs (E) show three distinguish groups according to number of incorporated nucleotides. (F) Peak amplitude (left) and AUC (right) as function of number of incorporated nucleotides with slope of (6.478 ± 0. 242) V and (0.899 ± 0.025) mC, both (mean ± standard deviation) for peak voltage and AUC, respectively. The nonlinearity is even smaller than the one for ≈1.25 pmol concentration as the correlation coefficient is near unity, ≈0.997 and ≈0.998 for peak voltage and AUC, respectively.

d. Concentration of ≈15 pmol of DNA

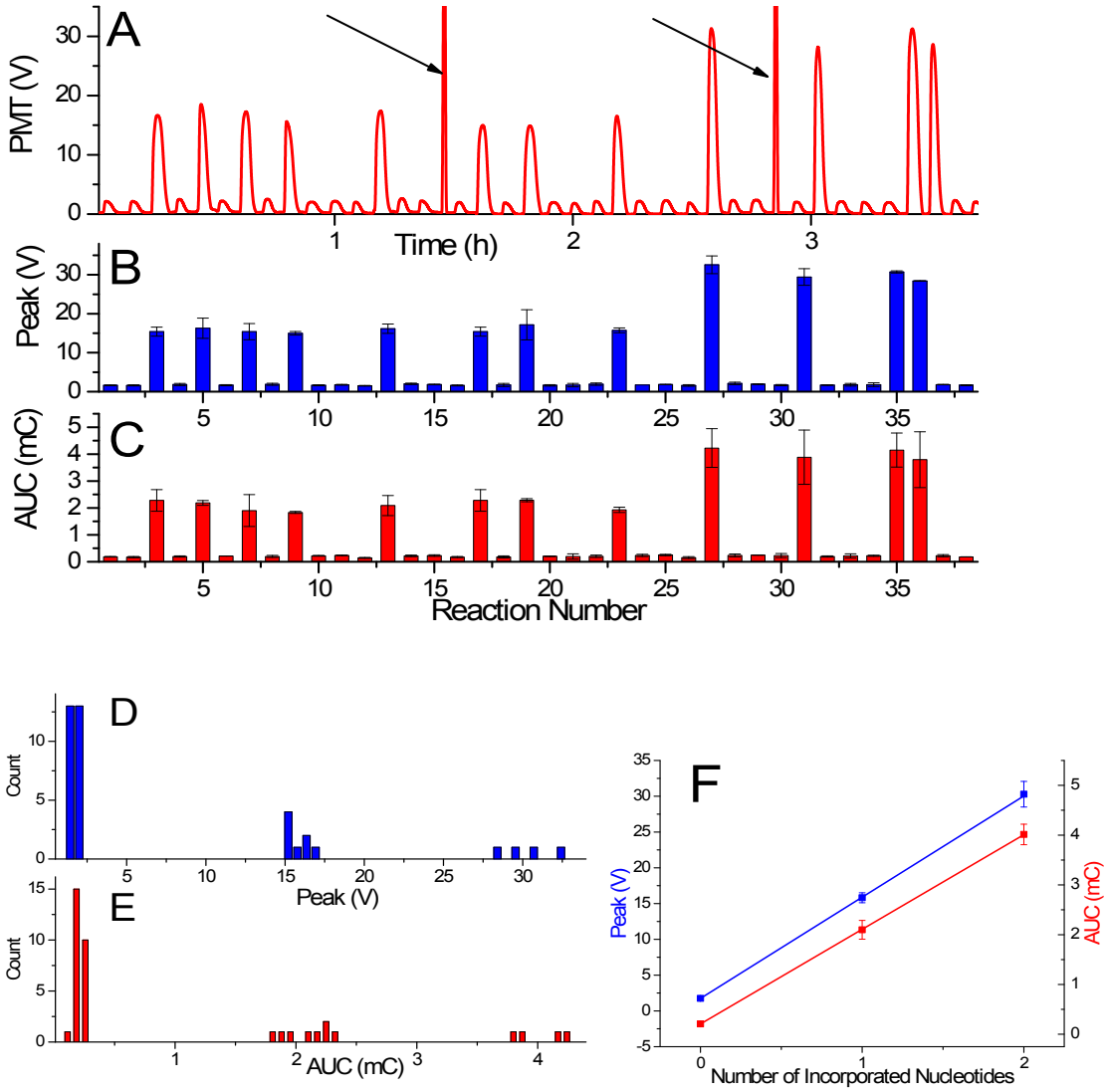

Figure *6* (A) A typical pyrogram consisting of 38 reactions performed on 3 glasses with two changes marked by black arrows. (B) Extracted peak voltages (mean value) with standard error as function of reaction number from three measurements. (C) Extracted AUC (mean value) with standard error as function of reaction number from three measurements. Frequency count of mean peak values (D) and AUCs (E) show three distinguish groups according to number of incorporated nucleotides. (F) Peak amplitude (left) and AUC (right) as function of number of incorporated nucleotides with slope of (14.144 ± 0.099) V and (1.900 ± 0.005) mC, both (mean ± standard deviation) for peak voltage and AUC, respectively. The nonlinearity is even smaller than the one for ≈1.25 pmol concentration as the correlation coefficient is practically unity, ≈0.999 for both, peak voltage as well as the AUC.

