Protocol: specimen preparation (brain dissection and region extraction) for using RT-qPCR to examine gene expression in brain regions of a fish

IN FULFILLMENT OF REQUIREMENTS FOR EDEN RESEARCH EXCHANGE SCHOLARSHIP

VISITING RESEARCH STUDENT: Jessica Nowicki

July 25- September 12, 2013

RATIONAL:

This protocol describes the steps taken to capture individual fishes in the wild, euthanize, dissect whole brains, and isolate specific brain regions. These steps can be incorporated into a RT-qPCR experimental workflow under the "sample validation and experimental data collection" theme (Nolan et al., 2006) (Figure 1.0) in order to quantify mRNA within brain regions of a teleost. *Ethical clearance and collection permits are likely to be required in order to use this protocol.

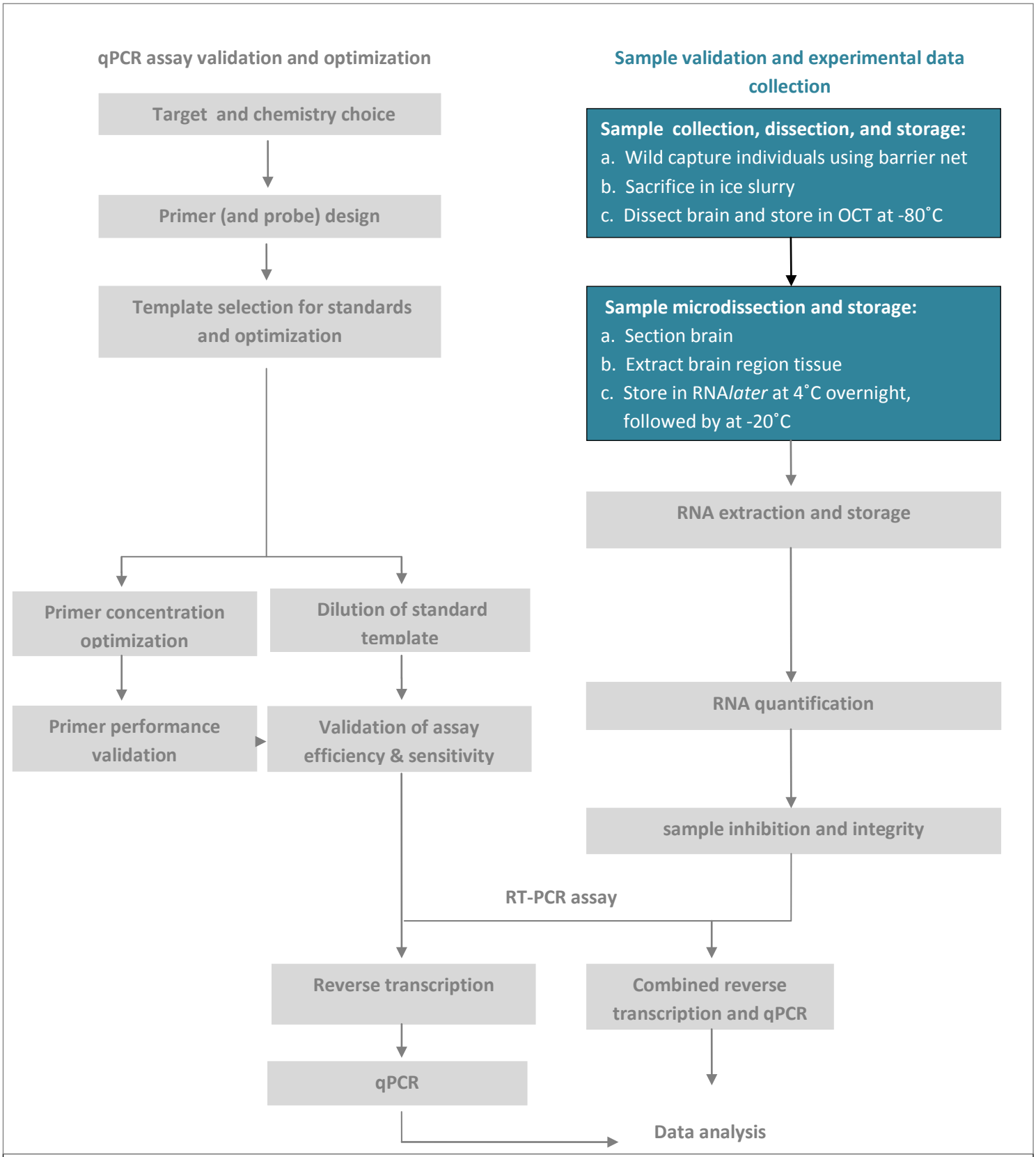

Figure 1.0 RT-qPCR experimental workflow, modified from Nolan et al., 2006. The four main themes of the workflow are assay validation and optimization, sample validation and data collection, RT-PCR assay, and data analysis. We present a partial protocol for the "sample validation and experimental data collection" theme that is customized for examining gene expression within brain regions of fishes (highlighted in blue).

