Report on ATAC-seq experiment

2016. 01. 27

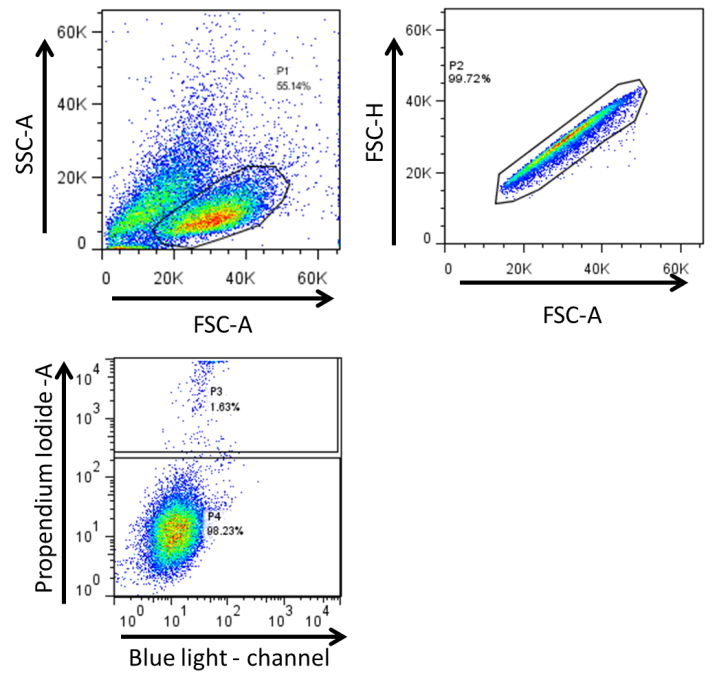
**Aims:** The goal of this experimentis to test the protocol provided by David Garfield/Bingqing Zhao (Furlong lab), we want to especially optimize the lysis and PCR-cleanup steps as well as to improve the cell viability (culture).

**1. Cell culture and harvest**

The cell viability was determined by propendium iodide staining, P1 population was selected to get a homogeneous cell population w/o any cell debris and disturbing particles (55%), P2 population was selected to get singlet cells (1 cell in 1 droplet to make sure that cell counting by FACS was accurate), P3 population (1.63%) was the dead population determined by propendium iodide staining, P4 was sorted out and further processed.

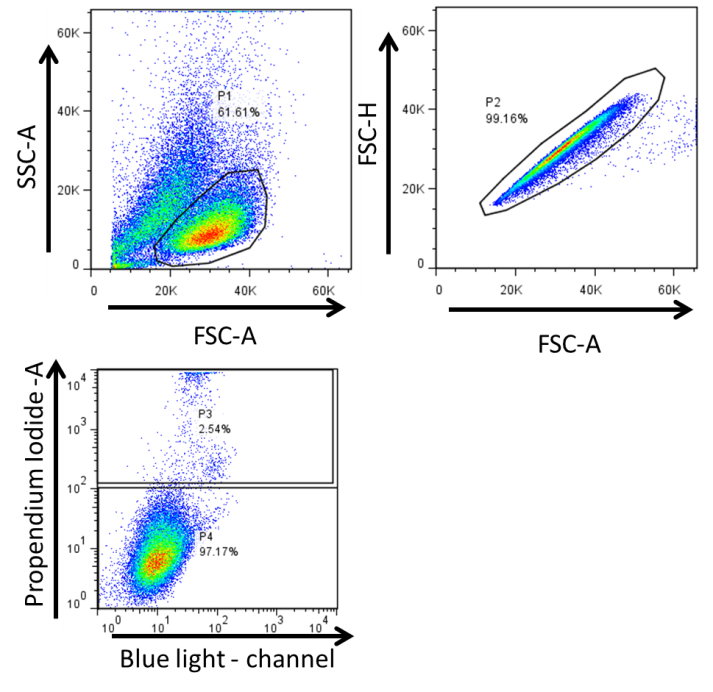
4 x 50’000 cells were sorted out and further processed

