Aberration-corrected ultrafine analysis of miRNA reads at single-base resolution: a k-mer lattice approach

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ABSTRACT

Raw sequencing reads of miRNAs contain machine-made substitution errors, or even insertions and deletions (indels). Although the error rate can be low at 0.1%, precise rectification of these errors is critically important because isoform variation analysis at single-base resolution such as novel isomiR discovery, editing events understanding, differential expression analysis, or tissue-specific isoform identification is very sensitive to base positions and copy counts of the reads. Existing error correction methods do not work for miRNA sequencing data attributed to miRNAs’ length and per-read-coverage properties distinct from DNA or mRNA sequencing reads. We present a novel lattice structure combining kmers, (k − 1)mers and (k + 1)mers to address this problem. The method is particularly effective for the correction of indel errors. Extensive tests on datasets having known ground truth of errors demonstrate that the method is able to remove almost all of the errors, without introducing any new error, to improve the data quality from every-50-reads containing one error to every-1300-reads containing one error. Studies on experimental miRNA sequencing datasets show that the errors are often rectified at the 5′ ends and the seed regions of the reads, and that there are remarkable changes after the correction in miRNA isoform abundance, volume of singleton reads, overall entropy, isomiR families, tissue-specific miRNAs, and rare-miRNA quantities.

INTRODUCTION

With rapid developments of sequencing technology, high-throughput platforms have inexpensively produced huge amounts of genomic reads at unprecedented speed (1), for example by whole genome sequencing, total RNA sequencing, mRNA sequencing and small RNA sequencing. Recently, sequencing of miRNAs (a special type of small RNA molecules containing about 22 nucleotide bases) has been widely used to examine tissue-specific expression patterns, to identify isomiRs (mature miRNA variants) and to discover previously uncharacterized miRNAs (2–6). As key regulators in various biological processes, miRNA dysregulation is implicated in many diseases for example cancer and autoimmune disorders (7–11). Numerous studies also reaffirm that miRNA regulatory functions are involved in post-transcriptional gene silencing (PTGS), transcriptional gene silencing (TGS) and transcriptional gene activation (TGA) (12,13), in which miRNAs bind to nascent RNA transcripts, gene promoter regions or enhancer regions and exert further effects via epigenetic pathways (8,14).

Fine-granulated analysis of miRNA reads at single-base resolution for uncovering their isoforms (isomiRs) and alternative splicing is one of the most frontier research areas in this field (4,15–20). IsomiRs vary in size and base content, due to the alternative and imprecise cleavage of Drosha and Dicer, or the turnover of miRNAs (20,21). IsomiRs have been classified into four categories: 5′ trimmed isomiRs, 3′ trimmed isomiRs, 3′ addition isomiRs, and polymorphic isomiRs (22). 5′/3′ trimmed or addition isomiRs are defined as those miRNA sequences which have one or more bases trimmed or added respectively at the 5′ or 3′ end from the canonical miRNAs, while polymorphic isomiRs usually have substitution mutations, causing one or more bases different from the canonical miRNA. For such broad range of miRNAome analysis, super high-quality sequencing data is demanded because the definitions are very

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sensitive to the base positions—one base difference can lead to entirely different read categorization. High-throughput sequencing technology produces short reads containing approximately 1% erroneous bases (1,23,24) such as aberrations of substitutions, base insertions, or deletions (indels). A previous study reported that the error percentage of most Illumina reads is \( \sim 0.5\% \) at best (25). These widely distributed errors or even erroneous bases fixed at only one position can cause lowered copy numbers for miRNA reads, and thus affect the calculation of miRNA expression levels and differential folds (26–30). Suppose a miRNA isoform has 100 copies in a diseased cell sample after library preparation, if there are substitution errors happened in five copies of them during the sequencing, three deletion errors in the other reads, and two insertion errors as well, then the reads count of this miRNA isoform would be tallied as 90 which is away from the ground truth 100. Further, the data may lead to wrong identification of isomiRs without correction of these errors. For example, those reads containing the deletion errors would be wrongly identified as a 5’ trimmed isoform of a canonical miRNA; If the errors occur at the seed region of a miRNA (conserved region of miRNAs), its target specificity analysis would be affected, potentially increasing the number of target transcripts (21,31,32). Although current research adopts ‘abandon ambiguity reads or noise reads’ to avoid misinterpreting erroneous sequence variants (ESVs) as isomiRs, the approach inevitably loses a part of the precious raw data (33,34). It is demanded to develop sophisticated algorithms to rectify these aberrations for truth-closer analysis of miRNA reads in a wide range of applications.

None of the existing error correction methods suits well for miRNA sequencing data, since they have not considered the unique characteristics of miRNA reads (short length and varying per read coverage). Besides, most of the methods, designed for DNA or mRNA sequencing reads, only focus on the correction of substitution errors and do not support indels error correction. So far, these methods have taken two streams of different correction ideas. The first one is a kmer-based error correction idea, represented by BCOOL (35), BFC (36), ACE (37) and BLESS (38). The key step is to examine the frequencies of kmers to distinguish between solid and weak k-mers according to a fixed global frequency threshold. Then the solid kmers (assumed as error-free) are referred as templates to rectify weak kmers (assumed as error-containing) to obtain correct reads. The second idea is a multi-alignment based error correction approach, represented by coral (39), ECHO (40) and Karect (41). These methods usually group those reads sharing the same kmer and then concatenate such a group of reads to form a long consensus contig. The contig is assumed error-free to correct erroneous bases. There are also a few methods designed for RNA sequencing reads error correction, for example Seeer (42) and Rcorrector (43). These approaches do not work for miRNA sequencing data error correction. For example, the consensus idea is not applicable to miRNA data because each read already encompasses one entire miRNA sequence. Our study did verify that the existing methods tend to significantly under-correct the errors and are prone to introducing tremendous number of new errors.

We present an error RE Ctification method for miRNA sequencing reads (named miREC), which is the first tool to address the problem of miRNA sequencing errors. Unlike the existing methods which have the primary goal of correcting substitution errors, our miREC concentrates more on insertion and deletion errors for excellent correction performance. The novel step of our method is the use of a 3-layer \( (k–1) \)-mer–\( k \)-mer–\( (k + 1) \)-mer lattice structure to maintain the frequency differences of the kmers (Figure 1). These superset-subset frequency differences are very effective to detect the errors especially the indel errors. The lattice structure is also a moving structure where \( k \) is set continuously from a small number to a big number 23 or 25 for a full coverage of error correction. Extensive tests on both simulated and experimental raw miRNA sequencing datasets show that miREC can excel performance in all of the precision, recall and gain.

**Figure 1.** A 3-layer kmer lattice structure. The red * symbol represents any nucleotide (A, G, T or C).

**MATERIALS AND METHODS**

A miRNA sequencing read \( r \) is a sequence \( r_1r_2\cdots r_n, r_i \in \Sigma = \{A, C, G, N, T\} \), where A, C, G and T stand for the nucleotide bases Adenine, Cytosine, Guanine and Thymine respectively, and the character N stands for an uncertain nucleotide; \( n \) is the length of \( r \). Usually, the length \( n \) of a miRNA read ranges from 15 to 28 in a dataset, but each read encompasses one entire miRNA. A kmer substring \( k \) is a contiguous subsequence in a read \( r \).
Neighborhood of a k-mer and a 3-layer kmer lattice structure

Given a miRNA sequencing read multi-set RS and a setting k, the copy count (or frequency) of a distinct read r in RS is the total number of its copies in RS, and the copy count (or frequency) of a distinct kmer in RS is the total number of its copies in RS. KMC3 (44) is used by this work as a kmer counter for these calculations.

