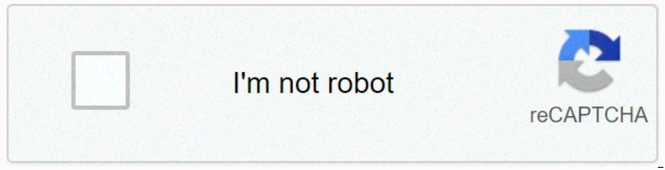

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What is annealing in pcr

To copy DNA, polymerases require a short sequence called a primer. PCR uses two primers, each complementary to opposite strands of the region of DNA, which have been denatured by warming. They can not be used to thread dna at temperature 95 degrees celsius, so the tube is cooled to 45 - 60 degrees C. The temperature at this stage depends on the melting temperature of the primer - mal hybrid. If the temperature is too high, the primers can not glow effectively, and if the glow temperature is too low, the primers may not bind specifically to the template. They are present in large excess, so this step can be repeated many times. The primers are designed so that each primer directs the synthesis of new strands of DNA towards the other (figure below). Thus primer green directs the synthesis of a thread of DNA that can then be primed by primer blue and vice versa. Back to What is PCR? Dominika Borek borek@leucine.ibch.poznan.pl The Department of Crystallography Faculty of Chemistry Adam Mickiewicz University Grunwaldzka 6 Poznan, Poland Back to DNA Reinforcement, PCR and qPCR Polymerase Chain Reaction (PCR) is a method of rapidly amplifying sequences of DNA. During a typical PCR, template DNA (which contains the area of interest) is mixed with deoxynucleotides (dNTPs), a DNA polymerase and primers. Primers are short segments of DNA that are complementary with the template DNA upstream of the region of interest and serve as recruitment sites for polymerase. PCR involves a number of temperature cycles that, although once performed by moving pipes through various water baths, are now controlled automatically using thermal cyclers, or thermal cyclers. Thermocyclers provide tight control over both the reaction temperature and the duration of each temperature stage, ensuring effective reinforcement. During a typical PCR, cycles of denning, glowing and extension are repeated to achieve exponential amplification of the target sequence. Denaturation consists of heating the samples usually between 94-98 °C to cause denaturation of the template DNA, disrupting hydrogen bonds and base stacking interactions that hold DNA strands together. When the threads are separated, the temperature is reduced to the glow temperature so that the primers can base pairs (or glow) to complementary regions of the template. The glow temperature (usually between 48-72 °C) is related to the melting temperature (Tm) of the primers and must be determined for each primer pair used in PCR. During the extension stage (usually 68-72 °C), the polymerase expands the primer to form a budding DNA thread. This process is repeated several times (usually 25-35 cycles), and because each new beach can also serve as a template for primers, the area of interest is exponentially amplified. The last step in PCR is a longer step with one temperature (often 5-10 min at 68-72 °C) that allows you to complete any partial copies and clearance of all replication machines from the emerging DNA. When the PCR is complete, the thermal cycler is set to 4°-10°C to maintain the integrity of the product until the pipes can be removed from the machine. See our brochure on PCR Reagents (polymerases, nucleotiides and DNA ladders) This overview will guide you through how Polymerase Chain Reaction (PCR) works. Learn how proofreading polymerases recognize and correct non-compliant bases. Open Access Peer-reviewed chapter By Marjanca Starčič Erjavec Sent: 4 December 2018Reported: 12 February 2019 Published: 11 February 2019 We used PCR to clone papA, papEF, papG and F17G genes of Escherichia coli isolated from fecal samples of dogs with diarrhea. Glow temperature of 55 °C was used in PCR. Nucleotide sequence analysis of 26 cloned PCR products showed that in PCRs with papA primers, six out of eight achieved PCR products were fake due to nonspecific binding of forward primer on both DNA strands; in PCRs with papEF primers, all seven obtained PCR products originate from specific binding of forward primer of 3' → 5' DNA