Poly(A) reamplification and purification for RNA-seq

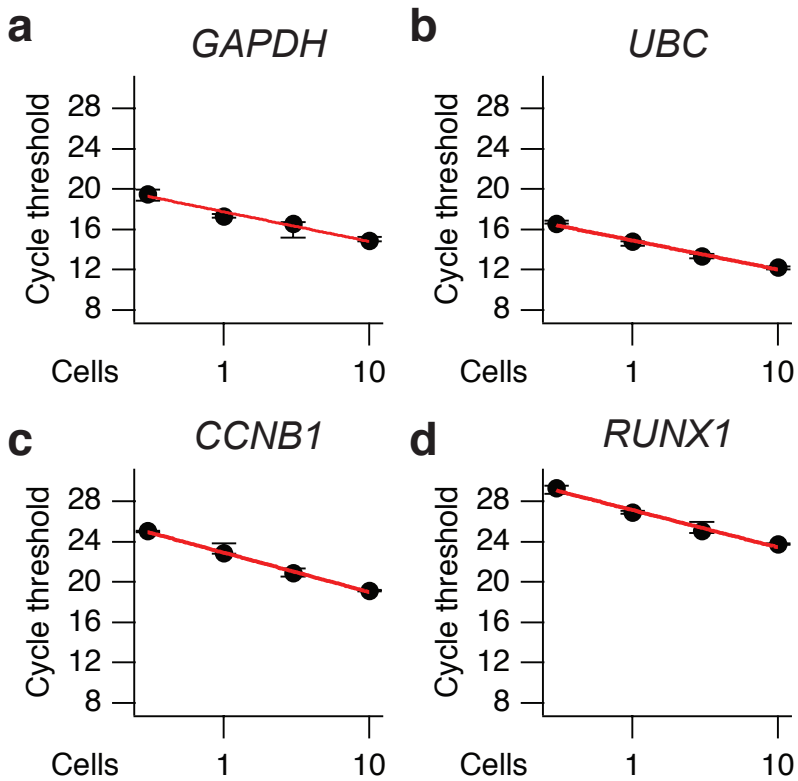
Learning objectives

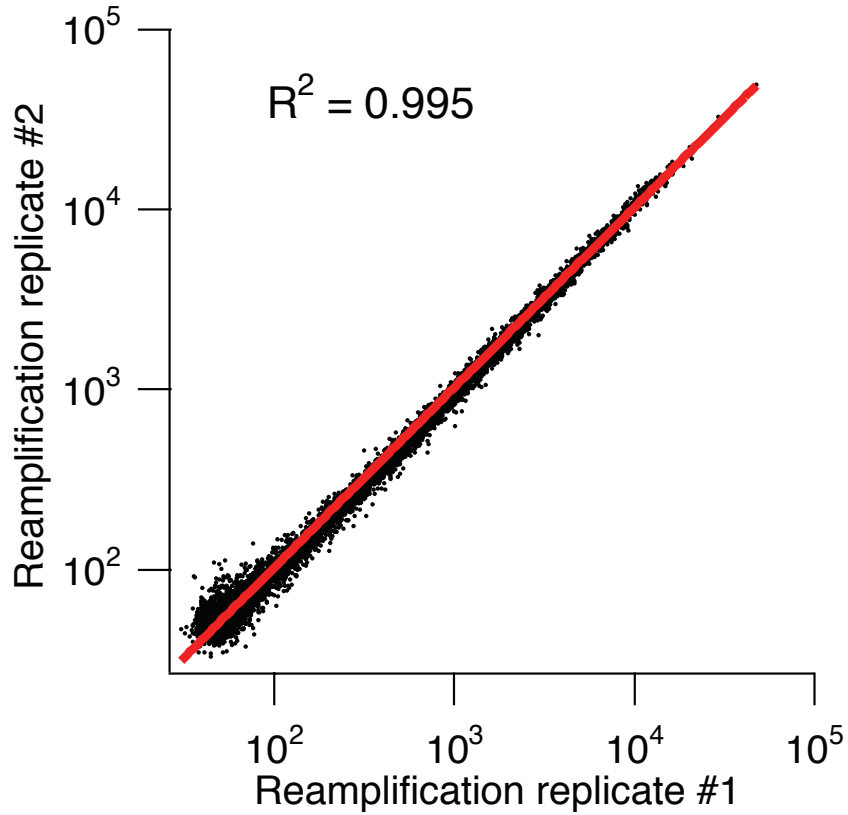
- Understand the chemistry and considerations of reamplification, purification, and tagmentation
- Appreciate the importance of maintaining quantitative accuracy in poly(A) materials
- Perform a pilot reamplification and a representative AMPure purification

Why reamplify?