Consider a kmer substringk, this kmer’s k-neighbourhood is defined as the set of kmers \(H(k, \text{substring})\) containing all possible distinct kmers of RS that each have only one base difference from substringk. Similarly, substringk’s (k−1)-neighbourhood is defined as the set of (k−1)mers \(H((k−1), \text{substring})\) containing all possible distinct (k−1)mers of RS each of which is an immediate subset of substringk, and substringk’s (k+1)-neighbourhood is defined as the set of (k+1)mers \(H((k+1), \text{substring})\) containing all possible distinct (k+1)mers of RS each of which is an immediate superset of substringk.

For example, when the kmer is given as GTC and assume that all its proper supersets and subsets exist in RS, then its (k+1)-neighbourhood \(H(4, \text{GTC}) = \{\text{AGTC, TGTC, CAGTC, GTAGTC, GTCTG, GTGC, GTCTC, GTCTC, GTTC, GTGC, GTCTC, GTCC, GTGC}\}\). Its (k−1)-neighbourhood \(H(2, \text{GTC}) = \{\text{TC, GC, GT}\}\). These three neighborhoods of kmer substringk can be combined and it is called a 3-layer kmer lattice structure of substringk.

A schematic example of this lattice structure is shown in Figure 1.

Error correction steps

The first step of the algorithm is to rectify substitution errors in RS. The algorithm traverses all of the distinct kmers. If a kmer substringk has a frequency lower than a threshold \(\tau\) (a small integer like 1, 2 or 3) and there exist at least one kmer in substringk’s k-neighbourhood \(H(k, \text{substring})\) whose frequency is larger than \(\tau\), we conjecture that substringk contains a substitution error. We choose the kmer with the highest frequency in \(H(k, \text{substring})\) as template to rectify the erroneous base in substringk. In the case where more than one kmer neighbors have the same frequency, we choose the smallest kmer according to the alphabetical order as the template. After the change in substringk, those reads in RS containing the original kmer are changed accordingly; some of them may become identical with other reads in RS. Again we use the double-checking strategy to decide whether we eventually accept the correction. We iterate these two steps by setting \(k\) from \(k_1\) (usually 8) to \(k_{end}\) (usually 20 or 25).

The second step is to rectify indel errors in the updated RS after the correction of substitution errors. The procedure is similar to correcting the substitution errors. But the concept is fundamentally different. We traverse all of the distinct kmers in the updated RS. If a kmer substringk has a frequency lower than a threshold \(\tau\) and there exist at least one kmer in substringk’s (k−1)-neighbourhood \(H((k−1), \text{substring})\) whose frequency is larger than \(\tau\), we conjecture that substringk contains an insertion error. If there exists at least one kmer in substringk’s (k+1)-neighbourhood \(H((k+1), \text{substring})\) whose frequency is larger than \(\tau\), we conjecture that substringk contains a deletion error. We choose the kmer with the highest frequency in \(H((k−1), \text{substring})\) or in \(H((k+1), \text{substring})\) as template to rectify the insertion error in substringk or to add the deleted base into substringk. After the change in substringk, those reads in RS containing the original substringk are changed accordingly; some of them may become identical with other reads in RS. Again we use the double-checking strategy to decide whether we eventually accept the correction.

We introduce a double-checking technique to decide whether we eventually accept the correction. We iterates theses two steps by setting \(k\) from \(k_1\) to \(k_{end}\). Setting a start \(k = 8\) is because of that we find low-frequency kmers (e.g. frequency equal to 1) at this k but we cannot find such low-frequency \((< \tau)\) kmers for \(k = 7\). Starting from \(k = 8\), we correct substitution errors first, then we perform the indel error correction, till \(k\) reaches \(k_{end}\). Our method is named miREC built from a 3-layer kmer lattice structure for effective correction of miRNA sequencing errors especially those insertion and deletion errors. The pseudo code of our algorithm is shown in Algorithm 1.

### Algorithm 1: miRNA Sequencing Reads Error Correction

**Input:** A read set \(RS = \{r^1, r^2, \ldots, r^n\}\), a frequency border \(\tau\), a k value region \((k_1, k_{end})\)

**Output:** A corrected read set \(S\)

```plaintext
Function Error_Correction (RS, (k1, k_end), \tau)
begin
    \(H_r[1 \ldots m] \leftarrow \) hash table ;
    \(H_a[1 \ldots m] \leftarrow \) hash table ;
    \(kmer \leftarrow \) kmer file; \(H[i] = \) an array; Each element of \(H[i]\) is a tuple composed of sequence and its frequency

    \(k = k_1 \) to \(k_{end}\) do
        \(H_a[1 \ldots m] \) to KMC3(RS, k)
        \(H_r[1 \ldots m] \rightarrow \) KMC3(RS, k − 1)
        \(H_a[1 \ldots m] \rightarrow \) KMC3(RS, k + 1)
    for \(i = 1 \) to \(n\) do
        \((s, f) \leftarrow \) Count(r^i
        Append \((s, f)\) to \(H_r[i], f\)
        \(C_kmer \leftarrow \) \(\emptyset\)

    for \(i = 1 \) to \(n\) do
        foreach kmer in \(r^i\) do
            if \(H_a[\text{kmer}] < \tau\) then
                \(C_kmer \leftarrow \text{FindNeighbor}(H_a)\)
                \(C_f \leftarrow \text{Replacekmer}(C_kmer, r^i)\)
                if \(H_r[\text{C_f}] > \tau\) then
                    \(r^i \leftarrow C_f\)
            for \(i = 1 \) to \(n\) do
                foreach kmer in \(r^i\) do
                    if \(H_a[\text{kmer}] \geq \tau\) then
                        \(C_kmer \leftarrow \text{FindNeighbor}(H_a, H_r)\)
                        \(C_f \leftarrow \text{Replacekmer}(C_kmer, r^i)\)
                        if \(H_r[\text{C_f}] < \tau\) then
                            \(r^i \leftarrow C_f\)
        \(S \leftarrow RS\)
    return \(S\)
end
```
Figure 2. The proportion of corrections varies at different lengths of \( k \)-mer.

Our miREC has been implemented as a software prototype. It provides several parameters for users to specify their tasks. Three most useful settings are: the error types, the frequency threshold \( \tau \), and the kmer range \( [k_1, k_{\text{end}}] \). miREC has two running modes: one is for the substitution error correction only, the other is for the correction of both indel and substitution errors. Based on our experience, the frequency threshold \( \tau \) is best recommended as 5 by default, and the kmer range parameter is set as \([8, 15]\). The higher frequency \( \tau \) is set, the bigger number of bases might be considered as errors. Thus, users should be cautious about using a too large frequency threshold to avoid over-correction.

Every iterative step of miREC with the increasing length of \( k \)-mer each time by 1 in the range \([k_1, k_{\text{end}}]\) actually corrects different amounts of errors. As shown in Figure 2, after five consecutive lengths of \( k \) are iterated, about 99.61% of substitution errors, 88.77% of insertion errors and 94.63% of deletion errors can be corrected on average over 12 wet-lab salmon datasets (Table 2) if \( k_1 \) is set as 8. With more loops of correction, more erroneous bases are detected and corrected. As each iterative loop consumes the same order of time complexity, users are suggested to narrow the kmer range (by setting \( k_{\text{end}} \) smaller) to shorten the program running time while correcting almost all of the errors for those miRNA sequencing datasets of huge size.

The source codes of miREC are publicly available online at https://github.com/XuanrZhang/miREC.

