Use of the Fluorescent Dye PicoGreen™ for Quantification of PCR Products after Agarose Gel Electrophoresis


Exact quantification of the amount of amplified polymerase chain reaction (PCR) product is a crucial element in quantitative PCR approaches. Normally, such quantification is based on the application of radioactive (2) or fluorochrome-labeled primers (4), HPLC (3) or densitometric-scanning of an agarose gel after staining with dyes like ethidium bromide (1). These quantification techniques often require the use of either labeled primers, highly specialized expensive equipment or both.

This paper describes a rapid, inexpensive method for quantification of DNA in single excised bands after electrophoretic separation in agarose gels. The method eliminates the use of labeled primers and may be used to quantify DNA directly from ethidium bromide-stained gels without any need for removal of the ethidium bromide. The principle of the method is fluorometric quantification of DNA in melted slices of low-melting agarose gels after staining with PicoGreen™. Fluorescence of the DNA-PicoGreen complex is measured directly in a standard laboratory fluorometer. In the present study, DNA quantities as low as 2.7 ng are measured after electrophoresis. However, with a lower detection limit of 27 pg of DNA, the method should have a potential for measurement of even lower quantities.

A standard PCR was set up by using a 431-bp amplicon as the template and two primers designed for specific amplification of a part of the 16S rRNA gene of the fish pathogenic bacterium *Aeromonas salmonicaida*. The reaction volume was 100 µL. The PCR mixture consisted of sterile water, buffer (standard 10× PCR buffer, Perkin-Elmer, Norwalk, CT, USA), dNTPs (each 200 nM), primers (each 1 µM), *Taq* DNA polymerase (2.5 U) and template amplicon (1.9 ng). After an initial denaturation step at 96°C for 6 min, the reaction mixture was cycled for 30 cycles in the following manner: 60 s at 95°C, 120 s at 55°C and 90 s at 72°C.

Before electrophoresis, the PCR product was diluted in a series of 1:2 dilutions with sterile water. Ten microliters of the undiluted reaction product and of each of five 1:2 dilutions were loaded onto a 2% low-melting NuSieve® agarose gel (FMC BioProducts, Rockland, ME, USA) with 10× sample loading buffer. Electrophoresis was performed in TAE buffer (40 mM Tris acetate, pH 8.8, 2 mM EDTA) in a 11-cm × 9-cm × 0.7-cm gel containing 1 µg/mL of ethidium bromide. The gel was run at 1 V/cm for 15 min and then at 5 V/cm for 3 h. The DNA-containing bands were visualized with ultraviolet (UV) transillumination. Bands representing the amplified PCR product were excised from the gel and transferred to preweighed plastic tubes.

Plastic tubes containing individual gel slices were weighed, and TAE buffer was added to a total volume of 1 mL (gel slice + buffer = 1 mL). The gel slice was melted by placing the tube at 70°C for 15 min.

One milliliter of a 1:200 dilution of PicoGreen (Molecular Probes, Eugene, OR, USA) in TAE buffer was added to the melted gel slice. After 2 min at room temperature, fluorescence was measured in a Perkin-Elmer LS5 Fluorescence Spectrophotometer under an excitation wavelength of 480 nm and an emission wavelength of 520 nm. Depending on the strength of the fluorescence signal, two different combinations of slits were used. For measurement of DNA in slices containing low amounts of DNA, the slits were set at 15 (excitation) and 20 (emission), while both slits were set at 2.5 when high amounts of DNA were measured. TAE buffer was used as a blank sample.

The exact amount of DNA in each sample was determined by using two standard curves, one for each of the two slit combinations. Standard curves were obtained by measurements of dilutions of a standard containing a known amount of bacteriophage λ DNA (Promega, Madison, WI, USA).