**Cell viability determined by FACS**



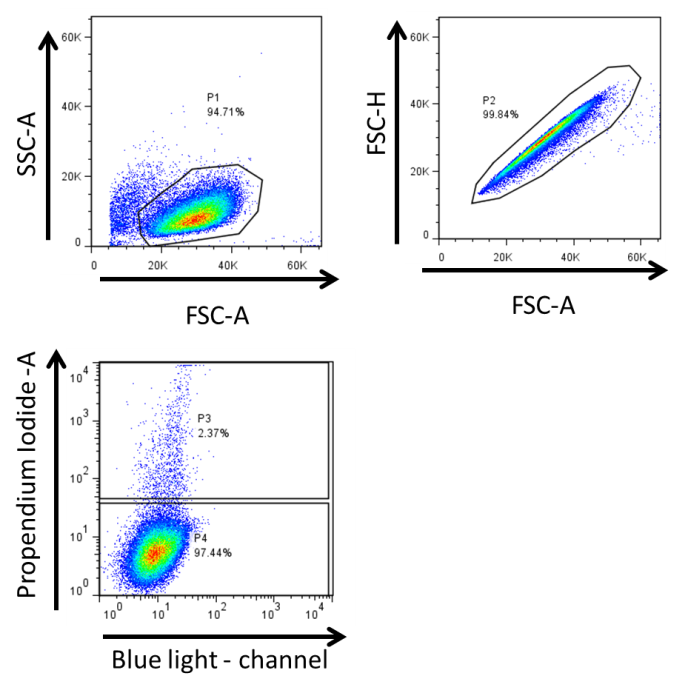
Another test was performed to determine the viability of the cells after FACS sorting (20 min after sorting, re-stained with propendium iodide)

**FACS-sorted cell population**



P1 (61.61%), P2 (99.16%), P3 (dead cell population 2.54%), P4 (live cell population 97.17%)

**Analysis of the sorted cell population (P4) 20 min after FACS sorting (re-stained with propendium iodide)**



P1 (94.71%), P2 (99.84%), P3 (dead cell population 2.37%), P4 (live cell population 97.44%)

Conclusion: the FACS sorting process can efficiently get rid of large part of cell debris and disturbing particles. However cells start to commit apoptosis after 20 min as shown by the plots above.

**2. Cell lysis and transposition**

**Aim:** The lysis condition with 0.1% digitonin (triplicates) was tested according to the protocol provided by David

**Method:**

* Centrifuge 50’000 cells in 1.5 ml Eppendorf tube @ 500xg for 5 min at 4°C
* 1x very gentle wash using 200 µl ice-cold D-PBS (no pipetting up and down)

