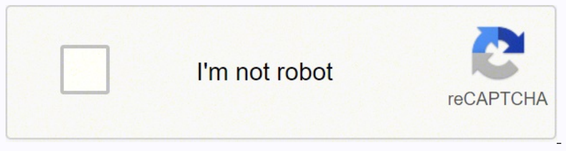
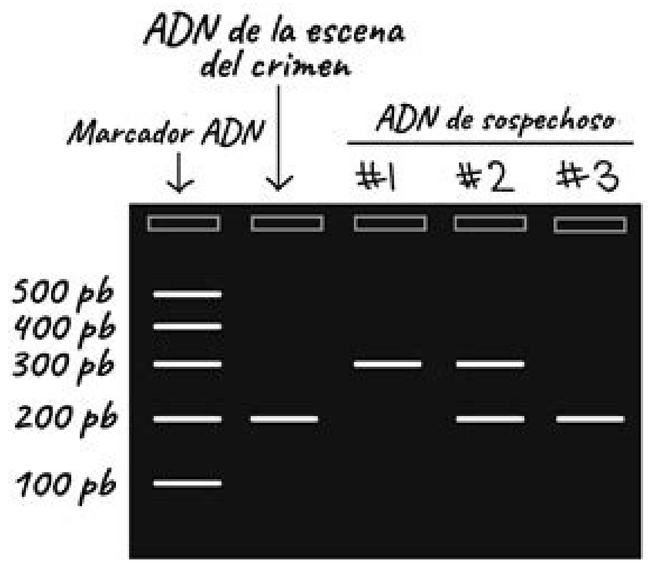


Open

Plasmid dna purification lab report



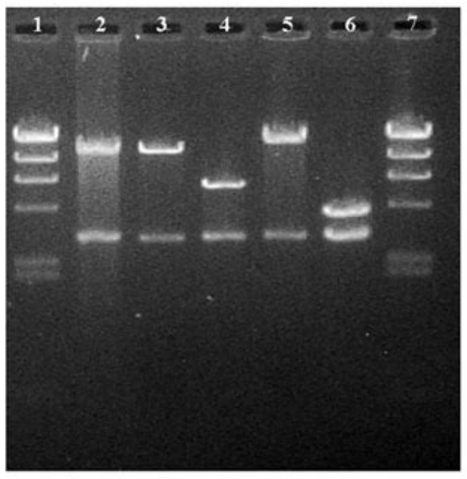
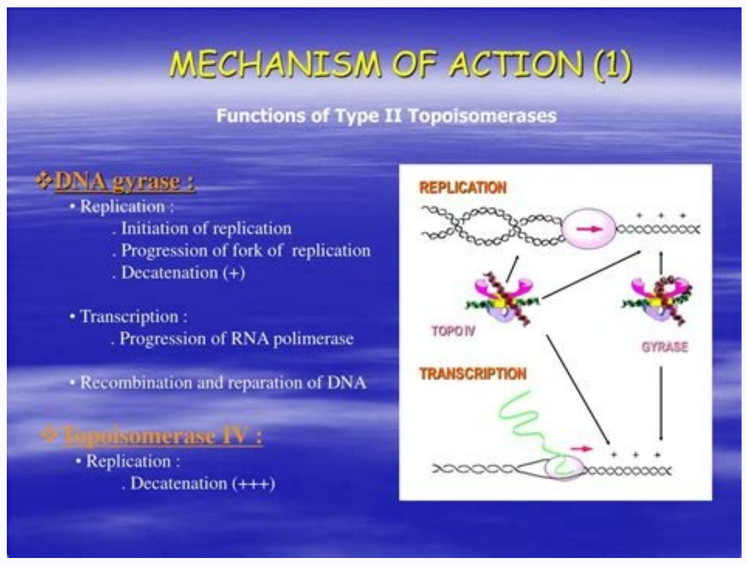


Figure 1: Agarose gel electrophoresis of recombinant plasmids carrying carotenoids biosynthesis genes.
