DIRECT SEQUENCING FROM CULTURE

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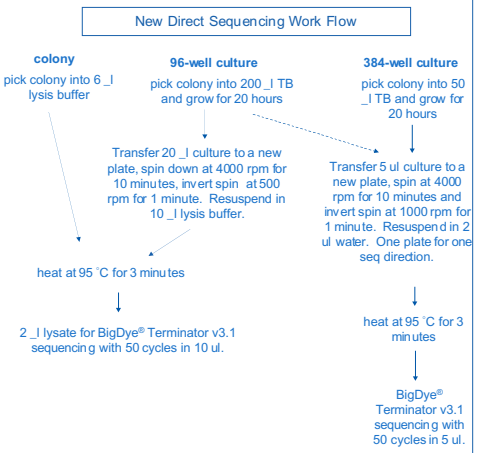
ABSTRACT

Genome centers are continually working to increase the throughput and decrease the reagent costs and manual labor of their sequencing process, including the plasmid template preparation step. Researchers have attempted to bypass this step by directly sequencing from bacterial cultures, but in the past the results have been considerably inferior to standard purified plasmid sequencing, with lower success rate and shorter read lengths. Using BigDye® Terminator chemistry on the Applied Biosystems 3730xl DNA Analyzer, we have recently obtained very promising results—we are able to produce read lengths and success rates comparable to high throughput purified plasmid sequencing. Here we will present the results of our studies to optimize direct culture sequencing from various template types, using 96 and 384-well format. We have also seen promising results with direct sequencing from colonies. This allows much faster screening of libraries for sequences of interest.

MATERIALS AND METHODS

The following conditions were used in this study:

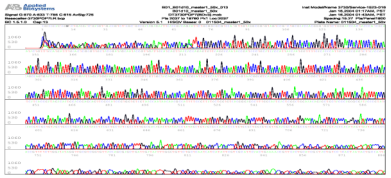
- Clones: ~2 kb inserts in pUC19 vector and DH5α cell or 0.5 to 7 kb inserts in pSport (high copy number vector);
- Chemistry: unless otherwise indicated, 0.8 ul BigDye® Terminator v3.1 Kit in 10 ul reaction;
- Analysis: 3730xl DNA Analyzer using LongSeq50_POP7_1 module with KB™ basecall [KB_3730_POP7_BDTv3.mob / kb.bcp] or ABI basecall [DT3730POP7{BDv3}.mob / Basecaller-3730POP7LR.bcp];
- Quality Assessment: KB20 or Phred20 Length of Read (LOR) with sliding window of 50 bases.



Direct sequencing from culture will produce more robust result.

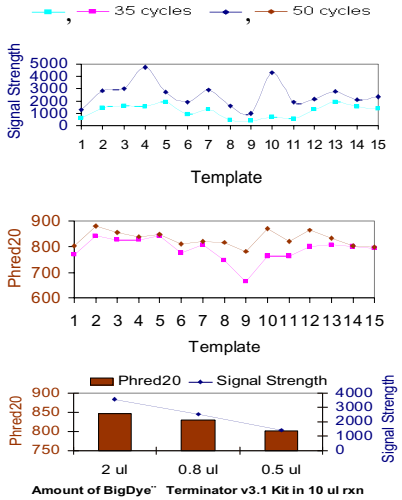
RESULTS

Direct culture sequencing



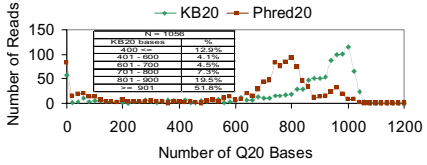
Direct culture sequencing conditions

Comparison of direct culture sequencing with 35 and 50 cycles. The signal strengths and Phred20 scores are improved with 50 cycles. As the amount of the BigDye® Terminator Ready Reaction Kit decreases, the quality of the sequence decreases.



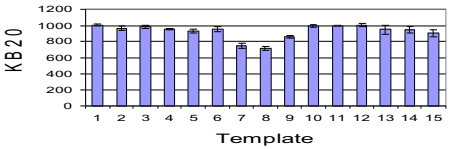
Direct culture sequencing of cDNA and BAC subclones

Libraries containing inserts from 0.5 to 7 kb in pSport or pUC vectors were directly sequenced from culture. Reactions were performed in 10 ul using 0.8 ul BigDye® Terminator v3.1 Kit for 50 cycles. After EDTA/Ethanol precipitation, the amplified fragments were resuspended in 10 ul Hi-Di Formamide.



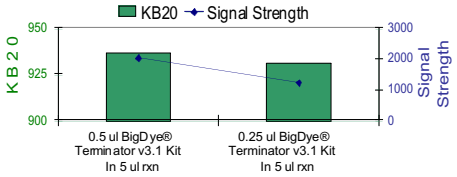
Direct colony sequencing

KB20 score of direct colony sequencing from 15 clones and 4 replicas for each template.



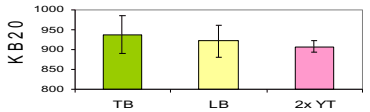
Direct culture sequencing in a 384-well format

Culturing and sequencing were applied in 384-well plates. At each point is an average of 120 templates or two batches. Both protocols can provide more than 900 KB20 bases. More BigDye® Terminator Kit gives better quality bases and signal strength.



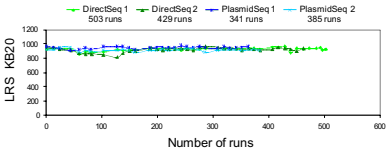
Effect of growth medium in direct culture sequencing

KB20 of cultures from 3 growth media 15 clones two replica each grow in a 20 hours culture in an incubator.



3730xl Analyzer array lifetime study of direct culture sequencing

For direct culture sequencing or plasmid sequencing, more than 300 runs have been loaded on each of four 3730xl DNA Analyzers. Out of every 11 runs, there are nine reaction loadings followed by two Long Read Standard loadings. The graph below tracks the performance of these intermittent Long Read Standard runs as an indication of array quality. Throughout the tracking of Long Read Standard, there are no statistical differences between the array lifetime of loading direct culture sequencing and the array lifetime of purified plasmid DNA sequencing.



CONCLUSIONS

We have developed protocols for direct sequencing from colony or culture in 96-well or 384-well format. Following these protocols, we have obtained good read lengths. The data quality from these protocols are comparable with high throughput plasmid sequencing for high copy number clones. From 3730xl DNA Analyzer array lifetime study, the direct sequencing will not accelerate capillary degradation.

TRADEMARKS/LICENSING