beach and unspecific binding of forward primed on 5' → 3' DNA beach; and in PCRs with F17G primers, four out of eight achieved PCR products were fake due to nonspecific binding of forward and reverse primer. The expected glow spots for nonspecific primer binding in analyzed nucleotide sequences are presented. In the case of PCR products obtained with papG-specific primers, all PCR products were reinforcing the papG sequence. When the glow temperature of papA PCRs was raised to 60°C, all achieved PCR products were reinforcements of the right DNA sequences.primer bindingannealing temperaturesequence analysisEscherichia coliadhesinP-fimbriaeF17-fimbriaeSince polymerase chain reaction (PCR) was invented in the mid-1980s, It has made its way into all molecular biology, genetic, microbiology or biochemistry laboratories, where it is, due to its simplicity and effectiveness, used in a very wide range of (PCR)-based techniques and applications [1 , 2]. In just a few hours with a certain amount of cycles consisting of three simple steps — DNA denning, glowing primers and extension [2] – the desired DNA sequence is multiplied approximately one million times [3]. The crucial step in PCR is the annealing of primers, where the glow temperature determines the specificity of primer annealing. The incandescent temperature in a standard PCR protocol is either 55°C [2, 3] or 60°C [4]. The selected temperature depends on the beach melting temperature of primers and the desired specificity. For greater stringency higher temperatures are [2]. PCR is often used to reinforce specific DNA fragments that are later cloned as inserts in plasmid vectors and are then used in subsequent experiments. Examples of such subsequent experiments are nucleotide sequencing, to determine the nucleotide sequence of effort or in vitro transcription, and translation, to achieve a specific protein. In our experiments, the goal was to determine the nucleotide sequence of several fimbrial genes from different Escherichia coli (E. coli) strains isolated from fecal specimens of dogs with diarrhea. The genes of interest were papA, papG, papEF of P-fimbriae and F17G of F17-fimbriae. Therefore, from a collection of 24 clinical hemolytic E. coli originating from fecal samples of dogs with diarrhea [5], genomic DNA was isolated and used as matrix DNA to reinforce these genes of interest with gene-specific primers with PCR. Moreover, the achieved PCR products were cloned into a TA cloning vector, and the nucleotide sequence was determined. Escherichia coli is one of the best studied organisms. It belongs to the family of Enterobacteriaceae. It is a Gram-negative rod-shaped bacterium, non-sporulating, nonmotile or motile of peritrichous flagella, chemoorganotrophic, faculty anaerobic, which produces acid from glucose, cata laser positive, oxidase negative and mesophilic [6]. It is a well-known commensal bacterium that is part of the gut microbiota of humans and other warm-blooded organisms. However, pathogenic strains of E. coli are also found and can cause a number of intestinal and extraintestinal infections in humans and many animal hosts. E. coli is considered to be one of the main pathogens; It is the most frequently isolated species in clinical microbiology laboratories [7]. Intestinal pathogens E. coli(IPEC) strains, also called diarrheagenic E. coli (DEC) strains, are divided into six different well-described categories, i.e. pathotypes: enteropatogene E. coli (EPEC), enterohaemorrhagic E. coli (EHEC), enterotoxigenic E. coli (ETEC), enteroaggregative E. coli (EAEC), enteroinvasive E. coli (EIEC) and diffuse trailer E. coli (DAEC) [8]. DEC causes diarrhea syndromes that vary in clinical presentation and pathogenesis, depending on the pathpathesis type of the strain [7]. E. coli strains involved in diarrhea diseases are one of the most important among the various etiological agents of diarrhea [9]. The extraintestinal pathogen E. coli (ExPEC) strain group consists of different E. coli associated with infections of extraintestinal anatomical sites [10]. Traditionally, expec isolates are divided into groups