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Book Descriptions:

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Questions 54 Publications 944 Questions related to Thermal Cycler Mohammed Ahmed asked a question related to Thermal Cycler How to choose the Tempreture in EVAGREEN Multiplexing digital droplet PCR. It was detected at thermal cycler annealing temperature 51 degrees with a 99 bp amplicon. The second gene has a master mix designed from BioRad and the recommended temperature is 58 degrees with an amplicon of 195 bp amplicons. How could I use the same well in ddPCR to detect both. Which Temperature should I use in my multiplex assay. Thanks all Best Relevant answer Katie A Burnette Aug 11, 2020 Answer Use gradient PCR to see if both primer sets have an overlap in usable range in annealing temp. Small products are generally easier to amplify at a range of temps because the ramping from the annealing temp to 72 will often hit the desired annealing temp along the way. If you dont have a gradient capable thermocycler, then youll just have to test several temps in several PCR runs. Good luck! View 24 Recommendations How to reboot unit in Multigene Mini Personal Thermal Cycler. When I look at the troubleshoot manual, the manual state to reboot unit. However, I didnt know how to do this. Can anybody tell me how to do it. View 4 Recommendations Marija Vukcevic asked a question related to Thermal Cycler How to calibrate Sacace SyCycler 96 RTPCR. Question 1 answer Mar 4, 2020 Hello everyone. My lab wants to accredit qualitative detection of WNV on Sacace SyCycler 96 RTPCR. For doing that, the instruments must be calibrated. How can we do it The distributor from who we bought it said that this instrument does not need to be calibrate. The short answer is maybe. There certainly IS something to calibrate. Many of the engineers I know think calibration is STRICTLY when a unit is adjusted to within specifications. Doubtless this is what your vendor is referring to.http://tortugafilms.com/adminfiles/canon-ir2016-and-ir2020-copiers-service-manual.xml

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What YOU NEED for your lab is an accredited certificate indicating the unit is performing to specifications on Temperature, Uniformity and time. About the only way I know to do this is with a multiplex data acquisition unit. DAQ. Youd need to have 89 temperature probes placed around the heating block all recording the data simultaneously. Top, middle, bottom, left, center, right. Take the temperature up and down across the range, stopping at whatever critical points you use, but making sure to get your minimum use and maximum use temperatures. Depending on criticality I usually use 59 testing points. Now your unit has ideally been connected to the same computer your DAQ is so you can compare the data across the range. This is why Labview is probably best to see this both in real time and to compare the set points to what the SyCycler 96 is saying the temp is, vs what the DAQ is recording. Is it adjustable Probably. But it might need to be repaired to fix it. That said, you can get a 3rd party Calibration with ISO 17025 accreditation to do this for you. The calibration lab would be have all the equipment and software to do this calibration for you in probably 23 hours and get you the paperwork either the same day or next day. You may need to request the graphs. Usually 3rd party labs do not provide that. But I like them. sacycler96tec hnical.pdf 795.47 KB View 0 Recommendations Muhammad Zakria asked a question related to Thermal Cycler Thermal Cycler

block temperature error, where is the problem. I have to switch the machine off and then restart, even then there is chance of error, and it is badly affecting my optimization.Question 3 answers Sep 12, 2012 I am using an Antarus MyCube PCR machine for high school student workshops and the laptop containing the operating software has died. The company has gone out of business so I am desperately seeking someone who might have a MyCube that could send me the software. Thank you!http://www.crigroups.com/home/content/45/11775545/html/admin/uploadsxxx/canon-ir1600-photocopier-user-manual.xml

