METHODS BETA

Polymerase Chain Reaction

Polymerase chain reaction is a method invented by Kary Mullis for creating multiple copies of DNA through repeated cycles of denaturing, annealing, and synthesizing driven by DNA polymerase.

From: Electrochemical Sensors, Biosensors and their Biomedical Applications, 2008

Related techniques:

Pyrosequencing, Exon, Indel Mutation, DNA Template

Protocols

From: Chapter 1 - The Polymerase Chain Reaction: PCR, qPCR, and RT-PCR,

Mehdi Jalali, Justyna Zaborowska, Morteza Jalali

Basic Science Methods for Clinical Researchers • 2017 • Pages 1-18

The protocol presented below [11] requires 30–60 minutes for reaction set up, around 3 hours to run the PCR program, and 1–2 hours for data analysis. It is based on the Quantitect SYBR Green PCR kit sold by Qiagen. The reaction mix contains HotStarTaq DNA polymerase, QuantiTect SYBR Green PCR Buffer, dNTP mix, SYBR Green I, ROX passive dye, and 5 mM $MgCl_2$.

1. Place these components on ice: Reaction mix (2×), 50 μM forward primer, 50 μM reverse primer, RNase-free water, cDNA.

   For each sample, the master mix consists of the following components:

   - Reaction mix (2×)—12.5 μL (final concentration 1×)
   - Forward primer—0.5 μL (final concentration 1 μM)
   - Reverse primer—0.5 μL (final concentration 1 μM)
   - RNase-free water—bring up to 25 μL
   - cDNA—1–5 μL

2. Calculate the total volume of master mix required according to the number of samples that need to be analyzed. Samples should be run in triplicate. Mix the master mix thoroughly and dispense equal aliquots into each PCR tube.
   TIP: Add 10% extra volume to your master mix to allow for pipetting error. Remember to include a positive control and no-template negative control.

3. Add cDNA to each reaction tube and cap tubes.

4. Place the tubes in the thermal cycler and set the PCR machine according to the manufacturer's instructions. Example of typical program is shown in Table 1.2.

Step	Time	Temperature
Initial denaturation	15 min	95°C
Denaturation	30 sec	95°C
Annealing	30 sec	45°C to 65°C (5° below primer Tm)
Extension	30 sec	72°C
Repeat cycles	35–45 cycles (depends on the amount of template)	
Final extension	5 min	72°C
Melt curve		45°C to 95°C

Table 1.2. Typical program for real-time cycler [13]

5. Run the PCR reaction in order to determine CT values for each sample.
6. The PCR specificity can be examined on 3% agarose gel using 5 μL from each reaction.

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74 h-index | 21,001 Citations | 390 Documents

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39 h-index | 21,971 Citations | 140 Documents

Relevant methods

Molecular genetic analysis of phosphomannomutase genes in Triticum monococcum

Chunmei Yu, Xinyan Liu, Qian Zhang,Xinyu He, Wan Huai, Baohua Wang, Yunying Cao,Rong Zhou

The Crop Journal • Volume 3, Issue 1 • February 2015 • Pages 29-36

> ### Quantitative RT-PCR
>
> T. monococcum accessions were grown in the glasshouse for four weeks. For each accession, leaf samples were collected from five individual plants and pooled together for isolating total RNA samples as described above. After cDNA synthesis (see above), quantitative RT-PCR was performed in an ABI 7500 Real-time PCR system with 7500 Software v2.0.4. The PCR mixture contained 2μL of diluted cDNA, 10μL of 2× SYBR Premix Ex Taq II (TaKaRa, Dalian, China), 0.4μL of 50×ROX Reference Dye II, and 400nmolL–1 of the appropriate primer set (Table S1) in a final volume of 20μL. Thermo-amplification was performed in a 96-well plate (Applied Biosystems, Carlsbad, USA) with the

following parameters: 30s at 95°C, followed by 40cycles of 5s at 95°C and 34s at 60°C. The specificity of the amplicon was verified by melting curve analysis (60 to 95°C) after 40cycles. Each assay included at least three technical replicates. The amplification of a wheat 26S rRNA gene served as the internal control for the assay [41]. Two independent assays were conducted for each gene (or primer set), with nearly identical results being obtained.

