

CLAIMS

1. A method of moving one or more stalled helicases past one or more spacers in a polynucleotide, comprising contacting (a) the one or more stalled helicases and the polynucleotide with a transmembrane pore and (b) applying a potential across the pore and thereby moving the one or more helicases past the one or more spacers on the polynucleotide.
2. A method of controlling the movement of a target polynucleotide through a transmembrane pore, comprising:
 - (a) providing the target polynucleotide with one or more spacers;
 - (b) contacting the target polynucleotide with one or more helicases such that the one or more helicases stall at the one or more spacers;
 - (c) contacting the target polynucleotide and the one or more stalled helicases with the pore; and
 - (d) applying a potential across the pore such that the one or more helicases move past the one or more spacers and control the movement of the target polynucleotide through the pore.
3. A method of characterising a target polynucleotide, comprising:
 - (a) carrying out the method of claim 2; and
 - (b) taking one or more measurements as the polynucleotide moves with respect to the pore wherein the measurements are indicative of one or more characteristics of the polynucleotide and thereby characterising the target polynucleotide.
4. A method according to claim 2 or 3, wherein providing the target polynucleotide with one or more spacers comprises modifying the target polynucleotide so that it comprises one or more spacers.
5. A method according to claim 3 or 4, wherein the one or more characteristics are
 - (a) selected from (i) the length of the target polynucleotide, (ii) the identity of the target polynucleotide, (iii) the sequence of the target polynucleotide, (iv) the secondary structure of the target polynucleotide; (v) whether or not the target polynucleotide is modified and (vi) whether or not the target polynucleotide is modified by methylation, by oxidation, by damage, with one or more proteins or with one or more labels, tags or spacers;

- (b) measured by electrical measurement and/or optical measurement; or
- (c) measured by a current measurement, an impedance measurement, a tunnelling measurement or a field effect transistor (FET) measurement.

6. A method according to any one of the preceding claims, wherein the one or more helicases control the movement of the target polynucleotide through the pore with the field resulting from the applied potential.

7. A method according to any one of the preceding claims, wherein:

- (a) the one or more spacers have a different structure from the polynucleotide;
- (b) the polynucleotide is deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) and the one or more spacers comprise peptide nucleic acid (PNA), glycerol nucleic acid (GNA), threose nucleic acid (TNA), locked nucleic acid (LNA) or a synthetic polymer with nucleotide side chains;
- (c) the one or more spacers comprise one or more nitroindoles, one or more inosines, one or more acridines, one or more 2-aminopurines, one or more 2-6-diaminopurines, one or more 5-bromo-deoxyuridines, one or more inverted thymidines (inverted dTs), one or more inverted dideoxy-thymidines (ddTs), one or more dideoxy-cytidines (ddCs), one or more 5-methylcytidines, one or more 5-hydroxymethylcytidines, one or more 2'-O-Methyl RNA bases, one or more Iso-deoxycytidines (Iso-dCs), one or more Iso-deoxyguanosines (Iso-dGs), one or more C3 groups, one or more photo-cleavable (PC) groups, one or more hexandiol groups, one or more spacer 9 (iSp9) groups, one or more spacer 18 (iSp18) groups, a polymer or one or more thiol connections;
- (d) the one or more spacers comprise a polypeptide or a polyethylene glycol (PEG);
- (e) the one or more spacers comprise one or more abasic nucleotides;
- (f) the one or more spacers comprise one or more chemical groups which cause the one or more helicases to stall;
- (g) the one or more spacers comprise one or more chemical groups which cause the one or more helicases to stall and the one or more chemical groups are one or more fluorophores, streptavidin and/or biotin, cholesterol, methylene blue, a dinitrophenol (DNP), digoxigenin and/or anti-digoxigenin or a dibenzylcyclooctyne group;
- (h) the one or more spacers are capable of stalling the one or more helicases in the presence of free nucleotides and/or the presence of a helicase cofactor;

(i) the one or more spacers are capable of stalling the one or more helicases in a salt concentration of about 100 mM or lower; or

(j) the one or more spacers are included in the polynucleotide and/or are not part of one or more blocking molecules hybridised to the polynucleotide.

8. A method according to any one of the preceding claims, wherein the method comprises (i) moving two or more stalled helicases past each spacer; (ii) moving two or more helicases attached to one another past each spacer; or (iii) moving two or more helicases covalently attached to one another past each spacer.

9. A method according to any one of the preceding claims, wherein (a) at least a portion of the polynucleotide is double stranded; (b) at least a portion of the polynucleotide is double stranded and the one or more spacers are included in a single stranded region or a non-hybridised region of the polynucleotide; (c) at least a portion of the polynucleotide is double stranded and the one or more spacers are included in a single stranded region which comprises a leader sequence which preferentially threads into the pore; (d) at least a portion of the polynucleotide is double stranded and the two strands of the double stranded portion are linked using a bridging moiety; or (e) at least a portion of the polynucleotide is double stranded, the two strands of the double stranded portion are linked using a bridging moiety and the one or more spacers are included in the bridging moiety.

10. A method according to any one of the preceding claims, wherein the one or more helicases are (a) one or more Hel308 helicases, one or more RecD helicases, one or more XPD helicases or one or more Dda helicases (b) one or more helicases derived from any of the helicases in (a); or (c) a combination of any of the helicases in (a) and/or (b).