Relevant answer A researcher Oct 23, 2019 Answer hi steven and carlos.Question 4 answers Jan 11, 2019 I have been used taq PCR kit 1000u so long time and may be I have used 10 kits 1000u until these days. I have 3 kits already but when I start to doing PCR, I can get successful results with 10x PCR buffer but I cant succed with coralload buffer. I used it in many times when kits arrived me but I couldnt work with it. When I do PCR both 10x PCR buffer and 10x coralload PCR buffer, I can see good result with 10x PCR buffer but not with coralload PCR buffer. Now I have about 50 coralload PCR buffer tubes and I want to use it. I use same protocol when I do PCR work. I prepare pcr master mix according to kit hand book and put them on thermal cycler. After PCR reaction I prepare 1% agarose gel adding ethidium bromide and directly load PCR samples containing coralload PCR buffer. I can see how much my DNA go on gel but when I want to see my PCR result on uv transsimulator, I couldnt get any band untill today. I wonder, should I prepare my agarose gel without ethidium bromide. When I dying my PCR product that was done with 10x pcr buffer, my samples can dye with coralload buffer but when I use it alone my PCR reaction doesnt work properly. I mean, coralload PCR buffer is working as gel loading dye but on PCR reaction it doesnt work. I have no problem about product storege and other condition. Because all solutions into kits are work when use. I wonder if coralload PCR buffer need specific PCR condition or imaging process or etc. I wonder your response immediately because Im continue doing pcr, so I want to use coralload PCR buffer to easy application. Relevant answer Muhammed Duman Jan 15, 2019 Answer Dear Paul, Thanks for your answer. I discard these coralload buffer on my work but I have about 50 corallaod PCR buffer and I want to use it with Taq DNA polymerase that was provided by different firm.

Because qiagen buffers are working very well with other PCR product of different firm. I used both 10x PCR buffer and coralload PCR buffer when I started to PCR master mix but my results were too bad than only 10x PCR buffer. As your suggestion, I will use these buffers as gel loading dye. But this is what I dont want to most. I will try different annealing temperature with coralload buffer. But I dont think about this negative result contact with annealing temperature. I asked this question to Qiagen technical support. If I get useful information about it, I will share. Best regards. View 5 Recommendations How best can I improve the efficiency of Atailing blunt PCR products for cloning into pGem T Easy. Question 16 answers Jul 11, 2016 I am amplifying a 5070bp gene with Phusion DNA polymerase and then try to clone it into pGem T Easy vector. All am getting at best are a few blue colonies without the insert. The positive control ligation reaction is working fine. That leaves me to suspect that the Atailing reaction is not working optimally. How best can I improve the efficiency of the Atailing procedure or is there something i am missing here. Relevant answer Fernando Cardona Jan 11, 2019 Answer Dear Thomas Only Taq Polymerase ad A overhang you can increase the final extensio time to at least 30 min to force Phusion pol to add A overhang View 0 Recommendations Alexandre Bueno asked a question related to Thermal Cycler After a qPCR using SYBR Green, with no melting curve, can I see the green color of the sample with naked eyes under ambient light. Question 9 answers Oct 30, 2018 Lets suppose I run a qPCR of some sample, with no melting curve in the end. The dye is SYBR Green and the thermal cycler accused a successful amplification. When I take out the sample, can I see its green color inside the tube with my naked eyes under normal light. Or the light emitted by fluorescence is too dim to be detected by the eyes.

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Relevant answer Alexandre Bueno Nov 13, 2018 Answer So, as far as I understood, I cant see the green light from the samples after qPCR because the light produced by fluorescence is too dim. But if I put more SYBR green than usual, would it be possible. Since its possible with ethidium bromide using the appropriated wavelength it should be possible also with the SYBR Green isnt it. View 0 Recommendations How to set temperatures within a gradient on BioRad T100 thermal cycler. Question 5 answers Oct 30, 2018 We have a BioRad T100 Thermal Cycler in our lab. Im trying to set up a gradient PCR but the machine wont allow me to choose the temperatures in the gradient beyond the front and back row low and high ends of range. I have 7 different PCR reactions that all have Tms between 5460C, and Im wanting to set up a gradient with the front row at 54C, next row at 55C, and so on up to 60C at row 7, so that I can run all 7 samples that have different Tms in one block in one night. However, the machine wont allow me to select the temperatures actually within the gradient only the first and last temperatures and I get undesired Tms when I set the first row at 54C and the last one at 60C, 61C, 62C, etc. Is it possible to manually determine all the temps within the gradient. Is the gradient step designed this way on purpose. Or is there a way to get the temps I want. Anything helps, thank you. Relevant answer Bin Mr Oct 31, 2018 Answer I dont have a such machine View 12 Recommendations PCR not working. Possible reasons Question 6 answers Aug 6, 2018 I genotype and sequnce zebrafish using a bit of the fin. Lately though, I have been struggling with PCR. So far I have tried the following but to no avail 1. Tried the primer pair in question with some control genomic DNA that I know to work. This has worked. 2. Checked the pH of my reagents. These are in place. 3. Reextracted the genome from the animals that Im having difficulties with and tried PCR with same primer set again.