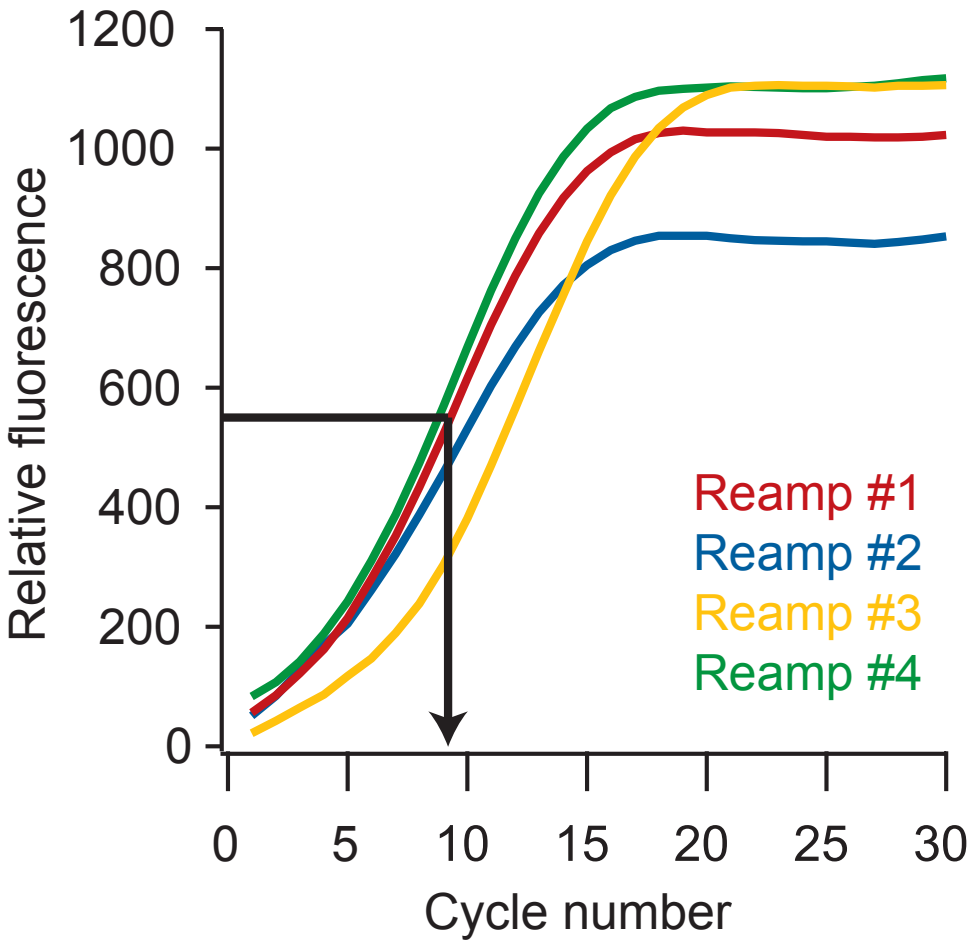
- Preserve stock poly(A) material to reuse at a later date (qPCR, resequencing, etc.)
- To generate enough starting material for microarrays (1 μg) or sequencing (1 ng)
- To dilute out high concentrations of AL1 primer that may interfere with downstream analyses

