Laser-capture microdissection and poly(A) amplification

Learning objectives

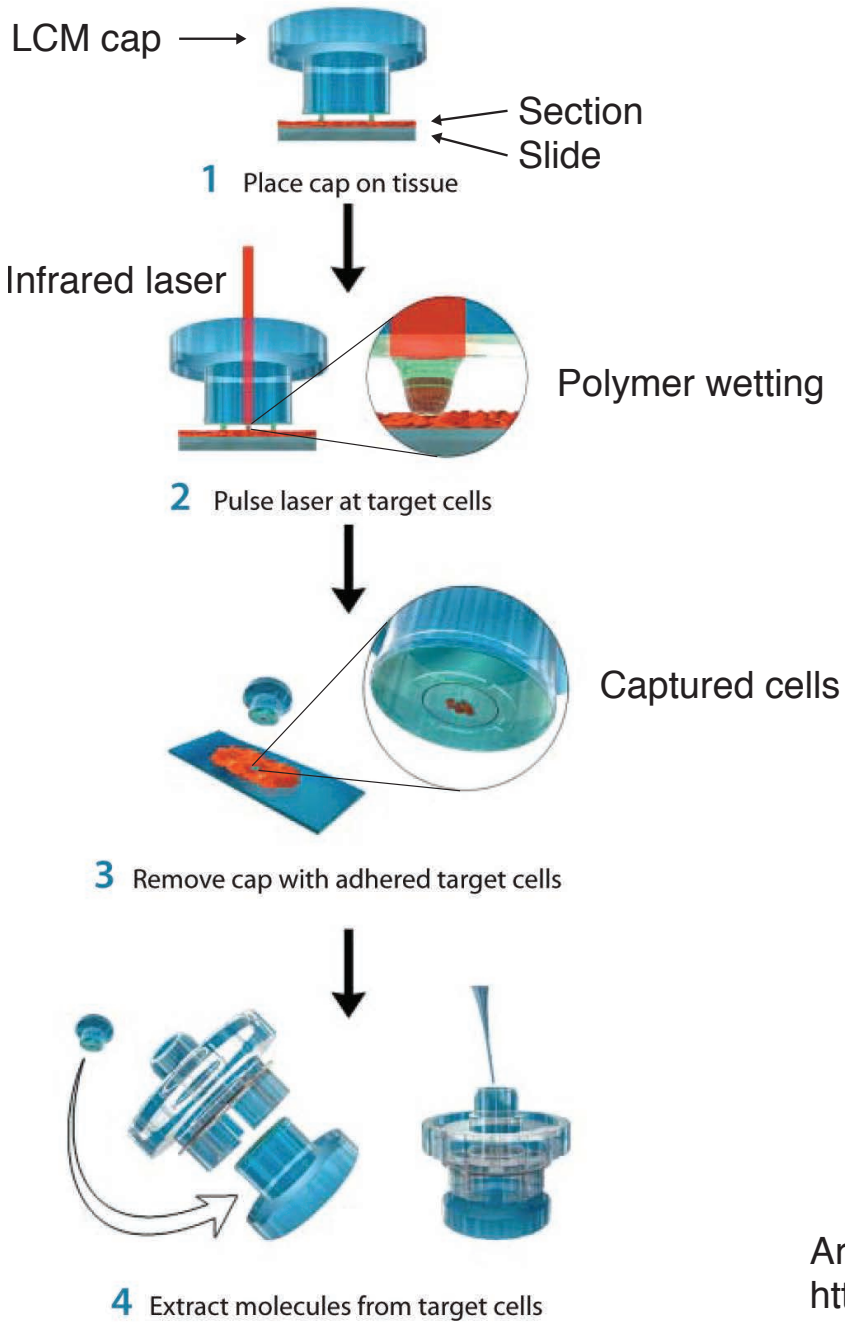
- Understand the distinctions between different LCM formats and the considerations for effective LCM

- Appreciate the rationale and critical steps for poly(A) amplification of microdissected material

