Sequence Rxn

Run template on agarose gel to macroscopically determine the amount of DNA.

- ➔ Use 11 μL of a decent to thick sized band.
- ➔ Use clean template; use qiagen kit (more than EtOH precipitation.)

11 μL template (usually plasmid or PCR product)

1 μL 0.8 pmol/μL primer

4 μL Big dye enzyme

4 μL 10X Big dye buffer

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20 μL

Mix; quick spin.

Add mineral oil.

Melt		96°C 30s
Anneal*	example	58°C 15s*This may change; be aware of your primer Tm!
Extend		60°C 4m

30 cycles

Clean up reaction with AutoSeq G-50 spin column, Amersham Biosciences product #27-5340-01. (Follow included instructions.)

Put clean sample into strip tube and bring to UWBTC for analysis.

Follow BTC computer instructions.

Select strip tube as sample type.

Place sample in freezer.

Expect 2-3 d for sample processing.

Refer to https://dna.biotech.wisc.edu/documents/Cycle_Sequencing_Protocols.htm for additional protocols.

Refer to https://dna.biotech.wisc.edu/documents/Pricelist.htm for pricing info.

Sequence Data & Analysis

before you begin: download bioedit and winzip from

https://dna.biotech.wisc.edu/index.asp

1. click access your data on the above webpage
2. user name Andes
3. password andeslab
4. download selected files
5. double click on downloaded file
    - ➔ agree to terms of winzip
6. file should upzip onto desktop
7. open unzipped file in bioedit

8. look at abi chromatogram to determine ~ where sequence starts and ends

    ➔ refer to https://dna.biotech.wisc.edu/documents/Troubleshooting.htm for additional troubleshooting.

9. minimize abi chromatogram and use dna sequence to copy selected sequence

10. paste sequence into word

11. print out and use chromatogram to resolve N's by hand on print out.

12. adjust N's in word document

13. copy resolved sequence

14. paste into http://us.expasy.org/tools/dna.html

    ➔ hint: determine which reading frame is correct by the stops and starts.

15. copy translated sequence and paste sequence into http://prodes.toulouse.inra.fr/multalin/multalin.html

16. paste in ncbi erg11 amino acid sequence, ACCESSION AF153850

17. select MultiAlin-Fast under sequence input format

18. scroll to bottom of page

19. adjust line length ~ 80; this enables you to be able to print your entire alignment.

    ➔ line length of 130 truncates ~30 bases at the end of each line

20. click start multalin!

21. if you doubt the accuracy of your alignment, use multalin to align your resolved dna sequence and the erg11 dna sequence, ACCESSION AF153850.

22. make appropriate adjustments to your resolved sequence and start again at step 14 with pasting your sequence into http://us.expasy.org/tools/dna.html.