

CLAIMS

1. A pool of nucleic acid standards of different lengths, wherein the nucleic acid standards comprise a unique molecular identifier (UMI) and:

- a. a 5' universal oligonucleotide, wherein the 5' universal oligonucleotide is the same for all standards;
- b. a 3' universal oligonucleotide, wherein the 3' universal oligonucleotide is the same for all standards; and
- c. at least one region between the UMI and the 5' universal oligonucleotide and/or between the UMI and the 3' universal oligonucleotide;

wherein the length of the at least one region determines the length of the standard.

2. The pool of standards of claim 1, wherein the pool further comprises a further nucleic acid standard that comprises a UMI and:

- a. a 5' universal oligonucleotide, wherein the 5' universal oligonucleotide is the same for all standards; and
- b. a 3' universal oligonucleotide, wherein the 3' universal oligonucleotide is the same for all standards;

wherein the further nucleic acid standard does not comprise at least one region between the UMI and the 5' universal oligonucleotide or between the UMI and the 3' universal oligonucleotide.

3. The pool of standards of claim 1, wherein the at least one region between the UMI and the 5' universal oligonucleotide and/or between the UMI and the 3' universal oligonucleotide comprise 0.2kb-10kb.

4. The pool of standards of any one of claims 1-3, wherein the 5' universal oligonucleotide and/or the 3' universal oligonucleotide each comprise an amplicon amplified from a sequence of interest.

5. The pool of standards of any one of claims 1 or 3-4, wherein the at least one region between the UMI and the 5' universal oligonucleotide and/or between the UMI and the 3' universal oligonucleotide each comprise an amplicon amplified from a sequence of interest.

6. The pool of standards of any one of claims 1 or 3-5, wherein the least one region between the UMI and the 5' universal oligonucleotide and/or between the UMI and the 3' universal oligonucleotide each comprise an arbitrary sequence.

7. A pool of nucleic acid standards of different lengths, wherein the nucleic acid standards comprise a UMI and:
- a. a 5' partially overlapping oligonucleotide, wherein the 5' partially overlapping oligonucleotide is identical over at least a portion of its sequence for all the standards; and/or
 - b. a 3' partially overlapping oligonucleotide, wherein the 3' partially overlapping oligonucleotide is identical over at least a portion of its sequence for all the standards;
- wherein the lengths of the 5' partially overlapping oligonucleotide and/or the 3' partially overlapping oligonucleotide determines the length of the standard.
8. The pool of standards of claim 7, wherein:
- a. the 5' partially overlapping oligonucleotide comprises at least a first portion of a sequence of interest; and
 - b. the 3' partially overlapping oligonucleotide comprises at least a second portion of a sequence of interest.
9. The pool of standards of any one of claims 7-8, wherein the 5' partially overlapping oligonucleotide and/or the 3' partially overlapping oligonucleotide each comprise a sequence that is 20bp-1kb smaller than a sequence of interest.
10. The pool of standards of any one of claims 7-9, wherein the 5' partially overlapping oligonucleotide and/or the 3' partially overlapping oligonucleotide each comprise an amplicon amplified from a sequence of interest.
11. The pool of standards of any one of claims 1-10, wherein the standards are double-stranded.
12. The pool of standards of any one of claims 1-11, wherein the standards comprise double-stranded DNA.
13. The pool of standards of any one of claims 1-12, wherein each standard comprises a different UMI.
14. The pool of standards of any one of claims 1-13, wherein the UMIs comprised in the pool of standards are a random set of sequences comprising 16-20 base pairs.
15. The pool of standards of claim 14, wherein the UMIs comprised in the pool of standards are a random set of sequences comprising 18 base pairs.

16. The pool of standards of any one of claims 1-15, wherein the pool of standards comprises 1×10^{10} or greater, 10×10^{10} or greater, or 100×10^{10} or greater standards, wherein each standard comprises a different UMI.
17. The pool of standards of any one of claims 1-16, wherein the number of standards in the pool is greater than the number of amplicons generated by an amplification reaction.
18. A pool of standards, wherein:
- a. at least a first portion of the standards are from any one of claims 1-6 or 11-17 and
 - b. at least a second portion of the standards are a pool of nucleic acid standards of different lengths, wherein the nucleic acid standards comprise a UMI and:
 - i. a 5' partially overlapping oligonucleotide, wherein the 5' partially overlapping oligonucleotide is identical over at least a portion of its sequence for all the standards; and/or
 - ii. a 3' partially overlapping oligonucleotide, wherein the 3' partially overlapping oligonucleotide is identical over at least a portion of its sequence for all the standards, wherein the lengths of the 5' partially overlapping oligonucleotide and/or the 3' partially overlapping oligonucleotide determines the length of the standard.
19. A method of generating a pool of nucleic acid standards comprising:
- a. providing multiple copies of at least one sequence of interest comprising nucleic acids;
 - b. providing a collection of oligonucleotides each comprising a UMI;
 - c. providing a collection of insertion oligonucleotides of varying lengths; and
 - d. ligating at least one sequence of interest of (a), at least one oligonucleotide comprising a UMI of (b), and at least one insertion amplicon of (c) to produce multiple nucleic acid standards of the pool of nucleic acid standards.
20. The method of claim 19, wherein the at least one sequence of interest and/or insertion oligonucleotide are prepared by amplification.
21. The method of claim 19 or claim 20, wherein the sequence of interest, the oligonucleotides each comprising a UMI, and/or the insertion oligonucleotides comprise a restriction enzyme cleavage site.

22. The method of claim 21, wherein the restriction enzyme cleavage site is proximal to the 5' and/or 3' end of the sequence of interest, the oligonucleotides each comprising a UMI, and/or the insertion oligonucleotides.
23. The method of claim 21 or claim 22, wherein the method further comprises cleaving the sequence of interest, the oligonucleotides each comprising a UMI, and/or the insertion oligonucleotides with a restriction enzyme before the ligating.
24. The method of claim 23, wherein the cleaving with a restriction enzyme produces sticky ends for the ligating.