

POSTER PRESENTATION

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Reflex™: a novel method to sequence barcoded long-range PCR products in a pooled population of hundreds of DNA samples

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We are developing novel methods to simultaneously analyze candidate genes or regions from multiple samples in a single experiment rapidly and cost-effectively. One method, we call *Reflex*, provides an elegant approach to sample preparation for long-range PCR (LR-PCR) amplicons. Current methods use LR-PCR for target enrichment then use random fragmentation of each sample followed by ligation of DNA barcodes before sequencing: this approach is expensive and labour intensive.

In the *Reflex* workflow, we perform LR-PCR on genomic targets using primers that also add a DNA barcode to the LR-PCR product. This allows us to then produce pools of the LR-PCR products of multiple, typically 384, samples. The equimolar pooled population of 384 LR-PCR products are then processed to generate tiled 'daughter' *Reflex* PCR products across the target, each carrying a copy of the cognate DNA barcode. This is achieved by an intramolecular fold-back between two inverted *Reflex* sequences, followed by polymerase extension, so copying the DNA barcode. The *Reflex* daughter PCR products are then equimolar pooled for sequencing where the DNA barcode allows identification of the originating DNA sample within the population pool.

We have developed *Reflex* workflows that are compatible with the Illumina, Ion Torrent and Roche-454 next-generation sequencing platforms and can index multiple pooled populations to sequence thousands of DNA samples in a single run. The workflow is robust and can be performed quickly and cheaply with small amounts of input DNA and with high specificity to discriminate between members of multigene families.

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