Simulated datasets (with known ground truth) and public wet-lab miRNA sequencing reads both for performance evaluation

To evaluate the performance of error correction methods, simulated datasets are required and the ground truth of the errors should be known. We introduce a novel process to generate simulated datasets that would have a close nature to wet-lab miRNA sequencing reads. We have two considerations in the process. One is to computationally replicate lab-verified miRNA sequences as templates to form the basic sequences of the simulated datasets, then we duplicate these basic sequences such that the copy counts of them follow a real distribution from a wet-lab dataset of miRNA sequencing reads. In fact, we replicated the mature miRNA sequences in miRBase (45) as the templates, and made the copy count distribution of these template sequences to follow the distribution drawn from a typical miRNA dataset (accession number SRR866573). In other words, the sequences in our simulated datasets are not random sequences (they are real lab-verified miRNA sequences); their copy count distribution is not random either. Then we injected errors into the simulated datasets under an error rate of 0.1% per base (24). Specifically, we randomly selected two reads from every 100 reads in the dataset; then for each selected read, we injected an erroneous base (substitution, deletion or insertion) randomly at any position of the read. We recorded all of these randomly and purposely injected errors for performance evaluation.

Considering that some existing methods only support substitution error correction, we synthesized 8 simulated datasets: four datasets containing substitution errors only (denoted as D_sub1, D_sub2, D_sub3 and D_sub4), and four datasets containing a mixture of 80% substitution and 20% indel errors (denoted as D_mix1, D_mix2, D_mix3 and D_mix4). More details of the simulated datasets are shown in Table 1.

Table 1. Description of our simulated datasets

| ID     | Total erroneous bases | Per read error rate |
|--------|-----------------------|---------------------|
| Simulated Datasets subs only | | |
| D_sub1 | 3071 | 3.03% |
| D_sub2 | 3022 | 2.98% |
| D_sub3 | 2973 | 2.93% |
| D_sub4 | 3124 | 3.08% |
| Simulated Datasets mix errors | | |
| D_mix1 | 1602 213 211 | 2.00% |
| D_mix2 | 1618 188 206 | 1.98% |
| D_mix3 | 1598 184 177 | 1.93% |
| D_mix4 | 1625 226 217 | 2.04% |

Notes: ‘subs’ means datasets contain substitution errors only and ‘mix’ means datasets contain both substitution and indel errors. Total erroneous bases list substitution, insertion and deletion errors respectively.
Table 2. 12 wet-lab salmon miRNA sequencing datasets and four human miRNA sequencing datasets.

| Tissue               | Total reads | Unique reads | Accession ID       |
|----------------------|-------------|--------------|--------------------|
| Liver                | 1 446 902   | 64 593       | SRR12881018        |
| Liver                | 1 647 133   | 75 273       | SRR12881030        |
| Spleen               | 8 597 057   | 295 940      | SRR12881030        |
| Spleen               | 2 236 013   | 89 165       | SRR12881030        |
| Kidney               | 10 065 660  | 243 430      | SRR12881030        |
| Head kidney          | 7 375 957   | 246 444      | SRR12881030        |
| Heart                | 2 812 993   | 118 366      | SRR12881030        |
| Brain                | 6 331 448   | 132 558      | SRR12881030        |
| Intestine            | 12 428 822  | 197 094      | SRR12881030        |
| White muscle         | 5 972 384   | 142 444      | SRR12881030        |
| Gills                | 6 240 735   | 132 038      | SRR12881030        |
| One day old individual| 18 041 561 | 172 048      | SRR12881030        |

human beta cells datasets

|                |               |              |                  |
|----------------|---------------|--------------|------------------|
| In low glucose| 63 008 516   | 5 803 166    | SRR13208981      |
| In high glucose| 33 444 257  | 1 856 318    | SRR13208980      |

human brain datasets

|                |               |              |                  |
|----------------|---------------|--------------|------------------|
| Sample of aged 75 | 11 849 807   | 635 169      | SRR12881030      |
| Sample of aged 94 | 17 250 812   | 361 039      | SRR12881018      |

Evaluation metrics

As the ground truth of the errors in the simulated datasets are known, we can use recall, precision and gain to compare the correction performance between different methods. On the wet-lab miRNA sequencing datasets, we measure the copy count changes of the reads, the entropy changes of the whole set of reads, and locations of the rectifications to understand the importance of error correction. There is no recall or precision performance on the wet-lab miRNA sequencing datasets, because the ground truth of error distributions is unknown.

Performance evaluation metrics on the simulated miRNA sequencing datasets. Precision, recall and gain rate are given as follows to assess the correction performance on the simulated datasets:

- Precision: \( TP / (TP + FP) \), shows the fraction of truly corrected bases among all the changed bases.
- Recall: \( TP / (TP + FN) \), shows the fraction of truly corrected bases among all the bases which are supposed to be corrected.
- Gain: \( (TP - FP) / (TP + FN) \), shows the fraction of removed errors without inducing additional errors.

where true positives (TP) correspond to corrected errors; true negatives (TN) correspond to initially correct bases left untouched; false positives (FP) correspond to newly introduced errors; and false negatives (FN) correspond to unidentified errors.

Metrics used for performance evaluation on wet-lab miRNA sequencing datasets. We examine the changes of miRNA copy counts and dataset entropy changes before and after error correction for multiple salmon fish miRNA sequencing datasets. Besides, we also summarize the position information of the corrections in the reads to record the proportion of corrections in the seed region. We define the miRNA count, dataset entropy and errors in the seed region as follows.

- miRNA count: the copy count of miRNA appearing in the datasets, which is corresponding to miRNA expression level or miRNA abundance.

- Dataset entropy: \( - \sum_{i=1}^{n} p_i \cdot \log p_i \), where \( p_i \) is the proportion of reads whose frequency is small than \( i \). We calculate the entropy for low-frequency reads and sum up to interpret the degree of disorder in the read dataset. When the entropy turns to be small, it means the certainty of the miRNA expression becomes higher.

- Errors in seed region: erroneous bases in the seed region, which is a conserved sub-sequence of miRNA (mostly situated at positions 2–8). Precise bases in the seed region are vital since the seed sequence must be perfectly complementary with its target mRNA to make the miRNAs function.

RESULTS

Our analysis and results are presented in five main parts. The first part is about the correction performance on the 8
simulated datasets; the second part is about correction performance on the wet-lab miRNA sequencing datasets after a small number of artificial errors are injected; the third part is about copy abundance recovery, entropy change and rectification site summary on the recently published salmon fish miRNA sequencing datasets after error correction; the fourth part provides detailed case studies on the change of isomiR families, tissue-specific isoforms, differentially expressed biomarkers and rare-miRNA quantity enhancement after the error correction on some of the wet-lab datasets, including the human miRNA sequencing datasets. The fifth part presents our verification results on sequencing reads datasets of 963 miRXplore Universal Reference miRNAs (three replicates) and their spike-in at eukaryotic cells.

**Gain, recall and precision performance on the simulated miRNA sequencing datasets**

The correction performance of our miREC is presented in Table 3 in comparison with algorithms Karect (41), Coral (39), BFC (36), Rcorrector (43) and Becool (35). Coral and Karect are multi-alignment based error correction methods. BFC is a representative of the k-mer based error correction methods. BFC requires a prior-setting of the k parameter; the best k in this work is 21 (namely, under other k settings, BFC did not exceed the performance of when k = 21). Karect is one of a few correction tools which supports the correction of indel errors. Rcorrector, a RNA reads error correction method, has a performance higher than another RNA correction method Seeer (42). Rcorrector also needs to set the k parameter and the best k in this work is 17. Even using the optimal k settings, only a few bases can be corrected by Rcorrector. A very recent error correction algorithm Becool (35), which uses a de Bruijn graph as the platform to correct errors, could not detect any errors in the simulated datasets. This surprising performance is not included in the table.

