

6  
2/7/09

## RACE 1st strand cDNA Synthesis

### RNA

- Start w/ *C. gigas* total RNA isolated from gill tissue 10/17/08

conc 2312.9 ng/ $\mu$ L      2.3  $\mu$ g/ $\mu$ L

↓  
add 0.5  $\mu$ L to rxn  
to hit 1  $\mu$ g target

1. 5' RACE

3' RACE

~~1  $\mu$ g RNA~~  
0.5  $\mu$ L RNA  
1  $\mu$ L 5' CDS primer A  
1  $\mu$ L SMART II A oligo  
2.5  $\mu$ L H<sub>2</sub>O

0.5  $\mu$ L RNA  
1  $\mu$ L 3' CDS primer A  
3.5  $\mu$ L H<sub>2</sub>O

5  $\mu$ L total

5  $\mu$ L total

incubate 70°C 2 min

cool on ice 2 min

spin

2. to both tubes add...

- 2  $\mu$ L 5x First strand buff'd
- 1  $\mu$ L DTT 20mM
- 1  $\mu$ L dNTP mix (10mM) ← not Prom kit!
- 1  $\mu$ L MMW RT (Promega)

• mix by pipetting, spin

3. Incubate @ 42°C for 1.5 hr in hot lid thermocycler