Lanes 1 and 7, Lambda DNA/ *Hin*dIII as size standard; lane 2, pXD-A1 (*idi*); lane 3, pXD-I10 (*crtE*); lane 4, pXD33.13 (*crtYB*); lane 5, pXD-C13 (*crtI*); lane 6, pXD-C19 (*ast*). The plasmids pXD-A1, pXD-I10, and pXD-C13 were digested with *Bam*HI (lanes 2, 3, and 5). The plasmid pXD33.13 was digested with *Eco*RV (lane 4), and plasmid pXD-C19 was digested with *Pst*I (lane 6).



Why is it so important to be sure you remove all the wash buffer from the column before the elution step? coli S17 containing the plasmid pARO180. The resulting conjugated HB101 cells will contain both the amp resistance gene and the strep resistance gene, so they will be able to grow on plates containing both amp and strep (they're double resistant). The water will elute the DNA, dissolving it off the column. If there is ethanol in your final DNA solution, the sample will have low density and will tend to float when you load the gel. Mobility of Plasmids. Finally the alcohol is removed and the pure DNA can be eluted, or dissolved off the glass matrix of the column, in pure water or TE buffer. Migration DistanceMigration Distance (cm) Log(DNA BasePairs) are pulled to the positive end of the gel base by current, however encounter resistance from the agarose gel. Conjugative plasmids and mobilizable plasmids are slightly different things; pARO180 is a mobilizable plasmid. How the experiment works You'll start with liquid cultures of with two strains, or genetic types, of E. Column Wash Solution. Transfer 800 µl of the supernatant into the Spin Column. "Miniprep" means that it starts with a single microcentrifuge tube of bacterial culture. Promega Wizard Plasmid Miniprep Instructions from Promega. Leave them in a rack. Label the plate containing the agar, not the lid (you might mix up the lids). Why do they work? 2 2 3 3 4 4 5 f(x) = − 0 x + 5² = 0. Test tube rack to hold the culture tubes 1 empty glass culture tube (for waste) One microcentrifuge tube (1.5 ml) for each sample and a rack to hold them Cell Resuspension Solution Cell Lysis (alkaline detergent) Solution. Purifying Plasmid DNA Using thProtocol jove/video/247/purifying-plasmid-dna-froe Qiagen Miniprep Kit |m- bacterial-colonies-using-qiagen-miniprep (accessed Sep 25, 2016). Add 250 µl of Cell Resuspension Solution to the microcentrifuge tube containing the bacterial pellet and completely resuspend the cell pellet by pipetting. In the end, the column step concentrates the DNA while purifying it, since the elution volume is smaller than the original volume of the lysate. Turn on your Bacti-cinerator when you have about 5 minutes left on your conjugation. If you're not sure, you can get an empty culture tube and pipet some water in there, 1 ml at a time, until the volume matches the LB tube you are using. Add 350 µl of Neutralization Solution and mix by inverting the tube 4 times. Incubate at room temperature for 30 seconds. Centrifuge for 1 minute at 13,000 rpm. Page 2 The Bio 6B lab explores bacterial plasmids and operons through a set of connected experiments over multiple lab days. Before you leave: sample storage and waste disposal You should have a tube of plasmid DNA from each of your samples (pARO180, pGLO, pGLO Blue mutant, pGLO non-fluorescent mutant) in a rack with everyone else's DNA in the freezer. Nucleic acids, which are highly water-soluble, remain in the aqueous (water) phase. Zhang, S.; Cahalan, M. The cells will take care of the conjugation on their own, but you need to give them enough time to complete the process. Why do we need controls? Gloves, paper towels, etc. You can use a plate spinner, but it's not necessary. Sterile water Method The instructions below describe the steps for a single culture; you should be doing two samples in parallel. Under these conditions, the DNA binds to the column. Why does plasmid DNA bind to the stationary phase in the column at one point in this experiment? Plasmids are circular double stranded DNA molecules that occur naturally in bacteria and come in variety of sizes. Also, once you do the calculation, you should realize that you're only adding a small amount of antibiotic solution; if you have 4 ml LB and you add a tiny amount of amp, the final volume will still be approximately 4 ml. How to inoculate the cultures To start a liquid culture from a plate, use a sterilized metal loop to scrape a tiny bit of a colony from the plate, then twirl the loop in the liquid LB. The liquid that went through the Spin Column is waste. I based the Bio 6B lab procedure on this article, but found it necessary to change some of the procedures in