Degenerated PCR with GoTaq Hot Start

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Working

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ABSTRACT

Degenerated PCR to test Y-linkage of genes in several Drosophila species. The reactions are made separately for males and females of 400 species and subspecies of Drosophila and related genera.

TAGS

Drosophila

PCR

Show tags

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

NAME	CATALOG #	
GoTaq(R) Hot Start Polymerase, 500u	M5005	by Promega
dNTP Mix, 10mM, 1000ul	U1515	by Promega
DEPC-Treated Water	#AM9906	by Ambion

Pre-Mix Preparation

1 Usually, we performing PCR tests in large-scale, testing several DNA samples from different species at once. We prepared a pre-mix stock to economy time in PCR experiments.

Reagent	1 reaction	1000 reactions
DEPC-Treated Water	11.6 uL	11.6mL
5x Green GoTaq Flexi Buffer	4.0 uL	4.0 mL
$MgCl_2$ 25mM	2.0 uL	2.0 mL
dNTP 10mM	0.4 uL	0.4 mL
TOTAL VOLUME	18 uL	18 mL

We divide the pre-mix solution in 1 mL aliquots and stocked at -20°C.

Final Degenerated PCR preparation

2 Normally, the DNA template concentration is 10 ug/uL or higher.

Reagent	1 reaction (20.1 uL)
Template	1 uL
Forward degenerated primer 40mM	0.5 uL
Reverse degenerated primer 40mM	0.5 uL
Premix	18 uL
GoTaq Hot Start Polymerase	0.1 uL

PCR Programs

3 We used different thermocyle programs, according to the primers. In all programs, the GoTaq Hot Start Polymerase was previous incubated for 2 minutes to be activated. The PCRs were performed in a Applied Biosystems Veriti™ 96-Well Thermal Cycler (Cat#4375786).

1) Degenerated PCR Program: Differently of the normal PCR thermocycler programs, the degenerated PCR have more time for annealing.

cycles	Denaturation	Annealing	Polymerization
1x	95ºC, 2:00 min	---	---
40x	95º, 0:30 min	xºC, 1:30 min	72ºC, 1:00 min /1000 pb of template
1x	---	---	72ºC, 7:00 min

xºC = optimal annealing temperature for the pair of primers.

2) Degenerated Touchdown PCR (TD-PCR) Program: In TD-PCR, we screen a range of annealing temperatures to try optimize the reaction in different species samples. So, we have a stage where the annealing temperature decrease -0.2ºC by cycle, in the end of this stage, the annealing temperature decreased -4ºC.

cycles	Denaturation	Annealing	Polymerization
1x	95ºC, 2:00 min	---	---
20x	95º, 0:30 min	XºC, 1:30 (Δ -0.2ºC by cycle)	72ºC, 1:00 min /1000 pb of template
25x	95º, 0:30 min	xºC, 0:30 min	72ºC, 1:00 min /1000 pb of template
1x	---	---	72ºC, 7:00 min

xºC = optimal annealing temperature for the pair of primers.

References:

Sambrook, J. & Russell, D. W. (2001) 'Chapter 8. Protocol 11. Mixed Oligonucleotide-primed Amplification of cDNA' in *Molecular cloning : a laboratory manual.* Cold Spring Harbor: Cold Spring Harbor Laboratory, p. 8.66-8.71.

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