

M3D5: Transcript & Protein Assays

Announcements

- Final quiz and final FNT -- next lab (Wednesday)
- **Module 3 report due M3D7, 5pm**
 - work in groups (tell me otherwise)
 - less formal, but clear and concise
- **Final project: Research Proposal Presentation M3D8**
 - More about scope of both next time -- *rubrics are available on wiki*
 - Day 7 lecture: Atissa is back (be there! -- presenting with a partner)
 - Day 8 lecture: special topics in TE (may also be D7)
 - Final lecture (5/16): class discussion/evaluation + Party!

Indirect
ELISA: Quantify CN I or CN II

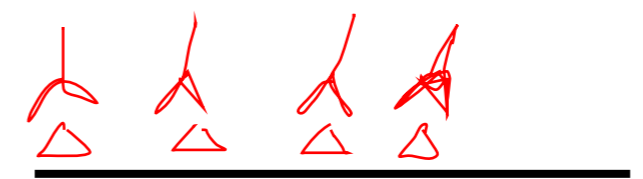
$\Delta = \text{CN}$

$\text{Y} = \text{Ab} = \text{Y}$

1 Add cell lysate
 ↓ protein binds



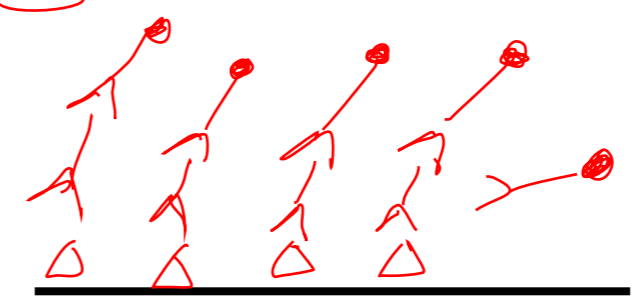
4 Wash w/ Tween-20



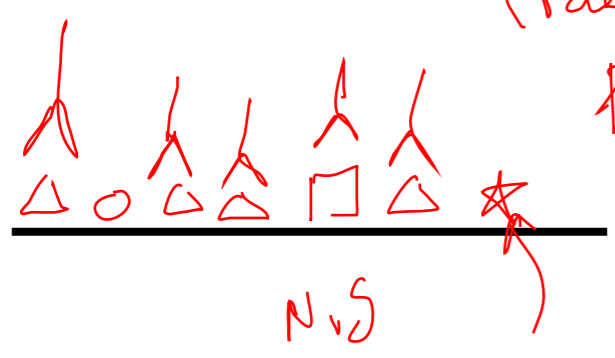
2 Block plate w/ Milk
 80 min



5 2° Ab + wash ● = AP
 alkaline phosphatase



3 Add 1° Ab αEN
 (rabbit)
 polyclonal



6 Add AP substrate
 Δ color \Rightarrow plate reader @ 430 nm



How will we quantify? CN I or CN II
 Standard curve

qPCR:

CNI & CNI1

mRNA \xrightarrow{RT} cDNA

www.5prime.com

Real time tracking [DNA].

Threshold Cycle (Ct)

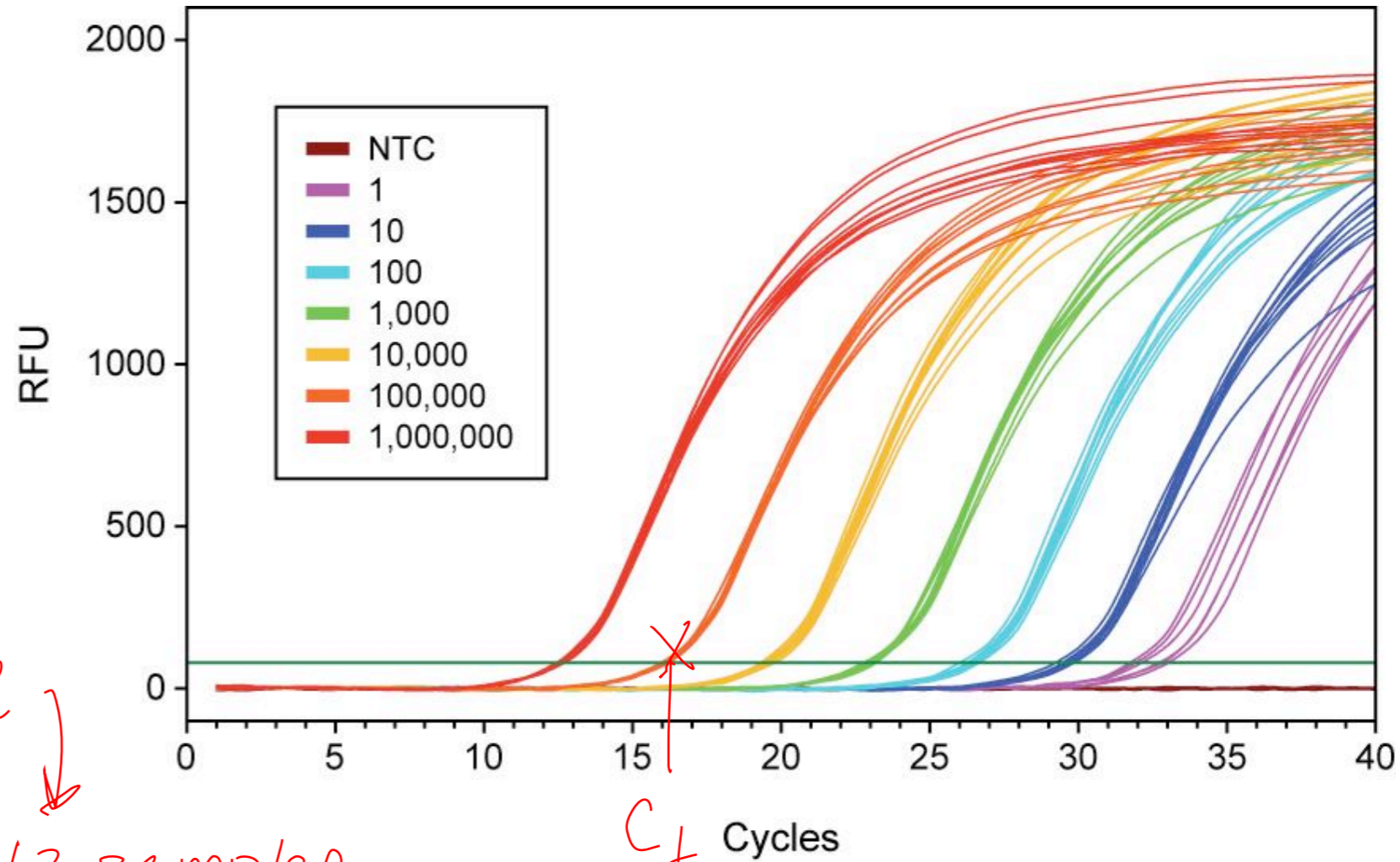
$C_t \uparrow \Rightarrow [DNA] \downarrow$

2 conditions x 3 genes x 2

CNI, CNI1, 18s RNA

12 samples.

C_t Cycles



Be very careful pipetting your samples today!