order to get results comparable to those presented in this article. Repeat the pipetting and spreading steps for all your plates. If there is, gently warm the tube and mix (but don't shake it up) until no precipitate is visible. coli HB101 containing the plasmid pARO180. Insert Spin Column into Collection Tube. go in the regular trash. Positive controls: S17 grows on amp, HB101 grows on strep. Not only will it eliminate proteins and other cell components from your plasmid DNA, it will also eliminate the chromosomal DNA so you end up with a very pure sample of plasmid DNA. Which control plates are necessary to prove that conjugation occurred? Label your empty culture tube "mix." Pipet 0.5 ml of E. This solution comes in a bottle with the kit, but it ethanol must be added to it before use. Both those techniques gave you the macromolecules you were looking for, but not in a pure form; you ended up using specific stains for proteins or nucleic acids on your gel so you could see your desired results. 5). If either strain already grows on both antibiotics, you will have no way of knowing whether conjugation occurred. Before you begin this experiment, read the bacterial strains and plasmids page. Not all the cells will conjugate, so the "mix" liquid culture will actually contain both original strains plus the new strain created by conjugation. The concentration of the stock solution might be 50 mg/ml, but you should check. Also, there are controls in this experiment, but there are no control groups. Calculating the amount of ampicillin Add ampicillin to your culture tube to a final concentration of 100 µg/ml. You can spin the tube without a lid, but balance the centrifuge carefully. Sterilize the loop again immediately after spreading. In both the DNA and protein columns, you can elute your macromolecules by washing away the chaotropic salts and dissolving the macromolecules in water. The neutral pH allows plasmid DNA to renature, returning to its original double-stranded state. Don't dump out the liquid; it will be sterilized and disposed of later. Results 0567891011 0 1 1. Biochemistry and Molecular Biology Education. The spin column was placed in a new clean 2 mL centrifuge tube. Review Terms and concepts Ampicillin & streptomycin Antibiotics and antibiotic resistance Conjugation Controls in this experiment: positive and negative Inoculate Liquid and plate media Plasmid Satellite colonies Selective media Review questions Compare and contrast ampicillin and streptomycin in this experiment. Why did we use each of these methods? There is a1% error. Biolabs, N. Satellite colonies are another potential problem with this procedure, specifically in the mixed culture. Don't scrape a large blob into your liquid; the culture will become overgrown. The plasmid purification technique used in this lab is much more specific. 13 minutes. Several companies make similar kits. Smillie et al., 2010. What are the mechanisms of resistance? These are the cultures you started in the previous lab period and left in the shaker incubator. If you're a chemistry minded-person, you might be interested in this: Water structure and chaotropicity: their uses, abuses and biological implications. We don't have the satellite colony problem with strep, because strep-resistant cells tolerate strep, but they don't destroy it. Sterilize your inoculating loop with the bacti-cinerator and use it to spread the 10 µl of liquid culture as evenly as possible, all over the plate. Let all 3 culture tubes (S-17, HB101, and mix) sit in the rack for 30 minutes, allowing conjugation to occur. The plasmid pBR322 contain 4,361 base pair. Our results indicate that plasmid pBR322 contains 4,448 base pairs compared to the literature value of 4,361 base pairs (Watson, 1988). Gene 1988, 70 (2), 399–403. If you don't get conjugated cells growing on A+S, you'll need these controls to help you understand what happened; these cultures will tell you whether the starting strains actually had the expected resistance. Transfer Spin Column to a new, sterile 1.5 ml microcentrifuge tube. Inoculating loop (metal) Optional: plate spinner Perform the conjugation and plate out the cultures Warning: glass culture tubes don't seal tightly! They are designed to allow air circulation. Peel off the tape labels and put the tubes in a tube rack in the used glassware tub on the cart. Add another 250µl Column Wash Solution to Spin Column and spin for 5 minutes. This long spin is important to eliminate all the ethanol from the wash solution. Keep the tubes upright by placing them in a test tube rack. The concepts