

## All-in-one sequencing: an improved library preparation method for cost-effective and high-throughput next-generation sequencing

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### Abstract

Next generation sequencing (NGS) has been widely used in biological research, due to its rapid decrease in cost and increasing ability to generate data. However, while the sequence generation step has seen many improvements over time, the library preparation step has not, resulting in low-efficiency library preparation methods, especially for the most time-consuming and labor-intensive steps: size-selection and quantification. Consequently, there can be bottlenecks in projects with large sample cohorts.

Here, We have described the All-In-One sequencing (AIO-seq) method, where instead of performing size-selection and quantification for samples individually, one sample one tube, up to 116 samples are pooled and analyzed in a single tube, ‘All-In-One’. The AIO-seq method pools libraries based on the samples’ expected data yields and the calculated concentrations of the size selected regions (target region), which can easily be obtained with the Agilent 2100 Bioanalyzer and Qubit Fluorometer. AIO-seq was applied to whole genome sequencing and RNA-seq libraries successfully, and it is envisaged that it could be applied to any type of NGS library, such as chromatin immunoprecipitation coupled with massively parallel sequencing, assays for transposase-accessible chromatin with high-throughput sequencing, and high-throughput chromosome conformation capture. We also demonstrated that for genetic population samples with low coverage sequences, like recombinant inbred lines (RIL), AIO-seq could be further simplified, by mixing the libraries immediately after PCR, without calculating the target region concentrations.

Thus, The AIO-seq method is labor saving and cost effective, and suitable for projects with large sample cohorts, like those used in plant breeding or population genetics research.

