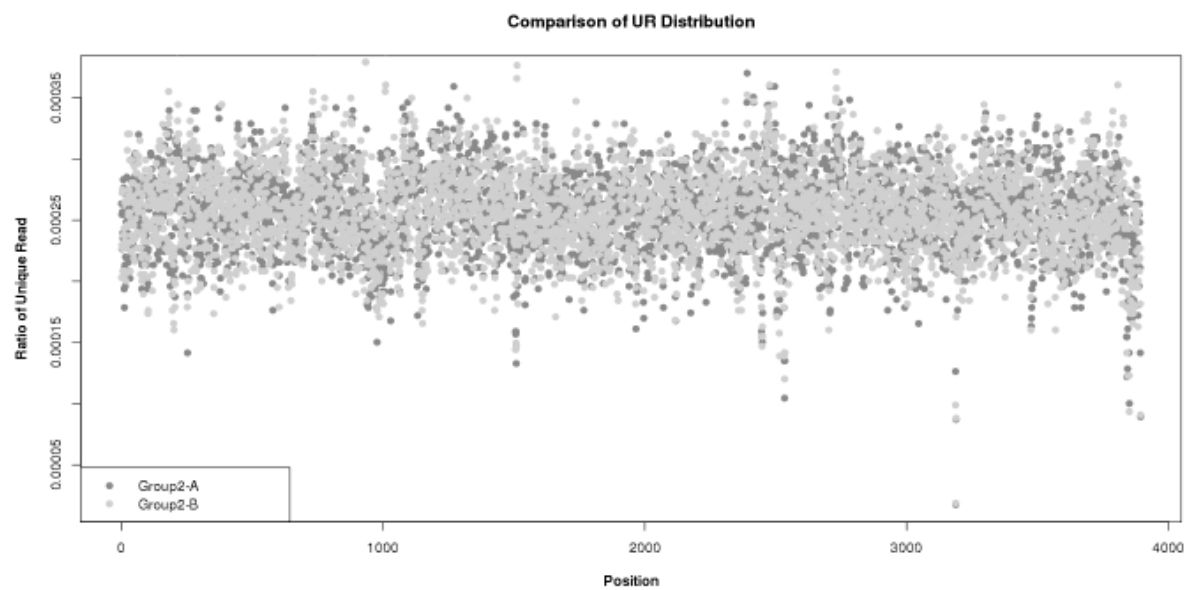


Abstract

The present invention relates to a method and kit for non-specific replication of DNA fragments. In general, the method disclosed in the present invention adds headers to DNA fragments that are expected to be replicated to generate ligated DNA fragments with header sequences. PCR primers are then used to amplify the ligated DNA fragments and the PCR products are cleaved to obtain a large number of DNA fragments which are substantially similar to the original DNA fragments before joining the headers.

Accompanying Drawing of the Abstract



1. A method of non-specific replication of DNA fragments, including:

Step B: Adding headers to processed DNA fragments that are expected to be replicated to generate ligated DNA fragments with headers;

Step C: Using primers that bind to the headers to amplify the ligated DNA fragments to obtain the PCR products;

Step D: Breaking the PCR products to obtain usable DNA fragments which are substantially similar to the DNA fragments that are expected to be replicated.

2. The method according to claim 1, wherein the header contains breaking areas, wherein the breaking areas are located near the connecting point between the header and the processed DNA fragments that are expected to be replicated;

preferably, wherein the breaking areas are cleavage sites; preferably, wherein the cleavage sites contain at least 1bp nucleotide sequence; more preferably, wherein the cleavage sites contains at least one enzyme cutting site or nucleotide sequence with at least one Thymine nucleotide;

preferably, wherein the headers with breaking areas are annealing products of nucleotide sequences such as shown in SEQ ID NO: 1 and SEQ ID NO: 2.

3. The method according to claim 1, wherein the primers contain breaking areas, wherein the breaking areas are located near the 3' terminal of the primers;

preferably, wherein the breaking areas are cleavage sites;

preferably, wherein the cleaving sites contain at least 1bp nucleotide sequence; more preferably, the cleaving sites contains at least one enzyme cutting site or nucleotide sequence with at least one deoxyuridine nucleotide;

more preferably, wherein the primers with breaking areas have a nucleotide sequence such as shown in SEQ ID NO: 3 and SEQ ID NO: 4.

4. The method according claim 1, wherein in said step D the breakings of the PCR product are achieved through reagent cleavage; preferably, wherein the reagent cleavage is performed by cleavage reagents to break the PCR product at least once,

preferably, wherein the cleavage reagents contain at least an enzyme with cleavage function,

preferably, wherein the enzyme with cleavage function is selected from one or more of the groups consisting of restriction endonucleases, exonucleases, enzymes with deoxyuracil cleavage function and enzymes with single-strand specific cleavage function,

preferably, wherein the enzyme with deoxyuracil cleavage function is the Uracil-DNA Glycosylase or a mixture of Endonuclease VIII and UDG; preferably, wherein the enzyme with single-strand specific cleavage function is the Mung Bean Nuclease and/or S1 Nuclease.

5. The method according to claim 1, wherein the quantity of the DNA fragments expected to be replicated in step B is 0.1 to 100 ng, and the size of the DNA fragments expected to be replicated is 80 to 1000 bp, preferably the size of the DNA fragments expected to be replicated is 100~250bp.

6. The method according to claim 1, comprise the step A-2 performed before the step B: end-repairing the DNA fragments expected to be replicated to obtain processed DNA fragments expected to be replicated; or end-repairing the DNA fragments expected to be replicated and subsequently or simultaneously adding the Adenine nucleotide to the 3' end of the DNA fragments to obtain the processed DNA fragments that are expected to be replicated,

preferably, wherein comprise a step A-1 performed before the step A-2: the sample DNA is disrupted to obtain the DNA fragments that are expected to be replicated.

7. A kind of usable DNA fragments obtained by the method of non-specific replication of the DNA fragments according to any one of claim 1 to 6.

8. A method of DNA library construction, which is used to construct libraries using the usable DNA fragments according to claim 7.

9. The method of DNA library construction according to claim 8, wherein the method includes the following steps performed after the step D:

Step E: End-repairing the usable DNA fragments to receive DNA fragments with blunt ends;

STEP F: Adding the adenine nucleotide to the 3' end of the DNA fragments with