Our method miREC has excelled in the correction performance:

- It did not introduce any new error, namely, it achieved the same gain and recall rates on all of the 8 datasets;
- It detected and corrected almost all of the errors including the indel errors; the recall rate ranges between 96.0–97.9%; the precision ranges between 98.6–99.5%.
- It improved the overall data quality remarkably: (i) from every 50 reads containing one error to every 1300 reads containing one error for the four error-mixed datasets and (ii) it improved the data quality from every 30 reads containing one error to every 1650 reads containing one error for the four substitution-only datasets.

The average recall and gain rate of miREC are much superior to Karect (the second-best method) respectively by 3.28% and 3.66% on the four substitution-only datasets, and by 19.44% and 28.7% on the four error-mixed datasets. Specifically, the average recall rates of miREC are 97.83% and 96.12% on the four Dmix datasets and on the four Dsub datasets respectively, which are 16.25%, 87.96% and 3.28% (on the Dsub datasets) and 27.86%, 87.21% and 19.44% (on the Dmix datasets) better than BFC, Coral and Karect. This implies that there are lots of errors undetected by these baseline methods meanwhile they introduced a lot of new errors (gains and recall not equal). The multi-alignment method performed worst on these miRNA datasets. A possible reason is that the alignment strategy could not differentiate miRNA reads well due to the short length of miRNAs. Rcorrector had a very low recall and gain performance as well, that means most of the errors were not detected by the method.

The performance of miREC is robust across all the 8 datasets including the four mixed-error datasets, in contrast to the baseline methods which exhibited a poor performance on the detection and correction of the indel errors. The gain rate of BFC drops from 80.83% (on the four Dsub datasets) to only 67.07% (on the four Dmix datasets), and the gain of Coral drops from 5.27% to 1.79%. It suggests that the performance of these methods on the substitution error correction was interrupted and affected by the addition of the indel errors into the datasets. As real-life wetlab sequencing reads more or less company with a small amount of indel errors, our miREC provides an unalterable advantage over the baseline methods for the correction of all types of aberrations.

### Table 3. Outstanding error correction performance by our miREC in comparison with the best available tools

| Dataset Type | miREC Gain (%) | BFC Gain (%) | Karect Gain (%) | Rcorrector Gain (%) | miREC Recall (%) | BFC Recall (%) | Karect Recall (%) | Rcorrector Recall (%) | miREC Precision (%) | BFC Precision (%) | Karect Precision (%) | Rcorrector Precision (%) |
|--------------|----------------|--------------|-----------------|---------------------|------------------|----------------|-------------------|----------------------|---------------------|-------------------|---------------------|--------------------|
| Dmix         | 95.96          | 95.73        | 96.02           | 96.76               | 96.18            | 96.23          | 96.51             | 96.87                | 97.83               | 97.61             | 98.58               | 99.63              |
| Dsub         | 96.12          | 96.76        | 96.24           | 96.97               | 96.73            | 96.43          | 96.54             | 96.87                | 97.86               | 97.98             | 98.78               | 99.48              |
| Notes: AVE indicates the average score over the four datasets. Bold font indicates the best result in the row. Cor, Kar and Rcor stand for the Coral method, the Karect method and the Rcorrector method respectively. Ds indicates datasets containing only substitution errors, while Dm indicates datasets containing mixed substitution, insertion and deletion errors. The underline 100 precision of Rcor on D_mix datasets. D_m1 and D_m2 and D_m3 stands for only one, two and one base is corrected respectively. |
Correction performance on wet-lab miRNA sequencing datasets injected with small numbers of artificial errors

We made 27 random base modifications (total 21 substitutions, 3 insertions and 3 deletions) at the salmon liver miRNA sequencing dataset (SRR866573). These manual modifications introduced/injected 18 genuine errors into the dataset, where a random base modification is not considered as a genuine error if its correspondingly modified read becomes identical with another read having a high frequency (i.e. copy count > 5).

Our algorithm corrected all of these 18 genuine errors (100% recall). For example, read @SRR866573.64765 (TG CGGACCAGGGGAATCCGACT) had a random manual deletion at base position 5, becoming TGGCGACAGGGGAATCCGACT; our miREC detected this error and restored it to its original base. As another example, read @SRR866573.212344 (AAGCTGCCAGCTGAGAAGACT) had a random manual substitution from C to G at position 8, becoming AACGTGCAGCTGAAGAAGACT; our miREC corrected it successfully.

Read @SRR866573.1103128 (AGACGGGGCCCGCCAAC TTCTGT) had a random manual insertion of G at position 16, becoming AAGCGGGGCCCCAAAAGCTCTCTG T; again, our miREC successfully detected this error and corrected it. For the remaining 9 randomly injected base modifications, they did not cause genuine errors because each of their reads was transformed into another read that has a high copy count in the same dataset. For example, read @SRR866573.360151 (ATGACCATATGAATTGACAGCCT) had a random manual substitution from T to C at position 21 (the last position). With this modification, the read becomes another read ATGACCATATGAATTGACA GCCC which has 156 copies. This modification was unable to be restored to its original base because every k-mer in ATGACCATATGAATTGACAGCCTC was highly frequent (at least 156 copies), namely, containing no error. Note that this modification should not be restored to ensure no over-correction would happen in practice, otherwise the correction would be of guilty. For performance comparison, the second-best method Karect was applied to the same error-injected salmon liver dataset, but it corrected only 5 of the 18 genuine errors.

We repeated this test with another round of manual base modifications at SRR866573 (total 28 modifications including 20 substitutions, 6 insertions and 2 deletions). Our miREC detected and corrected all of the 20 genuine errors (100% recall again). In comparison, Karect corrected only 9 of them.

Similarly, our miREC corrected all of the genuine errors caused by small numbers of random base modifications at other wet-lab miRNA sequencing datasets (40 substitutions, 3 insertions and 6 deletions; or second round 45 substitutions, 7 insertions and 7 deletions at the salmon heart dataset, 38 substitutions, 4 insertions and 4 deletions; or second round 43 substitutions, 6 insertions and 6 deletions at the salmon spleen dataset). However, Karect corrected only 8 of the 27 genuine errors or only 8 of the 35 errors on these two error-injected salmon heart datasets, and had similar performance on the two error-injected salmon spleen datasets.

On the two human brain datasets, our miREC achieved the same perfect performance (100% recall) to correct all of the genuine errors caused by small numbers of random base modifications (about 300 base modifications which had resulted in 130 and 120 genuine errors). However, Karect could only fix 12 or 20 genuine errors in these two datasets. Our source codes for the random error injection into wet-lab miRNA sequencing datasets and more detailed correction results are available at github link https://github.com/XuanrZhang/miREC.

Changes in isoform abundance, whole set entropy and base positions after error correction at the salmon fish miRNA sequencing reads

The perfect recall performance on the small numbers of errors injected into wet-lab miRNA sequencing datasets and the excellent gain performance on the simulated datasets are strong combined evidence to support our correction results on wet-lab datasets where the ground truth of errors are not available.

The salmon liver miRNA sequencing dataset (SRR866573) has a total of 900 814 reads, containing 32,972 distinct reads before error correction. After error correction by our miREC, there are only 27 299 distinct reads some of which gained plenty of abundance. In other words, most of the error-contained reads were corrected and turned to be identical with some other reads, making the abundance merging meanwhile the disappearance of the originally error-contained reads.