determined by the disease association, that will say uropathogen E. coli (UPEC), neonatal meningitis-associated E. coli (NMEC) and sepsis-causing E. coli (SEPEC), which names the main ExPEC groups. But ExPEC strains are also implicated in infections stemming from and pelvic sources (e.g. bile infections, infectious peritonitis and pelvic inflammatory disease) and also associated with skin and soft tissue infections and hospital infections [11]. Due to the genotypical and phenotypic diversity, E. coli is known as the paradigm for a versatile bacterial species [12]. The pathogenic strains have specialized virulens factors such as atheism, toxins, iron acquisition systems, polysaccharide coats and invasins not found in comenesal strains [7]. Adhesins play a very important role in host microbe interactions, as they convey adherence to epithelial cells, surface structures or molecules. Adhesion is the most important first step for most comsensale and pathogenic bacteria to colonize and persist in the host [13]. While adhesion to abiotic surfaces is usually mediated by nonspecific interactions, adhesion to biotic surfaces usually involves specific receptor-ligand interaction [14]. Adhesives are structures on the bacterial surface that help the bacteria bind to receptors on the host's cells (Figure 1). Scanning of electron microscopy of Escherichia coli strain 963 as a result of 19-day-old Caco-2 cells [5]. The fimbriale structures on the bacterial surfaces promote bacterial adherence to receptors on host cells. Adhesins are not only involved in compliance, but also in bacterial invasion, survival, biofilm formation, serum resistance and cytotoxicity [15]. Furthermore, they are also involved in bacterial motility and DNA transmission [13]. They differ in their architecture and receptor specificities. Types of adhesin vary depending on gram nature bacteria [15]. Adhesins are among the main virulens-associated properties of E. coli, as they are the main virulens factors for bacteria needed in bacterial colonization. There are two types of bacterial adhesives: fimbrial and afimbrial [16]. Fimbrialadhesins, it will will darken the fimbriae, are rod-like structures with a diameter of 5-7 nm. Each fimbria consists of several hundred copies of a protein, whose generic names are large subunits, and other proteins, present in one or very few copy numbers and called smaller subunits located either on the basis or on top of the fimbriae or intercalated between the large subunits [16]. Fimbriae may be even longer than 1 μm [13]. On the bacterial surface of wild-type E. coli strains, there are around 500 fimbriae [17]. P-fimbriae and F-17 fimbriae belong to the fimbrialadhesins. Non-fimbrialadhesins are monomeric or trimeric structures that decorate the surface of bacteria. These adhesives are anchored to the surface of the outer membrane, and due to their small size, the size of non-fimbrialadhesins is about 15 nm, allowing an intimate contact between the bacterial cell surface and specific substrates. One of the great classes of adhesins are autotransporteradhesins [13]. P-fimbriae are the most extensively studied athehesins. They are also the first virulens-associated factor found among uropathogen E. coli. These fimbriae bind to Gal(α1–4)Galβ moieties of the membrane glycolipids on human erythrocytes in the P blood group and on the uroepithelial cell fimbriae [18]. Additional receptors for P-fimbriae are present on erythrocytes from pigs, doves, birds, goats and dogs, but not on those from cows, guinea pigs or horses [19]. These fimbriae are encoded in pap operon, consisting of 11 different genes (see Figure 2A): papA (558 bp), papB (315 fps), papC (2511 bp), papD (720 bp), papE (522 bp), papF (504 bp), papG (1008 bp), papH (588 bp), papI (234 bp), papJ (582 bp) and papK (537 bp) [20]. The scheme of pap and F17 operon and annealing places of the used primers. Genes in operon are presented as cans. The positions of used primers to reinforce the studied genes are marked with arrows. (A) The scheme of pap operon. The scheme of pap operon was drawn based on genbank deposited