- Implement a pilot poly(A) optimization for a new biological context

Types of laser capture

- UV laser cutting (Leica, Arcturus):
  - Fantastic accuracy and precision but RNA destroyed at the edge
  - Requires special slides
- IR polymer wetting (Arcturus):
  - Requires extensive optimization for accuracy
  - Precision is local to the slide-cap placement
  - Very mild toward biomolecules
  - Requires special caps for pickup

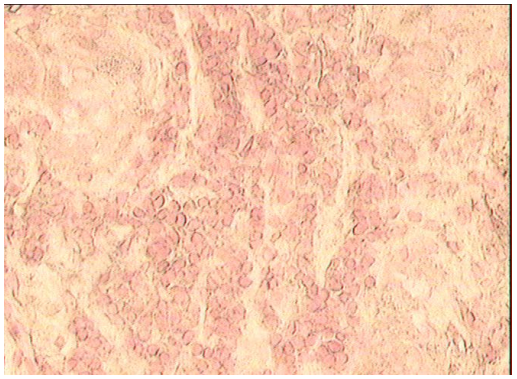


Arcturus Bioscience
http://www.arctur.com

Successes

Melanoma TILs

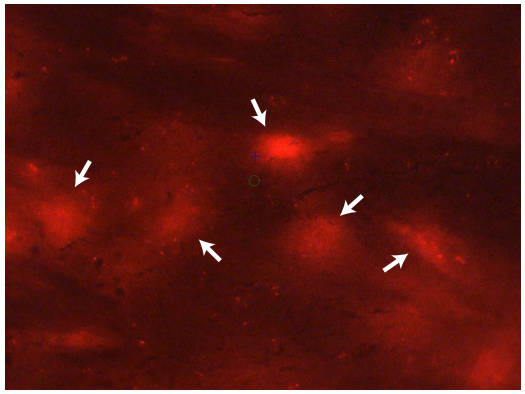
Before LCM



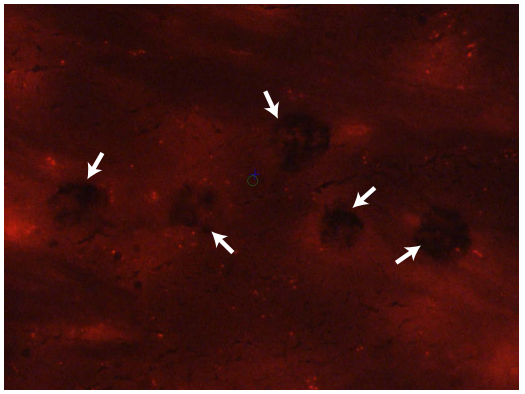
After LCM

Mouse OPCs

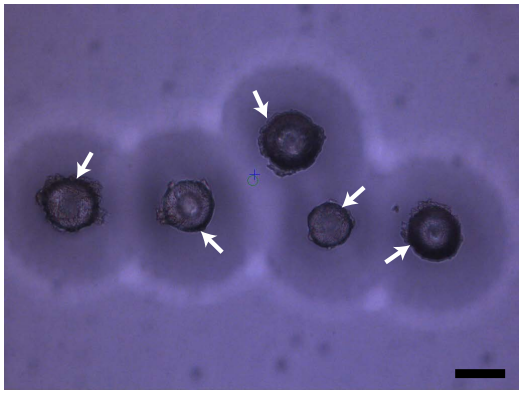
Before LCM



After LCM

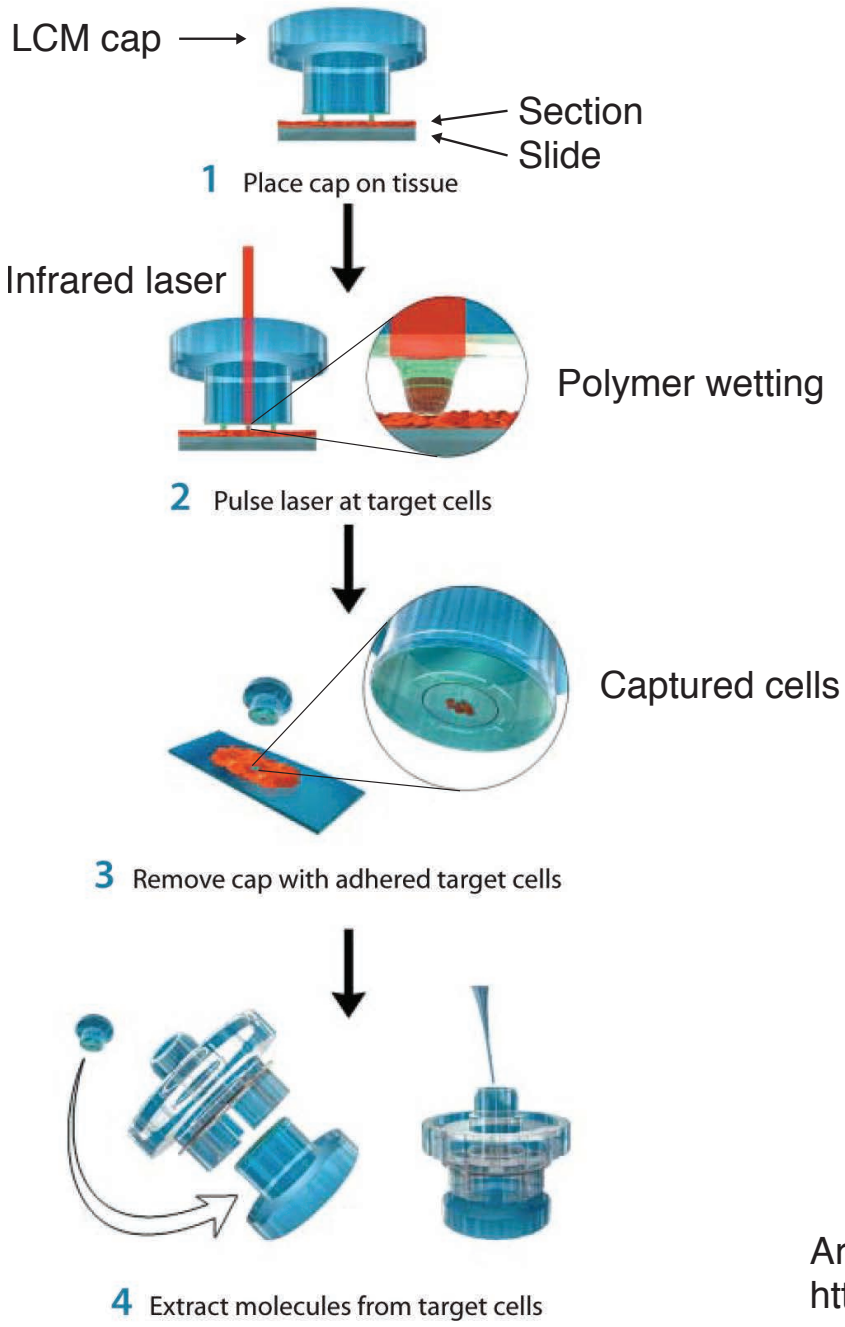


LCM cap



Key considerations for LCM

- (New) Cryosectioning at cold temperatures (≤20ºC and 24ºC if possible) to achieve opaque NEG50 on the slides
- Optimized dehydration of the sample
  - Too wet: no pickup
  - Too dry: excessive collateral pickup
- Laser parameters (voltage, duration) that determine the effective spot size