REQUIRED MATERIALS

Sample collection

1. 1 barrier net (1 M high X 4 M wide, monofilament). *This capture approach is best suited for benthopelagic fishes
2. 2 Hand nets (deep bodied)

Sample dissection

3. Ice slurry (1 part sea water to 8 parts shaved ice; total volume ~10 X that of fish)
4. Calipers
5. Scale
6. Dissection kit
7. Optimal cutting temperature (OCT) compound
8. Microcentrifuge vial (1.5 mL)
8. Liquid nitrogen or dry ice shavings

Sample microdissection and storage

9. Cryostat
10. OCT
11. Glass slides
12. Teleost brain atlas (one of a species within the same family or genus as your study species)
13. A hand-held micro-punching tool (e.g., Stoelting, model # 57401)
14. RNA*later*®
15. RNAse free microcentrifuge vials (0.65 mL)
16. 80% ethanol
17. 4 ˚C and -20 ˚C freezer

PROTOCOL

Sample collection, dissection, and storage:

a. Capture individuals in the wild using a barrier net and hand nets.

b. Sacrifice by gently submerging in an ice slurry for 5-10 minutes (ensure no gill/body movement to confirm death).

c. Measure total length and weight. Then, dissect whole brain, embed in optimal cutting temperature (OCT), and flash freeze at -80C within 10 minutes of sacrifice (Figure 2.0).

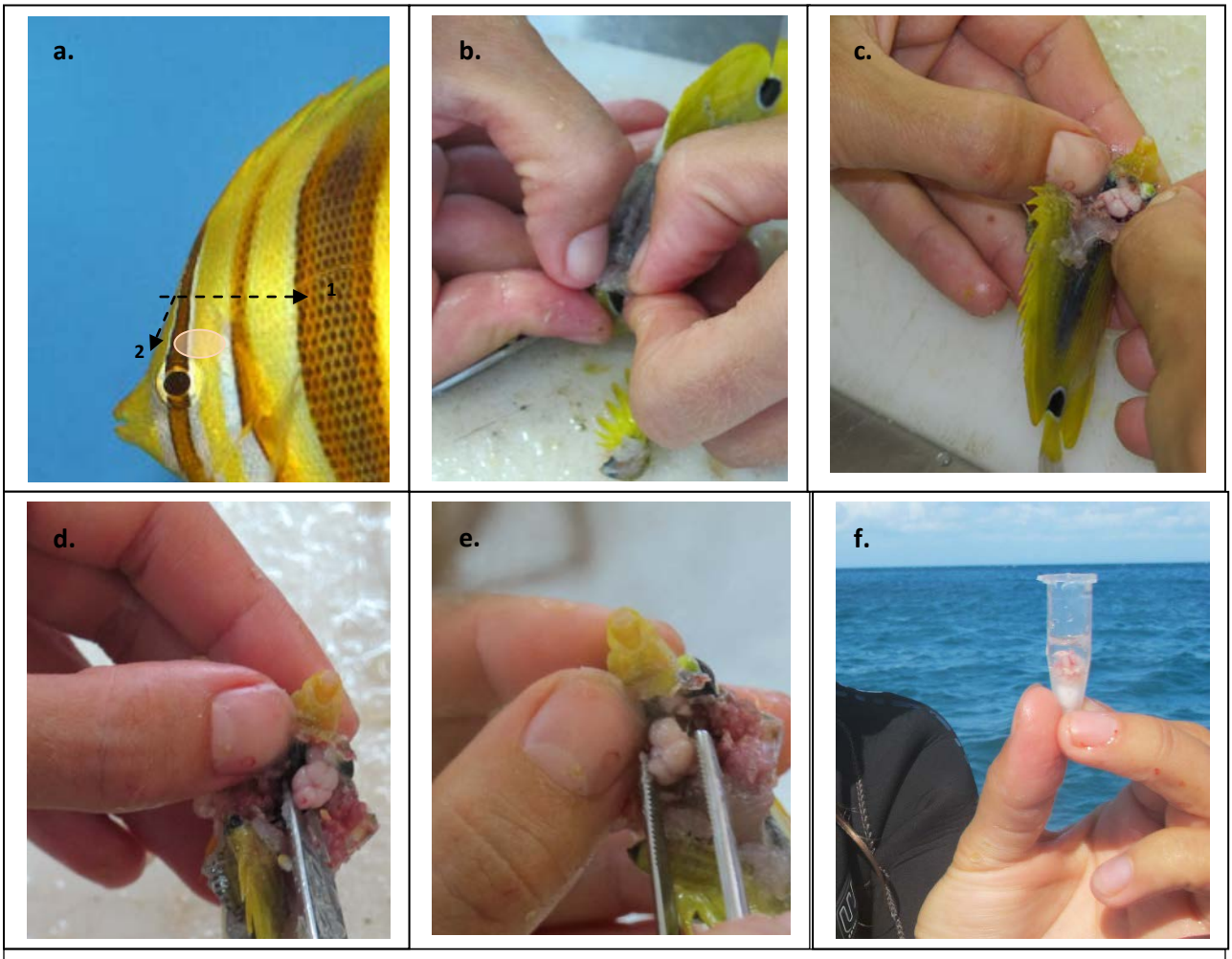

Figure. 2.0. Brain dissection workflow. a1) Along the anterior-posterior axis, cut an incision from above the eye to behind the gill cover. a2) Along the median sagittal axis, cut an incision from above the eye towards the eye. b,c) Manually pull apart the skull along the median sagittal plane to separate the cranium and fully expose the brain. d) Cut the brain free from the optical nerves and spinal cord. e) Extract the brain. f) Embed the brain in OCT compound (positioned on its side, so that the telencephalon is facing the bottom of the vial, and the rhombencephalon is facing towards the top of the vial) and freeze at -80 ˚C within 10 minutes of death.

