A Basic Polymerase Chain Reaction Protocol

Introduction

The polymerase chain reaction (PCR) is the cardinal laboratory technology of molecular biology. Arguably one of the most powerful laboratory techniques ever discovered, PCR combines the unique attributes of being very sensitive and specific with a great degree of flexibility. With the PCR it is possible to specifically address a particular DNA sequence and to amplify this sequence to extremely high copy numbers. Since its initial development in the early 1980's, dozens of variations in the basic theme of PCR have successfully been carried out. In fact, the very flexibility and application-specific variation of PCR make it seem like there are as many ways to do a PCR reaction as there are researchers doing them. Here, a basic, straight-forward PCR protocol is presented. Where appropriate, some of the choices for modifying this standard reaction that are routinely available to researchers are discussed.

Step 1: Choosing Target Substrates and PCR Primers

The choice of the target DNA is, of course, dictated by the specific experiment. However, one thing is common to all substrate DNAs and that is they must be as clean as possible and uncontaminated with other DNAs. Naturally, if the source material is an environmental sample such as water or soil, then the researcher must rely upon the specificity of the PCR primers to avoid amplification of the wrong thing.

Specificity in the choice of PCR primers should be an issue in any PCR amplification. The on-line IDT SciTools software OligoAnalyzer 3.0 and PrimerQuest are invaluable aids both in primer design and validation. PrimerQuest will assist in primer design and will permit the researcher to directly assess primer specificity via a direct BLAST search of the candidate sequences (see the Bioinformatics Tutorial). Taking candidate primer sequences into OligoAnalyzer will allow for each primer sequence to be assessed for the presence of secondary structures whether these are hairpins or homo- and hetero-dimers. Many of the basic analyses available in PrimerQuest and OligoAnalyzer are presented in the Polymerase Chain Reaction Tutorial.

Step 2: Setting Up the Reaction

Once you have chosen the appropriate substrate and your PCR primer sequences and you have them on hand, the basic reaction components are as follows:

Water
10x Reaction Buffer
$MgCl_2$
dNTPs
Forward Primer
Reverse Primer
Target DNA
Polymerase enzyme

The role that each of these components plays in a PCR reaction is discussed in the Tutorial entitled Polymerase Chain Reaction. One common choice available to the researcher is whether or not to use a Reaction Buffer that already contains the magnesium chloride. The vast majority of PCR reactions will work perfectly well at a 1.5 millimolar (mM) magnesium chloride concentration. For this reason PCR reaction buffers that do contain $MgCl_2$ are prepared so that the final concentration is 1.5mM in the 1:10 dilution. Occasionally, however, $MgCl_2$ final concentrations other than 1.5mM may be optimal. When this occurs it becomes necessary to use a reaction buffer that does not contain $MgCl_2$ and to add the $MgCl_2$ separately.

Step 3: Choosing the Reaction Conditions

The reaction conditions of a PCR amplification are composed of the total number of cycles to be run and the temperature and duration of each step in those cycles. The decision as to how many cycles to run is based upon the amount of DNA target material you start with as well as how many copies of the PCR product (amplicon) you want. In general, 25 to 35 cycles is the standard for a PCR reaction. This results in from approximately 34 million to 34 billion copies of the desired sequence using 25 cycles and 35 cycles respectively. Additional cycle numbers can be used if there is a small amount of target DNA available for the reaction. However, reactions in excess of 45 cycles are quite rare. Also, increasing the number of cycles for larger amounts of starting material is counter productive because the presence of very high concentrations of the PCR product is itself inhibitory (see Kainz, 2000).

