

# 16s rDNA

nikaeen@hlth.mui.ac.ir

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 R<sub>1</sub> Eubac27F

DNA Nested PCR

PCR μL DNA

dNTP 1X

Taq DNA / μM DNA μL polymerase

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

°C

(PBS)

freez-thaw

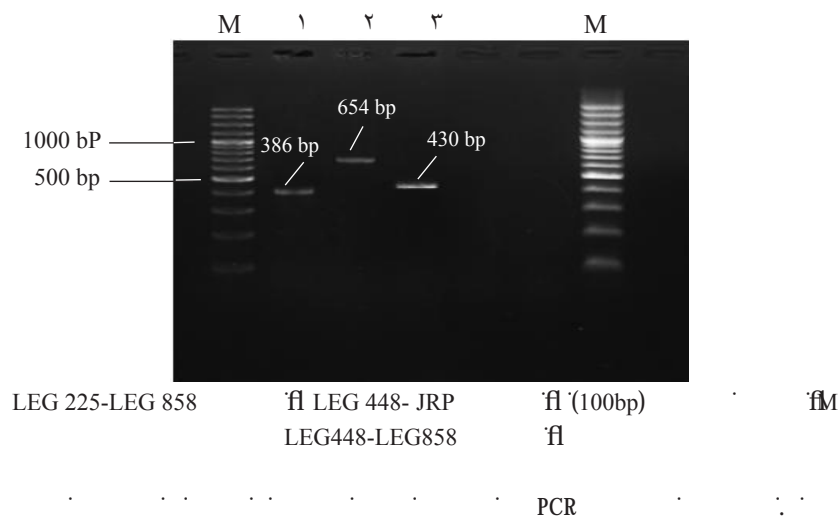
سایز محصولات (bp) PCR	ژن شناسایی	توالی پرایمرها	پرایمرها
حدود ۱۴۲۰ bp	16S rRNA	5'-AGA-GTT-TGA-TCC-TGG-CTC-A-<G>-3'	Eubac27 F 1429 R1
۶۵۴ bp	16S rRNA	5'-AAG-ATT-AGC-CTG-CGT-CCG-A-<T>-3'	LEG 225 LEG 858
۴۳۰ bp	16S rRNA	5'- AGG-GGT-TGA-TAG-GTT-AAG-AG-<C> -3'	LEG 448 LEG 858
۳۸۶bp	16S rRNA	5'- AGG-GGT-TGA-TAG-GTT-AAG-AG-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

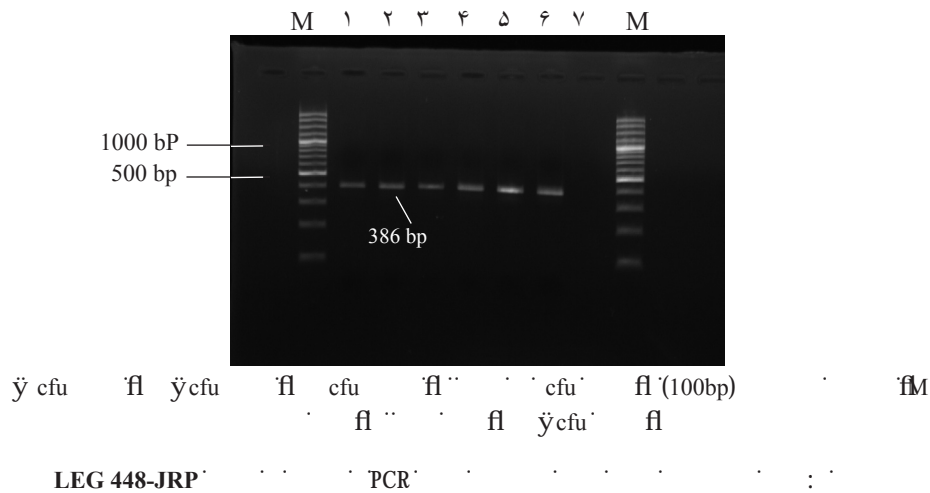
PCR

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)

