

CLAIMS

[Claim 1]. A method for generating extended sequence reads of long DNA molecules in a sample, comprising the steps of:

- (i) assigning a specific barcode sequence to each template DNA molecule in a sample wherein barcode-tagged molecules are obtained by PCR amplification using primers comprising template-specific sequences from opposite ends of the template molecule and a universal sequence at the 5' end, wherein one primer additionally comprises a barcode;
- (ii) clonally amplifying the barcode-tagged molecules;
- (iii) labelling the amplified barcode-tagged molecules with biotin;
- (iv) fragmenting the amplified barcode-tagged molecules to obtain barcode-tagged molecules by subjecting the amplified barcode-tagged molecules to unidirectional deletion from the barcode-distal end;
- (v) purifying the barcode-containing fragments using streptavidin-coated paramagnetic beads;
- (vi) juxtaposing the barcode-containing fragments to random short segments of the original DNA template molecule and circularising the barcode-containing fragments by intramolecular ligation, thereby generating a sequencing library of overlapping fragments of each template molecule with its assigned barcode; and
- (vii) obtaining demultiplexed reads from the sequencing library;
- (viii) assembling the demultiplexed reads to obtain extended sequence reads for each DNA template molecule.

[Claim 2]. The method according to claim 1, wherein the barcode-containing fragments have lengths ranging from about 300 base pairs to N base pairs, wherein N equals to the length of the original DNA template molecule.

[Claim 3]. The method of any preceding claim, further comprising the step of ligating sequencing adaptors onto the ends of the barcode-containing fragments prior to the step of juxtaposing the barcode-containing fragments to random short segments of the original DNA template molecule.

[Claim 4]. The method of any preceding claim, wherein the step of amplifying the barcode-tagged molecules is by circularizing the barcode-tagged molecules and performing rolling circle amplification.

[Claim 5]. The method of any preceding claim, wherein the extended sequences reads are compatible for sequencing on sequencing platforms.