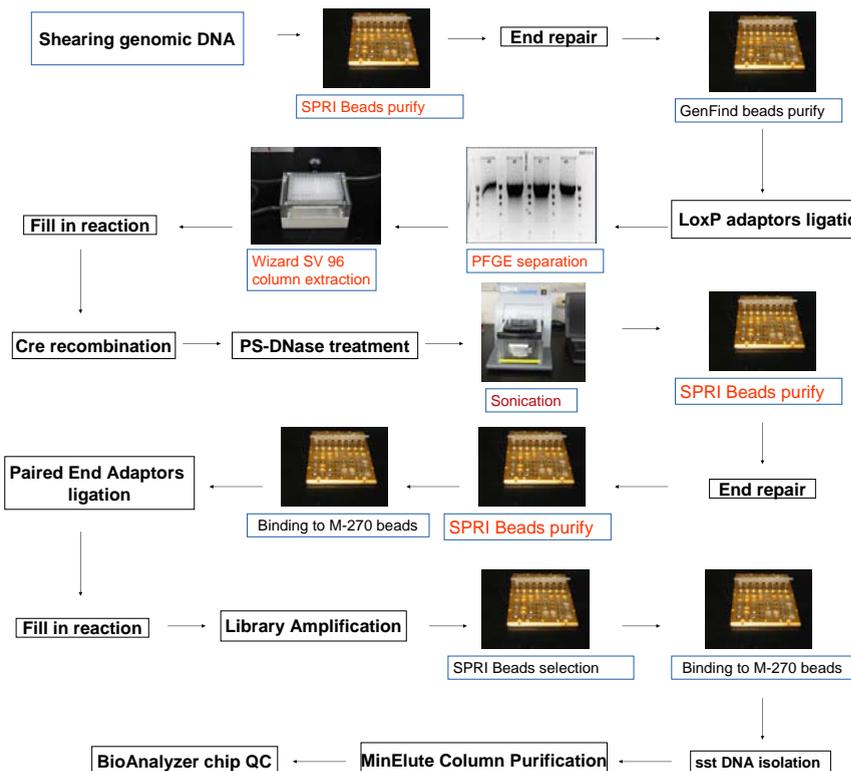


Abstraction

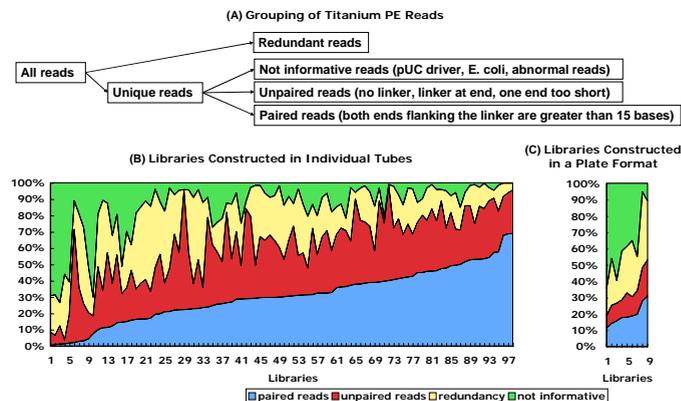
Paired-end library sequencing has been proven useful in scaffold formation during the *de novo* assembly of genomic sequences. The current protocol for constructing 454 Titanium paired-end libraries (Roche/454 unpublished) has allowed a routine production of libraries with 3 to 20 Kb insert sizes, which is suitable for assembling large eukaryote genomes with large segments of repeats. This protocol however was designed for manual operation that handles individual tubes, which is laborious and low in throughput. To increase the productivity, we modified the procedure by adopting an 8-strip tube, multiple channel pipette, and 96 magnetic O-ring plate. Other modifications include using the Promega Wizard SV 96-well column instead of gel electroelution after gel separation; replacing nebulization with sonication; and applying AMPure-Beads in certain steps for DNA purification. These changes could increase the productivity of our manual library construction process to 24 libraries a week. These modifications also make it possible for the future development of automatic or semi-automatic procedures. Furthermore, we have also modified some of the steps to improve the library quality and productivity. These steps include applying UV cross linked DNA as carrier DNA and using barcoded adapters in library construction for sequencing pooled libraries.

Modification of Titanium paired-end Protocol to 8-Strip tube Method

We do all enzyme reaction and beads binding and washing in 8-strip tubes and using multiple channel pipette.



Quality Assessment of the Titanium Paired-end Libraries



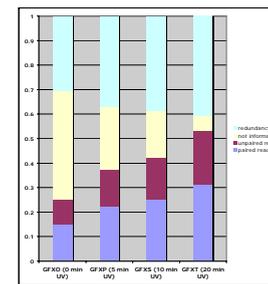
The quality assessment process of the Titanium PE libraries is shown in (A). We grouped the reads in 4 categories. The redundant reads are defined as any two reads with greater than 95% of sequence match and no more than 2 bases difference in sequence length. We examined sequences of 98 Titanium PE libraries constructed using the 454 Recomb PE protocol (B). Of them, 9 contain less than 10% paired reads, 30 contain 10-30% paired reads, and 58 contain greater than 30% paired reads. The average insert size is about 17Kb. The chimeric rate based on two finished genomes is less than 5%. We have also constructed 9 PE libraries using the 96-well plate format. Their paired reads percentage ranges from 11.5 to 30.9% (C). We are in the process of optimizing this process.

UV Radiation Effect on Carrier DNA Contamination

To decrease carrier DNA amplify in the library amplification step, different time UV radiated pUC19 DNA as a carrier has been tested using the 8-strip tube method. As show in (A), with the radiation time increase, more supercore DNA changed to be linear DNA; some inter-molecular cross links also appeared in 20 minutes radiation sample. Picture (B) shows carrier DNA contamination were dramatically decreased with the UV radiation time increase, indicating that UV induced DNA cross link prevent the carrier DNA amplification.



(A). Agarose gel electrophoresis of UV radiated pUC19



(B). Quality analysis of libraries constructed using UV radiated pUC19 as carrier

Conclusion

We have modified Roche/454 titanium paired-end library protocol to scale up production. Further improvement of library quality is needed.

Acknowledgements

We would like to thank Roche for providing early access to Titanium paired-end library construction reagents and protocol.