Plasmid templates

Considerations when cleaning up plasmid preps

Poor template quality is one of the most common reasons for bad sequence data, as mentioned above, and is a prime consideration when choosing a plasmid cleanup method to give DNA of optimal purity for automated sequencing. Plasmid template quality can be affected by a variety of factors and contaminants including the following:

* Salts or organics left over from template preparation
* Presence of cellular components such as RNA, proteins, polysaccharides or chromosomal DNA
* DNA that has degraded while in storage
* silica fines that carryover from template preparation kits that utilize loose resin or silica solutions

Here is a table of allowable contaminants and their acceptable concentration ranges that will still allow for good sequencing data:

CONTAMINANT	AMOUNT TOLERATED IN SEQUENCING REACTION
RNA	1 ug
PEG	0.3%
NaOAc	5-10mM
Ethanol	1.25%
Phenol	0%
CsCl	5mM
EDTA	0.25mM

Some comments on the effects of some of these contaminants are listed below:

RNA - As you can see from the value in the chart above, a significant amount of RNA can actually be tolerated in the sequencing reaction. It is a common contaminant in plasmid minipreps, especially when columns are overloaded and the capacity of the lysis buffers is exceeded - overloading can be a problem with Qiagen minipreps. One of the ways RNA can potentially interfere is when quantitating your DNA preps by spectrophotometer- RNA also absorbs at 260 and when there is a large amount present, it can really throw off the accuracy of your concentration. It's best, then, to treat your template preparations with RNase or high salt precipitation and also to quantitate your samples both by gel as well as spectroscopically. More on that in Methods for Quantitation.

PEG- the presence of residual polyethylene glycol in the template prep can have an inhibitory effect on the cycle sequencing Taq polymerase enzyme and lead to weak signal.

Salts- the processivity of the Taq polymerase used in the cycle sequencing reaction declines in the presence of high amounts of salts. Salt contamination in DNA preps may result from coprecipitation of salts in alcohol precipitations, insufficient removal of supernatant after precipitations or an incomplete wash of the pellet with 70% ethanol. Careful technique should be used when precipitating with alcohol. It has also been demonstrated that acetate ions, as opposed to sodium, potassium or chloride ions, are the most inhibitory in sequencing reactions. When using potassium acetate or sodium acetate, concentrations over 20 mM led to complete failure of the sequencing reactions, while concentrations of 60mM of sodium chloride were required before complete inhibition. Salts can be inhibitory when we sequence samples on our gel-based 377 but are even more problematic when running samples on our 3100 capillary system as these smaller, charged salts are preferentially injected and interfere with the migration of the DNA samples.

Ethanol- ethanol contamination can occur when the sample is insufficiently dried after precipitation or when carried over in an ethanol-containing wash buffer used in some DNA isolation procedures. Contamination with 10% or greater concentrations of ethanol usually leads to failure of the DNA sequencing reaction. Complete drying of the DNA samples is required to remove these traces of ethanol.

Phenol- phenol may be carried over from DNA alkaline lysis methods that utilize phenol and chloroform to remove proteins and other cellular contaminants from cell lysates. Phenol cannot be tolerated in the cycle sequencing reaction as it denatures proteins and will thus degrade the Taq polymerase enzyme used in the cycle sequencing reaction. Chloroform does not have the strong denaturing properties of phenol and doesn't appear to adversely affect the sequencing reaction.

Cesium chloride - When using a cesium chloride ultracentrifugation density gradient protocol, one can obtain DNA of very high quality suitable for automated sequencing. HOWEVER, it is strongly recommended that you either perform dialysis followed by ethanol precipitation (best method) or do a minimal room temperature isopropanol precipitation to remove all traces of residual cesium chloride as the cesium can inhibit the Taq polymerase used in the cycle sequencing reaction.

EDTA - EDTA can chelate the magnesium required by the Taq polymerase in the cycle sequencing reaction, so when submitting samples, it is best to always have them diluted or resuspended in sterile ddH20 or 1X Tris buffer. Suspension in TE buffer is not recommended, though people have done it and many times there is not a problem. However, providing template DNA in water is an easy thing to do and if there is a problem with your sequence quality, the fact that there is no EDTA in your sample is one potential problem we can eliminate right away.