11. A method according to any one of the preceding claims, wherein the pore is:

(a) a transmembrane protein pore or a solid state pore;

(b) a transmembrane protein pore derived from a hemolysin, leukocidin, *Mycobacterium smegmatis* porin A (MspA), MspB, MspC, MspD, lysenin, outer membrane porin F (OmpF), outer membrane porin G (OmpG), outer membrane phospholipase A, *Neisseria* autotransporter lipoprotein (NalP) and WZA;

(c) formed of eight identical subunits as shown in SEQ ID NO: 2 or a variant thereof in which one or more of the eight subunits has at least 50% homology to SEQ ID NO: 2 based on amino acid identity over the entire sequence and retains pore activity; or

(d) formed of seven identical subunits as shown in SEQ ID NO: 4 or a variant thereof in which one or more of the seven subunits has at least 50% homology to SEQ ID NO: 4 based on amino acid identity over the entire sequence and retains pore activity.

12. Use of a transmembrane pore and an applied potential to move one or more stalled helicases past one or more spacers on a polynucleotide.

13. Use of one or more spacers to stall one or more helicases on a polynucleotide before the polynucleotide is contacted with a transmembrane pore.

14. A method of controlling the loading of one or more helicases on a target polynucleotide, comprising:

(a) providing the target polynucleotide with one or more spacers; and

(b) contacting the target polynucleotide provided in (a) with the one or more helicases such that the one or more helicases bind to the target polynucleotide and stall at each spacer.

15. A method according to claim 14, wherein step (a) comprises modifying the target polynucleotide so that it comprises one or more spacers and/or wherein the target polynucleotide is provided with $(L-S)_n$ or $(S-L)_n$ in the 5' to 3' direction, wherein L is a single stranded polynucleotide or a non-hybridised polynucleotide, S is a spacer and n is a whole number.

16. A method according to claim 14 or 15, wherein step (b) comprises contacting the target polynucleotide provided in (a) with the one or more helicases such that the one or more helicases bind to the target polynucleotide and one helicase stalls at each spacer.

17. A method according to claim 16, wherein step (a) comprises providing the target polynucleotide with $(L^1-S)_n$ or $(S-L^1)_n$ in the 5' to 3' direction, wherein L is a single stranded polynucleotide or a non-hybridised polynucleotide which is only long enough for one helicase to bind, S is a spacer and n is a whole number.

18. A method according to claim 14 or 15, wherein step (b) comprises contacting the target polynucleotide provided in (a) with the one or more helicases such that the one or more helicases bind to the target polynucleotide and two helicases stall at each spacer.

19. A method according to claim 18, wherein step (a) comprises providing the target polynucleotide with $(L^2-S)_n$ or $(S-L^2)_n$ in the 5' to 3' direction, wherein L is a single stranded polynucleotide or a non-hybridised polynucleotide which is only long enough for two helicases to bind, S is a spacer and n is a whole number.

20. A method according to claim 18 or 19, wherein the two helicases stalled at each spacer are different from one another or wherein the two helicases stalled at each spacer are attached to one another or covalently attached to one another.

21. A method according to claim 20, wherein the method comprises:

(a) providing the target polynucleotide with $(L^2-S)_n$ or $(S-L^2)_n$ in the 5' to 3' direction, wherein L is a single stranded polynucleotide or a non-hybridised polynucleotide which is only long enough for two helicases to bind, S is a spacer and n is a whole number;

(b) hybridising a blocking polynucleotide to part of each region L^2 so that the remaining part of each region L^2 is only long enough to bind one helicase;

(c) contacting the target polynucleotide produced in (b) with one or more helicases such that one helicase binds to the remaining part of each region L^2 ;

(d) providing the one or more bound helicases in (c) with free nucleotides and a helicase cofactor such that they remove each blocking polynucleotide and stall at each spacer S; and

(e) contacting the target polynucleotide produced in (d) with one or more helicases which are different from those used in (c) such that one different helicase binds to each region L^2 and is stalled by each spacer and each helicase stalled in (d).

22. A method according to any one of claims 14 to 21, wherein the target polynucleotide is provided with two or more spacers and/or wherein the one or more stalled helicases are moved past the one or more spacers using a method according to any one of claims 1 to 11.

23. A complex for controlling the movement of a target polynucleotide, wherein the complex comprises (a) an adaptor comprising $(L-S-D)_n$ or $(D-S-L)_n$ in the 5' to 3' direction, wherein L is a single stranded polynucleotide or a non-hybridised polynucleotide, S is a spacer and D is a

double stranded polynucleotide and wherein n is a whole number and (b) one or more helicases stalled on each adaptor.

24. A complex according to claim 23, wherein (a) L is capable of binding only one helicase (L^1) or capable of binding only two helicases (L^2) or (b) wherein L is capable of binding only one helicase (L^1) or capable of binding only two helicases (L^2) and n is 1 and one or two helicases are stalled on the adaptor.

25. A kit for controlling the movement of a target polynucleotide, wherein the kit comprises (a) one or more spacers, one or more spacers as defined in claim 7 or one or more adaptors as defined in claim 23 or 24, (b) one or more helicases and (c) a transmembrane pore.