PCR on DNA samples (Chlamydomonas spp.) to test annealing temperature for the primers

Richard W Lambrecht[1], Manuela Spagnuolo[1]

[1]University of Konstanz

Richard W Lambrecht

MAR 30, 2023

OPEN ACCESS

Protocol Citation: Richard W Lambrecht, Manuela Spagnuolo 2023. PCR on DNA samples (Chlamydomonas spp.) to test annealing temperature for the primers. **protocols.io** https://protocols.io/view/pcr-on-dna-samples-chlamydomonas-spp-to-test-annea-cr5fv83n

Protocol status: In development
We are still developing and optimizing this protocol

Created: Mar 30, 2023

Last Modified: Mar 30, 2023

PROTOCOL integer ID: 79751

Keywords: PCR, annealing temperature test

ABSTRACT

Protocol developed to test the annealing temperature of primers (ITS, rbcL and 18S regions).

Initial observations

1 Make sure you had appropriate training/introduction to the laboratories and machines (From Dr.

Epp)

2 Book the laboratory benches and machines in advance

3 Respect the rules for each laboratory and the transit between the laboratories

Pre-PCR Lab

4 Take Primers, ultrapure water, MasterMix, and DNA out of the freezer and defrost completely

5 Primer dilution (Primer stock concentration 100 µM) to a 10 µM Primer

5.1 Take defrosted primer and mix by flicking the tube (do not vortex!!)

5.2 Aliquot 45 µl of ultra-pure water in a clean 1.5 ml Eppendorf tube and add 5 µL of the 100 µM primer to dilute it to a 10 µM concentration

6 Prepare the gradient reaction volume (for 14 reactions – 12 reactions + 2 extra)
* Number of reactions = number of wells per primer combination X 15 µL per well.
**Primer combinations list is on the last page of this protocol.

6.1 In 1.5 mL tubes:
- Water: 92.4 µL
- Hot Start Master Mix (TaqPolymerase): 105 µL: make sure the MasterMix is defrosted

completely and vortex 20 seconds before adding to ensure proper mixing
- Forward Primer: 4.2 µL: flick before adding
- Reverse Primer: 4.2 µL: flick before adding
- Template/DNA: 4.2 µL: flick the tube before adding

6.2 Flick all the 1.5 ml tubes with the mixed PCR reagents and spin them down using the quick-spin before adding them to the PCR plate

6.3 Seal plates using transparent sealing foil. Make sure that the plates are sealed correctly; otherwise, the samples will evaporate in the cyclers.

Post-PCR Lab

7 Put the plates in the cyclers in the post-PCR lab

7.1 Settings for the cyclers:
- Jena Analytics cycler - protocol name: richard (Temperature range: 46ºC to 56ºC)
- PeqLab2 – protocol name: 45-GRARICH (Temperature range: 58ºC to 68ºC)

a) 95ºC for 15 min (Hot Start for the Master Mix)
b) 35 cycles
  i) **[** 94ºC for 30 sec
  ii) Temperature gradient for 30 sec (Chosen temperature gradient)
  iii) 72ºC for 1:30 min (*for Taq DNA calculate 1 min per Kb of target DNA)**]**
c) 72ºC for 10 min.

8 Prepare the gel for the electrophoresis

8.1 [Fume hood] Prepare the gel tray by sealing the openings with lab tape. Make sure the tape is sticking properly and seals the openings, otherwise, your gel will leak

8.2 Put the combs with the number of wells you want to have in the tray (decide the amount of wells based on the amount of wells + at least 1 leader per row of wells)

8.3 For a 1.5% small gel:
i) Weigh in 0.75 g of agar (labeled with LB) and put it into a 100 ml Erlenmeyer flask
ii) Add around 60 mL of 1x TAE buffer (left side of the electrophoresis bench)

For a 1.5% big gel:
i) Weigh in 1.5 g of agar (labeled with LB) and put it into a 200 ml Erlenmeyer flask
ii) Add around 110 mL of 1x TAE buffer (left side of the electrophoresis bench)

8.4 Block the opening of the Erlenmeyer flaks with a small piece of paper. Make sure you are not completely blocking the opening. There should be still some air exchange possible. This will prevent you gel from boiling over in the microwave

8.5 Put the Erlenmeyer flask in the microwave until it is completely clear. Take the flask out every 30 seconds- 1 min to twirl it. This will ensure proper mixing

8.6 [Fume hood] Let the gel cool until you can touch the bottom of the flask for 5 seconds with gloves on otherwise the tape will not stick to the tray anymore. Twirl the flask every 2 minutes to ensure proper mixing

8.7 Add 5 µl of a 10 000X SYBR save (shelf on top of the microwave) to the small gel, for the big gel it is 10 µl to get a 1X concentration of SYBR safe

8.8 Twirl the flask to mix the SYBRsafe properly and immediately pour the gel into the tray to avoid clumping

8.9 Let it cool and harden (approx. 10 min) check with the broad end of a pipette tip if the gel is solid

8.10 Carefully remove the tape from the tray and place the tray with the gel in the electrophoresis chamber. Make sure the TAE buffer in the chamber completely covers the gel. The TAE should be 5 mm above the gel. If the TAE in the chamber is not enough pour some more from the bottle

8.11 Remove the combs carefully to not damage the wells of the cell. If you rip one of the wells, do not use it for loading anymore. Make sure that the TAE filled ever well of the gel

9 Stain the sample to load the gel for the electrophoresis

9.1 a) Aliquot approx. 1 µl of a 6X loading dye (green) to six small wells of a PCR loading tray (next to the sink) using a 20 µl pipette set to 8 µl

b) Add 5 µl of sample to each well. Mix by pipetting up and down 3 times and load it directly to the gel. Change the tip between every sample to avoid contamination

10 Load 3.5 µL of the leader (Gene ruler SM#0333) in the middle of each row of the gel for the electrophoresis, or in between samples to separate blocks. Change tips in between loading each ladder well

11 Running the gel

11.1 Close the electrophoresis chamber with the according lid. Make sure the connections are correct (red to red; black to black)

11.2 Mark on the tape on the lid that you ran a gel (TAE buffer in the chamber needs to be exchanged after 10 runs)

11.3 Connect the cables to the power supply

11.4 Chose the program with the voltage you want to run your gel (100 V should be program 1)

11.5 Time: Small gel (~30 min), big gel (~40 min). Make sure you check your gel in between. You do not want your samples to run it off the gel.

11.6 You can stop after the gel ran at least 2 cm from the well by switching the electrical device off

12 Gel analysis

12.1 Start the analysis computer

12.2 Open the “intas” software

12.3 Take the gel tray out of the electrophoresis chamber, use paper towels to dry the bottom of the tray and close the chamber after you are done

12.4 Place the gel with the tray on the table of the analysis device

12.5 Switch on the normal light in the device (NOT the UV light!!!!) and press “live” on the software. This allows you to see a live image to position your gel correctly

12.6 Change the position of the gel so that you can get an even image of your gel

12.7 You can focus and zoom in on your gel by turning the objectives of the camera on top of the analysis device.

12.8 When your gel is positioned correctly, close the door of the analysis device and switch on the UV light

12.9 Change exposure time until you get a clear image of your gel and your bands/ the ladder bands can be seen clearly

12.10 Press “freeze” on the software and switch off the UV light. This is protecting the UV light.

12.11 Save and print the picture of your gel

12.12 Take the gel tray of the device and discard the gel into the gel waste (white bin with blue lid)

12.13 Clean the tray, the Erlenmeyer flask and the plastic PCR sample tray with hot water