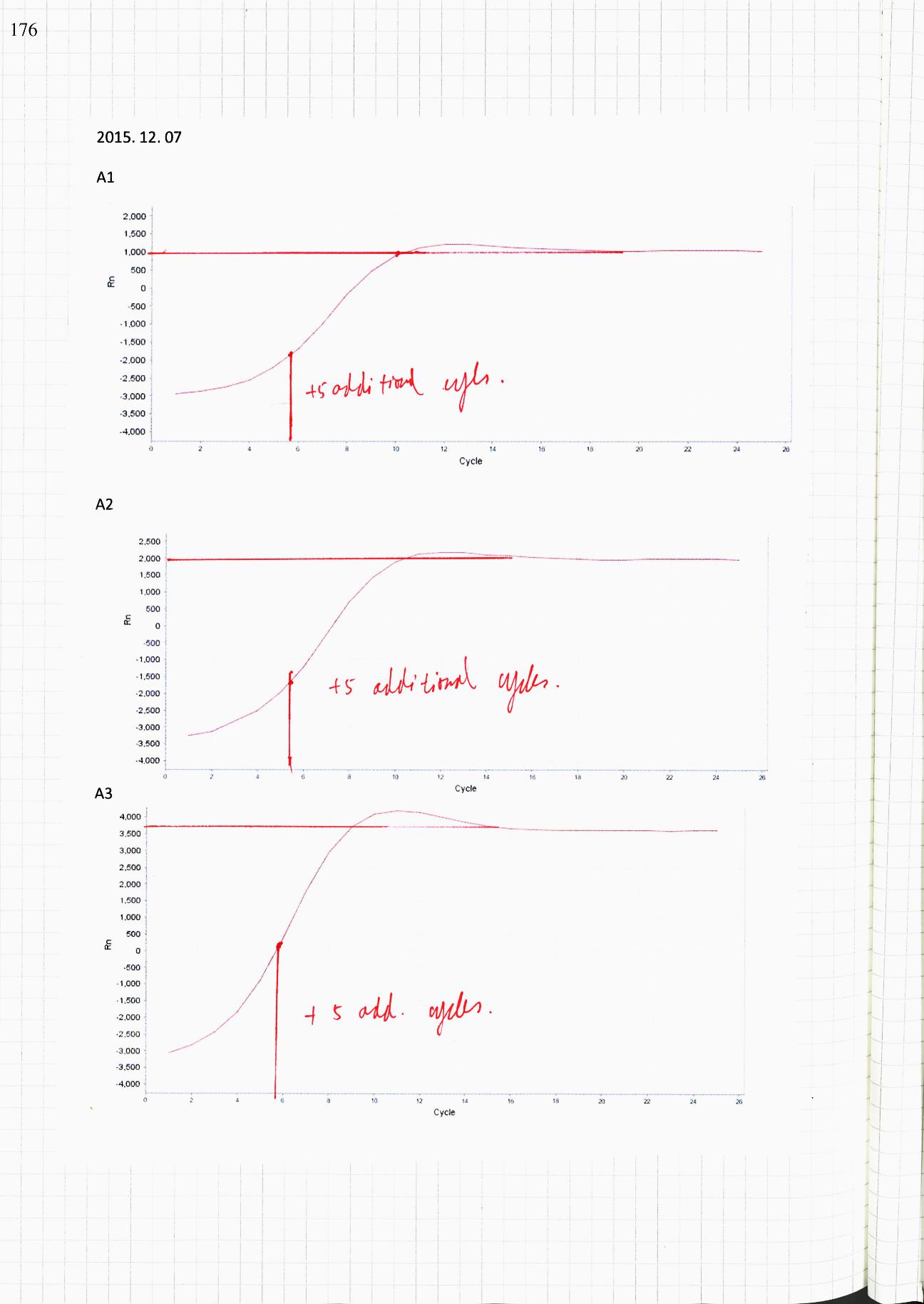
Remove D-PBS and addition of 50 µl tagmentation mix to perform the transposition as the following (no pipetting up and down):

* + - 25 µl TD Buffer
    - 22.5 µl H2O
    - 2.5 µl TDE
    - 0.5 µl 1% Digitonin
* Incubation @ 37°C for 30 min
* MinElute to stop tagmentation as described in the protocol