behind these labs are presented in a set of related pages on this site: In addition, there are multiple pages for the experimental methods, which you'll find in the menus. This could be as a result of losing some DNA during digestion and gel extraction. coli HB101 containing the mutated version of the plasmid pGLO that produces non-fluorescent colonies. A common plasmid-cloning vector in E is pBR322. Collection tubes. All the cells containing the pARO180 plasmid have the ampicillin resistance gene, so they are producing and secreting β-lactamase, the enzyme that breaks down ampicillin. The procedures shown here are only the beginning. To be sure that conjugation occurred, you need to see isolated colonies on the A+S plate, spread far away from the place where you initially transferred the culture liquid to the plate. The antibiotic plates will act as a selective medium, allowing some bacteria to grow while others can't. In Bio 6B, you will perform a multi-step examination of bacterial conjugation, which is the transfer of plasmid DNA from one cell to another. Under these conditions, the DNA binds to the glass matrix. Neutralization Solution Alkaline Protease Solution Spin Columns ("Wizard® SV Minicolumn"). How is chromosomal DNA eliminated? Kaiser & Suchman, 2013. Videos How to Harvest Plasmid DNA with Minipreps. Watson, N. Next, you use a wash buffer containing alcohol, which also decreases the solubility of DNA. Be careful not to spatter, and don't reuse the pipet tip. After studying and recording your plate results, extract total nucleic acid from your colonies, and save some plates as described in the instructions. Alkaline lysis and plasmid purification The detailed steps are described in the protocol section below, but it's worth taking a moment to consider what these steps are supposed to do. Chaotropic effects Both plasmid DNA purification and hydrophobic interaction chromatography (HIC) for proteins make use of chaotropic salts. You will perform a multi-step examination of bacterial conjugation, which is the transfer of plasmid DNA from one cell to another. coli S-17/pARO180 and 0.5 ml of E. Blanco September 26, 2016 Abstract Plasmid pBR322 was purified from an E cell using a Qiagen kit. Add 100 µl sterile water to the spin column. They don't tell us exactly what's in the solutions, but it's based on a classic technique known as alkaline lysis. You will need 9 plates, as listed above and shown in the results table below. For this experiment, you should look for the formation of new colonies: isolated dots of bacteria that form when a single cell grows into a large blob. It's always possible that some cells will lose their plasmids, simply by chance. (You can mix vigorously at this point because the DNA is still inside the cells.) Add 250 µl of Cell Lysis Solution to the resuspended pellet and mix by inverting the tube 4 times. What are the other controls for? Be sure there is no more wash solution in or on the spin column; it should be dry. Then 350 µL of N 3 buffer was added, mixed, and centrifuge for 10 minutes at maximum speed. Why do we use antibiotics in this experiment? How do you know whether conjugation occurred? The error could have resulted from the difficulty to measure the migration distance, accurately. liquid phase Review questions Why was it important to use antibiotics in the cultures? It also contains the genes for resistance to ampicillin and tetracycline as it shows in Figure 1 (pBR322 Vector). Microbiology and Molecular Biology Reviews. The TBE was diluted to obtain the required concentration. Centrifuge the Spin Column at 13,000 rpm for 1 minute. Don't put your tape label on the cap, because you might mix up the caps. This procedure described on this page uses the Promega Wizard Miniprep kit. If you google it, you'll find two kinds of explanations: those that are simple and clear, and those that are true. Repeat this process for the strep plates and the amp+strep plates. The alkaline pH disrupts the hydrogen bonding that holds the two strands of DNA together, so the DNA will be single-stranded.NeutralizeThe neutralization buffer restores pH to near neutral, and contains some ions. This experiment will be spread over six lab days and will include these steps: The steps of the conjugation lab will overlap with the pGLO lab. The molecular weight ofthe plasmid is approximately 2 x10 6 Daltons (Biolabs).Figure 1- pBR322 Vector speed. Once you've made your plates, you don't need the liquid cultures any more. DNA Purification: The basics of DNA isolation, plasmid growth and DNA quantitation from