e. Concentration of ≈20 pmol of DNA

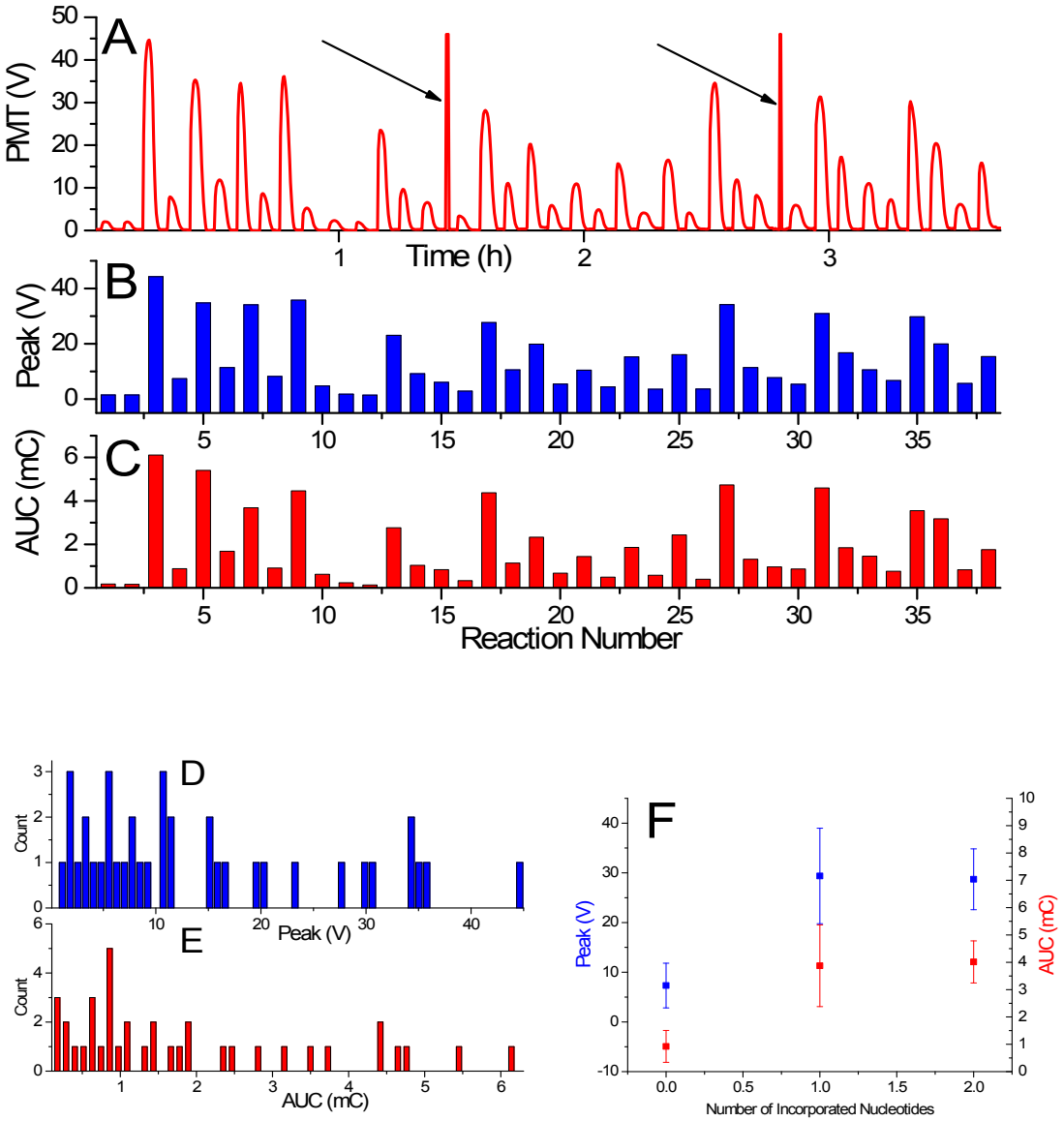

Figure 7 (A) A typical pyrogram consisting of 38 reactions performed on 3 glasses with two changes marked by black arrows. (B) Extracted peak voltages (mean value) with standard error as function of reaction number from single measurement. (C) Extracted AUC (mean value) with standard deviation as function of reaction number from single measurement. Frequency count of mean peak values (D) and AUCs (E) does not show distinguish groups according to number of incorporated nucleotides anymore. (F) Peak amplitude (left) and AUC (right) as function of number of incorporated nucleotides showing that this DNA concentration is not suitable for pyrosequencing.