### Workflow of AIO-seq

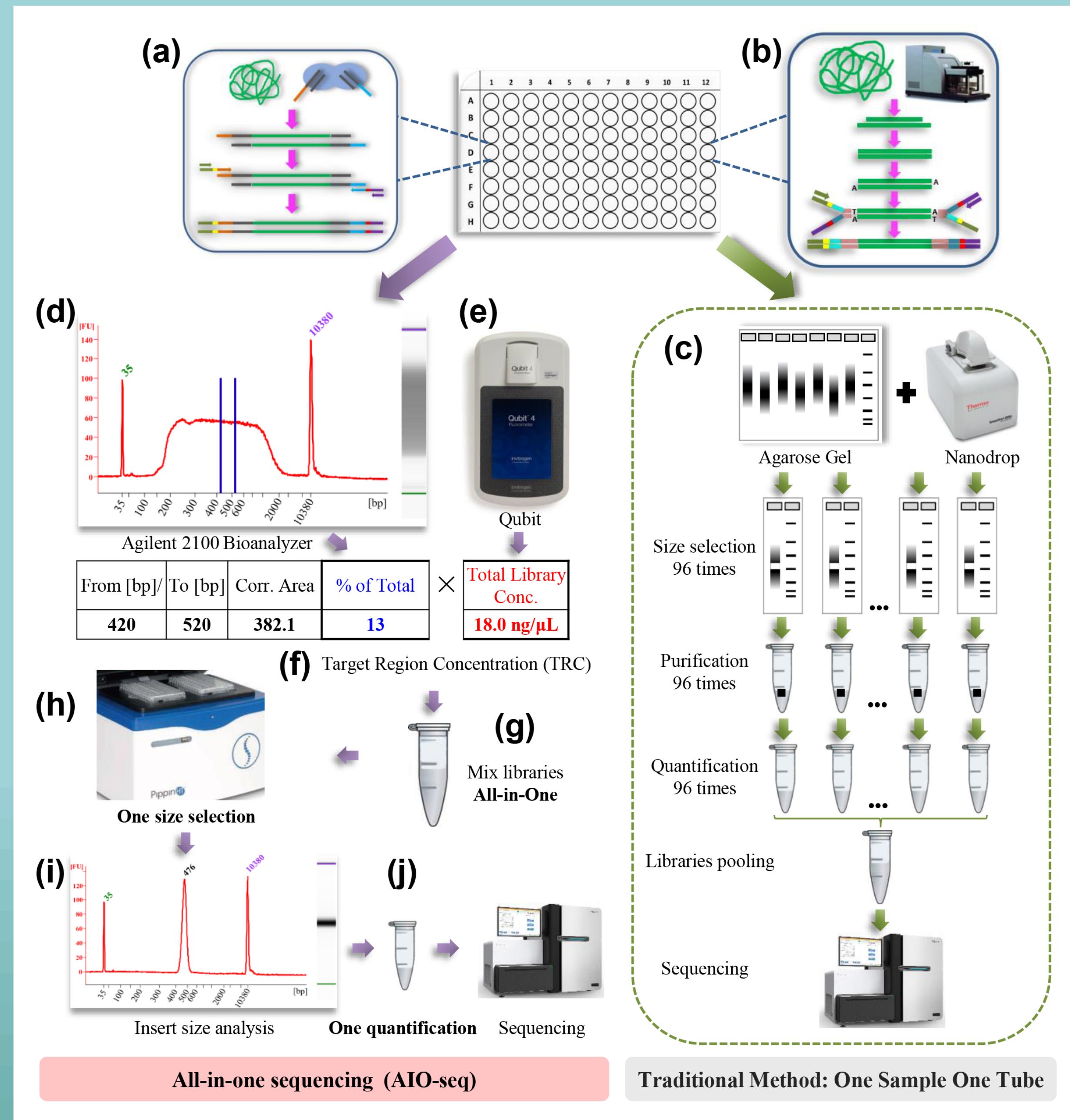


Fig.1 Flowchart for the All-in-One sequencing (AIO-seq) method

- (a) The libraries were prepared using Tn5 transposase.
- (b) The process of mechanical fragmentation was used to prepare the libraries.
- (c) In the traditional protocol, the size selection, and quantification were processed using a one sample one tube method.
- (d) With the AIO-seq method, the library analyzed by the Agilent 2100 Bioanalyzer will give the fragment distribution pattern and the ratio of the target region (between the two blue lines) to the total library.
- (e) The concentration of the total library could be obtained by Qubit™ 4.0 Fluorometer.
- (f) The target region concentrations (TRC) were calculated within each library by multiplying the proportion of the target region from (d) and the total library concentration from (e).
- (g) Mixing the libraries in one tube according to the calculated TRC and their expected yields of the sequence data.
- (h-i) One size selection by Sage HT.
- (j) Quantification of the selected fragment by qPCR and sequencing.

### Results

#### 1. AIO-seq could produce evenly distributed data outputs among multiple samples, and not impair the sequence quality for further analysis