Reamplification can be quantitatively accurate and highly reproducible

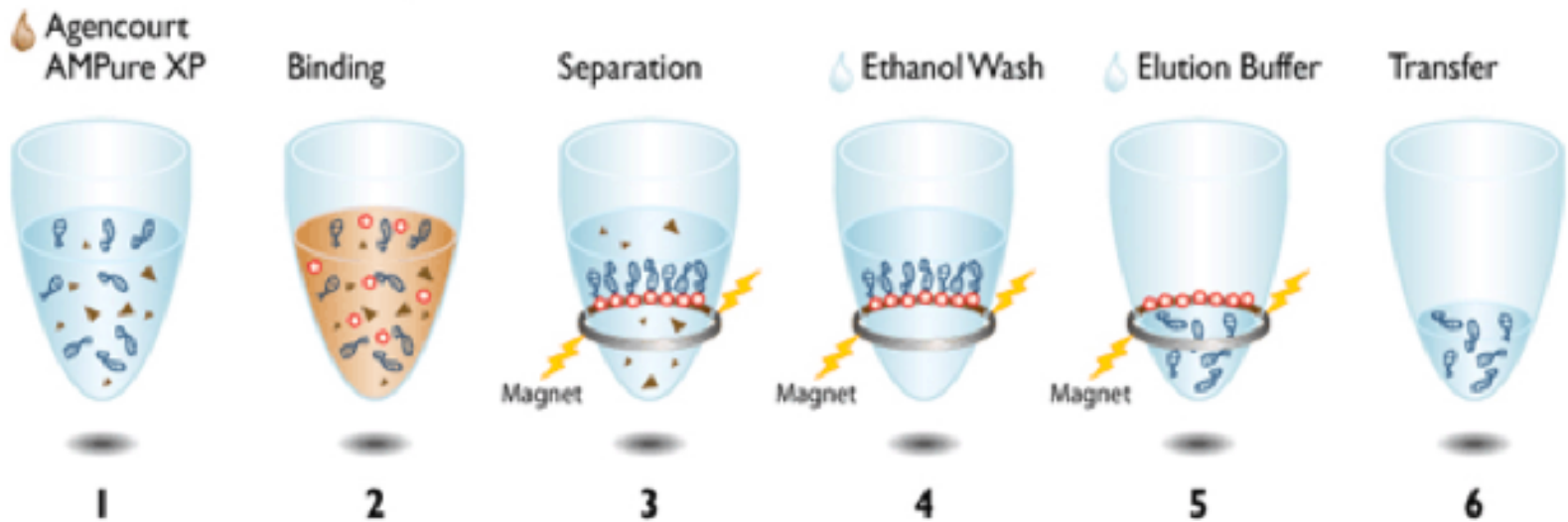


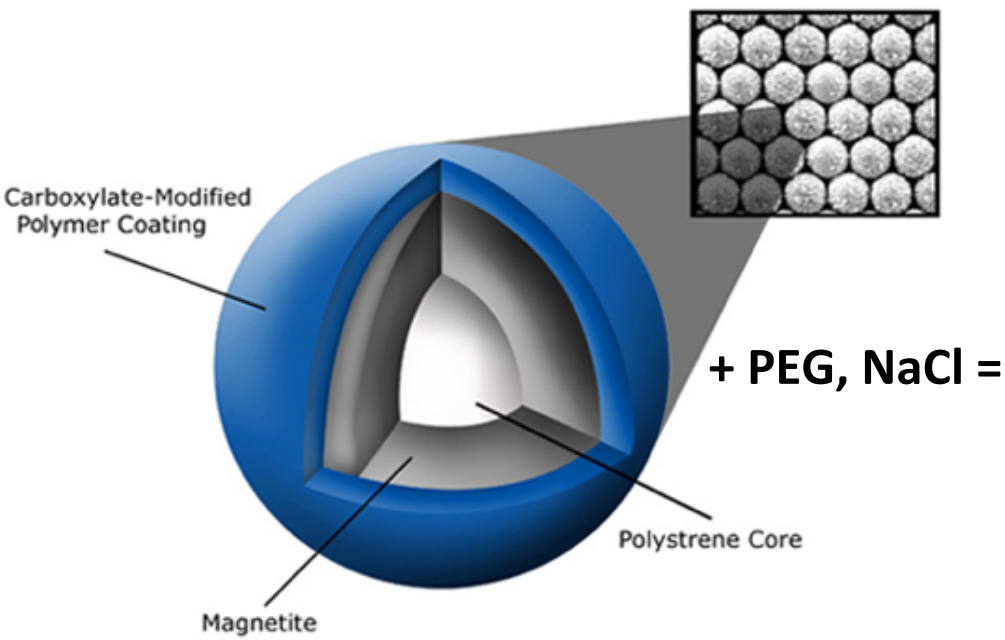