nucleotide sequence X61239.1 [20] and (B) scheme of F17 operon. The scheme of F17 operon was drawn based on GenBank deposited nucleotide sequence L77091.1 [26]. The product of the papA gene is the main subunit protein A (19.5 kDa) [19]. In papb, a regulatory protein (13 kDa) is encoded. PapB is required for activation of the papA expression [21]. PapC (80 kDa) is located in the outer membrane and forms the mounting platform for fimbrial growth. PapD (27,5 kDa) is present in periplasmaic space and is involved in the translocation of fimbriale subunits over the periplasma space of the outer membrane before assembly. PapE (16.5 kDa), PapF (15 kDa) and PapG (35 kDa) are smaller fibirial components. PapG is the adhesin molecule that provides the binding specificity [19]. PapH (20 kDa) ends fimbrial assembly and helps anchor the full-grown fimbriae to the cell surface [22]. PapI (12 kDa) is another regulatory protein involved in papA expression due to the activation of papb promoter [21]. PapJ (18 kDa) is a periplasmic protein required to maintain the integrity of P-fimbriae [23]. PapK (20 kDa) regulates the length of the tip fibrillum and turns it to the rod [24]. Many varieties of P-fimbriae exist. PapA molecules from different P-fimbrial serovariants have a high degree of similarity at N and C termini, while the central parts of the PapA show a large variation in the primary structure. This central part of papa is hydrophilic and exposed and thus under selective pressure from the host immune system. Significant heterogeneity is also between various smaller fimbriale subunits (PapE, PapF and PapG) [19]. In addition, also P-fimbria-related fimbriae, the so-called Prs-fimbriae, exists. Prs-fimbriae is encoded in prs (pap-related sequence) operon [18]. F17 fimbriae is on pathogenic E. coli strains, isolated from infections in domestic animals. They are detected mainly on cattle and ovine E. coli associated with diarrhea or septicemia, but also on E. coli from other hosts, including humans. F17 vedhesen binds to N-acetyl-d-glucosamine receptors of cattle intestinal cells; However, F17 subtypes were also found to bind to N-acetylg-d glucosamine receptors of human uroepithelial and intestinal cells [25]. F17 fimbriae is encoded in F17 operon, consisting of four genes: F17A (546 bp), F17D (723 bp), F17C (2469 bp) and F17G (1035 bp) (see Figure 2B) [26]. F17A protein (20 kDa [25]) is the structural component of F17-fimbriae (main subunit protein). The F17A protein is homologated to the PapA protein in P-fimbriae [27]. F17C protein (90 kDa) probably acts as a base protein on which the fimbriale subunits are polymerized. F17D protein (28 kDa) has a near homology to papd protein of P-fimbriae [28]. It acts as the periplasmic transport protein [29]. F17G protein (36 kDa [25]) is the smaller fimbrial component required for binding the F17 fimbriae to the receptor on the host cell [30]. Several variants of F17 fimbriae exist. The diversity is based on differences in F17A and F17G genes. The variant of F17 fimbriae found in humans is designated as G-fimbriae, encoded in gaf operon [25]. They analyzed 24 clinical haemolytic E. coli strains [5] originating from dogs with diarrhea and were isolated at the Veterinary Microbiological Diagnostics Centre of Utrecht University, Netherlands. A few more details about the tribes are provided in Table 1. As positive control strains, a dog uropatogen E. coli strain (strain 1473) and a cattle mastitis E. coli strain (strain E5) from Wim Gaastra's E. coli collection were used [31]. Strain numberDog's ageClinic charactersSerotypeMSHAMRHA297VerifiedUnknownO78K? E, P, CC333Young dogChronic diarrheaO6K53E, P, C, OP, O366UnknownUnknownNTB, E, P, C, OB, E, P, O375PupSepsisO20K? NoneP380UnknownUnknownO6K-B, E, P, C, OP, C, O442PupSepsis diarrheaO4K2B, P, C, OE, P, C, O450PupSepsis diarrheaO42K-E, P, CNone4675 monthsChronic diarrheaO6K1E, P, C, OP, C479PupSepsis diarrheaO23K1B, E, P, CP, C, O503UnknownUnknownO42K-C, ONone555PupSepsis diarrheaO4K53E, P, C, OO5673 monthsRestrøm diarrheaO4K? E, P, C, OP, C, O7273 monthsUnknownO139K? E, P, CP73418 