Conventional pcr using agarose gel electrophoresis detection

ASSIGN BUSTER

Conventional PCR using agarose gel electrophoresis detection According to Lewis " Agarose gel electrophoresis is the easiest and commonest way of separating and analyzing DNA". Conventional techniques used to detect mycoplasma involve culturing samples on selective media, which needs at least a week to obtain the results (HD BIOSCIENCES Co., LTD 2). The reason to add gel in this method is to either quantify the DNA or separate the particular band of DNA. With the addition of edhidium bromide, DNA becomes visible. This binds strongly to DNA by intercalating between the bases and is fluorescent meaning that it absorbs invisible UV light and transmits the energy as visible orange light (Lewis).

While addition of gel, the care for the percentage of it has to be taken as " a 0. 7% gel will show good separation (resolution) of large DNA fragments (5–10kb) and a 2% gel will show good resolution for small fragments (0. 2–1kb)." So, the percentage of the gel is kept between 0. 7% to 2%.

With intention to separate very tiny fragments, addition of high percentage (up to 3%), is not useful as a vertical polyacrylamide gel is more appropriate in this case. The medium percentage is always recommended as low percentage gel may break while trying to lift them and high percentage gels may often brittle not setting evenly. Lewis recommends 1% gel to use.

While suggesting for gel tank Lewis recommends, " Small 8x10cm gels (minigels) are very popular and give good photographs." For the applications of Southern and Northern blotting, larger gels are used. 30–50mL and 205 mL of agarose is required for minigel and larger gel respectively. While deciding the amount of DNA to be added to this solution, the nature of analysis has to be kept in mind. According to Lewis " Typically, a band is easily visible if it contains about 20ng of DNA."

After doing all the above preparation Lewis says, “ I usually digest and load 2–4µL of the 50µL obtained from a kit miniprep. But you see how it depends on the number and size of the bands expected. For PCR reactions, it depends on the PCR but in routine applications 10–20µL should be plenty to see the product on the gel.”

Depending on the volume of DNA being loaded and the number of samples, the design of comb is decided to include in the process. Lewis recommends, “ Combs with many tiny teeth may hold 10µL. This is no good if you want to load 20µL of restriction digest plus 5µL of loading buffer. When deciding whether a comb has enough teeth, remember that you need to load at least one marker lane, preferably two.” After having prepared this Lewis suggests the following process:

Making the gel (for a 1% gel, 50mL volume)

Weigh out 0. 5g of agarose into a 250mL conical flask. Add 50mL of 0. 5xTBE , swirl to mix.

Microwave for about 1 minute to dissolve the agarose.

Leave it to cool on the bench for 5 minutes down to about 60°C (just too hot to keep holding in bare hands).

Add 1µL of ethidium bromide (10mg/mL) and swirl to mix

Pour the gel slowly into the tank. Push any bubbles away to the side using a disposable tip. Insert the comb and double check that it is correctly positioned.

Leave to set for at least 30 minutes, preferably 1 hour, with the lid on if possible.

Pour 0. 5x TBE buffer into the gel tank to submerge the gel to 2–5mm depth. This is the running buffer.

Transfer an appropriate amount of each sample to a fresh microfuge tube.

Add an appropriate amount of loading buffer into each tube and leave the tip in the tube.

Load the first well with marker.

Continue loading the samples and finish of with a final lane of marker

Close the gel tank, switch on the power-source and run the gel at 5V/cm.

Check that a current is flowing

Monitor the progress of the gel by reference to the marker dye.

Switch off and unplug the gel tank and carry the gel (in its holder if possible) to the dark-room to look at on the UV light-box.

The loading buffer gives colour and density to the sample to make it easy to load into the wells. Also, the dyes are negatively charged in neutral buffers and thus move in the same direction as the DNA during electrophoresis. This allows you to monitor the progress of the gel. The most common dyes are bromophenol blue (Sigma B8026) and xylene cyanol (Sigma X4126). Density is provided by glycerol or sucrose.

Bromophenol blue migrates at a rate equivalent to 200–400bp DNA. If you want to see fragments anywhere near this size (ie. anything smaller than 600bp) then use the other dye because the bromophenol blue will obscure the visibility of the small fragments.

Xylene cyanol migrates at approximately 4kb equivalence. So do not use this if you want to visualise fragments of 4kb.

Advantages: and

Disadvantages:

As in next page

References:

HD BIOSCIENCES Co., LTD. “ Mycoplasma Detection Kit for Conventional PCR”. HD BIOSCIENCES Co., LTD, Unit 302, 303, 1011 Halei Road, Shanghai, P. R. C, Zip: 201203. 2005. May 15, 2009 www. hdbiosciences. com.

Lewis, M. “ Agarose Gel Electrophoresis (Basic Method)”. Department of Pathology

University of Liverpool. 2001. May 15, 2009. http://www. methodbook. net/dna/agarogel. html

PCR Station. “ PCR Limitations and Advantages” 2006-2007. May 15, 2009 http://www. pcrstation. com/pcr-limitations/

Wassenegger, M. “ Advantages and Disadvantages of Using PCR Techniques to Characterize Transgenic Plants.”. 2004-2007. Ion Channel Media Group Ltd. Medical literature citations were obtained from the National Center for Biotechnology Information. Protein database information was obtained from the European Bioinformatics Institute. http://www. epidna. com/showabstract. php? pmid= 11280933

Datar, R. H. and Bhisey, A. N., “ A sensitive method for permanent silver staining of DNA in agarose gels”. Indian Journal of Biochemistry & Biophysics. 1988. 25, 373-375.

Peats, S., “ Quantitation of protein and DNA in silver-stained agarose gels”. Analytical Biochemistry. 1984. 140, 178-182

Gottlieb, M. and Chavko, M., “ Silver staining of native and denatured eukaryotic DNA in agarose gels”. Analytical Biochemistry. 1984. 140, 178-182

Beidler, J. L., Hilliard, P. R and Rill, R. L. “ Ultrasensitive Staining of Nucleic Acids With Silver”. Analytical Biochemistry. 1982. 126, 374-380

Santillan-Torres, J. L. and Ponce-Noyola, P., “ A Novel Stain for DNA in Agarose Gels”, Trend genet. 1993. 9 (2), 40

Adkins, S. and Burmeister, M., “ Visualization of DNA in Agarose Gels as Migrating Colored Bands:” Applications for preparative gels and educational demonstrations, Analytical Biochemistry. 1996. 240, 17-23