16s Pyrotag PCR

Howard Ochman

Abstract

Modified from H. Ochman protocol: *Environ. Micro. 2009 Kunin, Engelbrekson Ochman & Hugenholtz*

Citation: Howard Ochman 16s Pyrotag PCR. **protocols.io**
dx.doi.org/10.17504/protocols.io.c5iy4d
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Guidelines

Protocol Using Takara Hot Start EX Taq (cat. no RR006A)

Pyrotag PCR Master Mix

Reagent	Volume µl
Water	18.25
10x EX Buffer II	2.5
dNTP mix (2.5mM ea)	2
F primer (10 µM)	0.5
R primer (10 µM)	0.5
Taq (5U/ µl)	0.25
DNA template	1

Cycling Conditions

Temp. °C	Time (min)	Cycles
95	3:00	
95	:30	25
55	:45	
72	1:30	
72	10:00	
15	Hold	

Protocol Using Sigma Taq (cat. no. D4545) and dNTP mix (cat. no. D7295)

Pyrotag PCR Master Mix

Reagent	Volume µl
Water	17.75
10X Buffer w/o MgCl2	2.5

MgCl2 (25mM)	2
dNTP mix (10mM ea)	0.5
F primer (10 µM)	0.5
R primer (10 µM)	0.5
Taq (5U/µl)	0.25
DNA template	1

Cycling Conditions

Temp. °C	Time (min)	Cycles
95	3:00	
95	:30	25
55	:45	
72	1:30	
72	10:00	
15	Hold	

Reconditioning PCR sample (from Nucleic Acids Research 2002 Thompson, Marcelino & Polz): Make a 1:10 dilution of PCR product into fresh PCR mix; cycle at 95°C for 3 minutes, then 3 cycles of 95°C for 30 second, 55°C for 45 seconds, and 72°C for 1.30 minutes, followed by 10 minutes at 72°C.

Howard's Protocol and Primers for 16s Pyrotagged PCR:

Pyrotag PCR Master Mix

Reagent	Amount µl
Water	39.25
10x Buffer	5
dNTPs (10mM)	1
F primer (10 µM)	0.5
R primer (10 µM)	0.5
BSA (10mg/mL)	1.5
Taq (5u/UL	0.25
DNA	2

Cycling Conditions

Temp. °C	Time (min)	Cycles
95	3:00	
95	:30	25
55	:45	
72	1:30	
72	10:00	
4	Hold	

Pyrotag Primers: 5' - emulsion PCR primer/ unique tag/ universal primer - 3'

926 forward primers:

926fA1 : gcc tcc ctc gcg cca tca g agc aaa ctY aaa Kga att gac gg
926fA2 : gcc tcc ctc gcg cca tca g atg aaa ctY aaa Kga att gac gg
926fA3 : gcc tcc ctc gcg cca tca g ctc aaa ctY aaa Kga att gac gg
926fA4 : gcc tcc ctc gcg cca tca g cag aaa ctY aaa Kga att gac gg
926fA5 : gcc tcc ctc gcg cca tca g tac aaa ctY aaa Kga att gac gg
926fA6 : gcc tcc ctc gcg cca tca g tcg aaa ctY aaa Kga att gac gg
926fA7 : gcc tcc ctc gcg cca tca g act aaa ctY aaa Kga att gac gg
926fA8 : gcc tcc ctc gcg cca tca g cgt aaa ctY aaa Kga att gac gg
926fA9 : gcc tcc ctc gcg cca tca g acacg aaa ctY aaa Kga att gac gg
926fA10 : gcc tcc ctc gcg cca tca g tgctg aaa ctY aaa Kga att gac gg
926fA11 : gcc tcc ctc gcg cca tca g cagcaaa aaa ctY aaa Kga att gac gg
926fA12 : gcc tcc ctc gcg cca tca g catgaaa aaa ctY aaa Kga att gac gg

1492 reverse primer:
1492rB1 : gcc ttg cca gcc cgc tca g agc tac ggY tac ctt gtt acg act t

Summary of PCR Conditions

Reagent	Howard Ochman	EX Takara HS	Sigma Taq
Total Volume	50 µl	25 µl	25 µl
MgCl2 conc.	unknown	2.5 mM	2 mM
dNTP conc.	each 0.2mM	each 0.2mM	each 0.2 mM
F primer conc.	0.1 µM	0.2 µM	0.2 µM
R primer conc.	0.1 µM	0.2 µM	0.2 µM
BSA conc.	0.3mg/ml	0	0
Taq conc.	1.25U/reaction	1.25U/reaction	1.25U/reaction
Template conc.	2 µl/50 µl	1 µl/25 µl	1 µl/25 µl

Protocol