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This has failed. 4. About to try the extracted genome with other primer pairs. If the last step fails, what can I do differently. I extract the genomic DNA using proteinase K and NP40 lysis buffer, in a thermal cycler at 55C. Relevant answer Simone Schindler Aug 6, 2018 Answer Hi Kunal, Maybe you are using too much template DNA. If there is too much template, you generaly see a smear on the gel. Have you tried diluting the genomic DNA template 110 for the animals in question and then repeated the PCR. Best Simone View 10 Recommendations Jasna BingulacPopovic asked a question related to Thermal Cycler Please your experiences with new Thermo Fisher basic PCR instrument miniamp plus thermal cycler. Question 3 answers May 24, 2018 We have offer to by this instrument, we would like to decide between thermocycler 2720 and new model. If you could compare it. Relevant answer Hassan Nima May 24, 2018 Answer follow View 4 Recommendations Ricardo Santander asked a question related to Thermal Cycler DNA running backwards on electrophoretic gel. Question 7 answers May 11, 2018 Hi. Im comparing the performance of two different thermocyclers. One of those is a commercial thermal cycler and the other is a prototype. For this test I prepared a 60 ul PCR mix and then separated it into two 30 ul tubes. A 30 ul layer of mineral oil was applied to both tubes at the end of the preparation. As soon as the PCR was finished in both thermocyclers, 10ul of each sample was mixed with loading buffer to put them in the electrophoresis gel The PCR sample of the commercial thermal cycler is the one on the left, the PCR sample of the prototype is the one on the right The other wells are empty. But at the time of putting the gel under UV light these were the results see attached images. I have a couple of questions. Was there any amplification in the prototype thermocycler.

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Many old pcr machines were deigned for specific total volumes and did not work well due to temperature overshoots and undershoots when the volume was much different from the designed volume View 14 Recommendations What is the best PCR condition PCR mix and cycles and

temperature for amplification of a WNT10A. Question 6 answers Mar 8, 2018 I have used the following procedures but am still having strong primer dimer bands while the desired product is absent. The cycling conditions for PCR program that i used were 5 min at 95C for activation followed by 35 cycles of 95oC for 30 s for denaturation, 55oC for 30 s for annealing, 72C for 30s for elongation and a final cycle 72C for 10min for final elongation the primer used are F CAGCCATACAGGGCATCCAG R ACAGATGGGTGTGTGGGGAT and expected pcr product size is 250 please i need a help about what is wrong Relevant answer Dalia Gamil Aseel Mar 14, 2018 Answer Hi I hope you are fine First, make sure of the nucleotide sequence from Blast Program online it is possible to give the time instead of 30 seconds to be 1 minute in all cycles denaturation 1 min, annealing 1 min and extension 1 min to give chance of primers for catch in gene target using dilut concentration of DNA Try different annealing temperatures of 50 to 65 gradient PCR Good luck View 0 Recommendations Need help with PCR condition. Can anybody help me. Question 3 answers Feb 28, 2018 Hi researchers, I have a problem with PCR condition interpretation for primer amplification. I have chose this primers from paper. The primers relates to SSR markers and they have a different temperature for anneling. Now i fill a bit confuse with PCR condition, it looks very complicated for me, and not only for me. I am just researcher, and this is my first time working with primers. Will be glad if someone can help with interpretation. Relevant answer Paul Rutland Feb 28, 2018 Answer I have not read the paper but the safe option is to run separate individual pcrs.

If 23 primer sets are differently dye labelled and also have the same Ta then these could be multiplexed but I expect that they ran individual amplifications and then mixed the different coloured pcr products and then analysed each sample one colour at a time. View 10 Recommendations Shadid Uz Zaman asked a question related to Thermal Cycler Is it possible to develop a rudimentary method of estimating gene expression using Normal PCR. Question 6 answers Dec 4, 2017 I have no expertise with PCR at all, but I do understand the principle behind PCR, qPCR and RTPCR. I want to estimate the expression of certain gene in bacteria. But right now, I only need a rudimentary estimation. If the results are encouraging I can buy the kits required for qRTPCR. I have two questions 1. Can I conduct RTPCR Reverse Transcriptase method in a regular thermal cycler. My limited knowledge tells me that it should be possible and I only need to add Reverse transcriptase to the master mix to get the cDNA from mRNA and add a phase for reverse transcription to occur. 2. Will it be possible to make quantification of the cDNA level of the final PCR product to estimate gene expression level. My understanding is that qPCR determines the amount of cDNA at the end of each cycle. I dont need that. I just need to know the amount at the end of PCR and compare it to a housekeeper to make a relative calculation, right. Say, I want to measure expression levels of NorA, I will evaluate the amount of NorA cDNA at the end of the PCR reaction and compare it with amount of cDNA of rpsL housekeeping gene. That should give me a ratio. Then I will measure the ratio for control bacteria as well and compare the ratio from sample bacteria to control bacteria to make a rudimentary guess about whether overexpression has occurred. Again I dont know all the intricacies of PCR, so any comment on this would be highly appreciated.