monthsChronic diarrheaO4K? E, P, C, OE, P, C, O7404 yearChronic diarrheaO25K13E, P, C, OP, C, O75912 monthsChronic diarrheaO4K? P, OP, O8579 monthsChronic diarrheaNT, C, OP, O897EkteChronic diarrheaO6K53B, E, P, C, OB, E, P, C, O912Old dogChronic diarrheaO4K-E, P, C, OE, P, C, O958PupSepsis diarrheaNTB, C, ONone963UnknownChronic diarrheaO4K? B, E, P, CB, E, P, C9964 monthsUnknownO4K? E, P, C, OO1205UnknownChronic diarronic O4K-E, P, C, OE, P, C, O12373 monthsBloody diarrhoeaO2K1B, E, P, OB, E, P, C, OCharacteristics of the 24 studied E. coli strains [5, 31]. MSHA is an abbreviation of mannose-sensitive haemagglutination, and MRHA is an abbreviation of mannose-resistant haemagglutination. Erythrocytes are abbreviated as follows: B, cattle erythrocytes; E, equine erythrocytes; C, dog; O, ovine; P, pigs. NT, non-typable. All used bacterial strains were stored at -80°C as suspension in a 1:1 mixture of L-broth and glycerol as published by Garcia et al. [32]. The strains were grown overnight on LB plates and in liquid LB medium at 37 °C. When grown in liquid LB medium, bottles of bacterial culture were incubated with aeration. Chromosomal DNA was isolated from all 24 clinical hemolytic E. coli strains [5] and strains used for positive controls [31] using a slightly modified protocol based on the protocol for miniprep of bacterial genomic DNA published by Ausubel et al. [33] To summarize, 2 ml of an overnight bacterial culture was centrifuged for 2 min at 14,000 rpm at room temperature. The obtained bacterial pellet was reused in 567 μl buffer TE and 6 μl of 0.5 M EDTA. The suspension was incubated for 15 min at -80 °C. After incubation at -80 °C, the suspension was thawed and 10 μl μl of 25 mg/ml proteinase K solution was added. The suspension was mixed thoroughly, and 30 μl of 10% SDS was added to the suspension and mixed thoroughly again. A 2-hour incubation at 37°C followed, and then 100 μl of 5 M NaCl was added to the suspension and mixed thoroughly. The next 80 μl CTAB/NaCl was added, mixed thoroughly again and incubated at 65°C for 10 min. After incubation, the suspension was treated with 200 μl chloroform/isoamyl alcohol and centrifuged for 5 min at 14,000 rpm at room temperature. Aqueous overnatant was transferred to a fresh microcentrifuge tube and treated with 100 μl phenol/chloroform/isoamyl alcohol and centrifuged for 5 min at 14,000 rpm at room temperature. Aqueous overnatant was transferred to a fresh microcentrifuge tube, and DNA in aqueous overnatant was triggered with the addition of 0.6 volume isopropanol. The preciated chromosomal DNA was transferred to a fresh microcentrifuger tube containing 100 μl μl of 70% ethanol. The expedited DNA of 70% ethanol was pelleted with centrifugation (10 min at 14,000 rpm at room temperature). 70% ethanol was then removed and chromosomal DNA pellet air dried at 37 ° C. Finally, the chromosomal DNA pellet was dissolved in 100 μl of sterile distilled water. A μl of the isolated chromosomal DNA was used in 50 μl PCR mixtures consisting of 20 pmol of each primer, 0.2 mM dNTP mixture and 0.625 U Taq polymerase in PCR buffer [5]. In PCR for P-fimbriale genes for positive control samples, the isolated chromosomal DNA of the dog uropathogenic E. coli strain 1473) was used. In PCRs for the F17 fimbrial gene for positive control samples, the isolated chromosomal DNA of the cattle mastitis E. coli strain (strain E5) was used. In all PCR negative control, sterile distilled water was used [31]. Primers used in the PCRs to reinforce the studied genes are listed in Table 2.PCR for gene(s)Primers (name, sequence of primer 5'→ 3')Tm (°C) of primerspapA2284.1ATGATGAATTCGGTTATTGCCGGTGCGG2368.3CTGAGAATTCAGGTTGAACSYMPTOMCGCpapEFPOP63.8CCACGTTTGAATTGACAT ATCGBAD72.9CCGTAGCAATGCCCGGGCPAPGGOD165.5ATGGTACCCAGCTTTGTTATTTTCCGOD262.4TGGCAATATCATGAAGCTTTF17GF17G-262.4TGGCAATATCATGAGAAGCTTTF 17GF17G-262.4TGGCAATATCATGAGAAGCTTTF17GF17G-262.4TGGCAATATCATGAAGCTTTF17GF17G-17G-262.4TGGCAATATCATGAGAAGCTTTF17G17G-17G-262.4TGGCAATATCATGAGAAGCTTTF17GF17G-17G-262.4TGGCAATA172.4CAGGCGGCAGTTTCATTTATTGGCF17G-276.4CCGGACTGAGGGTGACGTTTCCGTPrimers and their