Pilot reamplification to determine linearity of amplification

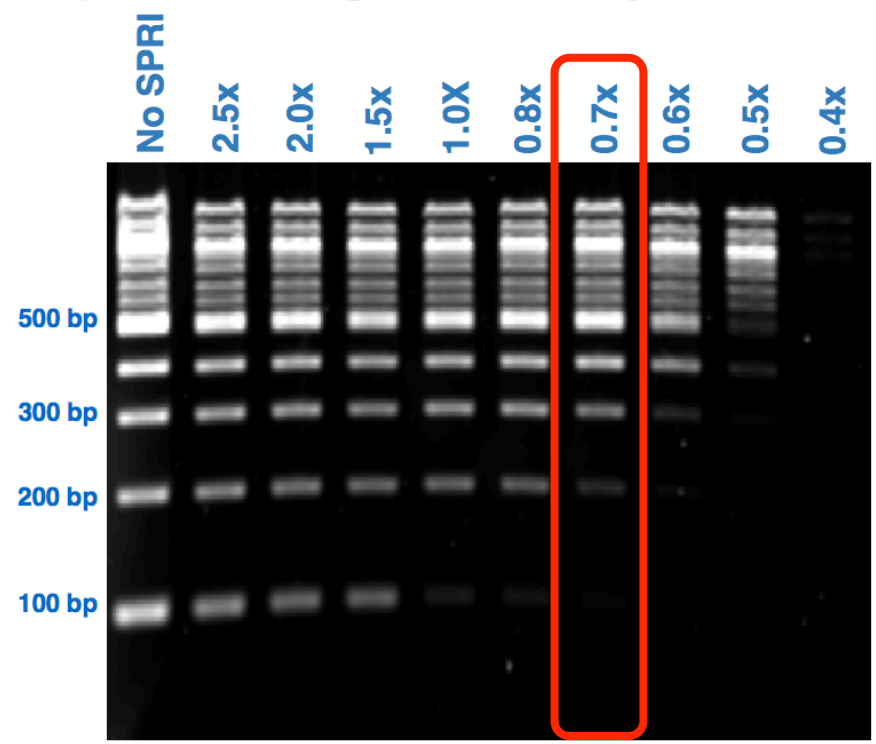


High-efficiency purification with AMPure SPRI beads

