

Microbial Genomics

Multiplex sequencing allows large numbers of DNA samples to be pooled and sequenced simultaneously during a single run on a sequencing instrument. This technique is particularly useful when sequencing bacteria because their relatively short genomes allows many bacteria to be read simultaneously within one flow cell's maximum yield capacity whilst still giving a decent depth of coverage. Multiplexing's main benefit is that it can reduce the cost of identifying each bacteria, yielding early, detailed information about Antibiotic Microbial Resistance (AMR) that is very treatment relevant.

In multiplexing, chemical barcodes are added to the ends of all the DNA molecules in each sample during the library preparation stage. All the material in each sample receives the same unique chemical barcode. All the barcoded samples are then pooled into a single tube and everything is sequenced. When the sequencing results are read out in the assembly software, all the molecules with the same barcode are collected together and each group is then assembled so the bacteria in each sample are identified. Having long DNA fragments of around 100,000 base pairs as starting material means that long overlaps between DNA fragments can be matched and the contiguous jigsaw picture of the complete bacterial genome, including the plasmids, assembled from only a relatively few reads. Fire Monkey extracts DNA fragments in a narrow, 'Goldilocks', range of long lengths which the long-read sequencing technologies can handle most efficiently. These Goldilocks DNA fragments enable the sequencing technologies to manage with smaller input samples, so more samples can be handled within the maximum capacity of each run, whilst also delivering faster results as well. Multiplexed long-read sequencing with Fire Monkey extraction means that the price of sequencing each individual patient's bacterial sample microbiome can be considerably reduced.

"Whole Genome Bacterial Multiplexing in a few hours for \$30 possible"

At present, Oxford Nanopore Technologies (ONT) sells 12 and 96 different chemical DNA barcodes for sequencing whilst Illumina sells 384 different DNA chemical barcodes.

The basic advantage of multiplexing is that it reduces the cost of sequencing each sample. This is particularly apparent with bacterial sequencing where the bacterial genomes can be about five million (5×10^6) base pairs long. This means that many samples can therefore be run together within the total sequencing capacity of 20 billion (20×10^9) base pairs that an ONT MinION flow cell is described as capable of yielding. With sufficient different chemical barcodes and assuming a few hours processing time, this system could in theory support multiplexing of up to some 96 samples in an automated combination of Fire Monkey extraction and a single ONT MinION flow cell run.

Barcoding or multiplexing is useful when the amount of data required per sample is less than the total amount of data that can be generated from a single flow cell: it allows a user to pool multiple samples and sequence them together, making more efficient use of the flow cell. Multiplexing samples onto one flow cell can reduce the cost per sample for a user. Below are some worked examples:

Cost/multiplex	No Bar code	6 barcodes	12 barcodes	96 barcodes
Fire Monkey price	\$20	\$120	\$240	\$1,920
Flow cell price	\$500	\$500	\$500	\$500
Library price	\$99	\$99	\$99	\$99
Barcode price	\$0	\$25	\$50	\$100
Price per sample	\$619	\$124	\$74.1	\$27.3

Price estimates based on RevoluGen and ONT's website quoted prices