melting temperatures (Tm) are used to reinforce the studied genes. Predicted primer annealing places of the used primers on target operons are shown in Figure 2.PCR amplification in all reactions for all studied genes were performed in the following stages: heating at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 ° C in 1 min, glowing at 55 ° C for 1 min, prolongation at 72 °C for 1 min and the final extension in 10 min at 72 °C.De expected sizes of PCR products were determined with the Primer-BLAST online tool (dataset nr organism Escherichia coli) on the Internet page of the National Center for Biotechnology Information, US National Library of Medicine ( ) as follows: papA-552 bp (GenBank deposited nucleotide sequence LR134092.1) , 555 bp (GenBank deposited nucleotide sequence CP025703.1), 534 bp (GenBank deposited nucleotide sequence CP018957.1) , 564 bp (GenBank deposited nucleotide sequence CP029579.1) and 561 bp (GenBank deposited nucleotide sequence CP024886.1); papEF-1372 bp (GenBank deposited nucleotide sequence CP027701.1), 1373 bp (GenBank deposited nucleotide sequence CP028304.1), 1367 bp (GenBank deposited nucleotide sequence CP026853.1) and 1371 bp (GenBank deposited nucleotide sequence LR134238.1); papG-1000 bp (GenBank deposited nucleotide sequence CP026853.1) and 1003 bp (GenBank deposited nucleotide sequence M20181.1); and F17G-888 bp (GenBank deposited nucleotide sequence AF055313.1) and 885 bp (GenBank deposited nucleotide sequence CP001162.1). Samples of isolated chromosomal DNA (5 μl isolated chromosomal DNA and 1 μl on 6 × loading dye) were subjected to analysis with agarose gel electrophoresis using 1% of agarose gels with 0.5 μg/ml ethidium bromide, running in 0.5 × TBEho electrore buffer. Samples of obtained PCR products (25 μl pcr products, 5 μl of 6 × loading dye) were subjected to analysis with agarose gel electrophoresis using 1% of agarose gels with 0.5 μg/ml ethidium bromide, run in 1 × TAE electrophoresis buffer. Used protocols for agarose gel electrophoresis were based on Sambrook al. [34]. For DNA increase lambda bacteriofag bacteriofag digested with the restriction endonuclease PSTI was used. Cloning of PCR products and DNA sequencing of cloned PCR products obtained in PCRs for P and F17 fimbrial genes was done as described by Starčič et al. [5]. In short, achieved PCR products were cut out of agarose gel, cleaned with GeneClean II Kit, inserted into TA cloning vector pMOSBlue and then transformed into electrocomponent E. coli pMOSBlue cells. Then plasmid DNA was isolated from pMOSBlue cells using the FlexiPrep Kit, and the nucleotide sequence was determined with the dideoxynucleotide chain's closing method using an automated laser fluorescence sequence sequencer. All procedures were carried out according to the manufacturer's protocols. Sequence analysis of cloned fragments, originating from PCR products obtained in PCRs for P and F17 fimbrial genes, was performed with the computer program BLAST on the Internet page of the National Center for Biotechnology Information, the US National Library of Medicine ( ) searches for homology in the GenBank no database. An incandescent temperature of 55°C was used in PCRs for reinforcement of the papa gene with primers 22 and 23. The achieved PCR products were all expected size (around 600 bp). However, the nucleotide sequence analysis of the eight achieved cloned PCR products showed that six clones had false, non-papA inserts. Four of these false clones originate from the amplification of part of the methylsocitrate glycase gene, and two clones derived from amplification of part of the RNA-binding protein Hfq gene and part of the GTPase HflX gene, as revealed by BLAST analysis. In both cases, although both primers, forward primer 22 and reverse primer 23, were added to the PCR mixture, primer 22 was used as forward, but also reverse primer. Further nucleotide analysis showed that in the case of amplification of part of the methylisocitrate glycase gene, The front primer glowed downstream from the c348059 position of 3'→ 5's beach and reverse primer upstream of 348539 position at 