Relevant answer Fabrice Chatonnet Dec 6, 2017 Answer Then go for the abovementioned semiquantitative RTPCR. If you have a good gel analyzer, you can have an idea of your gene overexpression by looking for the ratio between bands intensities. Good luck! View 10 Recommendations Can anyone recommend a 96well PCR plate. Question 3 answers Nov 17, 2017 Some specific requirements Compatible with the Veriti Thermocycler. Contains thinwalled tubes. Compatible with a 96well magnetic stand for ampure bead purification doesnt matter which magnetic stand. If you have a particular one in mind, please mention it. Relevant answer Yara Odeh Nov 20, 2017 Answer I use the MicroAmp Optical 96Well Reaction Plate Applied Biosystems Life Technologies Cat No N8010560 View 0 Recommendations If we accidentally set 35 cycle for PCR from Denaturation Step to Incubation Step, rather that it stop at Extension Step, does it affect the result. Question 3 answers Oct 23, 2017 PCR parameter input cycle. Relevant answer Furkan Alaraji

Nov 9, 2017 Answer Hi Yes i think yes it may effect the result.Recommend View 0 Recommendations Baiba Alksere asked a question related to Thermal Cycler What will happen if DNA samples are denaturated with random primers 2 times in a row. Question 3 answers Nov 8, 2017 There was an issue with my thermal cycler Relevant answer Paul Rutland Nov 9, 2017 Answer do you mean just the denaturation and annealing step was repeated or the whole process with cycling was duplicated. Doubling the denaturation step only will probably not make any difference because DNA and primers are very stable but you need to specify what enzyme is being used and what cycling parameters for people to give an accurate assessment View 4 Recommendations Laia Closa Gil asked a question related to Thermal Cycler Can anyone give us some tips to optimize a program with a new Veriti thermo.

And now we have changed to a Veriti Thermal cycler, emulating the 9600 ramp as well and using the same program as we have always done. The fact is that some routine analysis that worked well with the first thermal cycler started to fail with the Veriti, and the program that lasted 12 hours, with the Veriti lasts 10 2h difference. We suspect the problem is that the ramp time is different between thermos, as the program is exactly the same. Can anyone give us some tips to optimize this new thermo. Its normal this kind of diference. Thanks! Relevant answer Paul Rutland Oct 11, 2017 Answer I think that you are probably correct that the ramp conditions are different and some of your reactions may have relied on overshoot and undershoot of rapid ramping so that, for instance, when the sample moves from 72 to 94 the overshoot may momentarily take the reagents to 95 before settling at 94 and at annealing if set at 58c the fall from 94 to 58 may drop to 56 then come back to 58. If this fails you may have to run gradient pcrs again. I assume that you have checked the ramp rates of the 2 machines and set the ramp rate of the Veriti to match the 9700 but the 9600 and 9700s that you used previously may have been getting old and the temperature profile as set may not have been matched in reality by old circuitry and peltier blocks hence my suggestion to make the profiles a bit more conservative. Check also that the default volume for the Veriti is the same volume as the 9700 as some old machines had a default volume to calibrate temperature profiles of 50ul but the more modern machines may be set for 25ul liquid volumes as default. Changes in volume will change the rate of temperature change and even on rapid cycles the ability to reach the desired temperature in time View 6 Recommendations Supinya Thanapongpichat asked a question related to Thermal Cycler No turbidity in LAMP product tubes but found Ladderlike Band Pattern in agarose gel. What could be wrong with the protocol.