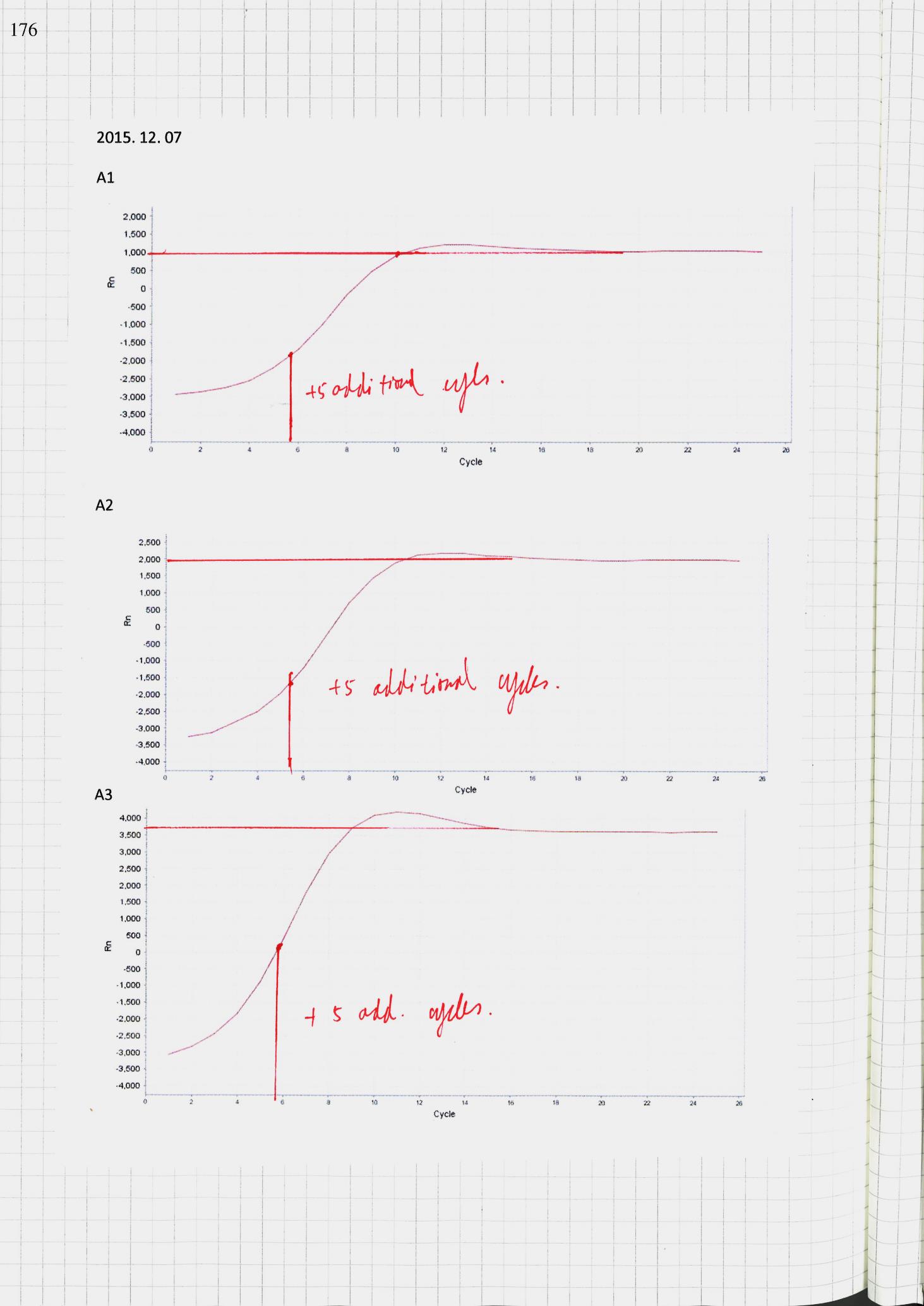
PCR-amplify the library for the first 5 cycles

