Streamlining HIV-1 antiviral resistance mutation detection with next generation sequencing

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INTRODUCTION

The UKHSA Public Health Laboratory Birmingham has a well-established HIV-1 antiviral resistance testing service.

Plasma samples are submitted from known HIV-1 positive patients and PCR is used to amplify protease and reverse transcriptase (PRT) and integrase (INT) genes (Figure 1) which are then sequenced.

The nucleic acid sequence allows the identification of HIV-1 clades, and mutations in the virus which could confer resistance to antiretroviral drugs including protease inhibitors (PI), Nucleoside reverse transcriptase inhibitors (NRTI), Non-nucleoside reverse transcriptase inhibitors (NNRTI) and integrase inhibitors. A summary of the process used is shown in figure 2.

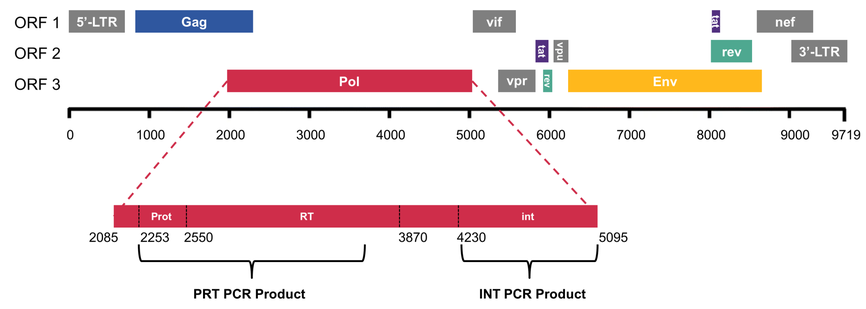


Figure 1: Schematic of the HIV-1 genome showing regions amplified by PCR[1]

AIMS

The process used in the laboratory for HIV-1 sequencing had been largely unchanged for over 20 years. We therefore sought to utilise next generation sequencing (NGS) technologies, automation, and expertise gained during the SARS-CoV-2 pandemic to update and streamline the laboratory process.

Our aim was primarily laboratory operational improvement. From the user's perspective, the same samples will be submitted and the same results received.

METHODS

The new sequencing methodology was optimised for performance based on previous SARS-CoV-2 sequencing and recommendations from the central sequencing laboratory at UKHSA Colindale. A summary of the updated process is shown in figure 3.

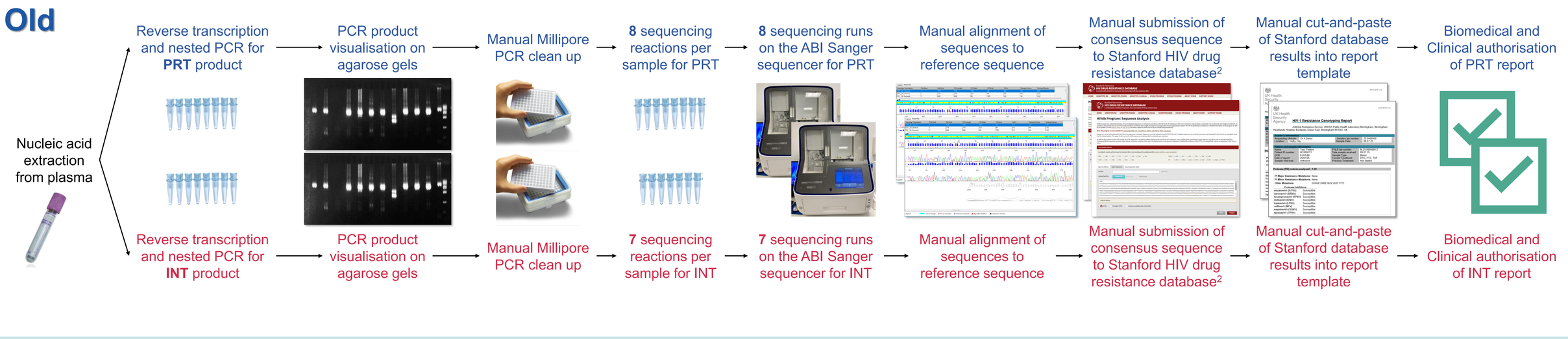
To validate the new method, HIV-1 PRT and INT PCR products from 270 previously sequenced samples underwent library preparation using the illumina DNA prep kit followed by sequencing of the libraries on the MiSeq, MiniSeq and NextSeq instruments. The DNA Prep kit was used at half the volumes specified in the kit instructions for use.