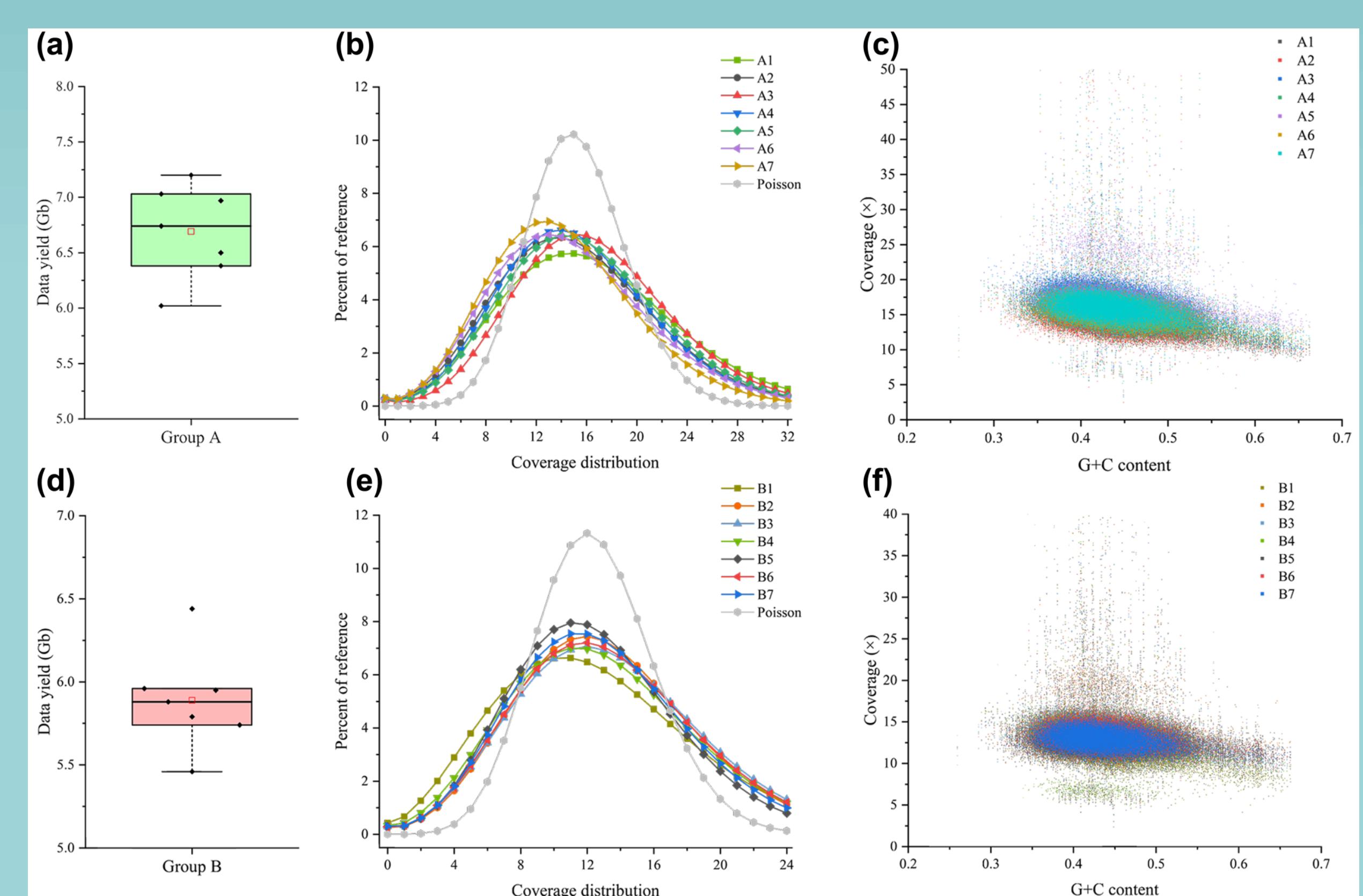


Fig.2 Data yield distribution and sequence coverage

#### 2. AIO-seq could obtain either the same or different expected data yields when more libraries were mixed in a single tube, and also worked well with RNA-seq

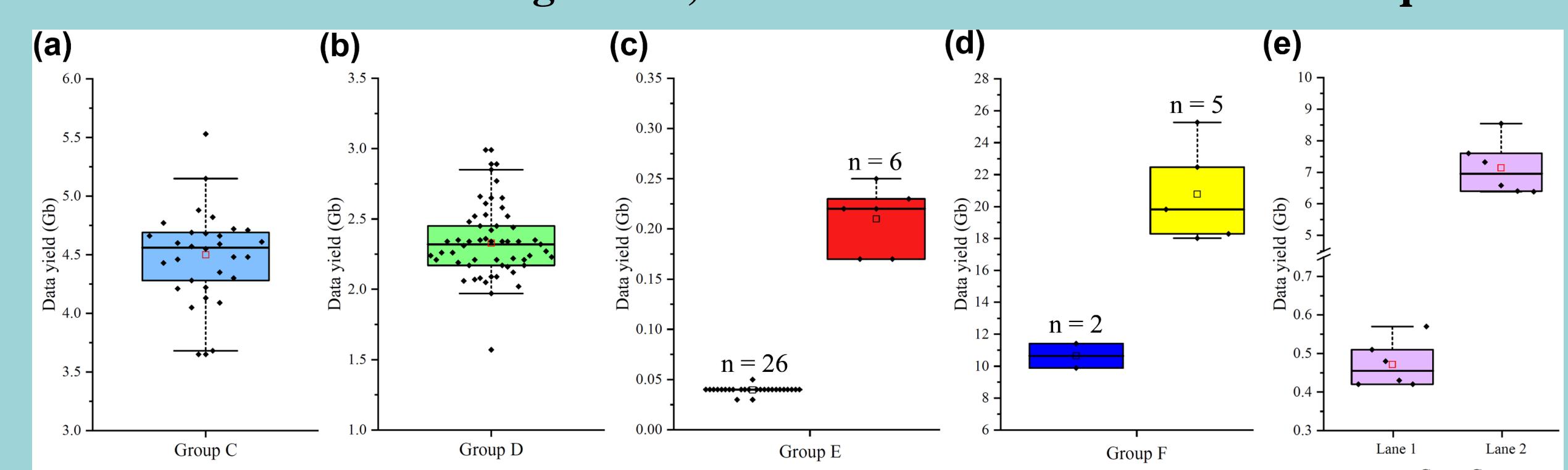


Fig.3 The distribution of data yields among the mixed samples in different groups

#### 3. Sequencing data derived from AIO-seq could be successfully applied to RIL populations for construction of linkage map and QTL mapping

