

5. heat @ 72 for 10min

4. Dilute to 100  $\mu$ L. Store @ -20 in Macs PCR reagents box.

17 Tricine  
EDTA buffer

\* 11

~~12/9~~

|                       |      |             |   |               |       |                |
|-----------------------|------|-------------|---|---------------|-------|----------------|
| PCR-Grade Water       | 34.5 | $\times 17$ | = | 586.5 $\mu$ L | 310.5 | <del>414</del> |
| 10x Adv. 2 Pce buffer | 5    | $\times 17$ | = | 85 $\mu$ L    | 45    | <del>55</del>  |
| dNTP (10mm)           | 1    | $\times 17$ | = | 17 $\mu$ L    | 9     | <del>5</del>   |
| 50x Adv. 2 Poly Mix   | 1    | $\times 17$ | = | 17 $\mu$ L    | 9     | <del>5</del>   |

41.5  $\mu$ L / rxn

5. add reagents per tables in manual (copy next page)

NOTE: 3' UPM negative control  $\rightarrow$  accidentally added 3' primer (no longer a neg control!)

6. NOTE: also included Sams 3', 5' RACE cDNA generated 6/19/08. This can be a pos. cDNA control since this material worked for COX  $\beta$ ACT RACE amplification

\* 11 ~~samp~~ rxns prepared (GSP1 = Reverse, 5') (GSP2 = Forward, 3')

1. Sams 5' cDNA
2. Mac's 5' cDNA
3. 5' GSP1+2 control
4. 5' UPM control
5. 5' GSP1 only control
6. Sams 3' cDNA
7. Mac's 3' cDNA
8. 3' GSP1+2 control
9. 3' UPM control (added GSP2!)
10. 3' GSP2 only control
11. extra for Vol. Recovery

7. 94C 30sec } 5 cycles  
72C 3min }

94C 30sec } 5 cycles  
70C 30sec }  
72C 3min }

94C 30sec } 25 cycles  
68C 30sec }  
72C 3min }

$\rightarrow$  then 4°C forever