

Outreach Biotechnology Kits – Frequently asked questions.

1. What is the purpose of the RNaseA using in step 15 of the mini-prep? When added does it need to be left in fridge overnight?

Answer: There is a lot of RNA in the mini-prep. It makes a smear on the gel. The RNase gets rid of it. It actually acts quite quickly. As little as 10 minutes at room temp is likely enough.

2. At the end of the mini-prep we should have 22uL of DNA, water and RNaseA in the tubes, correct?

Answer: YES

3. The purpose of the restriction digest then cuts the DNA from the Mini Prep so that when run on the gel we will see a banding pattern, correct?

Answer: YES or there would be multiple circular forms.

4. The Mix of the Restriction Digest should read 5uL of buffer, 10uL of DNA and 5uL of enzyme, correct? Is the DNA from the Mini Prep? Does this mixture need to be incubated for 2 hours?

Answer: Yes it is 10 ul not L. The DNA is from the miniprep. Less than 2 hours should work. I would not use less than 1 hr though.

5. Each team will then load two samples to gel, one of 11uL (plus 2uL of dye) uncut from the miniprep, and 22uL of cut DNA (including dye) from the digest, correct?

Answer: YES

6. I should be able to do all of this in 5 days, correct?

Answer: YES

How to shorten the protocol

After the Ligation and once you have your DNA product, freeze the DNA at -20. Keep the plates in a fridge over the weekend. Finish on Monday.

To complete things on Monday you will need 1 gel box, the power pack, and the transilluminator. Keep **just those** pieces of equipment. Read carefully through the protocol and keep just what you need to complete the last bit of the lab. Keep in mind you may need a pipettor to load the gel, you need 1 vial of loading dye etc. etc. You can dilute your 10X TBE and keep it in your own bottle until you make your gel on Monday.