In vitro transcription pf pFC8 plasmid

Reagents

Name	Volume (ul)
5x Buffer	4
100 mM DTT	4
2.5 mM NTP	1
DNA	2.12
H20	8.88

DNA concentration much lower than was marked on the tube.

Samples

- Control: Master mix only, no transcription
- Transcribed: Master mix + T7 Polymerase + RNAaseA
- Transcribed + RNAase H: Master mix + T7 + RNAaseA Polymerase + RNAaseH

Protocol notes

1. Measure DNA concentration using Nanodrop
2. Calculate volume of DNA sample required for ~ 200 ng per lane
3. Make master mix
4. Aliquot 5 ul for control and remaining sample into PCR reaction tubes.
5. Create thermocycler reaction profile; 37 C for 20 mins then 65 C for 10 mins.
6. Add 0.5 ul T7 Polymerase to the treatment tube (15 ul master mix). Run thermocycler profile.

7. Prepare 0.9 % agarose gel using 40 ml TBE buffer while thermocycler is running.
8. Add 0.5 ul RNAaseA to treatment tube incubate in thermocycler for 20 mins at 37 C.
9. Split treatment tube in half (7.5 ul remove to third PCR tube) and add 0.5 ml RNAaseH to the third sample.
10. Incubate at 37 C for 20 mins.
11. Add protease K to all samples to eat junk and incubate at 37 C for 10 mins.
12. Load samples into gel using purple loading dye.
13. Run get for 1 hr at 90 volts.
14. Remove gel from tray and place into temporary container (tuperware will work) and add 1 ul of ethedium bromide and aggitate on the spinner machine by the gel imager for at least 10 minutes.
15. Image the gel and pray.

Results

Not great lol. Somehow it looks like the DNA did not make it onto the gel.

IVT Plasmid pFC8 T1 T2

Summary

Redoing the IVT assay with different batch of the plasmid.

Reagents

Name	Volume (ul)
5x Buffer	4
100 mM DTT	4
2.5 mM NTP	1
DNA	4
H20	11

Protocol Flow

Note: When using more plasmids do not split the RNAse + and - cases until after the polymerase is added. Can keep these in one tube until then.

Results

There is actually DNA this time but no band shifting :(