Once the number of cycles is selected, it is necessary to choose the temperature and duration of each step in the cycles. The first step is the **DNA denaturation step** that renders all of the DNA in the reaction single stranded. This is routinely accomplished at 94^{o}C or 95^{o}C for 30 seconds. The second step is the **primer annealing step** during which the PCR primers find their complementary targets and attach themselves to those sequences. Here the choice of temperature is largely determined by the melting temperature (Tm) of the two PCR primers (see OligoAnalyzer 3.0 in IDT SciTools). Again, the usual duration is 30 seconds. Finally, the last step in a PCR cycle is the

polymerase extension step during which the DNA polymerase is producing a complementary copy of the target DNA strand starting from the PCR primer sequence (thus the term primer). The usual temperature of this step is 72 ^{o}C, considered to be a good optimum temperature for thermal-stable polymerases. A common rule of thumb for the duration of this step has been 30 seconds for every 500 bases in the PCR product. However, with the increasing quality of commercially available polymerase enzymes and the associated reaction components, this time can be significantly shortened but should be done in a systematic manner since the optimal extension time can be polymerase and sequence specific. In addition to these cycling conditions, it is often desirable to place a single denaturation step of three to five minutes at 94^{o}C or 95^{o}C at the beginning of the reaction and a final extension step of a few minutes at 72 ^{o}C. A convenient shorthand way of representing a complete set of reaction conditions is:

$$94^{o}C^{5:00}[94\,^{o}C^{0:30};\ 60\,^{o}C^{0:45};\ 72\,^{o}C^{2:00}]_{35};\ 72\,^{o}C^{7:00}$$

which means an initial denaturing step of five minutes at 94^{o}C followed by 35 cycles of 94^{o}C for 30 seconds, 60^{o}C for 45 seconds and 72^{o}C for two minutes and then a final extension at 72^{o}C for seven minutes.

Step 4: Validating the Reaction

Once your PCR reaction has run, there are two ways of determining success or failure. The first is to simply take some of the final reaction and run it out on an agarose gel with an appropriate molecular weight marker to make sure that the reaction was successful and if the amplified product is the expected size relative to the maker (see Gel Electrophoresis Tutorial).

The ultimate validation of a PCR reaction is to directly sequence the amplicon. This is often a choice that is not readily available since not everyone has access to a DNA sequencer nor will they have either the time or the funds to carry out such an analysis. One way to indirectly assess the sequence of an amplicon, however, is to carry out restriction enzyme digests on it. Given the vanishingly low likelihood that two well chosen primers will amplify an incorrect amplicon that matches the expected size, it is even more unlikely that an incorrect amplicon will give an expected pattern of restriction fragments (see Restriction Endonucleases Tutorial).

References and Resources

Kainz, P 2000 The PCR plateau phase- towards an understanding of its limitations. Biochem Biophys Acta 1494: 23-27.

There are a number of excellent resources for exploring the polymerase chain reaction and its variants. A few of these are listed below.

Bustin SA 2004 A to Z of Quantitative PCR. LaJolla, California: International Unniversity Line.

Chen B-Y, and HW Janes 2002 PCR Cloning Protocols, Second Edition. Totowa, New Jersey: Humana Press.

Dieffenbach CW, and GS Dveksler 2003 PCR Primer: A Laboratory Manual. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Harris E 1998 A Low-Cost Approach to PCR. Oxford: Oxford University Press.

Innis MA, DH Gelfand, JJ Sninsky, and TJ White (eds.) 1990 PCR Protocols: A Guide to Methods and Applications. San Diego, California: Academic Press.

McPherson MJ, SG Moller, R Beynon, and C Howe 2000 PCR: Basics from Background to Bench. Heidelberg: Springer-Verlag.

O'Connell J, and J O'Connell 2002 RT-PCR Protocols. Totowa, New Jersey: Humana Press.

Weissensteiner T, T Weissensteiner, HG Griffin, and AM Griffin 2003 PCR Technology: Current Innovations, Second Edition. Boca Raton, Florida: CRC Press.

In addition to reference materials, there are many web sites that can be consulted for applications and trouble-shooting. A few of these are listed below.

Animation:

www.dnalc.org/shockwave/**pcr**anwhole.html

http://www.dnalc.org/

http://www.people.virginia.edu/~rjh9u/pcranim.html

http://ls126.molsci.org/PCR_top.html

Applications and Trouble-shooting

http://biologi.uio.no/bot/ascomycetes/PCR.troubleshooting.html

http://info.med.yale.edu/genetics/ward/tavi/Trblesht.html

http://www.protocol-online.org/prot/Molecular_Biology/PCR/Real-Time_PCR/