Promega, the company that produces the kit that we use. The lysis buffer contains SDS, which solubilizes both membranes and proteins. Plasmids carry genes for the inactivation of antibiotics, production of toxins, and breakdown of natural product. Make sure all the liquid goes through the column for this and for subsequent spin column steps. When the spin is done, you'll have a cleared lysate. Before using, make sure there is no precipitate in this tube. Both cultures should contain ampicillin, because both plasmids contain the gene for β-lactamase, the ampicillin resistance gene. If you want to culture bacteria that contain the plasmid pARO, which plates could you use? A technique video from Synthetic Biology One. Philip Ball and John Hallsworth, 2012. Biochemistry. The column contains a matrix typically made of silicon dioxide (glass) particles. This solution is passed through a mini-column inside a microcentrifuge tube. ASM. If you put some of that culture liquid on the A+S plate, the excreted β-lactamase might break down the amp in the plate, allowing the non-amp-resistant HB101 cells to grow. The plasmid has a gene providing resistance to ampicillin, but the S17 strain isn't resistant to streptomycin. Microcentrifuge tubes and pipet tips go in the biohazard trash. Put the plates in the plate incubator to grow until the next lab period. Label your plates. So far, you have used lysates for proteins (in the SDS-PAGE lab) and for nucleic acid (for the total nucleic acid gel of the conjugation lab). (Fingertips courtesy of Jessica Gutierrez.) Get your lysate out of the centrifuge; you should now see a cleared lysate (the supernatant) and a white precipitate. How are proteins eliminated? How can you tell the difference between conjugated cells and the original plasmid donor? coli S-17/pARO180 (the plasmid donor) and HB101 (the plasmid recipient) in glass culture tubes. If you don't add the antibiotic, the no-plasmid cells, having less DNA to copy, will grow faster, and may eventually dominate the culture (especially if you grow the culture for more than 24 hours). Solid phase vs. An addition of 50 µL EB buffer was added to the spin column and held stationary for 1 minute and centrifuged for another minute. If one of the starting strains does grow on both antibiotics, it could either be because it's the wrong strain (already double resistant), or the cultures were cross-contaminated, or because the plates don't contain the antibiotics they're supposed to contain. Don't write directly on the tubes or caps, because we re-use them. Background Bacterial conjugation is the transfer of a copy of a plasmid from one bacterial cell to another. The antibiotics don't necessarily kill the cells, they only slow their growth. Procedure for day 2: plate results & total nucleic acid extraction Observe and record your plate results. If it doesn't close, pull or cut the lid off the tube and save it for later. If conjugation failed, you'll probably find that these colonies grow on strep and not on amp. There are some weak bands on the plasmid lane, such as, lane 1 and lane 2. Use C1V1=C2V2 to calculate the appropriate amount of antibiotic stock solution to add. L., Gatto, G. Your DNA should now be bound to the column. References Berg, J. coli: E. If you're unsure whether conjugation occurred, restreak some colonies from the A+S Mix plate onto a new A+S plate. Culture and pelletYou grow a dense culture of bacteria and spin it down into a pellet, concentrating the cells.ResuspendThe cell resuspension solution gently resuspends the cell pellet, so you can lyse the cells quickly and evenly in the next step.Lyse"Lyse" means to split the cells open (to cause lysis). You will use this technique two different times in Bio 6B, for the plasmids pARO180 and pGLO. In this lab procedure, you'll purify plasmid DNA from bacterial cultures. Genomic DNA Extraction: The Difference by Suzanne Kennedy. The essence of column chromatography is that there are two phases: a stationary phase (the glass matrix inside the column) and a mobile phase (the various buffers that you pass through the column). When I ask 6B students what a control is, they usually say that it's something you compare your experimental results to. pBR322 Vector promega/products/biochemicals-and- labware/nucleic-acids/pbr322-vector/ (accessed Sep 25, 2016). Don't set the tubes on their sides. The exact timing of the spin isn't important; the important thing is getting the liquid though the column. In HIC, you will also use chaotropic salts to weaken the interaction between water and protein, so the