

CLAIMS

What is claimed is:

1. A method for error correction of nucleic acid molecules, said method comprising:
 - (a) amplifying double-stranded nucleic acid molecules of a desired sequence;
 - (b) obtaining a first plurality of amplified double-stranded nucleic acid molecules comprising a) nucleic acid molecules of the desired sequence and b) nucleic acid molecules having at least one nucleotide mismatch relative to the desired sequence;
 - (c) fragmenting said first plurality of amplified double-stranded nucleic acid molecules by reacting said nucleic acid molecules with at least one molecule having unidirectional mismatch endonuclease activity;
 - (d) removing said at least one nucleotide mismatch by reacting said fragmented double-stranded nucleic acid molecules of (c) with at least one molecule having unidirectional exonuclease activity of the same directionality as the unidirectional mismatch endonuclease activity of (c) to provide a fragmented error-free double-stranded nucleic acid molecule of the desired sequence; and
 - (e) assembling a second plurality of double-stranded nucleic acid molecules comprising said fragmented error-free double-stranded nucleic acid molecule of (d), wherein the second plurality of double-stranded nucleic acid molecules has a higher proportion of nucleic acid molecules having the desired sequence and a decreased frequency of nucleotide mismatches compared to said first plurality of double-stranded nucleic acid molecules; and
wherein steps (a)-(e) are carried out in sequential order as listed.
2. A method according to claim 1, wherein said first plurality of nucleotide acid molecules comprises one or more synthetic nucleotide sequences.
3. A method according to claim 1, wherein said first plurality of nucleotide acid molecules comprises a mixture of one or more naturally occurring gene sequences and one or more synthetic nucleotide sequences.

4. A method according to claim 1, wherein obtaining a first plurality of nucleic acid molecules comprises synthesizing the nucleic acid molecules.
5. A method according to claim 1, wherein obtaining a first plurality of nucleic acid molecules comprises assembling the nucleic acid molecules from subsets and/or oligonucleotides.
6. A method according to claim 1, wherein step (c) and step (d) are performed as separate reactions.
7. A method according to claim 1, wherein step (c) and step (d) are performed as a one-step, simultaneous reaction.
8. A method according to claim 1, wherein said unidirectional mismatch endonuclease activity cuts 5' to said mismatch and said unidirectional exonuclease activity removes said nucleotide mismatch from the 5' end of said fragmented nucleic acid molecule.
9. A method according to claim 1, wherein said unidirectional mismatch endonuclease activity cuts 3' to said mismatch and said unidirectional exonuclease activity removes said nucleotide mismatch from the 3' end of said fragmented nucleic acid molecule.
10. A method according to claim 1, wherein said at least one molecule having unidirectional mismatch endonuclease activity is selected from the group consisting of RES I, CEL I, CEL II, an SP endonuclease, SP I, T7 endonuclease, T4 endonuclease, endonuclease V, a Mut protein, a variant of any thereof, and a combination of any two or more of the above.
11. A method according to claim 10, wherein said at least one molecule having unidirectional mismatch endonuclease activity is selected from the group consisting of: CEL I, CEL II, a variant of any thereof, and a combination of any two or more of the above.

12. A method according to claim 1, wherein said at least one molecule having unidirectional exonuclease activity is selected from the group consisting of exonuclease III, a DNA polymerase, lambda exonuclease, T7 exonuclease, T5 exonuclease, and a variant of any thereof.
13. A method according to claim 1, wherein said at least one molecule having unidirectional exonuclease activity is a polymerase with proofreading activity.
14. A method according to claim 13, wherein said polymerase with proofreading activity is selected from the group consisting of T4 polymerase, T7 polymerase, and phi29 polymerase.
15. A method according to claim 1, wherein
said at least one molecule having unidirectional mismatch endonuclease activity is selected from the group consisting of CEL I, CEL II, a variant of any thereof, and a combination of any two or more of the above; and
said at least one molecule having unidirectional exonuclease activity is selected from the group consisting of exonuclease III and a variant thereof.
16. The method according to claim 1 wherein the at least one molecule having unidirectional mismatch endonuclease activity is encoded by a nucleic acid sequence selected from the group consisting of:
 - a) a nucleic acid sequence exhibiting 90% or greater identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO: 09, SEQ ID NO: 12, SEQ ID NO: 15, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30-33, a complement of any, and a fragment of any; and
 - b) a nucleic acid sequence encoding a polypeptide exhibiting 90% or greater identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 28, and SEQ ID NO: 29.

17. The method of claim 1, wherein the molecule having unidirectional exonuclease activity is exonuclease III.
18. The method of claim 1, wherein the molecule having unidirectional mismatch endonuclease activity is CEL I or CEL II, and the molecule having unidirectional exonuclease activity is exonuclease III.
19. The method of claim 1, wherein the molecule having unidirectional mismatch endonuclease activity exhibits 90% or greater identity to SEQ ID NO: 10 or SEQ ID NO: 16.
20. The method of claim 19, wherein the molecule having unidirectional exonuclease activity is exonuclease III.
21. The method of claim 19, wherein the molecule having unidirectional mismatch endonuclease activity is SEQ ID NO: 10.
22. The method of claim 21, wherein the molecule having unidirectional exonuclease activity is exonuclease III.
23. The method of claim 19, wherein the molecule having unidirectional mismatch endonuclease activity is SEQ ID NO: 16.
24. The method of claim 23, wherein the molecule having unidirectional exonuclease activity is exonuclease III.