Plasmid DNA Sample Preparation and Purification Hints

Purpose

To submit good quality plasmid DNA samples for sequencing at the SegoliP Unit.

Before submission of Plasmid DNA Samples for Sequencing, the client should:

1. Ensure that the plasmid template supplied is of sufficient quality and quantity. The starting template DNA is the single most important determinant of the quality of the final sequencing data.
2. Verify the presence and size of the insert in the Plasmid DNA.

The Template Quality

1. The Qiagen[1] plasmid DNA kits are the most reliable way of isolating high quality plasmid DNA suitable for automated sequencing and we recommend their use for template DNA purification.

   Some common mistakes which compromise the final quality of sequence data include:

   a. The Isopropanol-precipitated DNA is not washed with 70% ethanol to remove excess salt.

   - Ensure that the DNA pellet is washed at least once as directed with 70% ethanol. Residual salt in the final template will interfere with the activity of Taq polymerase resulting in sequence data of less than 200 bases from the primer with a low signal.

   b. The template DNA is not dried completely before final resuspension in H_2O.

   - To remove residual ethanol, dry the DNA for 5 minutes in a functional speedvac.
   - If air-drying is preferred, a brief 15 min incubation of the open plate at 65^{o}C is often sufficient to completely dry the DNA.

   c. The directions for cell growth are not followed resulting in overloading the Qiagen resin.

   - A poor yield of plasmid DNA results, presumably due to competition with RNA fragments for binding to the Qiagen resin.
   - Use the recommended quantity of LB broth (don't use Terrific broth) for cell growth.

2. The Promega Wizard® DNA purification systems are good for consistent in preparing high quality template DNA.
   - Add an extra ethanol precipitation step at the end of the method for more reliable results.
3. The E. coli host strain used to propagate the plasmid can influence the quality of sequence data.

[1] This does not represent an endorsement of Qiagen products. There are numerous methods and plasmid preparation kits on the market which can give template of excellent quality.

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a. The commonly used strains, such as HB101, DH1, DH5a, and XL1Blue consistently produce high quality plasmid DNA.

b. Attention to good microbiological practices - using antibiotic selection when propagating plasmids and streaking out colonies from transformation plates to obtain "pure" single bacterial clones, is beneficial in obtaining good plasmid DNA yields and a homogenous population of plasmid DNA molecules (only one plasmid in the final DNA preparation).

The Template Quantity

1. The quantity of template is an important determinant of the accuracy and reliability of the final sequence data.
   a. Too little template results in reactions with little or no signal and poor or no basecalling.
   b. Too much DNA produces reactions which terminate prematurely, often with less than 250 bases of reliable sequence data.
2. The optimum amount of plasmid DNA for a BigDye Terminator cycle sequencing reaction is 100-300ng. Therefore, the accurate quantization of template DNA is an important step in the overall sequencing process.
3. We recommend Nanodrop spectrophotometry, in determination of Plasmid DNA concentration (recall that the absorbance @ 260 nm of a 50 µg/ml solution of ds DNA is 1) before submission to the unit.