M3D4: Cell Prep for Analysis

Announcements

- Lab Treat
- Today is last day in tissue culture!
- No lecture or lab Tuesday (4/29)
- M3D4 FNT due between 11am-12pm on 4/29 — 16-220 will be open for you to complete part of M3D5 if you would like to get started.
- **Module 3 report due M3D7, 5pm**
  - work in supergroups on Day 7
  - less formal, but clear and concise
- **Final project: Research Proposal Presentation M3D8**
  - More about scope of both next time -- *rubrics are available on wiki*

quantitative ≡ qPCR

qRT-PCR Overview

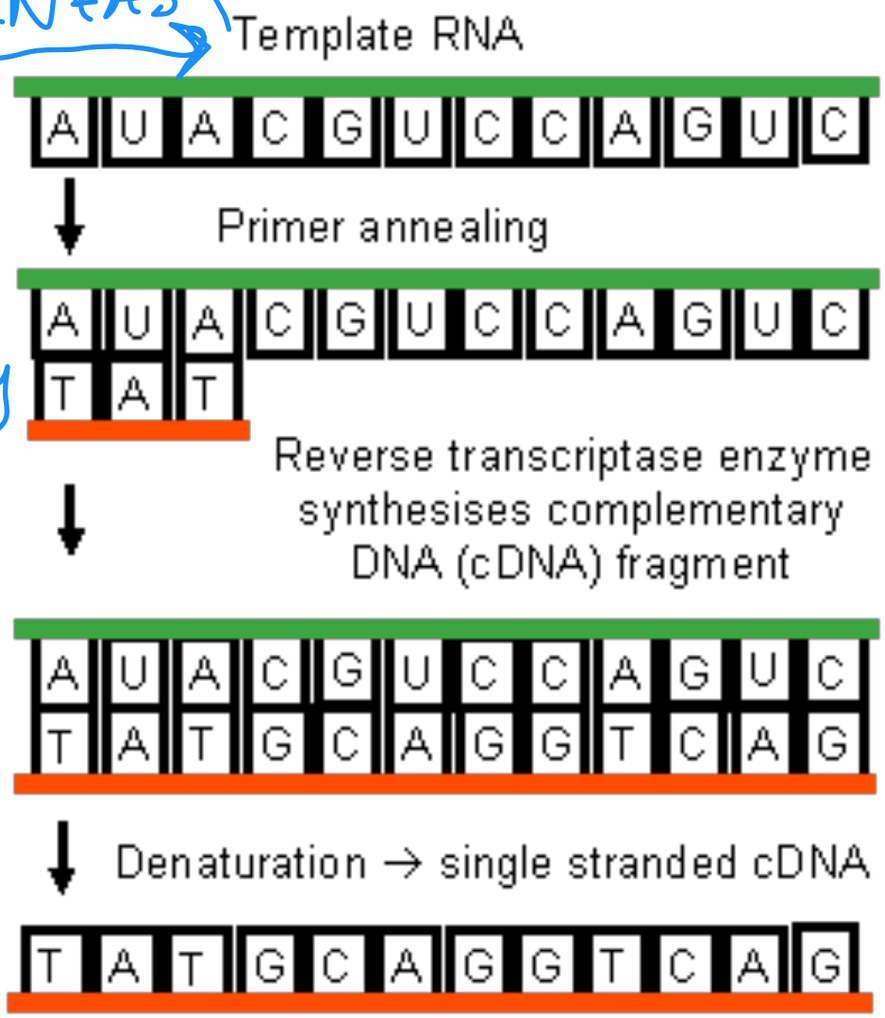
Goal: Quantify relative CN1 and CNII levels across conditions

1) Purity/Harvest RNA → Qiagen RNEASY

>200 nt ≡ enriches mRNA

RT = reverse transcriptase

2) random hexamers → cDNA library



Final step —>
PCR reaction with specific primer set

3) like in Mod1 → specific primers to amplify CN1 + CNII

http://wiki.biomine.skelleftea.se/biomine/molecular/index_07.htm

RT and qPCR controls:

RT Step:

No RT control: → contaminating / "impure" sample (cDNA / genomic DNA present)

No Sample control: → RNA contamination (you!, your reagents)

PCR Step:

Housekeeping gene: 18S rRNA (GAPDH or beta-actin)

Controls for: differences in input to your reaction

#Assume that RT reaction goes to completion & is 100% efficient

→ amt of RNA added to our reactions

Today in lab:

1. Yellow, Green, Blue, Pink — first group to TC
2. *Protocol may be slightly different in you have (ASK FIRST):*
    - *Not many beads*
    - *Beads that are falling apart.*
3. Working with RNA
    - Wipe down bench, pipettes, centrifuge with RNAse away
    - Wear gloves — tape off a ‘RNA-only zone’
    - Read the protocol — don’t throw away the lysate!
    - Be aware of tube changes

During downtime — work on your FNTs — start thinking about your research proposal.

-and- clean up your bench for the RNA harvest