Sequence analysis was performed using the UKHSA developed pipeline (see details in "Bioinformatics Pipeline" panel below).

Basic NGS quality parameters such as depth of read across the sequenced regions and the percentage of bases not assigned were reviewed.

Stanford database[2] outputs from the new process were compared with those from Sanger sequencing derived results.

SUMMARY OF OLD AND NEW HIV-1 SEQUENCING PROCESSES



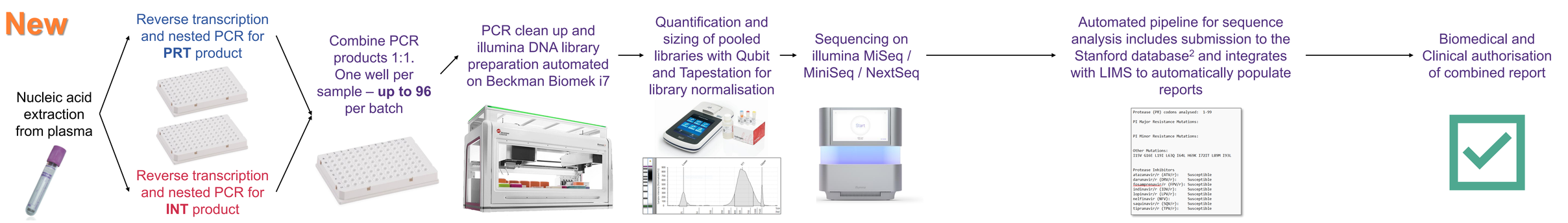


Figure 3: Summary of the updated methodology for HIV-1 sequencing and analysis based on illumina next generation sequencing technology

BIOINFORMATICS PIPELINE

The HIV-1 pipeline was developed by UKHSA's in-house bioinformatics department to:

- Check quality of individual short reads (filtering)
- Demultiplex samples via the indexes
- Map and align reads against reference HIV-1 genomes
- Report "fail" for samples don't produce sufficient filtered, mapped reads
- Generate files that return read depth for each nucleotide position and the % of each nucleotide at each position (quantification of mixtures)
- Generate consensus fas files for PR, RT and IN regions:
  - Remove sequence beyond the defined limits of the second round PCR products
  - Call mixed bases if the minority variant is 20% or more of the reads
  - Call any nucleotide with a read depth below 1000 N in to preserve high confidence
- Submits the 20% consensus fas files to the Stanford database
- Retrieves the output from Stanford and submits reports to the LIMS

RESULTS AND CONCLUSIONS

- Sequencing quality from all NGS platforms (MiSeq/MiniSeq/NextSeq) was very good (e.g. high read depth and low nucleotide ambiguity).
- Pipeline quality limits effectively removed spurious data providing high confidence.
- Report outputs were equivalent to those achieved with Sanger sequencing:
  - There were no discrepancies between the HIV-1 subtypes identified via the NGS pipeline vs Sanger sequencing.
  - For identification of mutations, 51% of samples showed complete concordance, 43% showed minor differences with no clinical impact, 1% (n=4) gave discordant calls at significant resistance mutation positions. When these reports were examined, these mutations would have been identified as suspicious/incongruous at technical validation or were at the 20% threshold for identification of mixed bases (identified by eye in Sanger sequencing). None of these discrepancies would therefore result in an incorrect report.
- The automated NGS/pipeline has significantly reduced hands-on practical and analysis time by Biomedical Scientist staff.

FUTURE WORK

- Once the service is fully implemented, we hope to also integrate our other antiviral resistance testing services for HIV-2, Hepatitis B Virus and Cytomegalovirus into the NGS workflow.
- The HIV-1 bioinformatics pipeline could identify minority variants in the quasispecies of HIV below 20% mixtures. With clinical correlation, this data could be used to investigate whether lower level mixtures have clinical significance[3].
- Updates are also being made to the mycobacterial sequencing service run by the National Mycobacterium Reference Service – Central and North, which will allow the NGS processes to be run in parallel with combined sequencing runs.

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