Poly(A) reamplification and labeling
Learning objectives

• Understand the chemistry and considerations of reamplification and fluorescence labeling

• Appreciate the importance of maintaining quantitative accuracy in poly(A) materials

• Perform a pilot reamplification and a representative labeling dye-coupling run
Single-cell cDNA reamplification
In-house modifications (part 3)

3.5 U High Fidelity polymerase
10–15 PCR cycles (94°C, 42°C, 72°C)

Different polymerase

Aminoallyl-dUTP: U–C=C–CH₂NH₂

Alexa Fluor 555 succinimidyl ester

Replaced
Minor improvements
Major improvements
Pilot reamplification to determine linearity of amplification

![Graph showing relative fluorescence over cycle number for different reamplifications.](image-url)
Tricks to stoichiometric purification

- Use Invitrogen PureLink columns
- Add NaOAc to lower pH after labeling to improve binding
- Heat the elution buffer to 65°C and elute in two steps
- Careful EtOH precipitations (+ glycogen) and thorough resuspensions of the cDNA pellet
Example labeling of poly(A) cDNA

A_{260} = 0.507

A_{555} = 0.168

DOL = 1.5

Instructions for microarray core

- Provide 1 µg of labeled poly(A) cDNA (effectively 500 ng of useful material)
- Denature at 95ºC and then add the sample warmed to 58ºC to the Illumina BeadChip
- Omit the “Block”, “Detect”, and “3rd Room-Temp Wash” from the Illumina protocol
  - Preps chips for adding streptavidin-Cy3
- Scan like any other Illumina BeadChip
Questions?