Question 4 answers Aug 21, 2017 I just working with LAMP Loopmediated Isothermal Amplification using target DNA. I designed 4 primers using PrimerExplorer V3. I do not see any turbidity in the tubes after 1 hour of reaction at 65 degree C in the thermal cycler BUT I can see multiple bands for my LAMP reaction products on the agarose gel. For negative control was clean. Why do I not see any turbidity. I also used HNB to detect the difference of color between positive control and negative control but It was not different. It is showing blue color. Could you please suggest what would be the possible ways to resolve the problem. Thank you for your help. Relevant answer Tom Edwards Aug 21, 2017 Answer The use of turbidity as a detection method is not particularly sensitive you need to have generated a lot of DNA amplicon before the byproduct is visible. Success also depends on pH if you are making your own buffer. I found HNB to be a bity hit and miss depending on the buffer. DNA binding dyes such as SYBR are a bit more sensitive in my experience, and if you have a realtime qPCR system you can optimise the assay using the realtime data which is a bit easier. Also, Id try not to run gels with the lamp products, the amount of amplicon is so high its really easy to contaminate the lab, even if you are a molecular biology ninja. View 4 Recommendations Potential Implications of Poor PCR Efficiency on Assay Design. Question 3 answers Jul 19, 2017 Dear all, I am currently designing an ARMSqPCR assay to detect mutant alleles at very low levels while diluted with a high number of wild type alleles in the same solution. I first did a gradient PCR on a real time PCR thermal cycler between 56 to 61.5 degrees Celsius to first determine the best annealing temperature

for my primers. This was determined by the slope of the exponential phase of the amplification plot in log view. I believe a steeper gradient implies greater PCR efficiency, which implies a better annealing temperature.

All annealing temperatures allow for a similar plateau height, so I believe the amount of amplicons produced at the different Tms are also similar. From that gradient PCR experiment, I chose 60C as the optimal annealing temperature. I then diluted the template and ran a qPCR experiment with that Ta, this time to determine the PCR efficiency. Between each 10 fold serial dilution, the Ct difference was 5. Consequently, the calculated PCR efficiency was poor 57%, but I had a very good R2 value of 0.99. Since I have already determined the optimal annealing temperature for the primers, is this truly the best PCR efficiency I can achieve with the current primers. From what I understand, poor efficiency can lead to poor analytical sensitivity high limit of detection. Is this true as well. Thank you in advance. What are you using as the standard. The reality is some amplicons are just difficult to PCR high GC content ect. Your temperature gradient is rather narrow, have you tried anything broader 5065C, getting at the GC issue. Also, are you using a commercial qPCR Master Mix or a homemade recipe. Last but not least, are you sure you did a 110 dilution. You dont know how many times Ive looked at an assay and been dismayed by my efficiency and then realized the dilution I did was different than I thought. Question 3 answers Sep 17, 2016 Dear colleagues, I have a problem in my RTPCR reaction, recently. I have used my PCR primers for ERK1 and ERK2 genes for several time and the results was OK.But recently I cant see any Bands in my PCR in both two genes while bactin band is always achieved.So what is the problem. I also can not see any bands in recent gradient reactions and I also have bought new primer pairs for Erk1 and Erk2 genes. Relevant answer Stacey Cranert Sep 18, 2016 Answer Have you changed samples or cDNA synthesis conditions. View 5 Recommendations What thermal cycler settings would you recommend for 16S V4 and 18S V4 Ion Torrent Sequencing.

Question 1 answer Mar 10, 2016 Im planning to perform PCR amplification on environmental DNA filtered microplankton biomass from seawater samples for both 18S and 16S, using Taq Hifi polymerase and barcoded Ion Torrent primers. My 18S forward primer Tms range from 72.1 o 72.6. The reverse primer is listed at 65.2 degrees. My 16S forward primers are 73.6 to 74.8 degrees, and the reverse primer has a Tm of 67.3 degrees. What Im more curious about are the optimal lengths for the denaturing, annealing, and extension steps. I am planning to test how well my samples amplify, perhaps using a temperature gradient, but Im wondering what kind of run settings you might suggest for a starting point. My primers are diluted to 10 uM. We do not typically use Ion Torrent, and Ive never performed PCR before using Ion Torrent primers, so Id appreciate it if someone could point me in the right direction. Relevant answer Eero Juhani Vesterinen Mar 12, 2016 Answer Just start with optimal annealing temperature for your polymerase enzyme and your primers. Perhaps use a gradient PCR to minimise primerdimers and wrong targets. I have successfully used Bioline MyTaq Hotstart ready mix, KAPA Robust ready mix and KAPA HiFi HS Readymix for Ion Torrent. View 0 Recommendations Linda Kothera asked a question related to Thermal Cycler What do you like or dislike about your realtime PCR machine. Question 3 answers Mar 10, 2016 We are in the market for a new realtime thermal cycler. We have a couple of CFX96s, which I like, but havent used anything else. Im using it for gene expression assays and species ID assays, both in multiplexes with fluorescent probes. Thanks for the input. Relevant answer Can Kiessling Mar 10, 2016 Answer If you are looking for nextgen type of PCR platforms consider looking into digital PCR platforms which have several advantages over the traditional PCR methods. I also used the CFX96 and very satisfied with it.

