Verifying indices of user-submitted Illumina library pools using Nanopore sequencing and cross-platform demultiplexing

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1) Background

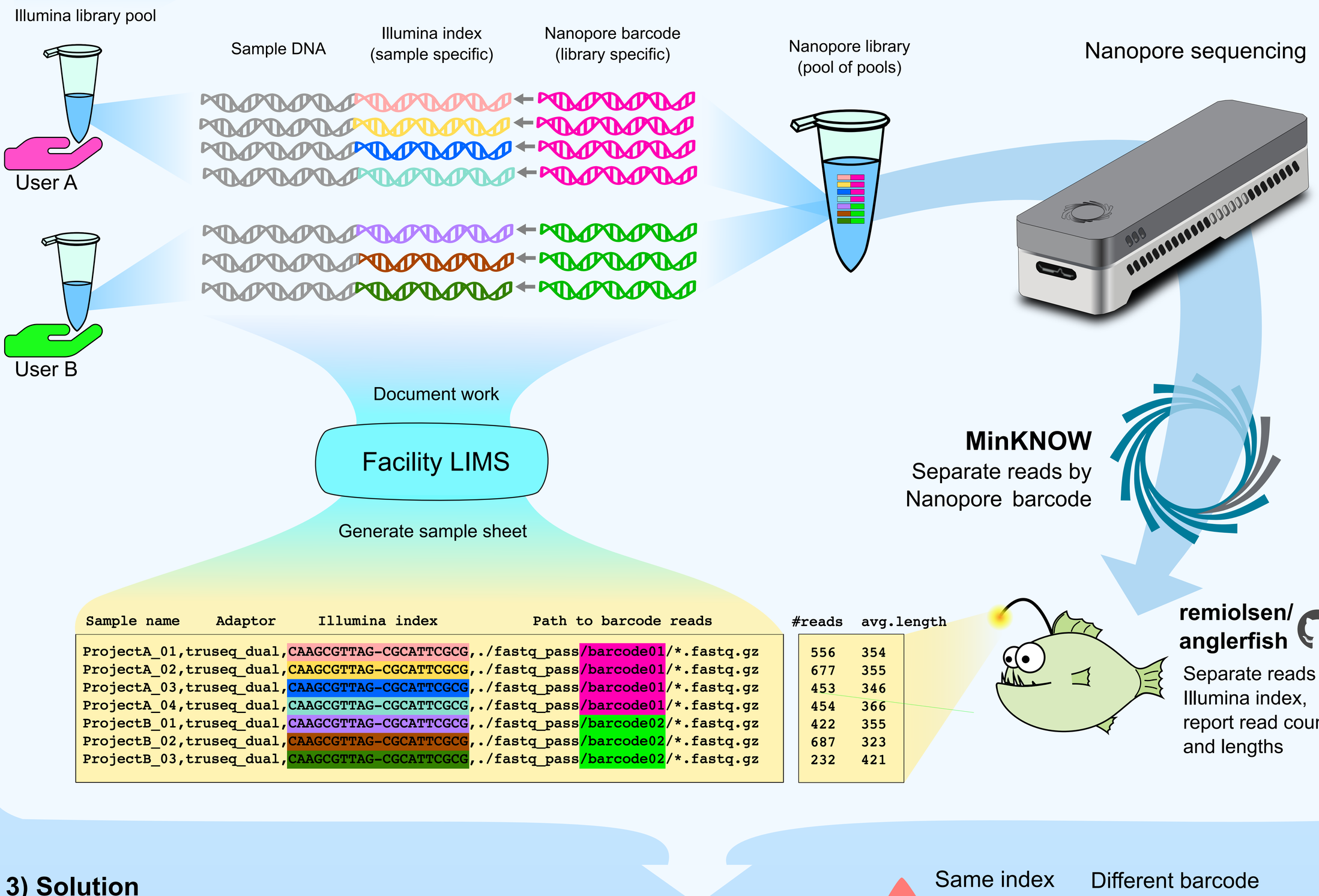
The National Genomics Infrastructure (NGI) at SciLifeLab is an accredited core facility offering a wide variety of library prep and sequencing services to researchers.

The highest thoughput of the facility consists of **user-prepared Illumina library pools.**

2) Problem

The increasing output of Illumina flowcells can improve the time- and cost efficiency of sequencing, provided that the library pools of different users can be sequenced together.

