Langerhans Lab Protocols

NC STATE UNIVERSITY

PCR primers used for *Gambusia* sequencing:

CytB—400 bp:

1.	L14724 (forward)	5'- CGA AGC TTG ATA TGA AAA ACC ATC GTT G- 3'
2.	H15149 (reverse)	5'- AAA CTG CAG CCC CTC AGA ATG ATA TTT GTC CTC A- 3'

Great efficacy across a wide range of species (*affinis*, *caymanensis*, *gaigei*, *hubbsi*, *lemaitrei*, *melapleura*, *nicaraguensis*, *nobilis*)

ND2—1100 bp:

1.	GamMet (forward)	5'- AAG CTT TCG GGC CCA TAC CC- 3'
2.	GamTrp (reverse)	5'- GCT TAG GGC TTT GAA GGC CC- 3'
3.	GamH4917	5'- ATG GTT AAT GCT ATT GCG- 3'
4.	GamL546	5'- AAT TGC CCA CCT CGG GTG AA- 3'
5.	GamH1047	5'- TAA RGG YRG GGG TAA YAG GG- 3'

3 is an alternate reverse primer for *G. hubbsi* used in the Evolution 2007 paper.

Overall good efficacy; a second band around 400bp is amplified in a few species (*nobilis*, some *affinis* [RBL 121], *nicaraguensis*) and around 1000 bp for *manni* specimens and some *G. hubbsi* specimens. Annealing temp for *G. nicaraguensis* of 66C is best to amplify upper (target) band.

Rag2—1000bp:

1.	1297R (reverse)	5'- TCG GTG GAG TAG TAA GGC TCC CA- 3'
2.	277F (forward)	5'- GAC CCC GAG YGY TAC CTC ATC C- 3'

Overall good efficacy, but requires gel extraction for many samples; almost all have a faint second band amplified around 400bp. Primers not effective for *B. belizanus* due to high non-specific binding.

S7—800bp:

1.	S7RP1F.RBL (forward)	5'- CTC TTC CTT GGC CGT CGC TG- 3'
2.	S7RP2R.RBL (reverse)	5'- TTA CCT GGG AGA TTC CAG ACT CAA A- 3'
3.	S7RPEX2R (reverse)	5'- AAC TCG TCT GGC TTT TCG CC- 3'
4.	S7RPEX1F (forward)	5'- TGG CCT CTT CCT TGG CCG TC- 3'
5.	S7RPEX3R (reverse)	5'- GCC TTC AGG TCA GAG TTC AT- 3' *from Chow and Hazema 1998
6.	S7RPEX1F.RKR (forward)	5'- CTC TTC CCA GGC CGT CGT TG- 3' *from Reachel Remington
7.	S7RPEX3R.10 (reverse)	5'- TCA GAG TTC ATC TCC AGC TC- 3' *from Peter Unmack

Carlos's nested approach with 4-7 gives best results and completely eliminates amplification of second band around 600bp. Primers 1-2 almost always amplify a second gene around 600bp in size, sometimes not amplifying the 800bp target at all; 3-4 give fair results.

PCR primers used for *Gobiomorus* sequencing:

ND2:

GOBYL4919	5'- CCC ATA CCC CGA AAA TGA TG- 3'
GOBYH6064	5'- CTC CTA CTT AGA GCT TTG AAG GC- 3'
GOBYL4575	5'- TGA GGN GGY YTM AAY CAA ACH CAA -3'
GOBYH5513	5'- GAG TAG GCT AGG ATT TTW CGA AGY TG -3'
GOBYL4NEW	5'- TGC AAG CTC TCA CTG ACT CC -3'
GOBYH986NEW	5'- AAA GGG TGA AGA GGG CAG TT -3'

GOBYL4 and GOBYH986 seem to be the most effective; a second 1500bp band will appear along with the desired 1100bp band at some annealing temperatures.