Watch for leaks!
Practice on an old cap

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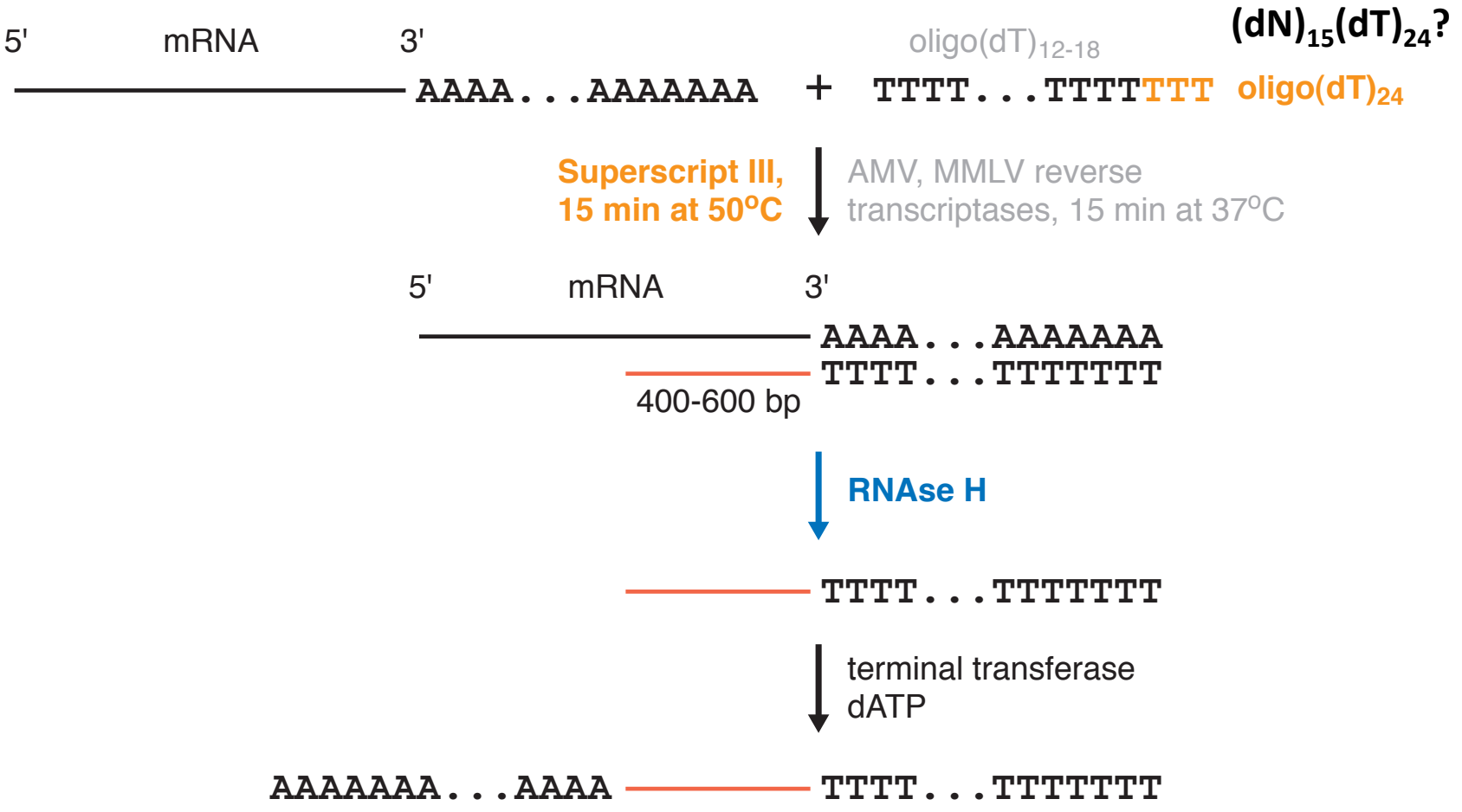
RNA elution from LCM cap

- 1 hr digestion with proteinase K at 42ºC
  - Frees mRNA from fixed polysomes
  - Degrades RNAses
- Centrifuge into large PCR tubes
- Stop digestion with high concentration of PMSF and supplement with RNAse inhibitors
  - Excess PMSF self-inactivates by hydrolysis
  - Other serine protease inhibitors will NOT substitute

