An. gambiae complex species identification by PCR

Fabio Gomes

Abstract

Scott, JA, WG brogdon, and FH Collins, *Identification of single specimens of the Anopholes gambiae complex by the polymerase chain reaction.* Am J Trop Med Hyg, 1993. **49**(4): p. 520-9

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Guidelines

Guideline for Identification

M Form: 370bp
S Form: 260bp

Before start

PRIMERS – 5' TO 3'		Size of fragment (BP)	Tm
Universal (forward)	**GTG TGC CCC TTC CTC GAT GT**		**58.3**
GA – gambiae (reverse)	**CTG GTT TGG TCG GCA CGT TT**	**390**	**59.3**
ME – merus & melus (reverse)	**TGA CCA ACC CAC TCC CTT GA**	**466/464**	**57.2**
AR – arabiensis (reverse)	**AAG TGT CCT TCT CCA TCC TA**	**315**	**47.4**
QD – quadriannulatus (reverse)	**CAG ACC AAG ATG GTT AGT AT**	**153**	**42.7**

Materials

HhaI - 2,000 units R0139S by New England Biolabs
Platinum PCR SuperMix 11306-016 by Thermo Scientific

Protocol

PCR

Step 1.
Assemble Mix

Reagent	**Per rxn:**
Platinum Supermix	16.5
UN (20uM)	0.5
GA (20 uM)	0.5
AR (20uM)	0.5
QD (20uM)	0.5
ME (20uM)	0.5
Template	1.0
Total	20ul

PCR

Step 2.
Run PCR setup

Hot Start: 94° for 2 min
30 cycles of: 94° for 30 sec
50° for 30 sec
72° for 30 sec
Final extension: 72° for 5 min
4° forever

PCR

Step 3.
Save 5uL for running a gel

Restriction Digestion

Step 4.
Take 10-15 uL PCR product, complete to 17.5 uL with NFW.
Add 2 uL 10x HhaI Buffer and 0.5 uL HhaI

Incubate at 37oC Overnihgt

Restriction Digestion

Step 5.
Run Agarose Gel using 100 bp ladder