protein wil bind to the stationary phase in the column. If some wash solution, which contains ethanol, remains in the column, you will not recover all your DNA and you not be able to load your DNA onto a gel later. Beakers used for waste tips: dump the tips into the biohazard trash and put the beaker into the dirty-glassware tub on the cart (if there's not a tub, check with the instructor). Centrifuge at 13,000 rpm for 1 minute. Offering a clear and simple overview of the technique, this is the best thing to read if you want to undersand the technique. One for each sample. Transfer of Conjugative Plasmids and Mobilizable Plasmids in Gram-Negative Bacteria. Be sure to use sterile water from a glass bottle, not deionized water from the tap (which isn't sterile). If both these positive controls grow as expected, it proves that you did actually add both strains to your "mix" tube. Log(DNA size) vs. In that case, some cell components had a higher affinity for chloroform, and they were pulled away from the aqueous phase. Through agarose gel electrophoresis we compare the purify plasmid with the prepared marker and calculated the base pair with 1% error. As positive controls, restreak some of the same colonies onto amp alone and strep alone. Be sure not to transfer any of the pelleted precipitate. (Alkaline protease is a protein-digesting enzyme that works in the alkaline conditions of the cell lysis solution. Adding the antibiotic ensures that only cells containing the plasmid can grow. Detailed background on the characteristics of plasmids that make them mobilizable. This neutralizes the highly alkaline pH of the lysis solution; if DNA is left too long in the lysis solution, it will be damaged. Pipet 1.5 ml of bacterial culture into a microcentrifuge tube. As with any experiment, it's important to understand the controls: Negative controls: S17 doesn't grow on strep (or A+S); HB101 doesn't grow on amp (or A+S). How To Perform a Plasmid Miniprep from Bio-Rad Explorer. Some of the cells are expected to conjugate, meaning that the plasmid pARO is copied from S17 to HB101. The molecule is double stranded circle with 4,361 base pairs length(Watson ,1988). The liquid that goes through the column contains your plasmid DNA. Add 10 µl Alkaline Protease Solution and mix by inverting the tube 4 times. (20171-73. The solution contains chaotropic salts, which weaken the hydrogen bonding in the water, and making the DNA less soluble. The chromosome contains a gene providing resistance to streptomycin, but without a plasmid, this strain isn't resistant to ampicillin. The supernatant was discarded from all three tubes and the pellets were combined and re-suspended with 250 µL of P 1 and P 2 buffers. Review Terms and concepts Alkaline lysis Chaotropic salts Column chromatography Denaturing DNA Lysis (noun, the process of breaking cells open); lyse (verb; to break open the cells); lysate (noun, the material released from cells during lysis). A control group would be used if you repeated the experiment multiple times with different individuals. Be sure the whole pellet gets resuspended – don't leave any chunks. Use a liquid culture in LB broth, because liquid cultures are the best way to produce large numbers of cells, and LB provides all the nutrients needed for growth. Why is the DNA released into the aqueous mobile phase (eluted) from the column at another point in the experiment? If isolated colonies grow, then they're resistant, meaning that conjugation occurred. The plasmid sample was prepared with 3 µL 6x dye, 6 µL EB buffer, and 10 µL of the plasmid. Physical Chemistry Chemical Physics. The plasmid purification method is also similar to hydrophobic interaction chromatography (HIC), which you will use as a protein purification technique in the pGLO lab. Pipet off the supernatant into an empty glass culture tube. Glass culture tubes with lids: peel off the tape labels and put the tubes in a tube rack in the dirty-glassware tub on the cart. Related techniques The purification step in the plasmid purification protocol is a form of column chromatography. If you grew your cultures in LB without arabinose, they won't be fluorescent. Background Purifying specific types of macromolecules is a fundamental technique in molecular biology and biochemistry. After the centrifugation, 800 µL of the supernatant was added to a spin column and centrifuged at same time and speed as prescribed above. If you don't get conjugated cells growing on A+S, it could be because the cells failed to conjugate, or because you failed to mix the two together when you were