A linear relationship was obtained between the calculated and measured values (Figure 1) for the DNA content of the individual bands from the agarose gel. The assay proved stable over a wide range of dilutions, and values ranging from 2.8 to 84 ng of DNA in single bands could easily be measured. Based on the measured DNA content of the band representing 10 µL of the undiluted sample (85.0 ng), the theoretical DNA content of the lowest dilution should be 2.7 ng, while the measured value of this dilution was 2.8 ng. This method also has a potential for the measurement of DNA content in bands that are too faint to be seen in gels stained with ethidium bromide. According to the manufacturer, the lower detection limit of the method is about 27 pg/mL of DNA, exceeding the sensitivity of, for example, Hoechst 33258 and ethidium bromide by 400-fold and 20 000-fold, respectively. However, when the quantity of DNA is very low, the present method is limited by problems with accurate excision of non-visible bands from the ethidium bromide-stained agarose gel. The manufacturer claims that the fluorescence response is linear over more than four orders of magnitude to 1000 ng/mL.

One big advantage of this technique is its possibility to measure the DNA content of single bands also from ethidium bromide-stained gels, since the presence of ethidium bromide in con-
Quantitation of T-Cell Receptor Transcripts Using a Wet Agarose Gel Method


Polymerase chain reaction (PCR) methods have been used to quantitate the relative expression levels of T-cell receptor (TCR) beta (B) and alpha (A) variable (V) genes in various tissues. In the initial report describing a PCR method to quantitate TCRBV transcripts by Choi et al. (2), each BV in a cDNA sample from T cells was separately amplified using a BV-specific primer and a BC primer end-labeled with 32P. An AC fragment was amplified as an internal control in each tube, and one AC primer was end-labeled with 32P. BV-BC and AC PCR products were separated in 2% agarose gels, which were dried and exposed to x-ray films. The autoradiographs were used to identify the PCR products, which were then excised from the gels and analyzed by liquid scintillation spectrometry. The BV/AC ratios were determined for each BV, and then the relative levels of each BV were calculated. This method to quantitate TCR transcripts has been used by various investigators to study human BV usage (3,4) and has been adapted for study of the BV repertoire in 48 individuals (unpublished observations), we used the BVPMs as follows: ten microliters of each PCR product were mixed with 3 µL of the corresponding BVPM and then electrophoresed in 2% agarose. The individual BV bands were readily visualized by ethidium bromide staining; it was unnecessary to use positional markers for AC bands as these are typically brightly fluorescent under UV light. To confirm that each BVPM identified the corresponding radiolabeled BV, a cDNA sample was PCR-amplified with each of the 23 BV primers and with the radiolabeled BC primer in separate tubes. After agarose gel electrophoresis with the BVPMs, the gel was dried and exposed to x-ray film. The position of each BVPM on the dried gel and the position of the corresponding bands in the autoradiograph were completely concordant.

Since the use of the BVPMs made gel drying unnecessary to detect agarose gel PCR products, we excised directly the BV-BC and AC bands from the wet gels immediately after electrophoresis, dissolved them in scintillation fluid (Ready Value™; Beckman Instruments, Mississauga, ON, Canada) and then determined the radioactive counts by liquid scintillation spectrometry. We evaluated the reproducibility of this wet agarose gel stained agarose gels especially when particular BVs were expressed at low levels. To detect BV PCR products, we developed a set of agarose gel position markers for 23 human TCRBVs, including BV families 1–20, BV5S1, BV5S2–3, BV13S1 and BV13S2. Each BV from a T-cell cDNA sample was PCR-amplified separately using 23 BV primers (2,8) together with a BC primer (2) that was unlabeled. A 2-µL aliquot of each BV-BC PCR product was then separately re-amplified in a second round PCR. The PCR products were chloroform-extracted and mixed in a 2:1 ratio with loading dye consisting of equal volumes of glycerol and 0.45 M Tris-borate/0.01 M EDTA, pH 8.0, containing 10 mg/mL bromophenol blue. Each BV-BC loading mixture was stored at -20°C for up to 1 year, then thawed when required and used as a BV-specific position marker (BVPM) to identify amplified BVs. During a study of the BV repertoire in 48 individuals (unpublished observations), we used the BVPMs as follows: ten microliters of each PCR product were mixed with 3 µL of the corresponding BVPM and then electrophoresed in 2% agarose. The individual BV bands were readily visualized by ethidium bromide staining; it was unnecessary to use positional markers for AC bands as these are typically brightly fluorescent under UV light. To confirm that each BVPM identified the corresponding radiolabeled BV, a cDNA sample was PCR-amplified with each of the 23 BV primers and with the radiolabeled BC primer in separate tubes. After agarose gel electrophoresis with the BVPMs, the gel was dried and exposed to x-ray film. The position of each BVPM on the dried gel and the position of the corresponding bands in the autoradiograph were completely concordant.