See Figure 3 for an average percentages of the distinct miRNAs over the 12 datasets that have a high- or low-level abundance recovery. There are around 47.3% of the distinct miRNAs whose copy counts have increased by more than 10% after the corrections, in particular, about 5.5% of the distinct miRNAs have obtained above 50% abundance increase. These corrections are useful to draw more reliable conclusions about miRNA discovery or isomiR classification or tissue-specific biomarker discovery (case studies presented later).

The reads abundance recovery of the miRNA isoforms after error rectification in a dataset implies that the numbers of distinct reads are decreased as reported above. We present Figure 4 to illustrate the overall entropy change of every entire dataset before and after the error correction to quantify this point. On average the entropy of the 12 datasets is shrank by 15.11% when the parameter $k$ of miREC ranges from 8 to 20, and the entropy score decreased by 14.51% when $k$ ranges from 8 to 25. These entropy declines (with slight variance) in the 12 datasets theoretically mean that the certainty of the miRNA expression levels is greatly improved. In other words, our miREC can enhance the data quality in the perspective of achieving a lower entropy or a higher certainty.

We found that the aberrations could occur at every base position of the reads. But, one-third of the errors are detected and corrected at the seed region of the miRNAs (Figure 5). These corrections at the seed region provide great benefits for miRNAs’ target prediction analysis. There are also a high percentage of the indel or substitution correc-
Case studies related to isomiR families, tissue-specific miRNAs and rare-miRNA quantity recovery

We report examples of miRNAs whose read counts have changed a lot after error correction. We also show examples of tissue-specific miRNAs after error correction, and describe the change in the ranking lists of differentially expressed miRNAs.

Case study 1: big abundance recovery. In the salmon heart dataset, read TTGGTCCCCTTCAACCAGCTGTAAT (mapped to miR-133a-1 in miRBase (45)) had 10 copies. Our miREC detected 13 erroneous reads related to this miRNA. Eight substitution errors happened at position 24 base A (sequenced to G or T), and five happened at position 25 base T (sequenced to A or G). After our correction, the abundance level of miR-133a-1 increased from 10 to 23, a 130% abundance recovery. Other two miRNAs (ssa-miR-133a-3p and ssa-miR-133a-5p) from the same miRNA family also recovered their abundance in the sequencing reads. See the read counts and change details in Table 4. We note that currently annotated functions of miR-133a-1 are related to conventional central osteosarcoma and heart conduction disease (47,49). With the refined abundance understanding, its functions can be re-examined more deeply.

Another example in Table 5, read ATCCCGGACGAGCCCCCAA, had 18 copies and its abundance increased to 31 after miREC correction. The aberrations include four deletion errors at position 1 (base A deleted), four substitution errors at position 19 base A (sequenced to C) and two insertion errors at position 1 and 2 (base A inserted). This error distribution implies that the sequencing mistakes can occur at multiple base positions with multiple times; and that our miREC is capable of correctly detecting these errors and performing accurate rectifications.

For comparison, we tested the second-best method Karect on this salmon heart dataset to see whether the same mistakes could be corrected. Take the cases in Table 4 as example, only three erroneous reads of the first read TTGGTCCCCTTCAACCAGCTGTAAT were detected by Karect (we detected 13); none of the erroneous reads of the other
Figure 4. Dataset-entropy changes before and after the error correction by miREC on the 12 salmon miRNA datasets. (A) when the continuous \( k \) settings from 8 to 20; (B) when the continuous \( k \) settings from 8 to 25.

Figure 5. The distributions of corrections at different positions.

Table 4. Changes in the read counts of some miRNAs

| Sequence                  | Read count before correction | Read count after correction | Abundance change percentage | Isoforms/editing events |
|---------------------------|------------------------------|----------------------------|------------------------------|-------------------------|
| TTGGTCCCTCTCAACCAGCTGTAAT | 10                           | 23                         | 130.00%                      | Substitution error      |
| TTGGTCCCTCTCAACCAGCTGTA-T | 44                           | 45                         | 2.27%                        | 5' deletion             |
| TTGGTCCCTCTCAACCAGCTGTA-  | 107                          | 111                        | 3.74%                        | 3' deletion             |

Related Erroneous Reads List

| Sequence                  | Read count before correction | Read count after correction | Isoforms/editing events |
|---------------------------|------------------------------|-----------------------------|-------------------------|
| TTGGTCCCTCTCAACCAGCTGTAGT | 4                            | 0                           | Error removal           |
| TTGGTCCCTCTCAACCAGCTGTA-T | 4                            | 0                           | Error removal           |
| TTGGTCCCTCTCAACCAGCTGAAA  | 3                            | 0                           | Error removal           |
| TTGGTCCCTCTCAACCAGCTGTAAG | 2                            | 0                           | Error removal           |
two reads in the table were detected. Only one of the four related erroneous bases was corrected by Karect, while all of the related erroneous bases were corrected by our method.

**Case study 2: miRNA isoforms and editing events.** Editing events and isoform variations at the cleavage sites can cause slight but important difference in many miRNA sequences (50). In the miRNA sequencing dataset (SRR866605) of salmon fish heart, canonical miRNA read \textit{ATCCCGGACGAGCCCCCAAA} co-exists with five isoforms having read counts 9, 1489, 16, 4 or 4; There are also three singleton CGAGCCCCCA that co-exists with five isoforms having read counts 9, 1489, 16, 4 or 4; There are also three singleton CGAGCCCCCAAA that co-exists with five isoforms having read counts 9, 1489, 16, 4 or 4; There are also three singleton CGAGCCCCCAAA. In our miREC grouped all of these reads and detected erroneous bases at multiple base positions. In particular, more than one third of erroneous bases happened at the seed region, important for gene target binding analysis.

\begin{table}[h]
\centering
\caption{isomiRNA detection}
\begin{tabular}{lcccc}
\hline
\textbf{Sequence} & \textbf{Read count before correction} & \textbf{Read count after correction} & \textbf{Abundance change percentage} & \textbf{Isoforms/editing events} \\
\hline
\textit{ATCCCGGACGAGCCCCCAAA} & 18 & 31 & 72.22\% & Deletion \\
\textit{ATCCCGGGACGAGCCCCC}- & 1489 & 1513 & 1.61\% & Deletion \\
\textit{ATCCCGGACGAGCCCCCAT} & 16 & 20 & 25.00\% & Substitution \\
\textit{ATCCGGACGAGCCCCCCAA} & 9 & 17 & 88.89\% & Insertion \\
\textbf{Related Erroneous Reads List} & & & & \\
\- \textit{TATCCCGGACGAGCCCCCAAA} & 4 & 0 & Error removal & Insertion \\
\- \textit{ATCCCGGACGAGCCCCCAC} & 4 & 0 & Error removal & Substitution \\
\- \textit{AATCCCGGACGAGCCCCCCAA} & 1 & 0 & Error removal & Insertion \\
\- \textit{ACCCCCGACGAGCCCCCCAA} & 1 & 0 & Error removal & Insertion \\
\- \textit{AATCCCGGACGAGCCCCCCAA} & 1 & 0 & Error removal & Insertion \\
\textbf{Notes:} After rank indicates the rank after error correction, while Before rank indicates the rank before error correction.
\end{tabular}
\end{table}

Case study 3: \textit{upside down change in differential expression analysis.} Analysis on tissue-specific uniquely expressed or top-ranked differentially expressed miRNAs in a specific tissue or at a disease stage is very sensitive to the sequencing data quality (9). Some uniquely expressed miRNAs can be identified only after error correction.

