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Degenerated PCR with GoTaq Hot Start

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We use this protocol and it's 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.

Materials

MATERIALS

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

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	10000 reactions
	DEPC-Treated Water	11.6 uL	116 mL
	5x GreenGoTaq Flexi Buffer	4.0 uL	40 mL
	$MgCl_2$ 25 mM	2.0 uL	20 mL

	dNTP 10mM	0.4uL	0.4mL
	TOTAL VOLUME	18uL	18mL

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

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	95°C, 2:00min	---	---
	40x	95°, 0:30min	x°C, 1:30min	72°C, 1:00min/1000pb of template
	1x	---	---	72°C, 7:00min

x°C = 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 -0.2°C by cycle, in the end of this stage, the annealing temperature decreased -4°C.

	cycles	Denaturation	Annealing	Polymerization
	1x	95°C, 2:00 min	---	---
	20x	95°, 0:30 min	X°C, 1:30 (Δ-0.2°C by cycle)	72°C, 1:00 min/1000 pb of template
	25x	95°, 0:30 min	x°C, 0:30 min	72°C, 1:00 min/1000 pb of

				template
	1 x	---	---	72°C, 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.