Toward pectin fermentation by Saccharomyces cerevisiae: Expression of the first two steps of a bacterial pathway for d-galacturonate metabolism

Eline H. Huisjesa, Marijke A.H. Luttik, Marinka J.H. Almering, Markus M.M. Bisschops, Dieu H.N. Dang, Michiel Kleerebezem, Roland Siezen, Antonius J.A. van Maris, Jack T.Pronka

Journal of Biotechnology • Volume 162, Issues 2–3 • 31 December 2012 • Pages 303-310

qPCR

The sampling procedure was based on the method used by Piper et al. (2002): exponentially growing S. cerevisiae shake-flask cultures on glucose were cooled, and 72mg of cells was harvested by centrifugation. The cell pellet was resuspended in 1.08ml ice-cold AE buffer and immediately 1.08ml acid phenol-chloroform (5:1, pH 4.5) and 108µl 10% (w/v) SDS were added. After vortexing vigorously, the tubes were placed in a water bath for 5min at 65°C. The content was homogenized by vortexing, and divided over 3 RNase-free tubes and stored at –80°C. RNA extraction was performed by the method of Schmitt et al. (1990) and cDNA was synthesized using the QuantiTect Reverse Transcription Kit (Qiagen, Dusseldorf, Germany). qPCR was performed in triplicate on three dilutions of the sample using the QuantiTect SYBR Green PCR Kit (Qiagen, Dusseldorf, Germany) with a primer concentration of 0.5µM in a total volume of 20µl in the Rotor-Gene Q (Qiagen, Dusseldorf, Germany). All qPCR primers are listed in Table 3. The PCR-efficiencies (E) were calculated from standard curves. The average efficiency over all product-yielding qPCR reactions was 1.8±0.1. CT values were determined with the Rotor-Gene Q Series Software by automatic scanning for the threshold setting which delivers optimal estimates of the given concentrations. IME083 ACT1 was always included as an internal standard. For each individual strain, the relative expression of uxaC, uxaB, uxaA, kdgK and kdgA was quantified by the method of Pfaffl (2001) using the following formula, with ACT1 as the reference gene: E∆CT(reference-target).

Major allergen Phl p Va (timothy grass) bears at least two different IgE-reactive epitopes

Bufe MD, Becker PhD, Schramm Petersen PhD,Mamat PhD, Schlaak MD

Journal of Allergy and Clinical Immunology • Volume 94, Issue 2, Part 1 • August 1994 • Pages 173-181

N-terminal and C-terminal recombinant peptides and PCR peptide

The C-terminal clone pPHLP5A1311 was detected by immunoscreening of the library as incomplete clone in correct reading frame, isolated, and sequenced. By restriction of a 5′end fragment of pPHLP5A1913 at a Xho I (CTCGAG) restriction site (at 418 bp in Fig. 1) and subcloning, we constructed an N-terminal peptide of 120 amino acids, which overlaps the C-terminal pPHLP5A1311 by 24 amino acids. A recombinant peptide (PCR peptide) of 59 amino acids was constructed from base pair 292 to 470 by PCR. Amplification was performed at 94° C for 30 seconds, 54° C for 30 seconds, and 72° C for 1.5 minutes for 30 cycles with pPHLP5A1913 as template. The sequences of the primers are: 5′end: ATATGGATCCGAGGCGCTCCGCATCAT 3′end: ATATAAGCTTAGACGGTGAACTTGTCGTTG The PCR product was purified from 1.5% agarose gel, restricted with BamH I and Hind III and ligated into the expression vector pQE9 (Qiagen, Chatsworth, Calif.). The correct reading frame was controlled by sequencing, and the recombinant protein was purified on a Ni2+-nitrilotriacidic acid-resin column according to the instructions of the manufacturer (Qiagen).

Related content

The diagnosis of microorganism involved in infective endocarditis (IE) by polymerase chain reaction (PCR) and real-time PCR: A systematic review

Reza Faraji, Mostafa Behjati-Ardakani, Seyed Mohammad Moshtaghioun, Seyed Mehdi Kalantar, Mohammadtaghi Sarebanhassanabadi

The Kaohsiung Journal of Medical Sciences • Volume 34, Issue 2 • February 2018 • Pages 71-78

Polymerase Chain Reaction (PCR)

G. Dorado, G. Besnard, T. Unver, P. Hernández

Encyclopedia of Biomedical Engineering • 2019 • Pages 473-492

Rapid PCR of STR markers: Applications to human identification

Erica L. Romsos, Peter M. Vallone

Forensic Science International: Genetics • Volume 18 • September 2015 • Pages 90-99

Chapter 1: The Polymerase Chain Reaction: PCR, qPCR, and RT-PCR

Mehdi Jalali, Justyna Zaborowska, Morteza Jalali

Basic Science Methods for Clinical Researchers • 2017 • Pages 1-18

Recent and emerging technologies for the rapid diagnosis of infection and antimicrobial resistance

Alexander J. Trotter, Alp Aydin, Michael J. Strinden, Justin O'Grady

Current Opinion in Microbiology • Volume 51 • October 2019 • Pages 39-45