In our differential expression analysis between the salmon heart and brain tissues (SRR866605 vs SRR866611), we found that 5,675 miRNA did not co-exist in the two datasets, and the number of common miRNAs was reduced from 16,443 to 10,768 after error correction. For example, read \textit{TGAGGTATGTGTTGTATGGTGG} (mapped to ssa-let-7-d-5p in miRBase), had four copies in the heart dataset and 26 copies in the brain dataset before error correction, while its read count was changed to zero in the heart dataset and changed to 30 in the brain dataset after error correction. Two more examples: read \textit{CTTTCTATGCTGTTGGTTTGACCA} (mapped to ssa-miR-30d-3p in miRBase) had 152 copies in the heart dataset and two copies in the brain data before correction, while its quantity was changed to 155 in the heart dataset and to zero in the brain dataset. Another read \textit{TGAGGTATGTGTTGTATGGTGG} (mapped to ssa-miR-153a-3p in miRBase), had three copies in the heart dataset and 14,343 copies in the brain dataset before error correction, while the quantity dropped to zero in the heart dataset but increased to 14,498 in the brain dataset after error correction.

Top-rank differentially expressed miRNAs can become low-ranked ones, and vice versa after error correction. The reason is that the expression folds of miRNAs between two tissue types or between two disease stages are sensitive to the copy counts after erroneous reads are corrected in the two classes. We compared the expression folds of common miRNAs between the salmon heart tissue and brain tissue before and after our error correction. Table 6 presents the list of 10 miRNAs whose expression folds between the two tissues are top-ranked after the error correction, in comparison with their ranking positions before the error correction. The two ranking lists are quite different. For example, the rank of ssa-miR-9a-5p (\textit{TCTTTGTTATCTCTAGCCTGTA}) is reverted from rank 8 to 4. Furthermore, the originally
Table 7. Ranking position change of tissue-specific miRNAs in the heart tissue (vs the liver tissue) before and after error correction

| miRNA sequence | Rank before correction | Rank after correction | Read count before correction | Read count after correction | Read count increase |
|----------------|------------------------|-----------------------|----------------------------|----------------------------|---------------------|
| TTAAGACTTGATGCAAGTTT | 1 | out of scope | 47 546 | 47 583 | 37 |
| TGGATGATGCAAGTTT | 2 | 1 | 12 650 | 12 728 | 78 |
| TTTGCTCCCTTCCACAGCTG | 3 | 2 | 4954 | 4985 | 31 |
| TTGGTCTCTTCTGACGCTG | 4 | 3 | 2322 | 2541 | 19 |
| TTAAGCTTCAGCTGATTT | 5 | 4 | 1663 | 1677 | 12 |
| ACAGCTCACTTTGACTGCT | 6 | 5 | 1174 | 1188 | 14 |
| TGGAATGTAAAGAAGTATGTA | 7 | 6 | 879 | 892 | 13 |
| AACATCCTTAAAGCTTCTGCT | 8 | 7 | 876 | 892 | 16 |
| TTGGTCTCCCTTCCACAGCTGTA | 9 | 8 | 835 | 856 | 21 |
| TGGAGTATGGTTGTATTGTTT | 10 | Out of scope | 780 | 791 | 11 |
| TGGAGGAGAATGCTGATAAGGG | 11 | 9 | 693 | 702 | 9 |
| TTAAGCTTCAGCTGATTT | 12 | 10 | 685 | 698 | 13 |
| TTGGTCTCCCTTCCACAGCTGTA | 13 | Out of scope | 659 | 666 | 7 |
| TGAGAGAATGCTGATAAGGG | 14 | 11 | 622 | 635 | 13 |
| TGGAATGTAAAGAAGTATGTA | 15 | 12 | 616 | 629 | 13 |

The two miRNAs only have one base difference at the 3′ end. The corrected top-one miRNA after error correction has one base trimmed at the 3′ end, compared to the original top-one ranked miRNA. The two miRNAs can be considered as 3′ end trimmed/addition isoforms each other.

- The original top-ranked miRNA and the corrected top-one miRNA have a huge abundance difference (31 316 copies = 48 092 − 16 776) in the brain tissue. One is extremely high-level expressed; the other is median-level expressed. This suggests that we would concentrate on wrong top-ranked miRNA biomarkers if the sequencing reads had not been cleaned by good error correction algorithms.

New top-ranked tissue-specific miRNAs (or called no-presence miRNAs or tissue- and disease-subtype dependent miRNAs by (9)) were found in the heart tissue (SRR866605) after error correction when the liver tissue (SRR866579) was compared. Table 7 presents two rankings of top-15 miRNAs specifically expressed in the heart tissue before and after error correction. Without our correction, the top-1, top-10 and top-13 tissue-specific miRNAs in salmon heart would be not detected because erroneous reads which are identical with these reads also exist in the liver tissue. Moreover, after our error correction, the quantity of the top-ranked miRNAs increases. These recovered read counts and accurate abundance measurement would help make more convincing conclusions in the down stream analysis.

Case study 4: class-specific miRNAs and rare-miRNA analysis for human miRNA sequencing datasets. Ranking positions of class-specific miRNAs and rare miRNA quantity recovery analysis are also conducted on human miRNA sequencing datasets (acquired from beta cells and brain samples).

The human beta cells were incubated with solution of low glucose or high glucose. It’s expected to reveal novel differentially expressed miRNAs between these two classes. We found that the number of distinct reads decreased by 8.85% from 5 803 166 to 5 289 466 in the low glucose solution cell, and reduced by 12.44% from 1 856 318 to 1 625 453 in the high glucose solution cell after error correction. For the top-ranked differentially expressed miRNAs between the two datasets, only slight rank changes were observed (Table 8). Some of the top-ranked miRNAs were just swapped ranking positions within top 10 after error correction. The copy counts of these top-ranked miRNAs all had small increases after the error correction. Note that these changes on glucose-level specific miRNAs in human beta cells after error correction is not as big as those changes made in the salmon heart-head tissue pair comparison by our error correction.

However, such big changes on age-specific miRNAs in brain samples can be observed again when we compared between miRNA sequencing reads of an Alzheimer’s disease patient aged 75 and a patient aged 94. The number of distinct miRNA reads decreased by 33.6% from 361 039 to 239 667 in the patient aged 75, and decreased by 16.1% from 635 169 to 532 708 in the patient aged 94, after error correction. Table 9 provides two rankings of top-10 age-specific miRNAs expressed only in the patient aged 94 before and after correction. New top-ranked age-specific miRNAs were identified in the patient aged 94. Without our error correction, top-1, 2, 3, 4, 6, 8 and 10 age-specific miRNAs would not be detected because erroneous reads which are identical with these reads also exist in the patient aged 75, with copy counts 3, 2, 2, 3, 4, 2 and 4 respectively.