View 4 Recommendations Why our T100 Biorad thermal cycler is not working with colony PCRs. Question 5 answers Feb 17, 2016 Hi everybody. We are having big problems trying to amplify DNA with colony PCRs with our 4y old BioRad T100 thermal cycler. We can amplify DNA from the same

colonies and same reagents just changing the thermal cycler old fashioned one.Otherwise we are able to have positive bands when our template its a purified plasmid. Do you have any idea of what is happening in our thermal cycler. Thanks a lot for you help!! Relevant answer Nuria Samper Feb 24, 2016 Answer Hi guys, thanks a lot for your help. Finally, I talked with a BioRad technician and he suggested me to change the ramp time as A Ali told me. In addition, spectra mapping emission at TAMs max 580 nm with excitation at FAMs max 480 nm looks very strange, sometimes with very little change of RLU across the temperature ramp. Ive attached a set of representative plots for reference. Any info would be greatly appreciated! RG FRE T.pub 405.50 KB Relevant answer Jose H Hodak Dec 3, 2015 Answer Tetra methyl rhodamine is quenched to different degrees by the different nucleotides in its proximity. Most likely your oligonucleotide is quenching the acceptor TAM and the emission does not report 100% of the energy transfer when the FAM is in close proximity to TAM. You can test if this is what is happening, because Quenchig would cause a lower emission quantum yield of emission for TAM in experiments where you directly excite the acceptor and monitor the emission at 580nm. Smears and multiple bands on agarose gel for negative control as well. Question 3 answers Sep 21, 2015 I recently started working with LAMP Loopmediated Isothermal Amplification using target DNA. I use LAMP Kit by Lucigen, Inc. For some reason I do not see any turbidity in the tubes after 1 hour of reaction at 70 degree C in the thermal cycler.

On the agarose gel, I can see multiple bands for my LAMP reaction products but I also see huge smears throughout the lanes along with thick band near the gel. My negative control reaction mixture without template gives me the same result. Can you suggest what is wrong with my protocol. Relevant answer Shazleen Mohtar Oct 13, 2015 Answer Sometimes, maybe due to contamination. I also faced the same problem. Can I know the volume of MgSO4 that have been added in the LAMP reaction.View 0 Recommendations Kate Mendez asked a question related to Thermal Cycler Any recommendations for PCR help. Question 6 answers Jun 24, 2015 I am currently running an experiment trying to detect environmental DNA for the species Hemimysis anomala or commonly known as bloody red shrimp.However, while running my controls using actually bloody red shrimp tissue, my gels were negative. I am using Qiagen Blood and Tissue Kits for DNA extraction. Amplifications were performed in 20 uL volumes containing 0.5 U of Hot Start Taq DNA polymerase Qiagen, 1 x buffer, 3 mM MgCLs, 0.2 mM dNTP, 10 pmoL of each primer and 10 ng of genomic DNA. PCR products were purified using QIAquick PCR cleanup kit and run through Agarose Gel Electrophoresis according to standard protocol. However, I am unable to detect any DNA and before I can begin my actual amplification of my eDNA filters, I need to be able to ensure that the primers and my technique can detect tissue of the H. anomala I am working with from the Finger Lakes region. Relevant answer Paul Rutland Jun 25, 2015 Answer when you say no measurable dna do you mean that you cannot see any bands after pcr. If so the problem should be isolated to dna preparation or improving the pcr process. Similarly borrow some dna that works for other people and run your primer set on it. Often I find that when primers do not work it is because one or both primers have degraded due to being dissolved in water not TE or degraded by freeze thawing too often.