Single-cell cDNA amplification

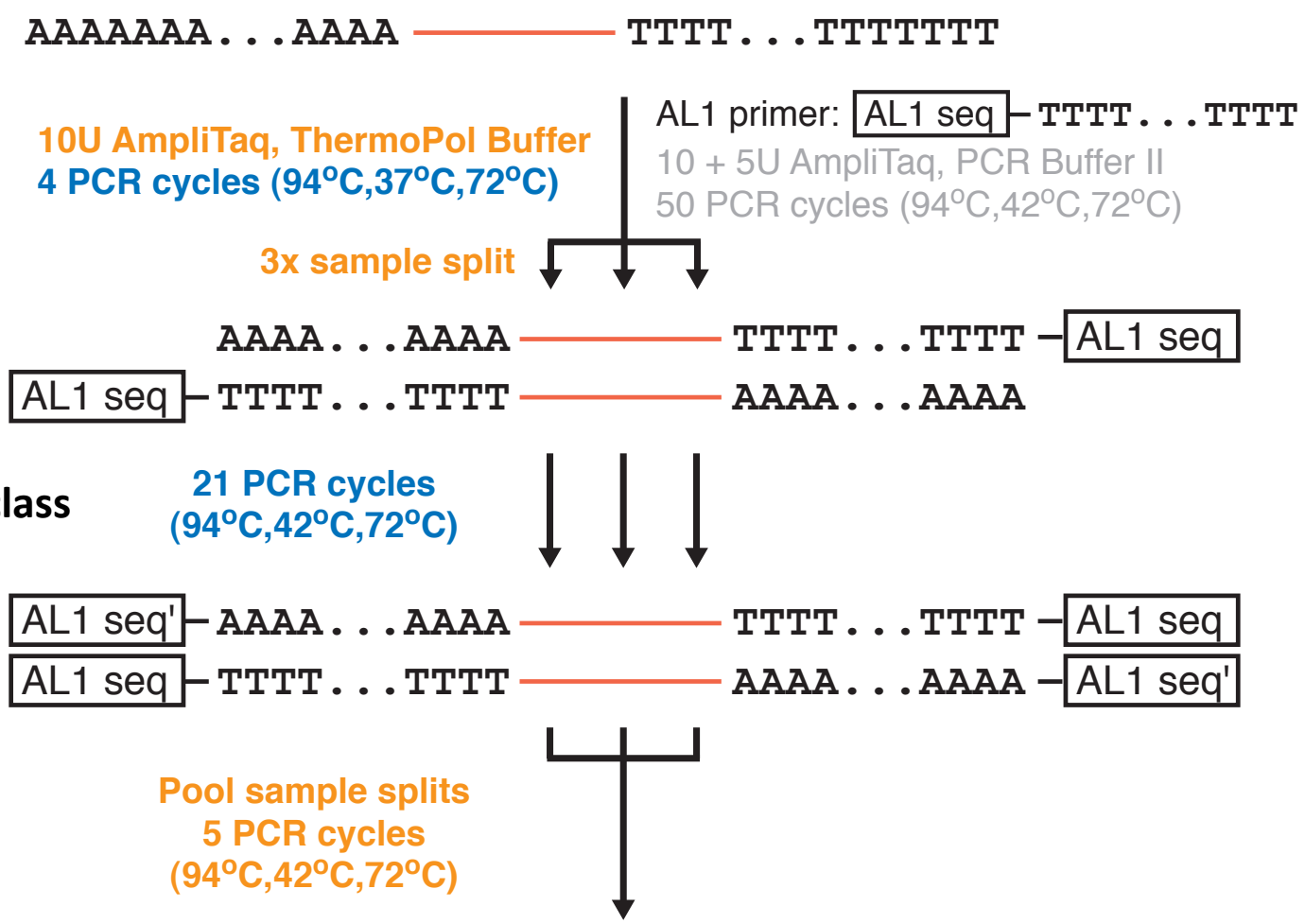
In-house modifications (part 1)



Replaced
Minor improvements
Major improvements

Single-cell cDNA amplification

In-house modifications (part 2)