**qPCR quality check** ---run 5 additional cycles

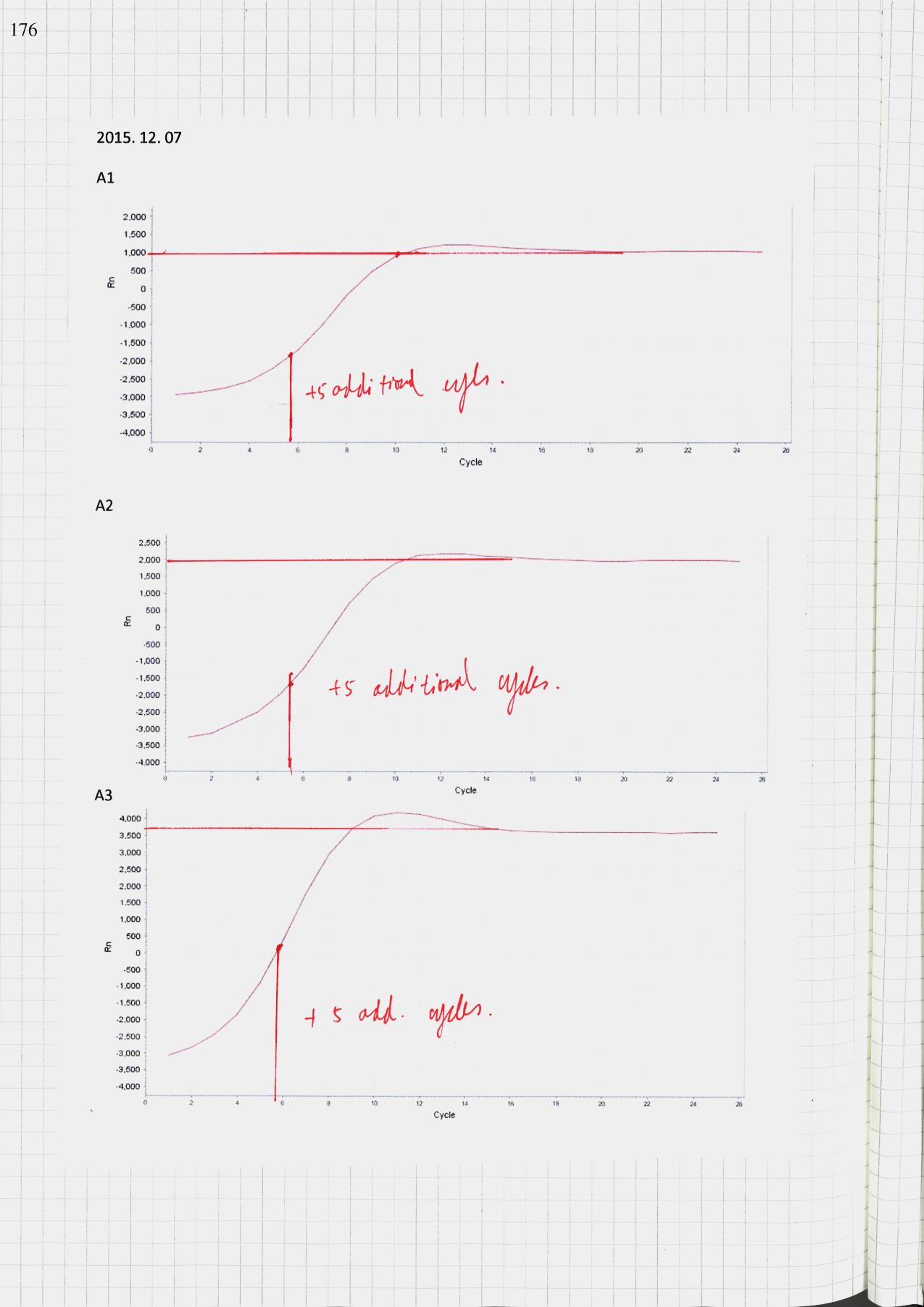
Replicate 1



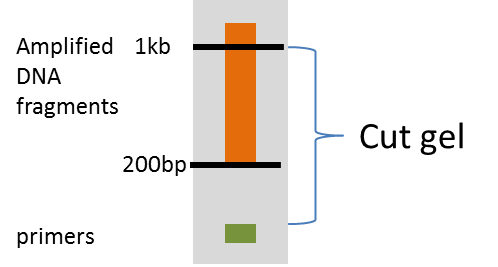
Replicate 2



Replicate 3



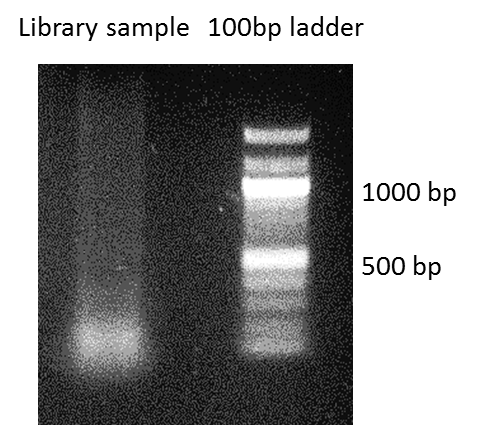
**3. Gel purification**

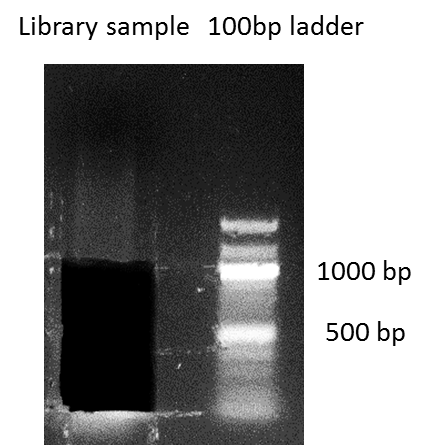


The gel purification step failed for the 1st time, the gel broke into pieces & 1 replicate sample was fully lost.