supposed to (which has happened many times in Bio 6B!). The sample was loaded into the well of the gel. However, the much-larger chromosomal DNA can't renature fully. The solution was microwaved for 2 minutes into electrophoresis chamber and hardened for approximately 30 minutes. J., & Stryer, L. Purification of plasmid DNA from E cells Michael J. coli HB101 containing the unmutated version of the plasmid pGLO, which produces green colonies. When you ran your total nucleic acid gel, you probably used a small sample of purified plasmid as a size marker. M., Tymoczko, J. Be sure all the liquid passes through the column. Now you can purify your own, and later analyze it with restriction digests and a gel. If you think this calculation requires a calculator, you might want to look at the amazing milli x micro trick on the Calculations page. This page includes an old-fashioned graphic that shows the difference. Procedure for day 1: conjugation Materials Test tube rack for glass culture tubes Your liquid cultures of E. Start this culture from the amp+strep plate from your conjugation experiment. Be sure all the liquid goes through. Here are the expected results (as shown in the diagram at the top of the page): PlateS17/pARO180HB101Mix Amp + — + Strep — + + A+S — — + "+" means that colonies grow; "-" means that they don't. Meanwhile, get your Spin Column ready. The experimental procedure is very simple; you simply need to mix the two bacterial strains together, let them conjugate, and then spread them on plates with antibiotics to see if they grow. Add 750 µl Column Wash Solution (which was previously diluted with ethanol) to Spin Column. I don't think you can have both. Positive controls: The mix culture grow on amp alone and on strep alone. The mechanisms by which chaotropic salts make macromolecules bind to the solid phase in a column make an interesting and somewhat controversial topic, which goes way beyond Bio 6B. It destroys enzymes that might damage the DNA.) Incubate 1 minute at room temperature. Label both column and tube. This is important for many downstream uses of plasmids, such as restriction digests, sequencing, and transformation. Swirl the S-17 culture gently, and pipet 10 µl of the liquid onto the S17/amp plate. The buffer content is: P1: 50 mM Tris/HCl, pH 8, 10mM EDTA, 0 mg/mL RNase P2: 200 mM NaOH, 1% SDS N3: .9 M at pH 4 acetate buffer PE: 10 mM Tris/HCl pH 7, 80% ethanol EB: 10 mM Tris/HCl pH 8. Spin at 13,000 rpm (high speed) for 5 minutes to pellet cells. coli HB101 containing the mutated version of the plasmid pGLO that produces blue colonies. If you put a lot of non-resistant cells on a plate, you might see some living cells after incubation. The wash solution that ends up in the Collection Tube is waste. In plasmid DNA purification, you reduce the affinity of the DNA for the aqueous liquid phase two different ways: first, you use chaotropic salts, which weaken hydrogen bonding and decrease the solubility of DNA. You should end up with each of your cultures (S17, HB101, and mixed) being cultured on three kinds of plates: amp, strep, and amp + strep. You'll use your purified plasmids from

today's procedure for restriction digests, but you didn't do that with the DNA from the total nucleic acid procedure. Each original strain is resistant to one antibiotic, but not to both, so you should be able to determine whether conjugation occurred by looking for colonies that grow on amp & strep together. The spin column was washed with 750 µL of PE buffer and was centrifuged again. Why? If they have greater affinity for the liquid phase, they are eluted (washed away) from the column. The mixture was mixed thoroughly. Also, in these conditions, the SDS and denatured proteins tend to aggregate and precipitate; the partially denatured DNA precipitates along with them and ends up in the pellet.Column purifyAfter a spin, the neutralized lysate contains plasmid DNA. When using glass culture tubes, label them by putting a piece of tape on the tube and writing on that. E. References Conjugation in Escherichia coli: A laboratory exercise Phornphisutthimas et al., 2007. After you perform the conjugation part of the experiment, you'll go on to analyze the DNA directly in a series of additional experiments. It needs to warm up until the inside of the tube is glowing orange. The wash solution that ends up in the Collection Tube is waste; transfer it to your waste tube before doing the next step. The lysate will go through the Spin Column, but the DNA sticks to the column. Note that each control serves a specific purpose. One empty