Tips for pure DNA preps

https://confluence.crbs.ucsd.edu/display/NCMIRWIKI/Sosinsky%27s+lab+protocols

Tips for highly pure DNA preps:

1- Make sure your stocks of media are not contaminated.

Always pass the top part of the bottle on a flame after pouring media or aspirating it, in order to prevent that the bacteria in the air can get inside the bottle and grow. Also always work close to a flame. That helps to keep away or burn the bacteria in the air!

2 - Make sure your antibiotic stocks are efficient.

Don’t thaw and freeze multiple times to avoid the antibiotic degradation.

Do not keep at room temperature. If you thaw a large aliquot and you have a large left over, store it in the refrigerator until you use it all. In the refrigerator the antibiotics can be stable for up to three months.

3 - After transformation, plate the bacteria (close to a flame) on fresh antibiotic plates.

If your plates have been sitting in the refrigerator for more than 3 months you can discard them and make new ones or spread few microliters of ampicillin and wait for it to adsorb completely. Usually bacteria survive to slight higher antibiotic concentrations than the ones recommended.

4 - Always try to plate bacteria before leaving for the day, so that the bacteria colonies won’t grow for too long in the incubator.

Avoid picking colonies when the satellite colonies (made by untransformed bacteria) over grew. It may be confusing to pick the right colonies! If you can’t pick the colonies that same day, have somebody parafilming your plate and putting it in the refrigerator in the morning. If you are doubtful about a colony you picked, you can first streak it on a plate and the day after, start your liquid cultures from single colonies.

5 - Avoid contact contamination at the bacteria inoculation step.

When inoculating the bacteria for growth in LB media for miniprep, make sure to use sterile tips or sterile tooth sticks. Avoid touching, even with gloves, any part of the tube or of the tips that will get in contact with the bacteria.

6 – Avoid liquid culture over growth, as that will cause plasmid lose!!!

Inoculate the bacteria always late in the afternoon, possibly as the last thing of the day. If you come late to the lab, have somebody moving your liquid cultures from the 37 degrees incubator to the refrigerator or at room temperature for you. It’s better to have the bacteria as pellet before the preparation, rather than overgrowing bacteria that will lose the plasmid you need to purify.

Bacteria overgrowing not only causes low yield of DNA after purification, but also contamination due to untransformed bacteria that might grow faster due to the ampicillin degradation.

Another way to avoid this problem is to use an equivalent selecting agent to ampicillin called carbenicillin.

“Carbenicillin demonstrates improved stability over ampicillin when used in growth media. It is more resistant to heat and low pH- induced degradation over time making it particularly useful for large-scale liquid culture growth. It also reduces the presence of satellite colonies seen with ampicillin. Carbenicillin is recommended as a substitute for ampicillin at the same concentration in molecular biology applications”.

I never tried carbenicillin myself, but it can be a good option.

7- Make sure your EB buffer has not contaminants or it’s no too old.

In my experience sometimes the EB that comes with the kit was sitting on my bench for too long and I started having weird DNA preps no matter what.

In this case you can:

1- Throw away the bottle and use a new one.

2- Check the pH of your bottle.
3- Make a streak on a plate without antibiotics and see what grows in it.

You can also filter it and see if it improves.

*As a general note try to use sterile conditions as much as possible and flame tube tops, caps, spatulas and whatever you are using that can be passed on flame.*