Editor Comments

Please sign the article license agreement. (Line 2)

We edited for language, clarity and structure to meet our Journal’s style. I have highlighted portions for filming (max 2.75 pages). Please check if appropriate. (Line 87)

We have revised the protocol to only include preparing the native and denaturing gels, running them and imaging them.

Gel staining solution? Please use consistent naming to avoid confusion.

Naming changed from 5X Gel buffer to 5X Gel Staining Solution to be consistent.

Bit confusing. Do you mean add 0.5+0.5 mL of BB and XC and 8.5 mL ddH2O? please clarify (Line 123)

Yes, this was changed to make it more clear. Both dyes are added into the loading dye solution.

Formula weight? (line 126)

This was changed to make it more clear. Previously said FW which is changed now to formula weight.

V:V? (Line 127)

What speed? (Line 127)

Maximum speed was used and has been clarified in text.

Please clarify, is solution C = 10X TBE? (Line 134)

This has been addressed this by adding 10X TBE in brackets next to solution C so as to imply that solution C is 10x TBE.

Which buffer exactly? The one from 1.1.2? Please be consistent with naming. (Line 148)

This has been addressed when all references to 5X Gel buffer were changed to 5X Gel staining in the third editor comments

Solution C? Please be consistent in terminology (Line 170)

Line 170 said 1X Buffer C which was changed to 1X Solution C to be consistent.

Aspirate? (Line 170)

The word “Blow” was changed to “aspirate” as per the editors request.

What ratio of samples to gel loading solution? (Line 173)

This was clarified to make the 2X denaturing gel loading solution to 1X.

Using a dry/water bath? (Line 174)

Both can be used so this was added.

Cool to what temperature? How long does cooling take? (Line 175)

Cool to touch. Should only be a few minutes. The samples can be cooled at room temperature

How much per well? (Line 175)

XXX This is highly dependent on the comb and the volume of the gel, not sure how to address this XXX

Unclear what is meant. Under which conditions do you skip to 2.1.1?? (Line 188)

Use of the word ‘otherwise’ here is confusing therefore I removed it

Do you mean 2.1.3? Mention exact step numbers, I think it should be 2.1.3-2.1.4 (Line 188)

Exact numbers are now mentioned. Changed from 2.1.1 to 2.1.3- 2.1.4, in order to increase clarity.

What ratio of samples to gel loading solution? (Line 191)

Make it 1X, this is the same as in step 2.1.5 which is addressed above.

Unclear which samples these are. Do you mean RNA samples?(Line 191)

‘Native gel samples” was changed to “RNA samples”

For how long?(Line 192)

Talking about the incubation time for the native gel samples which were incubated to 100 mins prior to running the gel therefore the time has been added.

When and how ate the above samples loaded?(Line 195)

XXX The samples are loaded using a pipette.. do we have to clarify this seems really obvious. XXX

Cannot be made into imperative voice so I have made this a note.(Line 202)

Add to the table of materials.(Line 203)

Reference?(Line 205)

Colors will not show up when the text is integrated on the jove website. Please use a different format or make this a figure instead. If you do so, reference this figure at the end of the last sentence (ending with “fold”) (Line 210)

XXX Should we make this into a figure or should we use different formatting?)

Commercially ordered from above note?(Line 222)

At the same concentration as in 1.2.2 correct? (Line 223)

Yes, added this for clarity.

Example %?(Line 227)

Addressed this by mentioning table 2 as well as giving an example.

Mention exact step numbers.(Line 231)

This was changed by adding the exact steps from just ‘step 2 to step 2.1.4-2.1.7’

When is the DNA loaded? (Line 233)

It was mentioned in the note above but in order to address this more clearly, this has been made into a step (4.1.3)

Add to the table of materials. (Line 234)

Fluorophore?(Line 235)

Change made.

What speed? (in g ) (Line 245)

Eluent? (Line 248)

Elutant was changed to eluent, this was a spelling error.

Please double check, you are mixing NTP to CTP, ATP, amd GTP to produce 5X NTP?? Does not add up (Line 258)

XXX UTP is missing XXX

This is confusing. Do you simply mean mix 100 mM NTP, 40 mM GTP, 25 nM CTP etc? (Line 258)

Make a 100 mM NTP stock made up of the following GTP, CTP, ATP, and UTP.

T7 transcription buffer?(Line 274)

Yes, added into the step for additional clarification

What volume? (line 275)

Depends on the final volume that the person wants to make. It says 10X to 1X so dilute accordingly.

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From where? Unclear which one this is Line 276)

See section 4, this is added to step 4.2.3.1

Check and update (Line 277)

Volume? (Line 277)

Depends on the final volume that the person wants to make. It says 10X to 1X so dilute accordingly.

Add to the table of materials. (Line 277)

Aluminium backed TLC plates added to the table of specific materials

Reference (Line 294)

What cell density? (Line 302)

It is mentioned in the step that the protocol is described elsewhere in detail with a reference. However, I have added in brackets that the cells are induced at an OD600nm  of 1.

Induced-E. coli Cell pellet? (Line 307)

Previously mentioned just pellet, clarified to ‘Induced-E. coli Cell pellet).

Concentration? (Line 307)

Not dependent on concentration. Is dependent on volume.

Speed and duration? (Line 308)

Max speed for 2 min.

You mean add phenol, vortex and then centrifuge? (Line 310)

Yes, fixed this to make it more clear.

Speed (in g) and duration? (Line 315)

Max speed for 2 min.

Centrifuge speed and duration?? (Line 318)

Max speed for 2 min.

Duration? (Line 319)

At least 30 min.

Add the kit to the table of materials. (Line 324)

XXX A kit was not used XXX the protocol is referenced

In which solution? (Line 327)

In ddH2O, since a protocol was followed, it is mentioned in the protocol but was added to the note to make it clear.

Add to the table of materials (Line 352)

T7 RNA polymerase added to the table of materials

Please avoid use of commercial names. (line 435)

“NEB protocol” was corrected to just a protocol, there is a reference for clarity

Remove the commercial name. (Line 464)

Commercial name of the imager has been removed to just say ‘fluorescence imager’

Remove the commercial name. (Line 496)

Commercial name of the imager has been removed to just say ‘fluorescence imager’

Should “acrylamide:bis" be “acrylamide:N,N’-methylenebisacrylamide” in the table? (Line 504)

This correction has been made in Table 3 as well.