- Announcements
- Quiz
- Luciferase review
- Microarrays: analysis, workflow
- Today in Lab (Mod 2 Day 7)

Announcements

- Steve will run much of lab today
- TC room update
- Discuss mid-term course feedback

Luciferase review

- Per sample, measure
  - Firefly: internal fx'n control
  - *Renilla*: target
- 4-6 replicates per expt'l sample
- Up to 32 replicates for others
- Representing your data+analysis

Microarray Workflow

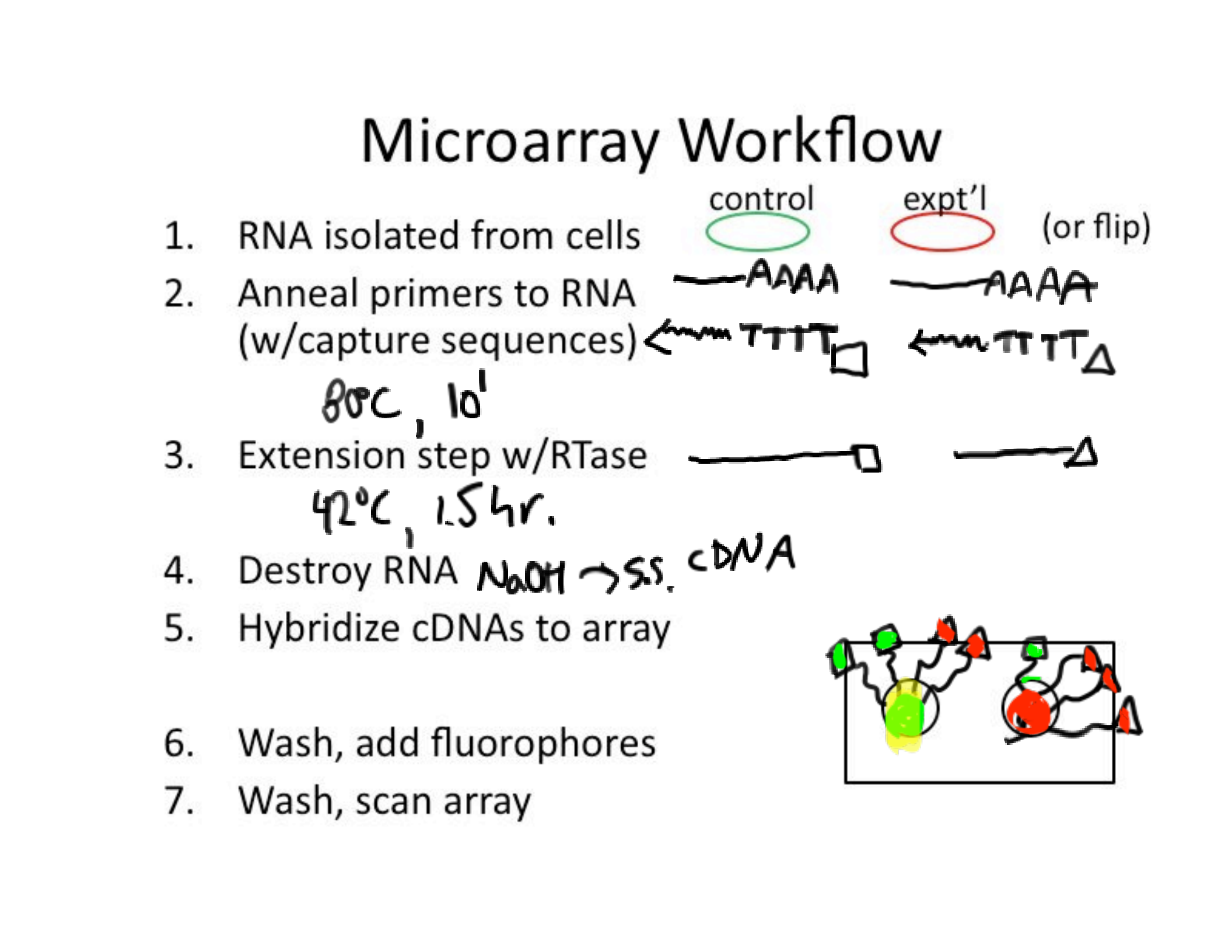
1. RNA isolated from cells
2. Anneal primers to RNA (w/capture sequences)

80°C, 10'