5'→ 3' DNA beach of E. coli K-12 MG1655 sequence as deposited in CP025268.1 nucleotide sequence [35]. The expected glow spots for nonspecific papA primer binding in this case are presented in Figure 3.Expected glowing sites for nonspecific papA primer binding in the methylisocitrate lyase gene. The displayed sequences are listed according to CP025268.1 GenBank deposited sequence [35]. The sequence and complement chromosome sequence are given. The sequence from 348033 to 348059 nt is displayed for the front primer, and for the reverse primer incandescent location, the sequence is displayed from 348539 to 348565 nt. The primer sequence is in the gray box. The arrows mark the direction of DNA extension in pcr. It the lyase gene is placed in the deposited sequence from 347733 to 348623 nt. In the case of amplification of part of the RNA-binding protein Hfq gene and part of the GTPase HflX gene, forward primer glowed downstream from c4402446 position on 3' → 5' DNA beach, and reverse primer glowed upstream from 4402903 position at 5'→ 3' DNA strand of E. K-12 MG1655 sequence as deposited in CP025268.1 nucleotide sequence [35]. The expected glow spots for nonspecific papA primer binding in this case are presented in Figure 4.Expected glowing sites for nonspecific papA primer binding in the RNA-binding protein Hfq gene (forward primer) and GTPase HflX gene (reverse primer). The displayed sequences are numbered according to CP025268.1 GenBank deposited sequence [35]. The sequence and complement chromosome sequence are given. The sequence from 4402419 to 4402446 nt is displayed for the front primer, and for the reverse primer incandescent location, the sequence from 4402903 to 4402934 nt. The primer sequence is in the gray box. The arrows mark the direction of DNA extension in pcr. The RNA-binding protein Hfq gene is located in the deposited sequence from 4402214 to 4402522 nt, and the GTPase HflX gene is located from 4402598 to 4403878 nt. In the PCRs for the papEF reinforcement, the glow temperature of 55 °C was also used. Seven PCR products, all expected size, of around 1400 bp, were cloned, and the achieved insertion sequences were analyzed. All seven clones had the enhanced papEF-related sequence, the PRSEF sequence of Prs-fimbriae (GenBank X61238.1 [36]); but in all seven cases, only forward POP primer glowed to the correct complementary sequence from c27 to c48 nt on 3' → 5' DNA beach of X61238.1, while the reverse primer was not as expected BAD primer, but again pop primer, which glowed on another partially complementary sequence of the PRSEF gene from 1357 upstream on 5'→ 3' DNA beach. Further BLAST analysis showed that the BAD primer has only a partially complementary region of nine nucleotids in position 3229 to 3237 in 5' → 3' DNA beach and in position c3238 to c3230 in 3' → 5' strand of X61238.1 sequence. The expected glow spots of the POP primer on the analyzed X61238.1 nucleotide sequences are presented in Figure 5.Expected glowing locations of primer POP in the PRSEF sequence. The displayed sequences are numbered according to the X61238.1 GenBank deposited sequence [36]. The sequence and complement chromosome sequence are given. For the front primer glow site, the sequence is displayed from 27 to 48 nt, and for the reverse primer the glow site, the sequence is displayed from 1357 to 1375 nt. The primer sequence is in the gray box. arrows mark the direction of DNA extension in PCR. The PRSE gene is located in the decomposeed sequence from 79 to 600 nt, and the PRSF gene is located from 676 to 1179 nt. In PCRs for papG reinforcement, also the glow temperature of 55 ° C was used. Three PCR products, all expected size, of around 1,000 bp, were obtained and cloned, and the achieved insertion sequences were analyzed. All three clones had the expected papG sequence. In all three PCR reinforcements, both primers, the front GOD1 and the reverse GOD2 primer, glowed at the expected positions. The expected incandescent locations for specific papG primer binding of GOD1 and GOD2 as revealed by analysis of the nucleotide E. coli sequence CP027701.1 [37] are presented