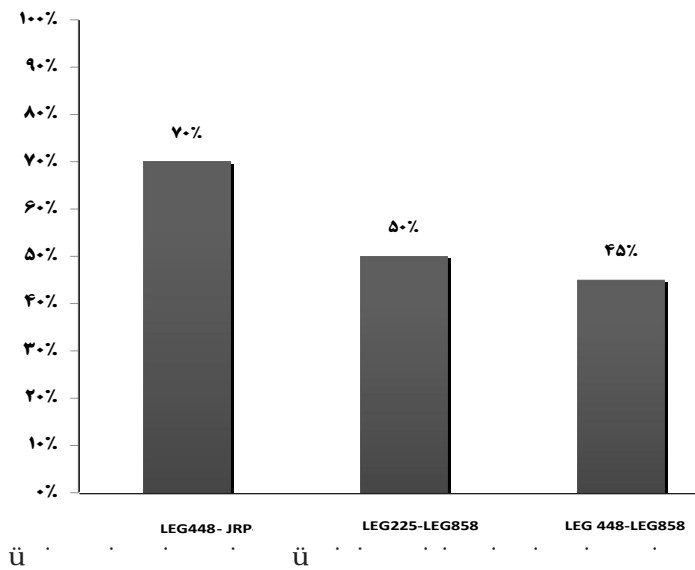
Loading Buffer / DNA  
 DNA (UV Tech, France)  
 DNA Nested PCR  
 DNA PCR  
 DNA PCR

DNA for center HPA ,FEPTU) NCTC 12821, United pneumophila.L ,infections Kingdom, London





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)

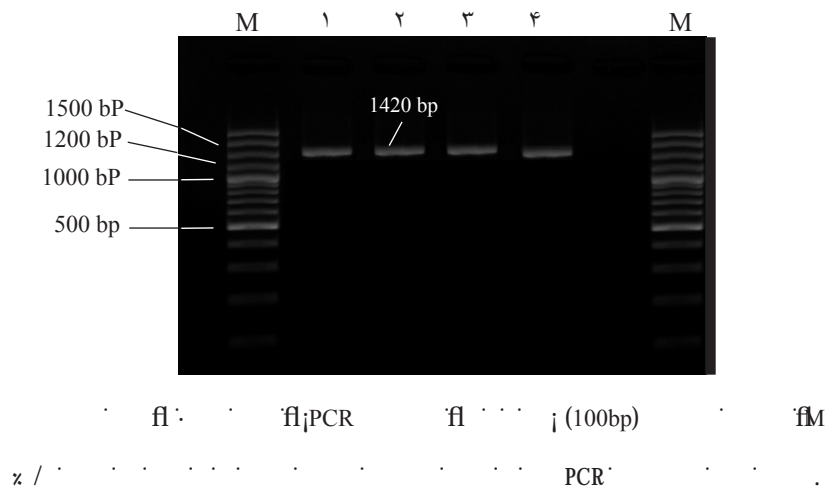
LEG448-LEG858 LEG225-LEG858

(P = 0.001)

LEG448- JRP

(LEG448-LEG858 LEG225-LEG858)

PCR



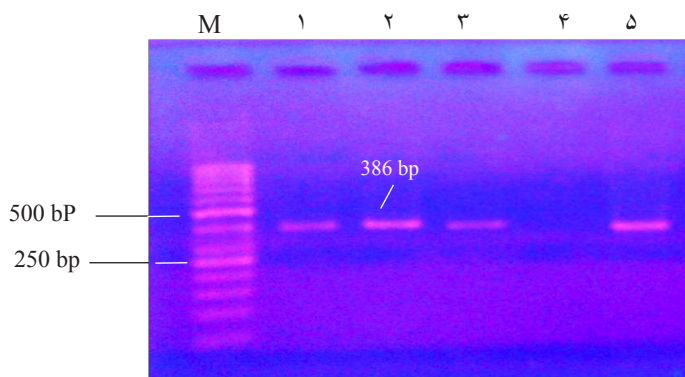


Figure 1: Agarose gel electrophoresis showing PCR products. Lane M: DNA ladder (500 bp, 250 bp). Lane 1: JRP LEG448. Lane 2: LEG448-JRP. Lane 3: Nested PCR. Lane 4: LEG448-JRP. Lane 5: Nested PCR. A band at 386 bp is indicated.

Sharpness

LEG448-JRP

n

yy

Nested PCR

DNA

LEG448-JRP

PCR

n | LEG225 - LEG858

"

( L

PCR

DNA

PCR

( L

PCR

PCR

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

**Farzaneh Baghal Asghari<sup>1</sup>, Mahnaz Nikaeen<sup>2</sup>**

<sup>1</sup>Department of Environmental Health Engineering, Faculty of Nursing and Health, Urmie University of Medical Sciences, West Azarbaijan, Iran

<sup>2</sup>Department of Environmental Health Engineering, School of Health, Isfahan University of Medical Sciences, Isfahan, Iran

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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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\*Corresponding Author: [nikaeen@hlth.mui.ac.ir](mailto:nikaeen@hlth.mui.ac.ir)

Tel: +98 311 7922660, Fax: +98 311 6682509