NOTE: This protocol was taken from the website of another DNA Sequencing Facility – http://www.analyticalgenetics.ca/PlasmidSample.php. We believe it represents valid information, but we have not personally tested its recommendations.

Plasmid Sample Preparation: Plasmid templates can be prepared using a variety of protocols. Commercial columns (Qiagen or equivalent) and standard alkaline lysis methods are acceptable. Do not use a boiling preparation method unless the samples are phenol extracted to remove the protein! Excess protein in samples causes smearing and reduces the lifetime of capillary arrays. The cleaner the DNA, the better the sequence! Miniprep kits do not ensure cycle-sequencing grade DNA, especially if yield is low! Here are some suggestions for preparation of your plasmids.

- **1. Competent cell selection:**
  Certain strains of *E. coli* are better for plasmid production. Recommended strains are: XL1-Blue, DH5(alpha), DH1, C600 SURE, NM294. Other strains such as JM83, JM101, NM522, NM544, TB1, TG1, BL21, MC1061, and Y1088 often yield DNA that is of low quality, and additional purification steps (such as the minimal isopropanol precipitation described below) will be necessary.

- **2. Growing up cells:**
  Do not overgrow *E. coli* cells especially in simple LB, as the cells decline rapidly after reaching stationary phase (usually by low pH inhibition). Overgrowth can result in release of chromosomal DNA and increases in polysaccharides that co-purify with plasmids and inhibit sequencing reactions.

- **3. Prewash:**
  A 0.5 M NaCl wash of the cells prior to lysis can be very helpful in removing polysaccharides and media components that often copurify with the DNA and inhibit sequencing reactions. Simply resuspend the cell pellet completely by vortexing, then re-spin.

- **4. Remove salt from column:**
  Remove salt from plasmid prep columns by washing at least 2X with the washing buffer (e.g., Qiagen buffer PE). As little as 10-20 mM salt can inhibit a sequencing reaction! It is also beneficial to wash 2-3X with the binding solution to remove more inhibitors. It is important to remove the alcohol from the column before DNA elution as too much inhibits the reaction later.

- **5. Remove RNA contamination:**
  The majority of the RNA should be removed from miniprep DNA, either by RNase, high salt precipitation or column purification.

- **6. Avoid inhibitors:**
  For standard alkaline or boiling minipreps, it is important to avoid the co-precipitation of inhibitors from the cell lysate, especially when columns are not used for purification. For some samples, one or more isopropanol precipitations helps to remove inhibitory compounds. To a fairly dilute DNA solution (<100 ng/µl), add 0.5X volume of 7.5 M ammonium acetate or 0.1X volume of 3 M sodium acetate; mix well; add 0.6X volumes of room temperature isopropanol (calculated using the new volume of DNA and salt); mix well; and, spin after 5 minutes at room temperature. Follow with a full-tube 70% ethanol-wash of the pellet. Please note that standard 2X volume ethanol or 1X volume isopropanol precipitation protocols may not be sufficient to precipitate marginal templates.