

16s rDNA

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16S rDNA

16S rDNA

PCR

(PCR)

Nested PCR

(LEG448-JRP) (LEG225- LEG858) ; (LEG448-LEG858)

LEG448-JRP

LEG225-LEG858

LEG448-LEG858

LEG225- LEG858

DNA

Nested PCR

PCR

promega ,Wizard® Genomic DNA Purification Kit, Madison, USA (Promega

DNA PCR PCR

DNA mL

16s rRNA R₁ Eubac27F

DNA Nested PCR

PCR μL DNA

dNTP 1X_i

Taq DNA / μM DNA μL polymerase

$$n=z^2s^2/d^2$$

°C

(PBS)

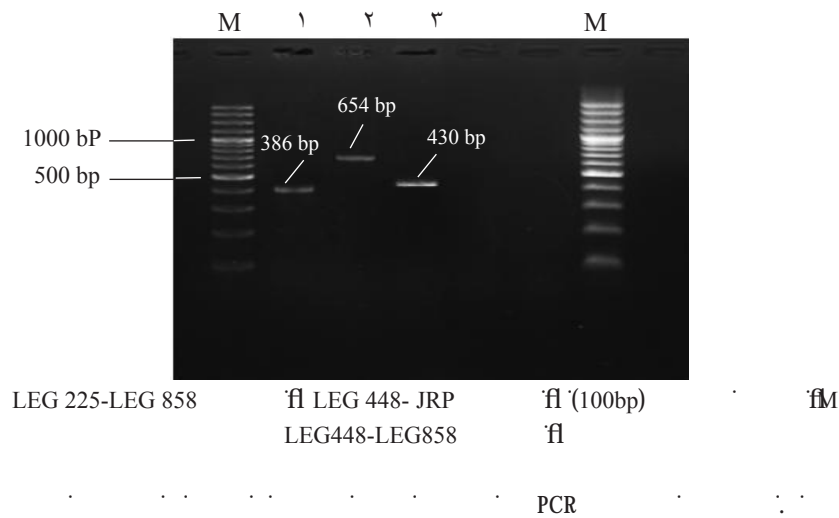
freez-thaw

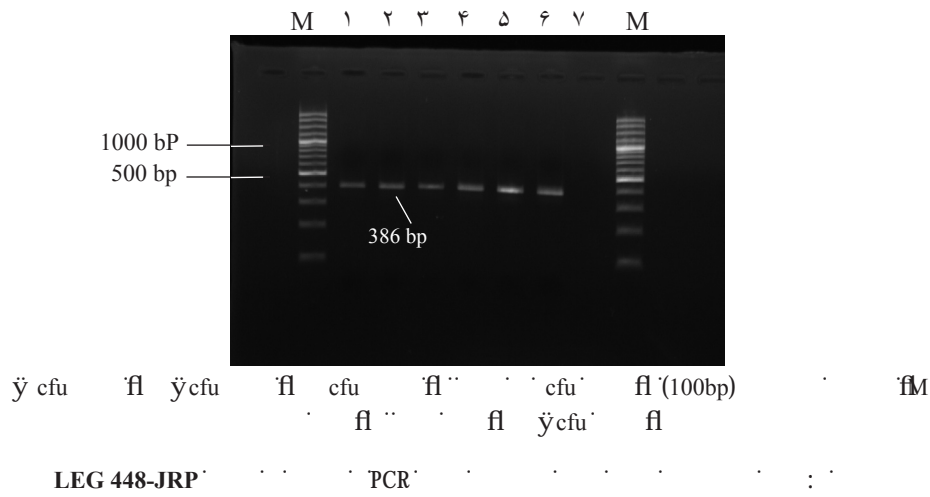
سایز محصولات (bp) PCR	ژن شناسایی	توالی پرایمرها	پرایمرها
حدود ۱۴۲۰ bp	16S rRNA	5'-AGA-GTT-TGA-TCC-TGG-CTC-A-<G>-3'	Eubac27 F 1429 R1
۶۵۴ bp	16S rRNA	5'-AAG-ATT-AGC-CTG-CGT-CCG-A-<T>-3'	LEG 225 LEG 858
۴۳۰ bp	16S rRNA	5'- AGG-GGT-TGA-TAG-GTT-AAG-AG-<C> -3'	LEG 448 LEG 858
۳۸۶bp	16S rRNA	5'- AGG-GGT-TGA-TAG-GTT-AAG-AG-<C> -3'	LEG 448 LEG JRP

PCR

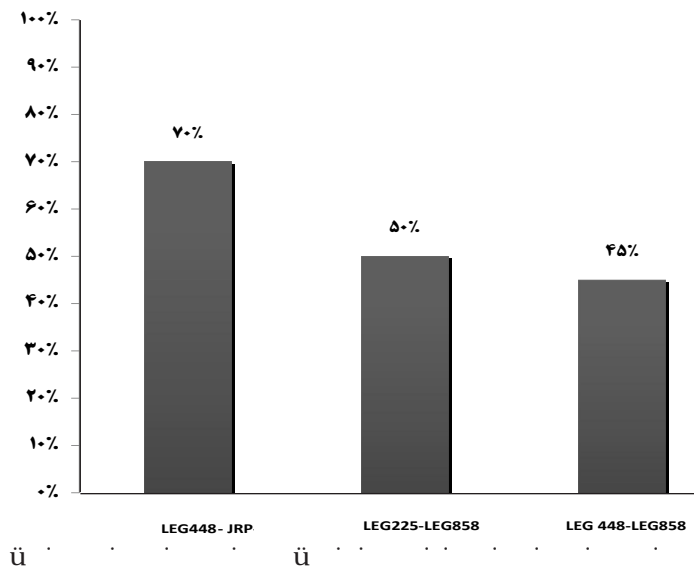
زمان	درجه حرارت	تقسیمات فرعی هر مرحله	تعداد مرحله و سیکل ها
۵min	۹۵°C	Pre- Denaturation	مرحله اول (۱ سیکل)
۴۵s	۹۴°C	Denaturation	
۱min	۵۵°C	Annealing	مرحله دوم (۳۰ سیکل)
۱/ ۳۰min	۷۲°C	Extention	
۵min	۷۲°C	Final Extention	مرحله سوم (۱ سیکل)
۳min	۴ °C	Cooling	مرحله چهارم (۱ سیکل)

