

WHAT IS CLAIMED IS

1. A method to evaluate the seeding efficiency of a flow cell with polynucleotides, comprising:
 - seeding a flow cell with polynucleotides for at least 1 minute, and
 - (i) contacting the flow cell with a labelled agent that binds to or incorporates onto seeded polynucleotides and determining the amount of label present in the flow cell thereby determining the seeding efficiency; or
 - (ii) collecting the supernatant; quantifying the polynucleotides in the supernatant by using step (a) or (b):
 - (a) amplifying the polynucleotides in the supernatant using qPCR and/or droplet PCR; or
 - (b) reseedling the supernatant using a second flow cell and counting clusters generated after bridge amplification of the polynucleotides; and
 - (c) determining seeding efficiency of the flow cell by comparing the number of polynucleotides quantified in the supernatant vs. the number of polynucleotides used to seed the flow cell.
2. The method of claim 1, wherein one channel of a flow cell is evaluated for polynucleotide seeding efficiency.
3. The method of claim 1, wherein more than one channel of a flow cell is evaluated for polynucleotide seeding efficiency.
4. The method of any one of the preceding claims, wherein the flow cell comprises a plurality of primers bound to the surface of the flow cell.

5. The method of claim 4, wherein the bound primers comprise P5 primers which have the sequence of SEQ ID NO:1 and/or are P7 primers which have the sequence of SEQ ID NO:2.
6. The method of claim 4 or claim 5, wherein the plurality of primers are randomly bound to the surface of the flow cell.
7. The method of claim 4 or claim 5, wherein the plurality of primers are bound to specific areas of flow cells.
8. The method of claim 7, wherein the plurality of primers are bound to the surface of an array of wells that are patterned on the flow cell surface.
9. The method of any one of the preceding claims, wherein the flow cell is used in a next generation sequencing device.
10. The method of any one of the preceding claims, wherein the polynucleotides comprise adaptors.
11. The method of claim 10, wherein the adaptors are bridge PCR compatible.
12. The method of any one of the preceding claims, wherein the polynucleotides comprise a DNA library.
13. The method of claim 12, wherein the DNA library is generated using a library preparation kit.
14. The method of claim 13, wherein the DNA library is prepared according to a method comprising the steps:
 - (A) simultaneous fragmenting and adding primers to isolated DNA using transposomes;

(B) amplifying the fragmented DNA using reduced-cycle PCR, wherein the PCR amplification primers comprise index and adapter sequences; and

(C) washing and pooling the amplified DNA fragments to form a DNA library.

15. The method of claim 14, wherein the transposomes are linked to beads.

16. The method of any one of claims 13 to 15, wherein the DNA library is generated from genomic DNA isolated from a human subject.

17. The method of any one of the preceding claims, wherein the polynucleotides are seeded in the flow cell from 5 min to 60 min.

18. The method of claim 17, wherein the polynucleotides are seeded in the flow cell for 10 min to 40 min.

19. The method of any one of the preceding claims, wherein the qPCR comprises a double stranded binding dye that allows for quantification of a double stranded amplified product based upon the level of fluorescence.

20. The method of claim 19, wherein the double stranded binding dye is selected from SYBR® Green I, BRYT Green® Dye, PicoGreen, YOYO-1 iodide, and SYBR® Gold.

21. The method of claim 18, wherein the qPCR comprises a sequence specific probe that is labeled with a fluorescent reporter and a quencher molecule that binds to a DNA template.

22. The method of claim 21, wherein the quencher molecule is a dark quencher that absorbs light over multiple wavelengths and does not emit light.

23. The method of claim 22, wherein the dark quencher is selected from Dabsyl, Black Hole Quenchers, Iowa Black FQ, Iowa Black RQ, IRDye QC-1, and Qx1 quenchers.

24. The method of any one of claims 1 to 18, wherein the second flow cell is used to quantitate the polynucleotides in the supernatant is different from the flow cell that is seeded with polynucleotides.

25. The method of claim 24, wherein the second flow cell provides up to 12 Gb of sequence data per run while the flow cell that is seeded with polynucleotides provides up to 120 Gb of sequence data per run.

26. The method of any one of the preceding claims, wherein the method is performed multiple times using flow cells that were seeded with the same concentration of polynucleotides but with different seeding lengths of time.

27. The method of claim 26, wherein the seeding efficiency of a flow cell with polynucleotides is evaluated over various time points in time-lapse fashion.

28. The method of claim 1, wherein the labelled agent comprises labelled dNTPs that are incorporated onto a seeded polynucleotide by a polymerase.

29. The method of claim 1, wherein the labelled agent comprises a labelled nanoparticle or labelled dendrimer that

binds to a complementary oligonucleotide on a seeded polynucleotide.

30. The method of claim 1, wherein the labelled agents comprises a labelled adapter or labeled complementary oligo to a seeded polynucleotide.

31. The method of claim 1, wherein the labelled agent comprises a labelled structure grown from an end of a seeded polynucleotide.

32. The method of any of claims 1 or 28-31, wherein the label is a luminescent or fluorescent detectable label.

33. The method of claim 1, wherein if the seeding efficiency is insufficient, the flow cell is reseeded and the seeding efficiency is measured again.

34. Use of the method of any one of the preceding claims, to engineer flow cell surfaces that have improved seeding efficiencies for polynucleotides.