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

James Casbon, Andrew Slatter, Esther Musgrave-Brown, Robert Osborne, Conrad Lichtenstein*, Sydney Brenner

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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.

Population Genetics Technologies Ltd, Babraham Research Campus, Cambridge CB22 3AT, UK

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