Summary of Q&A (last updated on 09/19/2016)

1. During Extraction

Q#1. What are the results expected for the isolation of DNA, and the yield of the Isolated DNA? What are possible reasons that would cause genomic DNA degradation during isolation?

A. I found a good example for the DNA gel in the below picture.

For 1.5ml overnight cell culture, typically I can obtain 5-10 ug DNA. For *E. coli* genomic DNA, you expect to see bands with smear patterns from high to low MW range, although most of DNA fragments are accumulated at high MW) on the gel. So, if you see most of DNA fragments are small, very likely your DNA got degraded. Two possible reasons that may cause DNA degradation in this protocol: make sure do not vigorously vortex and pipet of DNA solutions at step6 or subsequent steps. It may have some DNases contamination in your sample. DNases released from the lysed cells are supposed to be removed by phenol and chloroform extraction (involves protein denaturation too). So, make sure reagents and materials you use are DNase –free ("Molecular Biology" grade reagents), especially RNase. You can try to check your DNA on the gel before you treat it with RNase. As mentioned at step 15 above, you should be able to see (try to see the tube against light) DNA precipitation (white color) floating in the solution after mixed with cold ethanol DNA precipitation. If not, that means you have lost the DNA before that. If you do see it, you can keep the tube at -20 degree overnight, which will increase the yield. Another way to increase the yield is that you could use more cell culture (e.g. 5 ml). Another option is to try some commercial kits, like QIAamp DNA Mini Kit. They should be good, but expensive. (I personally nerve tried them though)
Q#2. If we use RNAase in the step 7, incubate with the protease and SDS. Will the protease or SDS denature or degrade my RNAase. I think that use the phenol/chloroform to get rid of protease and then incubate with RNAase then repeat again with phenol/chloroform to eliminate the RNAase.

A. You can inactivate protease K at 95 °C for 15 min.

Q#3. What are the purposes of each reagent used in this protocol including proteinase K, SDS, sodium acetate, EDTA, DNases, Phenol/chloroform and Ethanol in this protocol?

A. In lysis buffer, proteinase K is to digest proteins including membrane proteins, and SDS facilitates digestion of cells by denaturing and solubilizing membrane proteins. EDTA is used to inhibit DNases. Phenol/chloroform is used to remove proteins from DNA sample. Ethanol with 2.5-3 vol. (stored at -20 C) is used to precipitate DNA. High concentration of sodium acetate (3M) can be used to precipitate high molecular weight molecules including genomic DNA. I did not use it in my experiments. But, it might help to increase the DNA yield by using sodium acetate.

Q#4. Why is DNA unaffected by phenol/chloroform?

A. Phenol/chloroform, used in the above protocol are used to denature proteins, not nucleic acids. However, oxidized phenol can damage the nucleic acids. For some related references, you may find it at http://en.wikipedia.org/wiki/Phenol%E2%80%93chloroform_extraction.

2. Post Extraction

Q#5. How to know the difference between the DNA, RNA and plasmid bands on an agarose gel under UV?

A. You may be able to tell the difference based on their patterns and sizes. For genomic DNA, most of them are very big and do not migrate far from well. And, since they often consist of DNA fragments with different sizes, you can bands with the big range of sizes (bands are smear if many got degraded).

For plasmid DNA, when they are uncut, they often appear more than one band on the gel (due to different topology). To confirm the plasmid DNA of your interest, you can cut the DNA with known restriction enzymes before run the gel.

For RNA, I do not have much experience on running specific RNA samples on agarose gels (may need denaturing agarose gel). But, for RNA contamination found in your DNA prep (without RNase treatment), RNA bands (often very bright) migrate toward the bottom of the gel.
Q#6. How to carry out calculation to know the purity of genomic DNA isolated from E.coli.

A. You can quickly get some idea (not accurate estimation, though) about the purity of genomic DNA by the following three ways:

1) For checking protein contamination, you can take the reading of A260nm/A280nm. If A260nm/A280nm is about 1.8-2.0, it indicates that your DNA sample is quite pure--not much protein contamination.

2) For checking contamination of some organic compounds or salts (phenol would be the main source of this type of contamination by using the above protocol), take the reading of A260nm/A230nm. For a good DNA prep sample, A260nm/A230nm is about 1.5-1.8.

3) For checking RNA contamination, you can check it on a DNA gel. As mentioned in the protocol, RNA contamination can be easily removed by RNase.

Please see below for some recommended references related to above suggestions:


3. Comparisons with other protocols

Q#7. Will this protocol work if I want to isolate bacterial genomic DNA together with plasmid DNA?

A. While I never tried it, theoretically I think it should work. If you try it with a high-copy plasmid, you may be able to distinguish plasmid DNA from genomic DNA on a DNA gel if it works.

Q#8. Why Glucose/Sucrose is added during plasmid DNA isolation and not during genomic DNA?

A. For the protocol "Plasmid DNA Extraction from E. coli Using Alkaline Lysis Method", Glucose is added (in resuspension solution) to make the solution isotonic. In fact, isotonicity is not required for bacteria with cell walls (i.e. E. coli) because bacteria with cell walls can withstand wide range of solution concentration. Thus, glucose may not have to be included in the resuspension buffer. I never tried the one without glucose, though. So, please share your experience with us if you try it without glucose.
Q#9. *Why the process can work without CTAB (hexadecyltrimethyl ammonium bromide) that used in other protocols.*

A. From the original paper about CTAB method by Kate Wilson ([http://onlinelibrary.wiley.com/doi/10.1002/0471142727.mb0204s56/abstract](http://onlinelibrary.wiley.com/doi/10.1002/0471142727.mb0204s56/abstract)), it seems that CTAB can help to remove polysaccharide contamination in DNA prep. This would be useful since "exopolysaccharides can interfere with the activity of enzymes such as restriction endonucleases and ligases." (Quoted from the original paper).

4. **Genomic DNA extraction from other materials**

Q#10. *Could you suggest a protocol for S.aureus genomic DNA extraction or for gram positive bacteria?*

A. I have asked Prof. Chikara Kaito at the University of Tokyo for help with your question. Below is his response: "The extracting protocol of S. aureus genomic DNA has been written in Novick RP, Methods in Enzymology, Vol 204, 587-636. The method is written on page 589-590. For convenience, we are usually using the extraction Kit from Qiagen (QIAamp DNA Blood Mini Kit, Cat No. 51106) after lysing S. aureus cells with lysostaphin."

Q#11. *How could we isolate the DNA from onions?*

A. Sorry, I've never done that experiment. Please use Bio-protocol RaP service ([http://www.bio-protocol.org/RaP.aspx](http://www.bio-protocol.org/RaP.aspx)) to request such a protocol.

Q#12. *Do you have protocol for whole genome extraction from soil sample?*

A. We have a protocol "Isolating RNA from the Soil" from Jorge Vivanco group (Colorado State University) published online, please the link [http://www.bio-protocol.org/e903](http://www.bio-protocol.org/e903), you can find it by searching some key words, like "RNA, soil" (in this case) on the Bio-protocol home page, which may be interesting to you.

Q#13. *Where can I find a DNA extraction Kit and PCR mastermix to isolate E. coli DNA in groundwater samples?*

A. There are a number of companies (i.e. Qiagen) providing the related commercial products with detailed manufacturer's instructions. Thus, Bio-protocol does not publish this type of protocols as a separate publication. You could either find the instructions on the company websites or contact them directly.