Since the use of the BVPMs made gel drying unnecessary to detect agarose gel PCR products, we excised directly the BV-BC and AC bands from the wet gels immediately after electrophoresis, dissolved them in scintillation fluid (Ready Value™; Beckman Instruments, Mississauga, ON, Canada) and then determined the radioactive counts by liquid scintillation spectrometry. We evaluated the reproducibility of this wet agarose gel stained agarose gels especially when particular BVs were expressed at low levels. To detect BV PCR products, we developed a set of agarose gel position markers for 23 human TCRBVs, including BV families 1–20, BV5S1, BV5S2–3, BV13S1 and BV13S2. Each BV from a T-cell cDNA sample was PCR-amplified separately using 23 BV primers (2,8) together with a BC primer (2) that was unlabeled. A 2-µL aliquot of each BV-BC PCR product was then separately re-amplified in a second round PCR. The PCR products were chloroform-extracted and mixed in a 2:1 ratio with loading dye consisting of equal volumes of glycerol and 0.45 M Tris-borate/0.01 M EDTA, pH 8.0, containing 10 mg/mL bromophenol blue. Each BV-BC loading mixture was stored at -20°C for up to 1 year, then thawed when required and used as a BV-specific position marker (BVPM) to identify amplified BVs. During a study of the BV repertoire in 48 individuals (unpublished observations), we used the BVPMs as follows: ten microliters of each PCR product were mixed with 3 µL of the corresponding BVPM and then electrophoresed in 2% agarose. The individual BV bands were readily visualized by ethidium bromide staining; it was unnecessary to use positional markers for AC bands as these are typically brightly fluorescent under UV light. To confirm that each BVPM identified the corresponding radiolabeled BV, a cDNA sample was PCR-amplified with each of the 23 BV primers and with the radiolabeled BC primer in separate tubes. After agarose gel electrophoresis with the BVPMs, the gel was dried and exposed to x-ray film. The position of each BVPM on the dried gel and the position of the corresponding bands in the autoradiograph were completely concordant.

Since the use of the BVPMs made gel drying unnecessary to detect agarose gel PCR products, we excised directly the BV-BC and AC bands from the wet gels immediately after electrophoresis, dissolved them in scintillation fluid (Ready Value™; Beckman Instruments, Mississauga, ON, Canada) and then determined the radioactive counts by liquid scintillation spectrometry. We evaluated the reproducibility of this wet agarose gel stained agarose gels especially when particular BVs were expressed at low levels. To detect BV PCR products, we developed a set of agarose gel position markers for 23 human TCRBVs, including BV families 1–20, BV5S1, BV5S2–3, BV13S1 and BV13S2. Each BV from a T-cell cDNA sample was PCR-amplified separately using 23 BV primers (2,8) together with a BC primer (2) that was unlabeled. A 2-µL aliquot of each BV-BC PCR product was then separately re-amplified in a second round PCR. The PCR products were chloroform-extracted and mixed in a 2:1 ratio with loading dye consisting of equal volumes of glycerol and 0.45 M Tris-borate/0.01 M EDTA, pH 8.0, containing 10 mg/mL bromophenol blue. Each BV-BC loading mixture was stored at -20°C for up to 1 year, then thawed when required and used as a BV-specific position marker (BVPM) to identify amplified BVs. During a study of the BV repertoire in 48 individuals (unpublished observations), we used the BVPMs as follows: ten microliters of each PCR product were mixed with 3 µL of the corresponding BVPM and then electrophoresed in 2% agarose. The individual BV bands were readily visualized by ethidium bromide staining; it was unnecessary to use positional markers for AC bands as these are typically brightly fluorescent under UV light. To confirm that each BVPM identified the corresponding radiolabeled BV, a cDNA sample was PCR-amplified with each of the 23 BV primers and with the radiolabeled BC primer in separate tubes. After agarose gel electrophoresis with the BVPMs, the gel was dried and exposed to x-ray film. The position of each BVPM on the dried gel and the position of the corresponding bands in the autoradiograph were completely concordant.