4. Base calling in the homopolymeric stretches

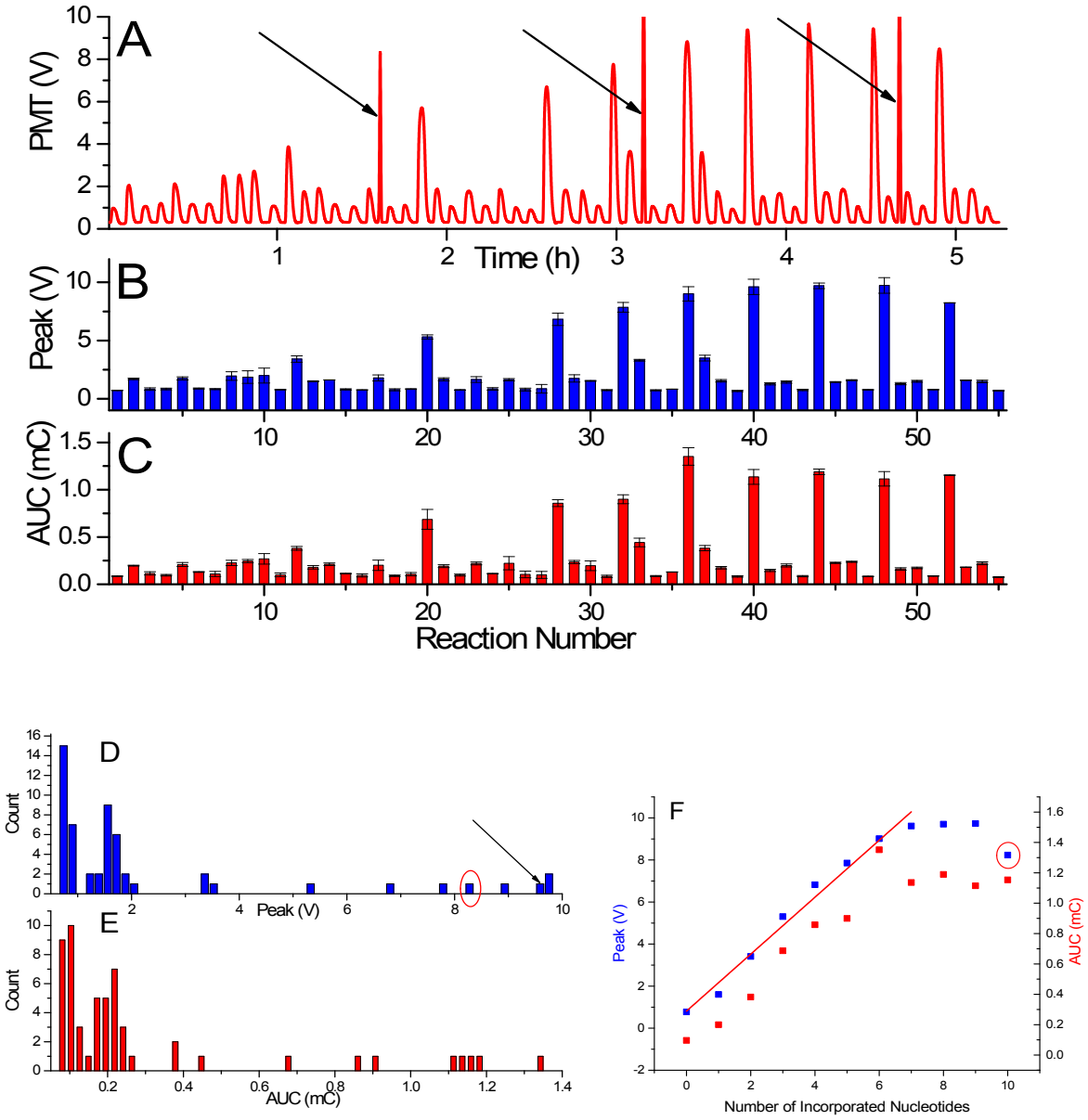

Figure 8: The sequence B-5‘-ATGGGGGGGGGGATGGGGGGGGGGATGGGGGGGGGATG GGGGGGATTGGGGGGTTGGGGGATGGGGTCTGGTATGGATGTAAACGCCTGGTAT CTTTATAGTCCA-3‘ was determined using the sequencing-primer 5‘-GGACTATAAAGAT ACCAGGCGTT-3’. This was to explore the limitations of the technique by presenting extreme cases such as 10 nucleotides in succession. (A) A typical pyrogram consisting of 55 reactions performed on 4 glasses with three changes marked by black arrows. (B) Extracted peak voltages (mean value) with standard error as function of reaction number from two measurements. (C) Extracted AUC (mean value) with standard error as function of reaction number from two measurements. (D) frequency count of mean peak values showing grouping according to number of incorporated nucleotides from 0 up to 7 (marker by a black arrow). 10 incorporated nucleotides is inside red circle. (E)) frequency count of mean AUC showing grouping according to number of incorporated nucleotides from 0 up to 5 demonstrating that the peak values are providing more consistent data. (F) Peak amplitude (left) and AUC (right) as function of number of incorporated nucleotides. Here we demonstrate that the glass pyrosequencing can be used for up to 7 nucleotides with (1.354 ± 0.075) V (mean ± standard deviation) per incorporated nucleotide.