in Figure 6.Expected glowing spots of primer GOD1 and primer GOD2 in the papG sequence. The displayed sequences are numbered according to CP027701.1 GenBank deposited sequence [37]. The sequence and complement chromosome sequence are given. The sequence from 507724 to 507748 nt is displayed for the front primer, and the sequence from 508702 to 508723 nt is displayed. The primer sequence is in the gray box. The arrows mark the direction of DNA extension in pcr. The PapG gene is placed in the deposited sequence from 507716 to 508726 nt. At the glow temperature of 55°C with primers specific to the F17G gene, eight PCR products, all expected sizes of about 900 bp, were achieved and cloned. Nucleotide sequence analysis of all eight clones showed that four ended up correct and four had false bets. All four false bets were, as BLAST revealed, sequences of the protein rtn gene of E. coli K-12 MG1655 chromosome, which deposited in cp025268.1 nucleotide sequence [35]. Further nucleotide analysis showed that in the case of rtn gene amplification, forward primer F17G-1 glowed downstream from c2275331 position at 3' → 5'DNA beach and reverse primer F17G-2 upstream of 2276103 position at 5' → 3' DNA beach of E. coli K-12 MG1655 CP025268.1 nucleotie sequence. The expected glow spots for nonspecific F17G primer binding in analyzed nucleotide sequences are presented in Figure 7.Expected glow locations for nonspecific F17G primer binding in the rtn gene. The displayed sequences are numbered according to CP025268.1 GenBank deposited sequence [35]. The sequence and complement chromosome sequence are given. For the front primer, F17G-1, the glow site, the sequence from 2275306 to 2275331 nt is displayed, and for reverse primer, F17G-2, incandescent location, the sequence from 2276103 to 2276129 nt is displayed. The primer sequence is in the gray box. The arrows mark the direction of DNA extension in pcr. The Rtn gene is located in sequence from 2274762 to 2276318 nt. The main goal of our research was to determine the sequences of selected P- and F17-fimbriae genes among E. coli isolated from stool samples of diarrhea dogs. As we assumed that the fimbriae of such E. coli strains, due to already known variations of P- and F17 fimbriae, may have nucleotide differences, the glow temperature of 55 ° C in PCRs was used. To our surprise, even if only PCR products of expected sizes were cloned, many of the achieved PCR clones, in the case of PCR products obtained with papA primers 75% and in the case of PCR product obtained with F17G primers 50%, conducted false bets. Nucleotide sequence analysis showed that also in the case of papEF clones, although cloned inserts were as hoped for fimbrial inserts, even if they were Prs-fimbrial genes, binding instead of reverse primer was not the expected. The high percentages of counterfeit PCR products were achieved when high melting temperature PCR primers (Tm) were used at the glow temperature of 55°C – primer 22 has Tm of 84.1°C, and 75% of fake PCR products were obtained with this primer; F17G-1 and F17G-2 primers have Tm of 72.4°C and 76.4°C, respectively, and 50% of fake PCR products were obtained with them. In the subsequent PCR reinforcements with primers 22 and 23, the glow temperature was raised to 60 °C, and from these PCRs several PCR products were obtained, namely 16. All 16 were cloned and analyzed, and all clones were with the correct bets (data is not displayed). To conclude, we all know that with PCR we can get fake unspecified products, and we believe that such PCR products will differ from the right PCR products, because the fake PCR products will not be of the correct expected size; However, our results showed that even PCR products of expected size can be fake PCR products. Therefore, to avoid false positive PCR results, it is important to use the correct glow temperature that should not be too different from the temperature of the primer. The author is very grateful to Wim Gaastra for primer nucleotide sequences. This analysis was supported by the Slovenian Research Agency (P1-0198). The author has no conflict of interest.