**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 processes, 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 consistently 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 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.

**RESULTS**

**Direct culture sequencing**  
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 colony sequencing**  
KB20 scores 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 grown in a 20 hours culture in an incubator.

**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 pJ70 (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 LysingCFO POP7, 1 module with KB20 basecall [KB_20], POP7, POP7_SOD v3.0 and KB20_proj, 1 module with ABI basecall [DT3730POP7{BDv3}.mob / Basecaller-3730POP7LR.bcp];
- **Quality Assessment**: KB20 or Phred20 Length of Read (LOR) with sliding window of 50 bases.

**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**

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