sterile glass culture tube Pipetters, tips, and beaker for waste Plates: 3 LB with ampicillin, 3 LB with streptomycin, 3 LB with both amp and strep. A 1% agarose gel solution for electrophoresis was prepared. That tube could potentially contain three different strains: S17/pARO180, HB101, and conjugated cells (HB101 with pARO180). pBR322 Vector neb/products/n3033-pbr322- vector (accessed Sep 25, 2016). Do your results prove that conjugation occurred? Compare and contrast the total nucleic acid extraction procedure with the plasmid purification procedure. Unlike the plasmid DNA column, the HIC column is hydrophobic, and the proteins bind by hydrophobic interactions. References Plasmid purification The Basics: How Alkaline Lysis Works by Nick Oswald on Bitesize Bio. coli HB101 with no plasmid. You'll purify four different plasmids, so you'll need to start four different liquid cultures: E. Pour off the wash solution from the Collection Tube. A very detailed guide to the general principles involved as well as the specific products. Don't put it in your beaker with your waste tips. How do the cells get lysed in this procedure? no growth is simple, but plate results can sometimes be difficult to interpret. Centrifuge the lysate for 10 min at 13,000 rpm. How do these antibiotics act? Also add your name and the date. If the lid of the microcentrifuge tube closes on top of the spin column, close it. The Qiagen kit contains the necessary buffers and instruction to purify plasmid pBR322. The solution contained .5g of agarose in 50 mL of 1mM Tris/Borate/EDTA (TBE). In this experiment, which plates are controls? For the 3 amp plates, label them S17, HB101, and mix. This is true, but it's not meaningful; the question is, what do you learn from the comparison? Column chromatography is similar to the liquid-liquid extraction that you performed with chloroform. Pipet the thick gob of stuff at the bottom of the culture tube to get as many cells as possible. Do not combine liquid waste and solid waste. Starting the cultures You'll start the cultures one lab period and let them grow until the next. Use a glass culture tube as a waste container for the liquid; you can either pour it or pipet it from the collection tube into the waste tube. Don't use a bottle directly out of the kit if the ethanol hasn't been added yet. In principle, growth vs. Plasmid purification protocol Materials Your tubes of liquid bacterial culture containing pARO180 and pGLO plasmids (including regular pGLO and mutant pGLO). Introduction Plasmid purification is used to isolate and purify DNA from genomic DNA, proteins, ribosomes, and bacterial cell well, such as, E. Don’t leave the tubes in a beaker. It also contains a strong base such as NaOH. Detailed manual, including troubleshooting. Where are the genes located (chromosome or plasmid)? In this experiment you'll allow conjugation to occur, then verify that it occured both by checking for the transfer of antibiotic resistance from one cell to another and by directly examining the cells' DNA. Mix gently at this point because the cells have lysed and the DNA is free in solution and has been denatured to a single-stranded form. The DNA molecules can be damaged by excessive pipetting or mixing. If you can see any cells transferred into the liquid, it's plenty. Other relevant articles on the same site include How DNA Extraction Kits Work in the Lab and Plasmid vs. This experiment will be spread over six lab days and will include these steps: Plasmid purification is also part of the pGLO lab. The chaotropic salts are washed away with a buffer that contains alcohol; this wash buffer dissolves the salts but not the DNA. The solution goes through, but the DNA remains stuck to the Spin Column. Keep these tubes upright; don't lay them down anywhere, ever. The volume of LB broth in the tube varies; often it's 4 ml. Throw away the spin column, close the tube containing the eluted DNA, and save it in the freezer until next lab period. If molecules have greater affinity for the solid phase than for the liquid phase, they bind to the column. Don't pour out the liquid culture medium; the tubes will be autoclaved to kill the cells. Only the conjugated cells will grow on amp + strep plates. Finally you elute the DNA using pure water, which readily dissolves DNA. coli HB101 into this tube, mixing the two cultures so they can conjugate. You'll mix liquid cultures of these two strains to make a new liquid culture: "Mix." The two strains mixed together in one culture tube. That's it for today; you will see your plate results in the next lab.