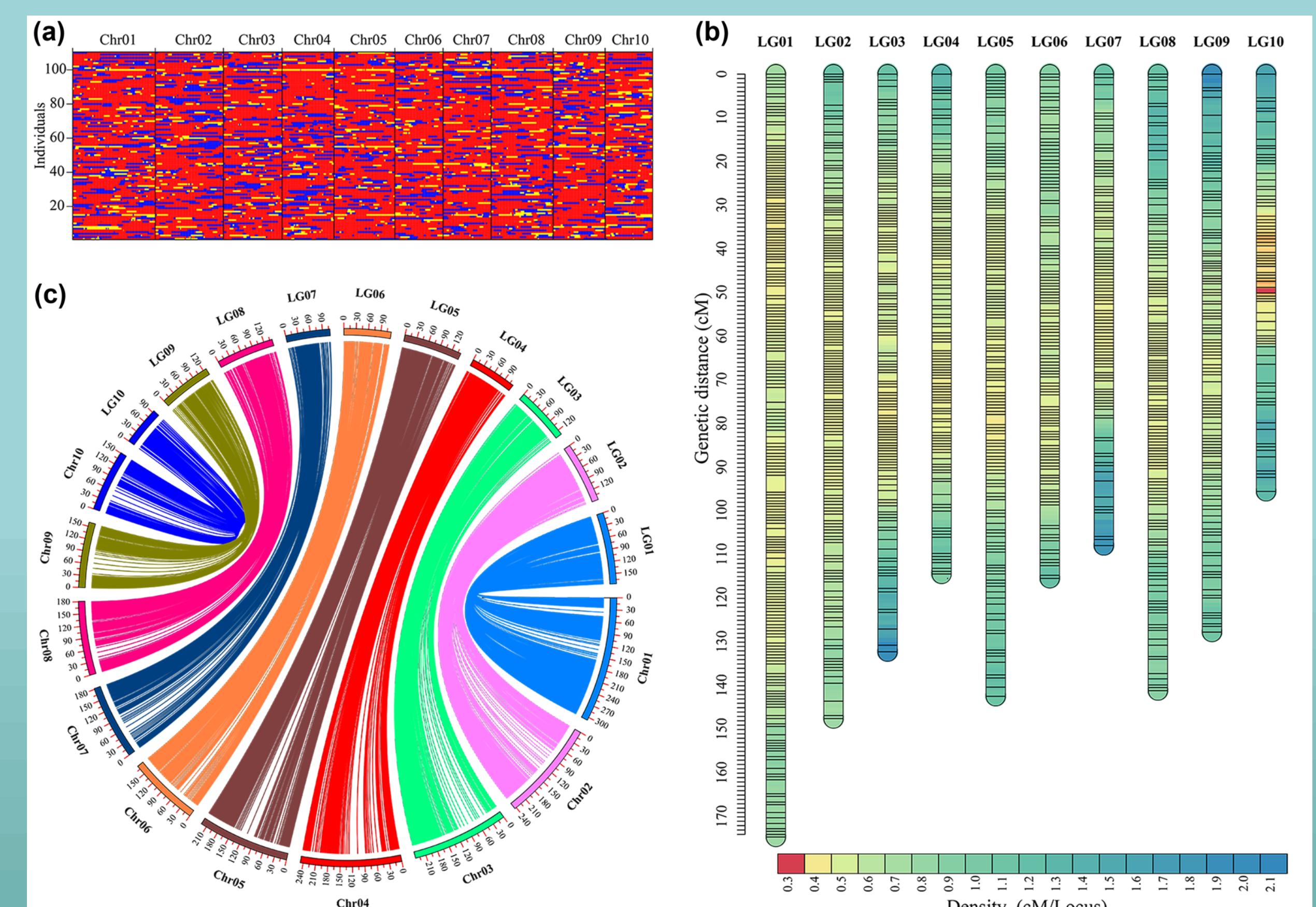


Fig.4 Map construction and collinearity analysis of a maize BC<sub>1</sub>F<sub>4</sub> population

### Conclusions

1. AIO-seq method was developed and presented here, which is a highly efficient and cost-effective improvement for the preparation of NGS libraries. It replaces the standard ‘one sample, one tube’ method for the size selection and quantification step, with a multiple samples ‘all-in-one tube’ method.
2. We have demonstrated the practicability of AIO-seq method for multiplexing WGS libraries, where the mixed samples in a single tube had either the same or different expected data yields, to sequence in a whole or partial HiSeq X lane.
3. AIO-seq also worked for an RNA-seq library and has the potential to be applied to numerous other NGS libraries.
4. As sequencing library preparations are limiting steps for large sample cohorts, our method has a great perspective on population genetic studies and plant breeding research.