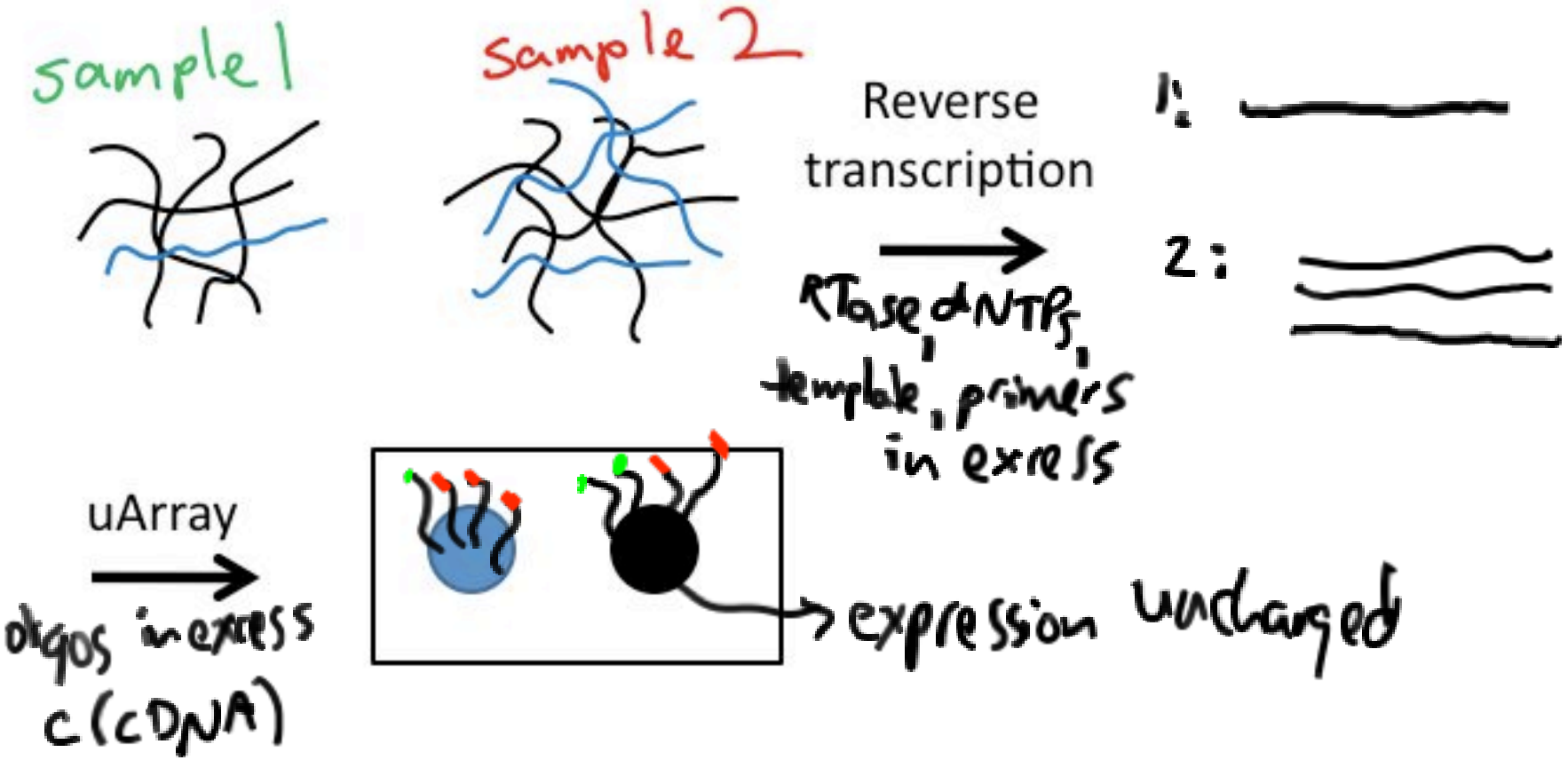
3. Extension step w/RTase

42°C, 1.5 hr.

4. Destroy RNA NaOH → ss. cDNA
5. Hybridize cDNAs to array
6. Wash, add fluorophores
7. Wash, scan array



Microarray Principle



Intensity of fluorescence at a particular spot is proportional to the original amount of the relevant mRNA in that sample. Next time you'll analyze 44,000 green and red intensity values!

Microarray Analysis

Analysis	What/why/how	Typical #'s
Background Subtraction	 $S_I = I - B_I$ make a cutoff $= IF(S_I > 0, S_I, 0)$ intensity b/w spots is noise	bkgnd 50-100 signal 10^2-10^5
Normalization	$R_I = k G_I$ C_y5: less stable but higher quantum yield of C_y3	$\angle 2$-fold
Ratio of GI:RI, Log2 Transform	→ change in expression → "readability"	~10 — +10 (-1 — +1)

Analysis examples

	Ex. 1	Ex. 2	Ex. 3
Green value	2000	200	160
Red value	1000	100	80
Background (assume green and red same)	80	80	80
Ratio w/out bkgnd subtraction	2	2	2
Ratio with subtraction	2.1	6	undefined

Today in Lab

- Set up RT-PCR
  - With RNase-free technique!
  - If possible, use 1-5 µg of RNA
  - **Save rest of your RNA in case others need it**
- Meanwhile practice uArray analysis
- Hybridize cDNA to uArray slides
  - Watch demo first, be careful with slides