If users do not report the indices of their library pool correctly, this may cause **missing or unexpected indices** as well as **index collisions** between library pools. Thus, the need arises to **confirm the Illumina index identity within and between user-prepared library pools prior to sequencing**.

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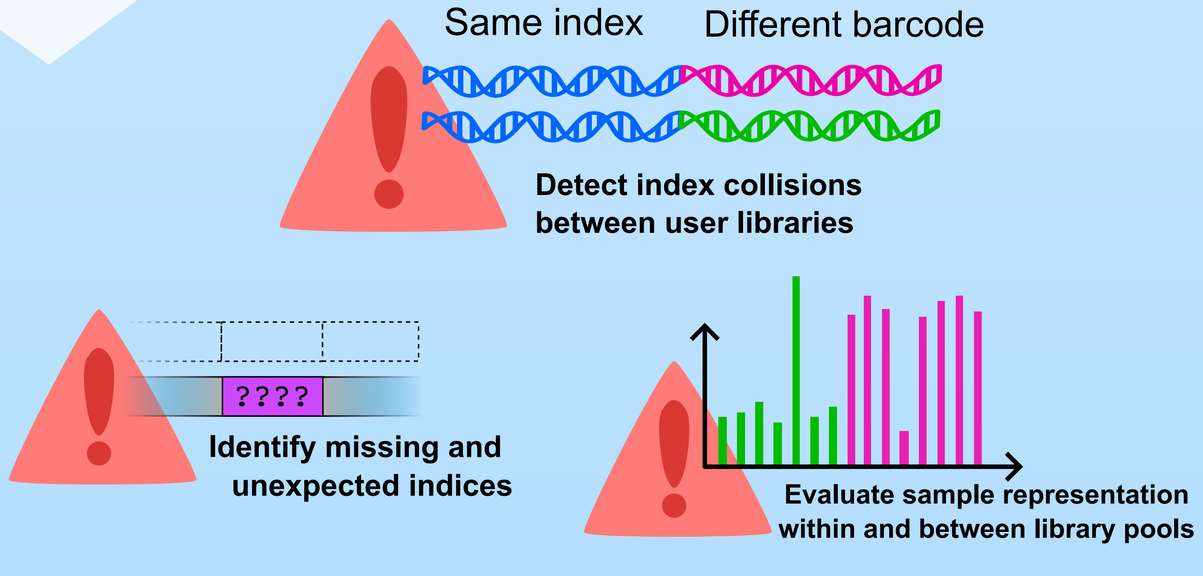
Sample name     Adaptor      Illumina index           Path to barcode reads                                  #reads  avg.length
ProjectA_01,truseq_dual,CAAGCGTTAG-CGCATTCGCG,./fastq_pass/barcode01/*.fastq.gz   556     354
ProjectA_02,truseq_dual,CAAGCGTTAG-CGCATTCGCG,./fastq_pass/barcode01/*.fastq.gz   677     355
ProjectA_03,truseq_dual,CAAGCGTTAG-CGCATTCGCG,./fastq_pass/barcode01/*.fastq.gz   453     346
ProjectA_04,truseq_dual,CAAGCGTTAG-CGCATTCGCG,./fastq_pass/barcode01/*.fastq.gz   454     366
ProjectB_01,truseq_dual,CAAGCGTTAG-CGCATTCGCG,./fastq_pass/barcode02/*.fastq.gz   422     355
ProjectB_02,truseq_dual,CAAGCGTTAG-CGCATTCGCG,./fastq_pass/barcode02/*.fastq.gz   687     323
ProjectB_03,truseq_dual,CAAGCGTTAG-CGCATTCGCG,./fastq_pass/barcode02/*.fastq.gz   232     421
```

3) Solution

User-prepared Illumina library pools are carried through a Nanopore library prep, where-in each user-prepared pool can be assigned it's own Nanopore barcode.

The barcoded pools are combined and sequenced on a cheap, low-throughput Nanopore flowcell. Each sample can then be demultiplexed on a user project level using the Nanopore barcode and on sample level using the original Illumina index.

Looking at read counts for a given combination of Nanopore barcode and Illumina index allows us to qualitatively and **quantitatively detect index issues.**



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