# Langerhans Lab Protocols

NC STATE UNIVERSITY

## PCR Reaction Cocktail:

Multiply each volume below by the number of PCR reactions +1 (i.e., for 10 reactions, multiply volumes by 11) to make a Master Mix, aliquot 21 µl into each PCR tube, and add 4 µl of template DNA. Reaction volume is 25 µl.

12.5 µl SuperMix
0.5 µl UltraPure $H_2O$
4 µl forward primer (10 µM)
4 µl reverse primer (10 µM)
+ 4 µl template DNA

from mid-2012, Jennifer used different Master Mix. Lower template DNA volume helps reduce interference in PCR by impurities in extracted DNA. Lower primer volume recommended by Qiagen tech support. Multiply each volume below by the number of PCR reactions +1 (i.e., for 10 reactions, multiply volumes by 11) to make a Master Mix, aliquot 23 µl into each PCR tube, and add 2 µl of template DNA. Reaction volume is 25 µl.

12.5 µl SuperMix
4.5 µl UltraPure $H_2O$
3 µl forward primer (10 µM)
3 µl reverse primer (10 µM)
+ 2 µl template DNA

Protocol for RAG2 (DMSO enhances reaction by disrupting base pairing in GC-rich regions; Mg improves amplification but decreases specificity):

12.5 µl SuperMix
3.75 µl UltraPure $H_2O$
0.25 µl DMSO
0.5 µl 25mM Mg++
3 µl forward primer (10 µM)
3 µl reverse primer (10 µM)
+ 2 µl template DNA

DMSO source: Borski lab chemical cupboard
25 mM Mg++ source:

# PCR Cycles

***Activation temperature (step 1) should be adjusted according to the polymerase or SuperMix being used in the reaction. Most will be 94-96°C.

## Genes: CytB, GNRH

Protocol 3STP58 (primary protocol, good starting protocol for trying to optimize new loci); also called CYTBLANG in PTC-100 Thermocycler in DCL room 386

1. 95°C 3 min
2. 95°C 30 sec
3. 58°C 30 sec
4. 72°C 1 min 30 sec
5. 72°C 4 min

Start cycle with #1, repeat #2-4 30 times, close with #5, hold at 4°C

## Gene: RAG2

Protocol _____

1. 95°C 3 min
2. 95°C 30 sec
3. 57°C 30 sec
4. 72°C 1 min 30 sec
5. 72°C 4 min

Start cycle with #1, repeat #2-4 30 times, close with #5, hold at 4°C

## Gene: ND2

For GamMet + GamTrp in *G. hubbsi*, use annealing temp of 56°C
For GamMet + Gam4917 in *G. hubbsi*, use annealing temp of 48°C
For GamMet + GamTrp in *G. nicaraguensis* , use annealing temp of 66°C to amplify upper (target) band.
Protocol RBLND2

1. 94°C 3 min
2. 94°C 30 sec
3. 56°C 30 sec
4. 72°C 1 min 30 sec
5. 72°C 4 min

Start cycle with #1, repeat #2-4 30 times, close with #5, hold at 4°C

Protocol Evolution2007 (worked well for *G. hubbsi* in Langerhans et al. 2007 *Evolution* paper)

1. 94°C 2 min
2. 94°C 35 sec
3. 50°C 35 sec
4. 72°C 1 min 30 sec
5. 72°C 5 min

Start with cycle #1, repeat #2-4 40 times, close with #5, hold at 4°C

**Gene: ND2 for Gobys**

Protocol GOBY56 (preferred protocol)

1. 95°C 3 min
2. 95°C 30 sec
3. 56°C 35 sec
4. 72°C 1 min 30 sec
5. 72°C 7 min

Start with #1, repeat #2-4 35 times, close with #5, hold at 4°C

## Gene: S7

Protocol TD67 (touchdown from 67 to 57)

1. 95°C 3 min
2. 95°C 30 sec
3. 67°C 30 sec
4. 72°C 1 min 30 sec
5. 72°C 4 min

Start with #1, repeat #2-4 while lowering #3 by 1 degree each cycle until 57 is reached, repeat #2-4 an additional 25 cycles (with #3 maintained at 57°C), close with #5, hold at 4°C

Protocol Carlos (nested PCR approach using 4 different primers) (PREFERRED PROTOCOL)

Begin using primers 1f and 3r:

1. 95°C 3 min
2. 95°C 30 sec
3. 53°C 35 sec
4. 72°C 1 min 30 sec
5. 72°C 4 min

Start with #1, repeat #2-4 35 times, close with #5, hold at 4°C

Dilute 1:49, and use the product with primers 1f.rkr and 3R.10 using the following protocol:

1. 95°C 3 min
2. 95°C 30 sec
3. 53°C 35 sec
4. 72°C 1 min 30 sec
5. 72°C 4 min

Start with #1, repeat #2-4 30 times, close with #5, hold at 4°C