LCM time for class

Replaced

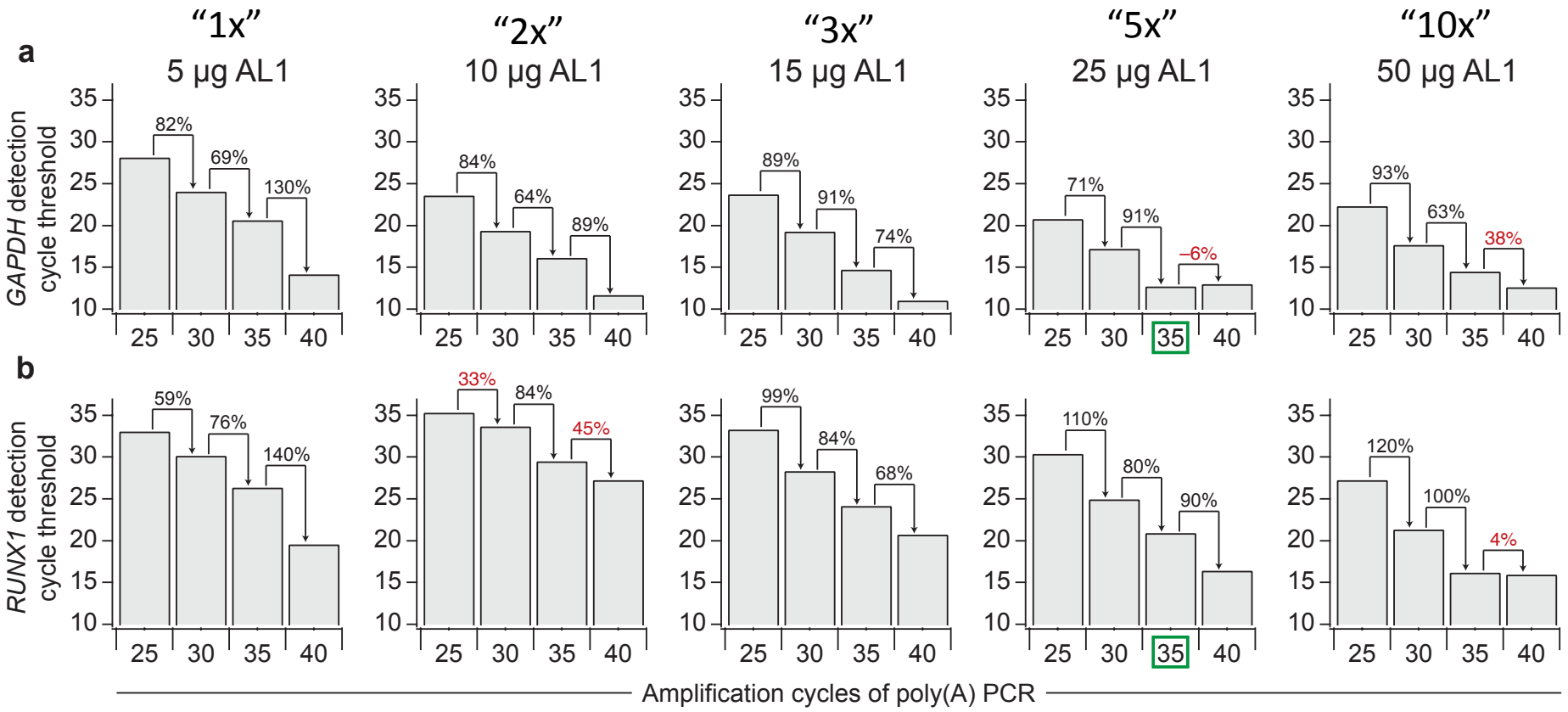
Minor improvements

Major improvements

Optimizing poly(A) PCR for a biological context

- Two key amplification parameters: number of amplification cycles and amount of AL1 primer
- Number of amplification cycles:
  - Yields more poly(A) cDNA with each cycle
  - Efficiency declines to zero when saturated
  - Saturated amplifications are not quantitative
- Amount of AL1 primer:
  - Promotes amplification efficiency
  - May cause high-abundant targets to saturate early

An example of poly(A) optimization



qPCR of poly(A) samples will be introduced tomorrow (Chun-Chao)

Questions?