Loading Buffer / DNA
 DNA (UV Tech, France)
 DNA Nested PCR PCR
 DNA PCR
 DNA PCR





درصد فراوانی نمونه های مثبت با استفاده از پرایمرهای مختلف جهت بررسی لژیونلا



PCR

LEG225-LEG858) ، (LEG448-JRP

PCR

PCR

LEG448-LEG858

LEG225-LEG858

LEG448- JRP

JRP LEG448

McNemar

LEG448- JRP LEG225-LEG858

LEG448- JRP LEG448- LEG858

(P < 0.05)

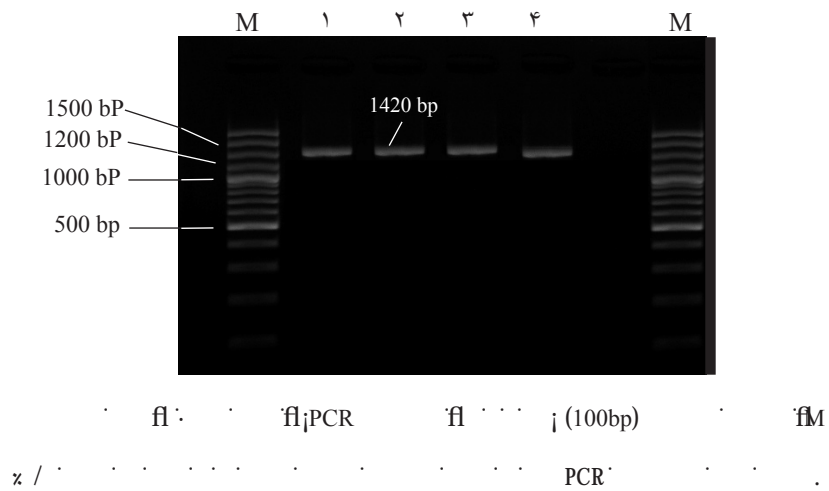
LEG448-LEG858 LEG225-LEG858

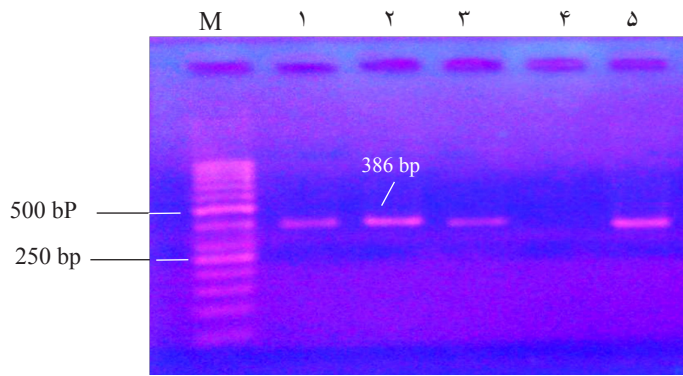
(P = 0.001)

LEG448- JRP

(LEG448-LEG858 LEG225-LEG858)

PCR





500 bp
250 bp
386 bp
M 1 2 3 4 5
JRP LEG448 PCR

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sharp nessL
LEG448-JRP
Nested PCR
DNA PCR LEG448-JRP
LEG225 - LEG858
PCR
DNA PCR
PCR
PCR

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Sensitivity Comparison of Different 16s rDNA- Specific Primers for Detection of Legionella Species in Aquatic Samples

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ABSTRACT

Background and Objectives: Legionella are gram-negative bacteria widely dispersed in natural and man-made water sources. Some Legionella species are pathogenic and could cause respiratory infections. Cultivation technique is the conventional method for the detection of Legionella spp. in aquatic samples. However, the method has low sensitivity and require prolonged incubation period. Therefore, Polymerase chain reaction (PCR) as a rapid method with extreme sensitivity is used. The present study was designed to evaluate the feasibility and sensitivity of PCR method for detection of Legionellas pp. in aquatic samples using three sets of primers.

Materials and Methods: In this study, 60 water samples were investigated for the presence of Legionella species using Nested- PCR technique. The sensitivity of this technique was evaluated for the detection of Legionella species in aquatic samples using three primer sets, including (LEG225-LEG858), (LEG448-LEG858), and (LEG448-JRP).

Results: The nested PCR assay revealed that detection percentage of Legionella in samples was 70 when LEG448-JRP primers were used, whereas this percentage reduced to 50 and 45 when we applied prime sets of LEG225-LEG858 and LEG448 - LEG858, respectively.

Conclusion: The results of the study showed that contamination of aquatic samples to the Legionella spp. could be easily and rapidly detected by nested PCR. However, selecting appropriate method for DNA extraction and choosing the primers are important factors in efficiency and sensitivity of detection method.

Keywords: PCR, Water, Detection, Legionella

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