[redacted]

21 November 2019

Oxford Nanopore Technologies' views on Illumina's revised remedies proposal of 19 November 2019

ANNEX

ONT provides below a non-exhaustive list of applications which may be excluded from Illumina's proposed licences on the basis of the limited field of use.

ONT further notes that, in the event of a narrow definition of the licenses' applicable field of use, Illumina's revised remedies proposal would not offer any legal certainty to ONT in relation to its current litigation against PacBio before the District Court of Delaware. Indeed [redacted].

1. **"*Native*" sequencing**

Assuming that "*native*" excludes amplified DNA as well as complementary DNA ('cDNA') synthesized from single-stranded RNA, the proposed field of use would exclude at least the following applications:

- **Amplicon panels**: amplicons are fragments of DNA or RNA that are a result of amplification of specific sections of DNA/RNA. Sequencing often takes the form of amplicon panels, where the experiment pulls out multiple, specific (targeted) regions of interest of the genome and those areas of the sample are sequenced. This method is commonly used in for example:
  - Cancer diagnostics;
  - Inherited disease diagnostics;
  - Drug resistance characterisation in infectious disease;
  - Genotyping of plants and animals; and
  - HLA typing
- **Single-gene amplicons**: this method is commonly used in relation to the 16S and CO1 genes, for example in:
  - species identification, e.g. pathogen detection in infectious disease; retroviral integration sites
- **Short tandem repeats (STR's)**: this method is commonly used in for example, human identification in forensics
- **RNA analysis**: where the original RNA is converted by reverse transcription to cDNA for sequencing. cDNA analysis represents approximately one third of current sequencing for research purposes
- **Enrichment**: which relates to the selection of DNA of interest from a 'background' sample, including for example selecting pathogen DNA from a human tissue sample in order to provide an infectious disease diagnostic
- **Instances where only a small original sample is available**: for example in biopsies or for ancient DNA samples, environmental sampling, single cell applications, cell-free applications, and flow sorted chromosomes
- **Low frequency variants**: where researchers perform amplification to make it easier to analyse a rare variant, e.g., in cancer
- **Clinical metagenomics** (viral, bacterial) typically currently requires amplification either of the DNA or cDNA (for viruses). The majority of work related to pathogen-agnostic infectious disease analysis would therefore be 'not native DNA'

- **Bait capture panels** e.g. exome analysis typically includes rounds of PCR (Polymerase Chain Reaction) amplification
- **Reproductive health:** preimplantation genomic screening for aneuploidy in embryos requires whole genome amplification, as well as non-invasive prenatal testing ('NIPT')
- **Newborn screening** from blood spots, would be another example of a low input, amplicon or bait panel application where non-native DNA is sequenced

2. "*Long read*" sequencing

The term "*long read*" has not been defined in Illumina's revised remedies proposal – and indeed the length of DNA or RNA in a sample may be a spectrum from short to ultra-long rather than a binary 'short' or 'long' – it is therefore hard to know exactly what would be excluded but ONT believes the proposed field of use would likely exclude at least the following applications:

- **Circulating DNA**, which is typically present in shorter fragments
  - E.g., in cancer, where circulating tumour DNA may be obtained from the blood and sequenced to gain insight into a disease state
  - NIPT, where foetal DNA is obtained from maternal blood for aneuploidy diagnosis
- **FFPE** ('Formalin Fixed Paraffin Embedded', a standard practice for tumour samples), which is a form of preservation and preparation for biopsy specimens that aids in examination, experimental research and diagnostic/drug development. Cross linking, as part of this process, makes the DNA fragments very short
- **Human RNA** which typically averages ~1kb in length. If so-called 'long read' excludes this, then all RNA analysis (which currently represents one third of the research market) would be excluded from the proposed licences
- **Liquid biopsy**: screening for cancer diagnostics or recurrence is carried out on short DNA fragments from samples such as blood, urine and cerebrospinal fluid
- **Low pass whole genome sequencing**: counting for depth (so-called 'CNV analysis') usually starts with shearing down to short fragments
- **Amplicons** which are **limited in read length** by the amplification (PCR) process
- Any **sample that is not 'fresh'** is likely to be fragmented into smaller fragments (e.g., for ancient DNA, and environmental DNA)
- Any sample where the **original length** of the DNA has **not** been **carefully preserved**

3. "*Single-molecule*" sequencing

Again the term "*single-molecule*" has not been defined and it is difficult to assess the applications and/or competitors which would be included or excluded from the proposed licences on this basis. For example, Genapsys recently launched their sequencer, a technology which is based on the amplification of short read multi molecule detection which would likely be outside the scope of the proposed licenses.[1]

[1] See https://www.genapsys.com/products/