Discovery of rare miRNAs is of strong interests. We examined the read counts of low-expression miRNAs (or rare miRNAs) before and after error correction in the Alzheimer’s disease patient aged 94. Note that all these rare miRNAs here are defined to have no expression in the patient aged 75. Table 10 shows top-10 read-count greatly-changed rare miRNAs before and after error correction. It suggests that the read counts of these rare miRNAs were all enhanced by about 2 or 3-fold after error correction.
### Table 8. Read count changes and ranking changes of top-10 differentially expressed miRNAs in the high glucose incubated human beta cell after error correction, and those in the low glucose incubated human beta cell after error correction

| miRNA sequence | Rank Among high glucose | Read count After | Read count Before | Read count Increase | miRNA sequence | Rank Among low glucose | Read count After | Read count Before | Read count Increase |
|----------------|-------------------------|------------------|-------------------|---------------------|----------------|------------------------|------------------|-------------------|---------------------|
| GTGGGGCCACGAGCTGAGTGCTG | 1 | 86 | 92 | 6 | CGCCCGTCCCCGCCCCTT | 1 | 640 | 651 | 11 |
| AGCAAGGGTGGGCGCTGTTAGTA | 2 | 68 | 69 | 1 | CGCCCGTCCCCGCCCCTT | 2 | 582 | 586 | 4 |
| GAGTTCGCGCTTTCCCCT | 3 | 61 | 62 | 1 | GCCGCAGGGTCGGGCC | 3 | 565 | 572 | 7 |
| GTGGAGGCGGTCGCTTAAT | 4 | 60 | 61 | 1 | GCCGCAGGGTCGGGCC | 4 | 484 | 489 | 5 |
| TCGGAAGCTAAGCAGGGTGCGGCC | 5 | 57 | 57 | 0 | GCCGCAGGGTCGGGCC | 5 | 428 | 436 | 8 |
| GTCTACGGGCTCCTCCACCTTGACG | 6 | 55 | 56 | 1 | GCCGCAGGGTCGGGCC | 6 | 371 | 376 | 5 |
| GTCTACGGGCTCCTCCACCTTGACG | 7 | 54 | 55 | 1 | GCCGCAGGGTCGGGCC | 7 | 317 | 324 | 7 |
| GCCGCAGGGTCGGGCC | 8 | 51 | 54 | 3 | GCCGCAGGGTCGGGCC | 8 | 292 | 300 | 8 |
| GCCGCAGGGTCGGGCC | 9 | 50 | 51 | 1 | GCCGCAGGGTCGGGCC | 9 | 297 | 298 | 1 |
| GCCGCAGGGTCGGGCC | 10 | 49 | 51 | 2 | GCCGCAGGGTCGGGCC | 10 | 272 | 278 | 6 |

### Table 9. Ranking position changes of age-specific miRNAs in brain tissue from an Alzheimer male patient aged 94 (vs a patient aged 75) before and after error correction

| miRNA sequence | Rank after correction | Rank before correction | Read count before correction | Read count after correction | Read count increase | Original read count (aged 75) |
|----------------|-----------------------|------------------------|-----------------------------|-----------------------------|---------------------|-----------------------------|
| TTTCTCTACTGCACTTGACTGCT | 1 | Out of scope | 1416 | 1477 | 61 | 3 |
| TTTCTCTACTGCACTTGACC | 2 | Out of scope | 839 | 877 | 38 | 2 |
| TTTCTCTACTGCACTTGAC | 3 | Out of scope | 817 | 857 | 40 | 2 |
| TTTCTCTACTGCACTTGACTAG | 4 | Out of scope | 726 | 761 | 35 | 3 |
| TTTCTCTACTGCACTTGACA | 5 | 1 | 719 | 746 | 27 | 0 |
| TTTCTCTACTGCACTTGACTAGT | 6 | Out of scope | 593 | 628 | 35 | 4 |
| TTTCTCTACTGCACTTGACTA | 7 | Out of scope | 521 | 533 | 12 | 0 |
| TTTCTCTACTGCACTTGACTA | 8 | Out of scope | 381 | 410 | 29 | 2 |
| TCTGGTAGTATAGTAGTATAG | 9 | 3 | 365 | 375 | 10 | 0 |
| TGAAGATGATGTTAGATGAT | 10 | Out of scope | 339 | 352 | 13 | 4 |

**Figure 6.** A rare miRNA in the Alzheimer’s disease patient aged 94 showing significant copy count change after error correction.
Table 10. Copy count enhancement of 10 rare miRNAs after error correction in a human brain dataset acquired from an Alzheimer’s disease patient aged 94

| miRNA sequence | Read count before | Read count after |
|----------------|-------------------|------------------|
| TCATTGGTATCTAGCTGTATGC | 6 | 18 |
| TAGAACCTCGTCGAGTACGCTC | 9 | 26 |
| AAAAACTGGGTTGAGAGGCCGTTGA | 6 | 17 |
| AGCAAGGGCGTGGCCATGGA | 8 | 22 |
| TGAGGCGATAGGTGTTGTTTATT | 6 | 16 |
| TCCAGCATGAGTTTGTG | 6 | 16 |
| TCAGACAGAGCCCCGTCCTTTT | 6 | 16 |
| GTGGTCGAGGTGGTCG | 6 | 16 |
| TCCCCGACCCTCCACCAT | 9 | 23 |
| AGGAATCGGAATAGGACGTTGA | 8 | 20 |

Notes: After indicates after error correction, while before indicates before error correction.

Figure 6 depicts how the read quantity of a rare miRNA is enhanced from 14 copies to 23 in the correction process. The corrections were involved with four types of erroneous reads: four reads with a deletion error (labeled in blue), two reads with an insertion error (labeled in green), one read with a substitution error from A to G at position 11 and two reads with a substitution error from A to T at position 20 (labeled in orange). Our miREC can detect all of these erroneous reads and corrected them to recover this rare miRNAs quantity.

Verification results on the sequencing reads of the 963 miR- xploré universal reference miRNAs (pure control and spike-in)

Our algorithm was tested on the sequencing reads of an equimolar mixture of synthetic miRNAs from the miRXplore Universal Reference that consists of 963 miRNAs from human, mouse, rat and viral sources (three replicate samples miRXploreUR rep1-3 corresponding to GSE139936.GSM4149813, GSE139936.GSM4149814 and GSE139936.GSM4149815 (51)). The test was to verify

- whether our detected erroneous reads can be each corrected into one of the 963 miRNA sequences, and
- whether any new sequences are introduced into the read dataset after the correction.

An ideal performance should be: every error-corrected read is turned to be an exact copy of one of the 963 miRNA sequences, and previously non-existing reads are never created by the correction step.

The correction performance by miREC in comparison with Karect (the best literature method (41)) are shown in Table 11. On the sequencing dataset named D18-6962 1 of GSE139936.GSM4149813, our algorithm detected a total of 43362 errors. After correction, the correspondingly rectified reads were each exactly matched with one of the 963 miRNA sequences. The total read count of the 963 miRNAs was therefore increased by about 19.59% (see Supplementary file S1 for details). During this correction step, previously non-existing reads were never generated/created. In fact, the number of distinct reads was decreased from 259867 to 212093. On the other hand, almost all (99.22%) of the remaining unchanged 231792 distinct reads were not considered as the erroneous reads of the 963 miRNAs by our algorithm. This is reasonable because each of them has a minimum editing distance of 2 or bigger with any of the 963 miRNA sequences. These remaining reads also have an extremely low counts such as 1, 2 or 3. They can be considered as noisy reads which may be caused by the library preparation noise or contaminates.

Karect detected total 127 642 errors, but only 18 225 of them were corrected into the sequencing reads of the 963 miRNAs, increasing their read counts by 8.22% in total. Meanwhile, the other base modifications have introduced a pool of 37 678 new sequences which did not exist in the dataset before Karect’s correction.

From these comparisons, we note that our algorithm miREC has corrected almost all of those reads which should be rectified and that miREC has never introduced previously non-existing reads. This is true for all other datasets listed in Table 11. However, Karect introduced large pools of new reads which have never existed in the original reads set; also Karect corrected less than half of those reads which should be rectified.

On a spike-in sample of the 963 miRNAs at human cells (GSE159434.D19-10246.assembled.fastq (52)), our algorithm detected 89 301 erroneous reads of the 963 miRNAs. After correction, their read counts increased by 15.66% in total. The algorithm did not generate any previously non-existing reads, but decreased the number of distinct reads by 45 189, greatly diminishing the uncertainty/entropy of the data set. On the other hand, Karect detected and corrected 15885 erroneous reads of the 963 miRNAs, making their read counts increased by 7.62% in total. However, Karect created 14 462 new reads which were non-existing previously.

