A Cost Effective, Rapid, and Portable Approach to Characterize HPV L1 Genomic Variability for Population Screening Using Nanopore Sequencing

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Abstract: Human papillomavirus (HPV) is associated with cervical cancer, as also cancers of the head and neck, penis, vulva, vagina, and anus. Per the GLOBOCAN 2018 [1] estimates of cancer incidence and mortality—there were 570000 cases, and 311000 deaths due to cervical cancer worldwide. Similarly, 600000 cases of head and neck cancer (HNC) were reported in 2018. HPV is linked to a subset of head and neck squamous cell carcinoma (HNSCC), particularly oropharyngeal cancer. Not all HPV infections progress to cancer. Persistent infection with HPV high-risk genotypes; and genomic variability and integration are critical in HPV induced carcinogenesis. We have developed a cost-effective approach for characterisation of HPV L1 genotypes and variability using Nanopore sequencing. The assay consists of amplification of 450 base HPV L1 region by PCR using degenerate HPV primers followed by sequencing of positive samples using long read Nanopore sequencing. This is followed by analysis of sequence variants and deriving a risk score based on the variants. For bench marking and validation of the method, we used cell lines (n=3), and positive clinical samples in HPV16, 18, 31, 33 and 45. Portability of the sequencer, flexibility of scale, and low reagent cost of the assay [21 USD per sample] make Nanopore sequencing a valuable tool for large scale HPV screening programs in high risk populations.

Methodology / Workflow:

Step 1: 1 hour → Step 2: 3 hours → Step 3: 9 hours → Step 4: 2 hours

gDNA Extraction:

gDNA was extracted using commercial kits.

Oral rinse specimens [collected with, or without cytobrush epithelial scraping]; Cervical swabs, FFPE tissue, or tissue in normal saline were acceptable clinical samples.

PCR:

PCR was run using consensus primers MYO09/11, and GP5+/GP6+ [2].

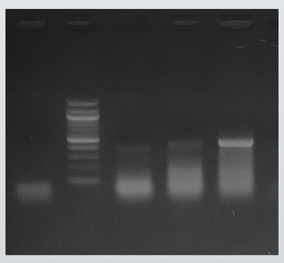

Fig. 1. The MY09/MY11 PCR.
Lane 1: Negative Control;
Lane 2: 100 bp ladder;
Lanes 3-4: Representative clinical samples;
Lane 5: Positive control.

Library Preparation, and Sequencing:

The 450 bp MY09/11 product from each sample was PCR barcoded using PCR Barcoding Expansion 1-96 with the Ligation Sequencing (EXP-PBC096 & SQK-LSK109) kits. Libraries from 92 barcoded, and pooled samples were sequenced using MinION MK1B device with MinION FLO-MIN106/107 flow cells. Sequencing was carried out to generate approx. 50000 reads.

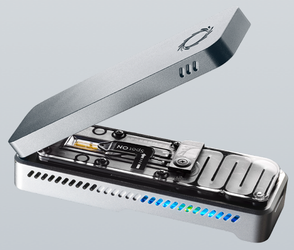

Fig. 2. MinION MK1B device

Analysis

We used automated analysis, using the following steps:

Raw Data >> BaseCalling>> Raw Data QC >> Mapping to HVP L1 >> Mapping QC >> Error Correction >> Variant Calling >> Consensus calculation >> Genotyping >> Reporting

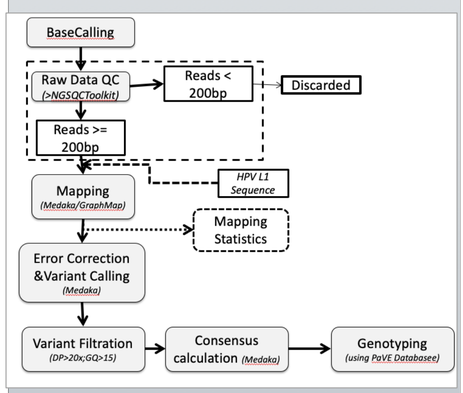

Fig. 3. The data analysis algorithm.

Results: Table 1. Nanopore Sequencing of 12 representative clinical samples.

Sample	Total Reads	Total HPV mapped reads	Mapping %age	Variants Detected	Genotype
1	52000	59	0.11%	0	Negative
2	76000	107	0.14%	14	Most similar to HPV16 with 95.94% identity
3	63953	81	0.13%	0	Negative
4	56000	21576	38.53%	29	Most similar to HPV16 with 92.69% identity
5	68000	4050	5.96%	7	Most similar to HPV16 with 98.41% identity
6	44000	49	0.11%	0	Negative
7	36000	97	0.13%	4	Most similar to HPV16 with 94.54% identity
8	28000	38	0.14%	0	Negative
9	56000	66	0.12%	17	Most similar to HPV16 with 97.54% identity
10	24000	28	0.12%	24	Most similar to HPV16 with 98.64% identity
11	44000	39	0.09%	36	Most similar to HPV16 with 90.01% identity
12	44000	57	0.13%	34	Most similar to HPV16 with 90.41% identity

Summary: Cervical cancer ranks as the 2nd leading cause of female cancer in India, with >469.1 million women at risk. Per GLOBOCAN 2018, annually there were 96,922 new cases, and 60,078 cervical cancer deaths in India; similarly, there were 17,903 new cases, and 14,953 deaths due to oropharyngeal cancer. These statistics point to the dire need for reliable population based HPV screening measures. We have developed a highly portable Nanopore sequencing based assay, with a reagent cost of 21 USD/sample, and a workflow of 24 hours. With as low as 50000 reads per sample, we were able to reliably detect HPV with 0.1% mapped reads.

References:

1. Ferlay J, Colombet M, Soerjomataram I et al. Global and Regional Estimates of the Incidence and Mortality for 38 Cancers: GLOBOCAN 2018. Lyon: International Agency for Research on Cancer/World Health Organization; 2018.
2. Shen-Gunther, J., and Yu, X. (2011). HPV molecular assays: defining analytical and clinical performance characteristics for cervical cytology specimens. Gynecol. Oncol. 123, 263–271.

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