Sample microdissection and storage

a) Acclimate brains to temperature of cryostat (-16 to - 20 ˚C) for 1 hour before sectioning. Following, from anterior to posterior, section brains on a coronal plane at 110 µm thickness using the cryostat, thaw mount onto glass slides (Figure 3), and store at -80˚C.

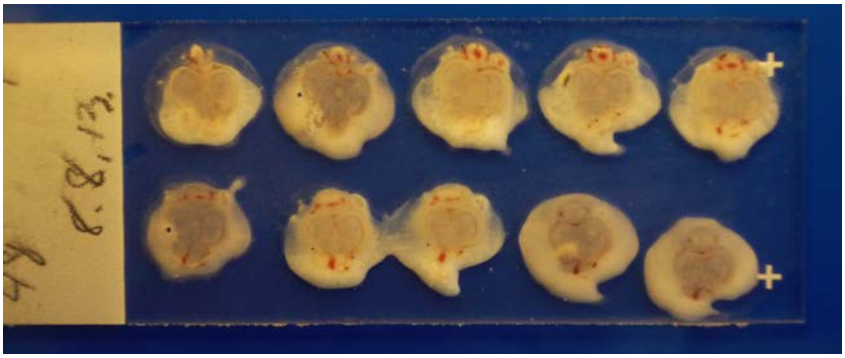

Figure 3.0. Coronal sections thaw mounted onto a glass slide.

b) Choose brain regions in which you wish to measure mRNA (e.g., Table 1). Acclimate brain sections to between -35 and -28 ˚C by placing them inside the cryostat for 1 hour prior to region extraction. Following, identify regions on coronal sections by referring to a teleost brain atlas (preferably one of a species within the same family or genus as your study species) (Figure 4a).

Table 1.0 Brain regions of interest, among which gene expression level will be examined.

	Teleost brain regions
1	Central part of the ventral telencephalon (Vc)
2	Dorsal part of the ventral telencephalon (Vd)
3	Medial part of the dorsal telencephalon (Dm)
4	Lateral part of the dorsal telencephalon (Dl)
5	Posterior tuberculum (TPp)
6	Supracommissural part of the ventral telencephalon (Vs)
7	Preoptic area (POA)
8	Cerebellum (Cereb.)

Following, manually extract the whole brain region from sections using a hand-held micro-punching tool (Stoelting, model # 57401) at -35˚ C (Figure 4b).

c) Depress extracted brain regions from the mimcro-punching tool into RNA*later* ® and incubate at 4˚C overnight, and then store at -20˚ C (can be stored indefinitely at this temperature). *Ensure to sterilize the tool in between samples using 80% ethanol.

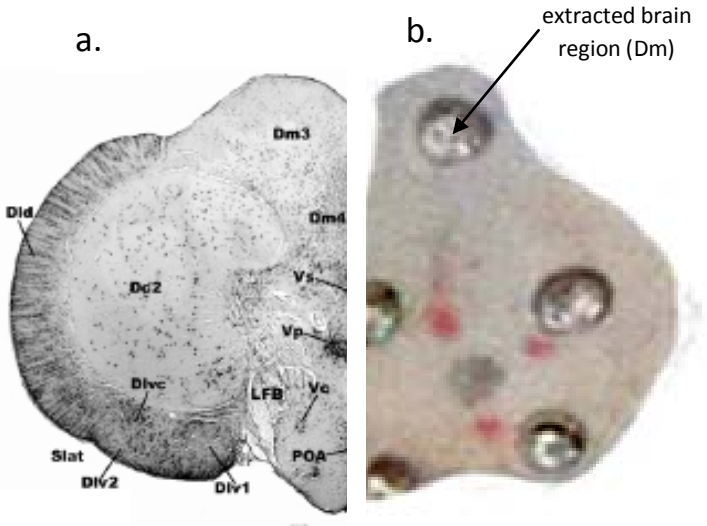

Figure 4. A comparative picture of some brain regions of interest, which were identified using a brain atlas of a species of Butterflyfish, *Chaetodon multicinctus* (A. Dewan Ph.D. thesis)(a) and punched out for mRNA analysis (b).

References:

Dewan, A. K., Tricas, T.C. (XXXX). Cytoarchitecture of the telencephalon in the coral reef multiband butterflyfish (*Chaetodon multicinctus*: Perciformes). *Ph.D. Thesis,* pp 37.

Nolan, T., Hands, R. E., & Bustin, S. A. (2006). Quantification of mRNA using real-time RT-PCR. *Nature protocols*, *1*(3), 1559-1582.