These comparative results on both the control and spike-in sample demonstrate that our modified reads are genuine correction and that our algorithms do not generate any previous non-existing reads after the correction process.

DISCUSSION

There are several aspects of complexities in miRNA sequencing datasets which can limit the performance of error correction algorithms. For example, some miRNAs (A versus B) can be very similar (just one base different) and have similar abundance in the sample. In this case, erroneous reads of A can be sometimes exactly the same sequence as B (or, erroneous reads of B can be sometimes exactly the same sequence as A). Such an error is unable to be detected by any error correction algorithm because A and B have similar abundance level (see examples in Section - Correction performance on wet-lab miRNA sequencing datasets injected with small numbers of artificial errors). However, we note that such sequencing errors (without correction) would not affect much about the true read counts of A and B. The reason is that the count of As such erroneous reads should be at the same level of the count for B’s such erroneous reads.

Some miRNAs have very low-level abundance and are prone of errors in sequencing. In this case, the read count of such a miRNA can be zero, meaning all of its reads are wrongly sequenced (see examples in the ‘Editing distance = 1’ column of Table 11). Our algorithm is unable to detect
Table 11. Correction performance by miRECa in comparison with Karect \(^b\) (41) on the sequencing reads of the 963 miRXplore Universal Reference miRNAs (pure control and spike-in)

| Dataset\(^c\) | Total read count | Method | Number of detected bases for correction | Total read count of the 963 miRNAs | Distinct reads with the same frequencies before and after correction | Minimum editing distance ## |
|---|---|---|---|---|---|---|
| | | | before correction | after correction | Het(%) | increased | new sequences |
| | Count | Pct(%) | Count | Pct(%) | Count | Pct(%) |
| D18-6962\(^1\) | miRECa | 43,362 | 221,657 | 265,076 | 19.59 | 0 | 231,792 | 24 | 0.004 | 1,809 | 0.7804 | 47,949 | 20.6862 | 182,030 | 78.5230 |
| (544,056) | Karect | 127,642 | 239,882 | 8.22 | 37,678 | 33 | 233,418 | 23 | 0.0099 | 1,813 | 0.7767 | 47,832 | 20.492 | 183,780 | 78.7214 |
| D18-6962\(^2\) | miRECa | 43,122 | 223,921 | 267,994 | 19.28 | 0 | 233,418 | 23 | 0.0099 | 1,813 | 0.7767 | 47,832 | 20.492 | 183,780 | 78.7214 |
| (547,087) | Karect | 129,410 | 241,979 | 8.06 | 38,508 | 34 | 235,988 | 24 | 0.0116 | 1,821 | 0.7872 | 47,924 | 20.8355 | 183,790 | 79.1188 |
| D18-6963\(^1\) | miRECa | 35,211 | 150,886 | 186,126 | 23.36 | 0 | 181,380 | 35 | 0.0093 | 1,310 | 0.7222 | 47,810 | 20.4389 | 183,710 | 79.1188 |
| (423,49) | Karect | 108,583 | 167,678 | 11.13 | 30,885 | 29 | 124,456 | 11 | 0.0088 | 8,058 | 0.4674 | 13,726 | 6.1208 | 108,261 | 53.7641 |
| D18-6963\(^2\) | miRECa | 35,060 | 152,201 | 187,237 | 23.02 | 0 | 180,491 | 35 | 0.0094 | 1,301 | 0.7219 | 47,803 | 20.3903 | 183,731 | 79.0125 |
| (40,407) | Karect | 109,344 | 168,894 | 10.97 | 31,590 | 30 | 123,387 | 11 | 0.0089 | 8,040 | 0.5161 | 13,704 | 6.1065 | 101,632 | 62.3685 |
| D18-6964\(^1\) | miRECa | 39,369 | 192,095 | 213,512 | 20.52 | 0 | 215,161 | 25 | 0.0116 | 1,372 | 0.6337 | 39,194 | 18.5508 | 173,830 | 80.8000 |
| (40,577) | Karect | 113,706 | 229,543 | 9.08 | 32,412 | 31 | 154,167 | 12 | 0.0078 | 9,488 | 0.6544 | 17,004 | 6.1208 | 127,662 | 82.8083 |
| D18-6964\(^2\) | miRECa | 39,210 | 194,177 | 233,441 | 20.22 | 0 | 213,499 | 26 | 0.022 | 1,401 | 0.6562 | 39,802 | 18.6427 | 178,720 | 80.6889 |
| (46,8317) | Karect | 115,845 | 211,474 | 8.91 | 33,398 | 32 | 151,458 | 11 | 0.0073 | 9,513 | 0.6289 | 17,069 | 6.1208 | 124,385 | 88.4430 |
| D19-10246 | miRECa | 89,301 | 208,219 | 280,828 | 15.66 | 0 | 77,335 | 16 | 0.0207 | 1,782 | 2.3043 | 11,129 | 14.3906 | 64,408 | 83.2844 |
| (70,7426) | Karect | 85,491 | 224,089 | 7.62 | 14,462 | 14 | 78,927 | 7 | 0.0089 | 9,750 | 12.3532 | 14,004 | 17.7430 | 55,166 | 69.8950 |

\(^a\) The parameter of kmer range of miRECa: \([8\, 25]\).

\(^b\) The parameters of Karect: \(-matchtype=hamming\, -celltype=haploid\).

\(^c\) D18-6962\(^1\) (180719Ded\(_{1}\)D18-6962\(_{1}\)sequence.fastq) and D18-6962\(^2\) (180719Ded\(_{2}\)D18-6962\(_{2}\)sequence.fastq) from GSE139936.GSM4149813; D18-6963\(^1\) (180719Ded\(_{1}\)D18-6963\(_{1}\)sequence.fastq) and D18-6963\(^2\) (180719Ded\(_{2}\)D18-6963\(_{2}\)sequence.fastq) from GSE139936.GSM4149814; D18-6964\(^1\) (180719Ded\(_{1}\)D18-6964\(_{1}\)sequence.fastq) and D18-6964\(^2\) (180719Ded\(_{2}\)D18-6964\(_{2}\)sequence.fastq) from GSE139936.GSM4149815; D19-10246 (D19-10246.assembled.2NN.fastq) from GEO accession GSE159434.
these errors for correction. If the read count of a miRNA is not zero but only 1, 2 or 3, again our algorithm is unable to detect the erroneous reads of this miRNA for correction (see examples in the ‘Editing distance = 0’ column of Table 11).

Although having these challenges, in this work, we have proposed an effective miRNA sequencing error correction method named miREC, which is the first tool to address the error correction problem in the area. The novelty of the method is a 3-layer k-mer–(k + 1)mer–(k – 1)mer lattice structure to hold the kmer’s supersets and subsets’ frequency differences which underline the locations of the errors and the correcting templates. Our miREC has showed excellent performance to rectify not only substitution errors but also indel errors at both simulated and real miRNA sequencing datasets. The experiments conducted with different running parameters showed that the miREC is insensitive to datasets and it has good robustness to guarantee high-quality correction performance. Our error correction performance have been also verified on the control and spike-in sequencing datasets of the 963 synthetic miRNAs from the miXplore Universal Reference. With the precise aberration correction and free of new error introduction, we are able to conduct ultrafine analysis on miRNA sequencing data at the single base resolution. The method is immediately applicable to miRNA sequencing datasets from the fields of plant biology and cancer biology which are worth future investigation in detail.

DATA AVAILABILITY

The computational programs and datasets used in this work are available at https://github.com/XuanrZhang/miREC.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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