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Degenerated PCR with GoTaq Hot Start

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Antonio Bernardo de
Eduardo Dupim¹, Carvalho¹

¹Universidade Federal do Rio de Janeiro

Eduardo Dupim

Universidade Federal do Rio de Janeiro

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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

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

Protocol status: Working
We use this protocol and it's working

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15128

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Degenerated PCR

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	100 reactions
DEPC-Treated Water	11.6 uL	11.6 mL

5 x GreenGoTaqFlexi Buffer	4.0 uL	4.0 mL
MgCl ₂ 25 mM	2.0 uL	2.0 mL
dNTP 10 mM	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.

Reaction (20 μ L)	1 μ L	0.5 μ L	0.5 μ L
Reagent	Template	Forward degenerated primer 40 mM	Reverse degenerated primer 40 mM

P r e m i x	1 8 u L
G o T a q H o t S t a r t P o l y m e r a s e	0 · 1 u L

PCR Programs

- 3** We used different thermocycle 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.

c y c l e s	D e n a t u r a t i o n	A n n e a l i n g	P o l y m e r i z a t i o n
1 x	9 5 ° C, 2: 0 0 m in	---	---

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

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.

	c y c l e s	D e n a t u r a t i o n	A n n e a l i n g	P o l y m e r i z a t i o n
	1 x	95 °C, 2:00 min	---	---

20 x	95°C, 0:30 min	x°C, 1:30 min (Δ - 0.2°C by cycle)	72°C, 1:00 min / 1000 pb of template
25 x	95°C, 0:30 min	x°C, 0:30 min	72°C, 1:00 min / 1000 pb of template
1 x	---	---	72°C, 7:00 min

x°C = 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.