The gel purification step was repeated again by pooling the 2 remaining samples.

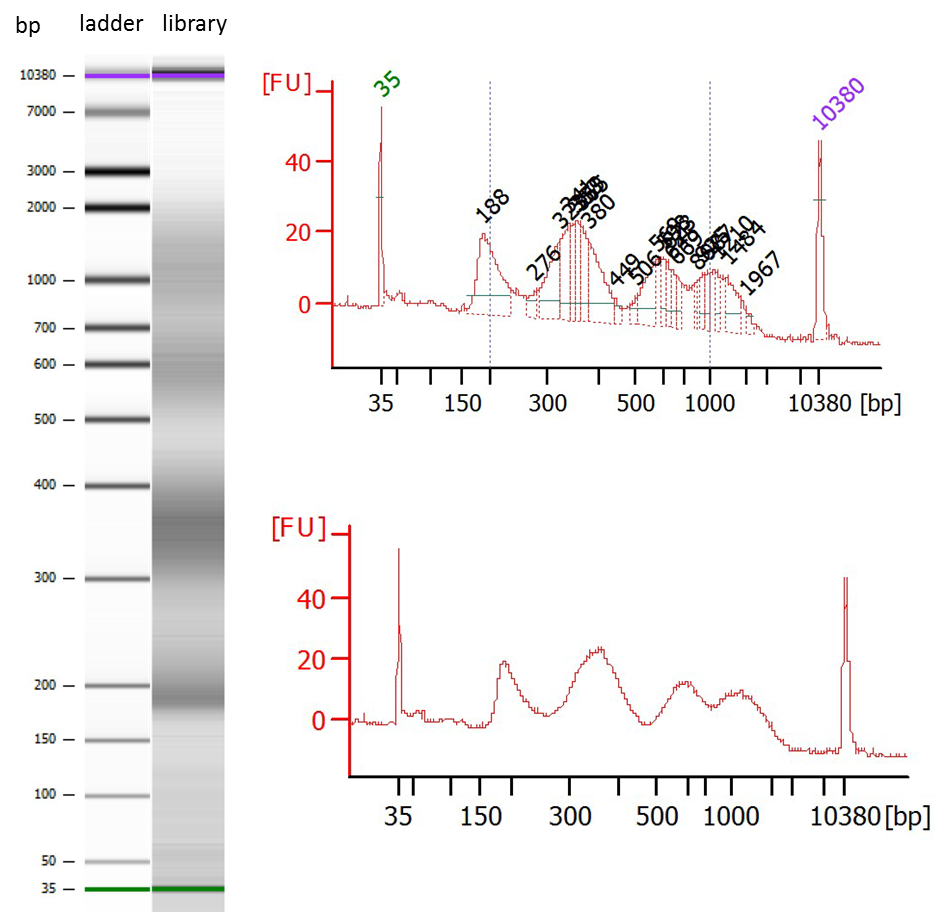
In total 30 µl was loaded on the gel





After a MinElute gel purification, the sample was loaded on Bioanalyzer and analyzed as the following:

**Quality check on BioAnalyzer (profile)**



**4 peaks observed:**

1st peak at 188 bp: 421.30 pg/µl

2nd peak at 380 bp: 185.60 pg/µl

3rd peak at 569 bp: 94.68 pg/µl

4th peak at 1,484 pg/ µl: 53.62 pg/µl