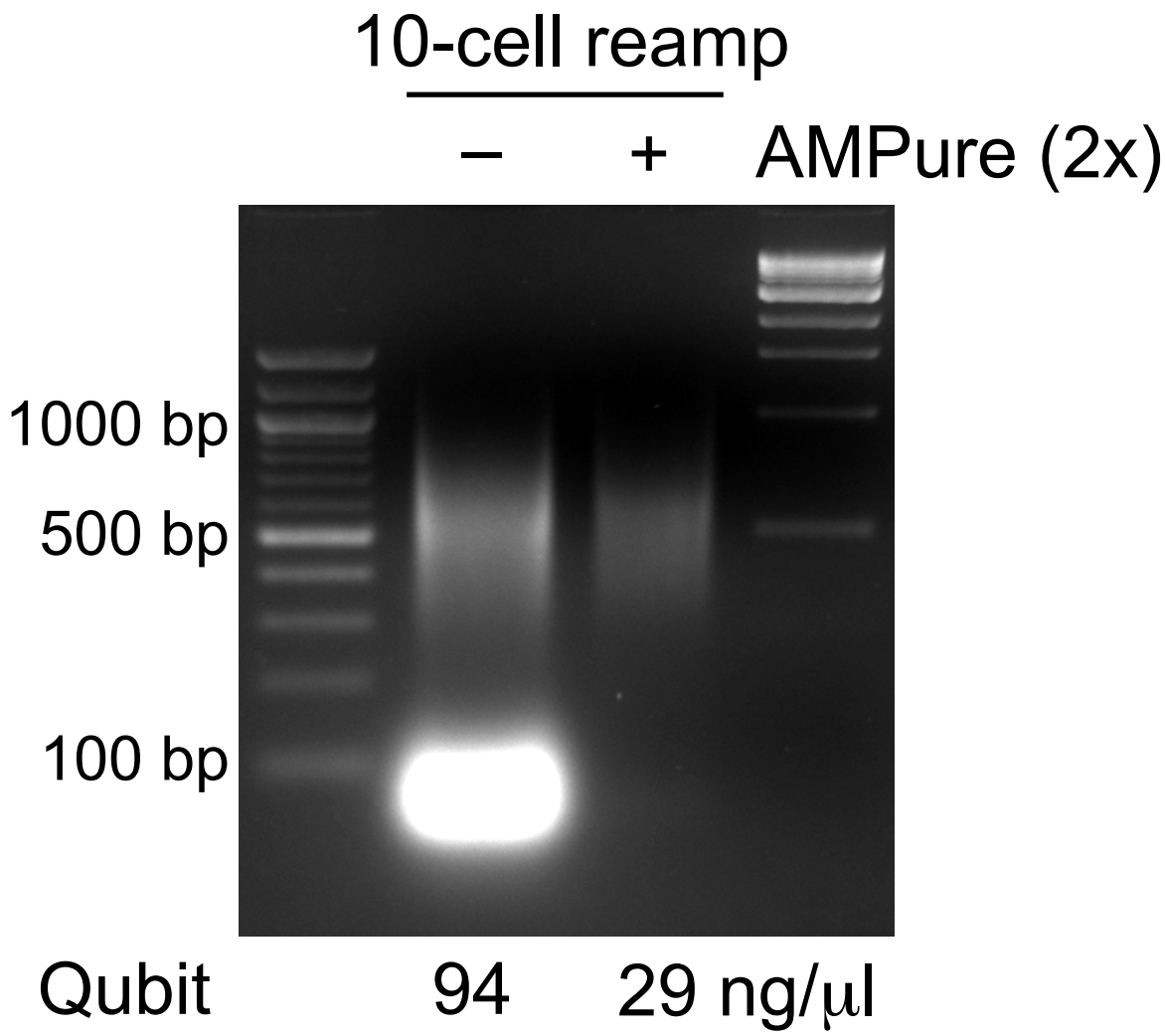




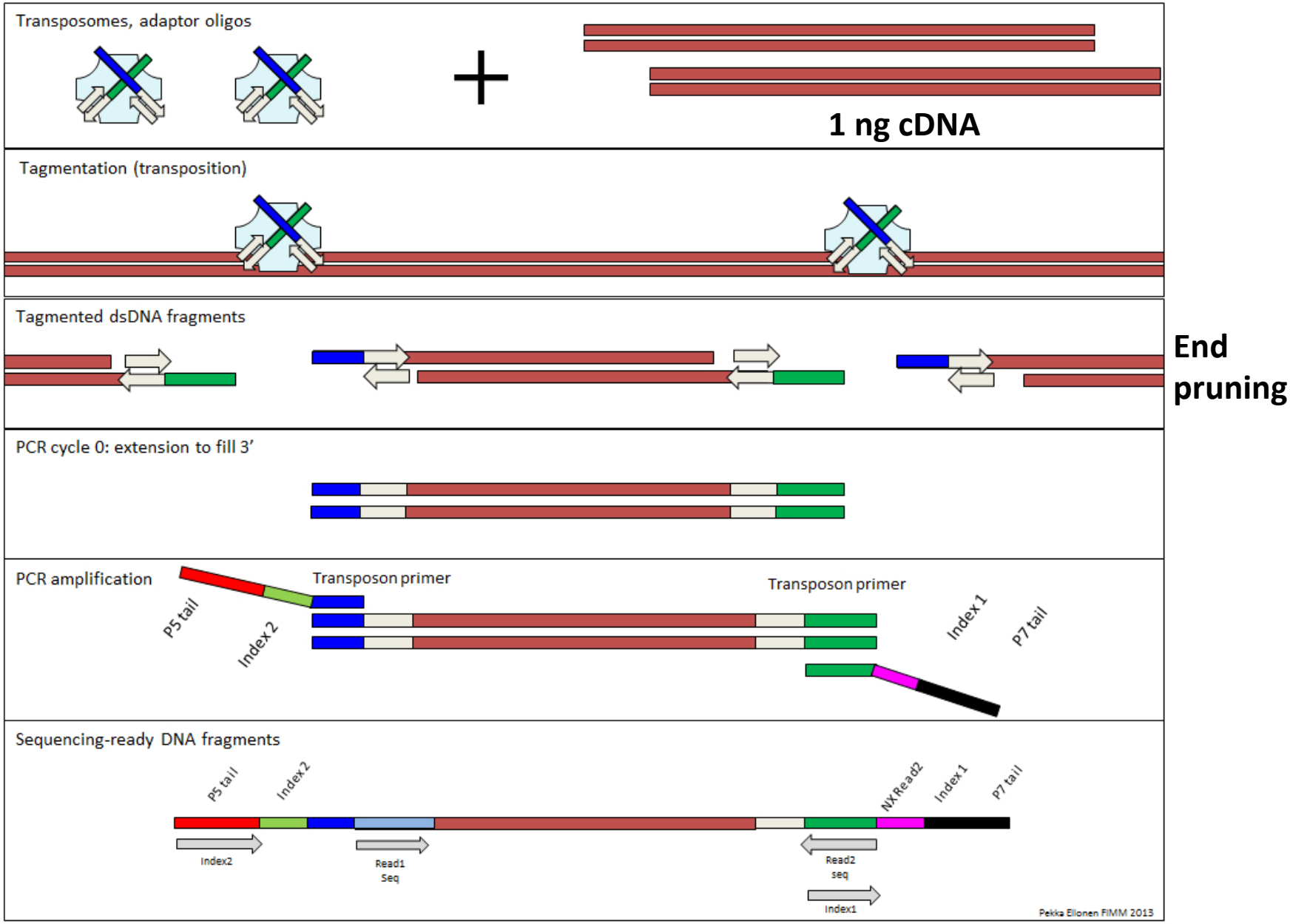
+ PEG, NaCl =



Expected results from today



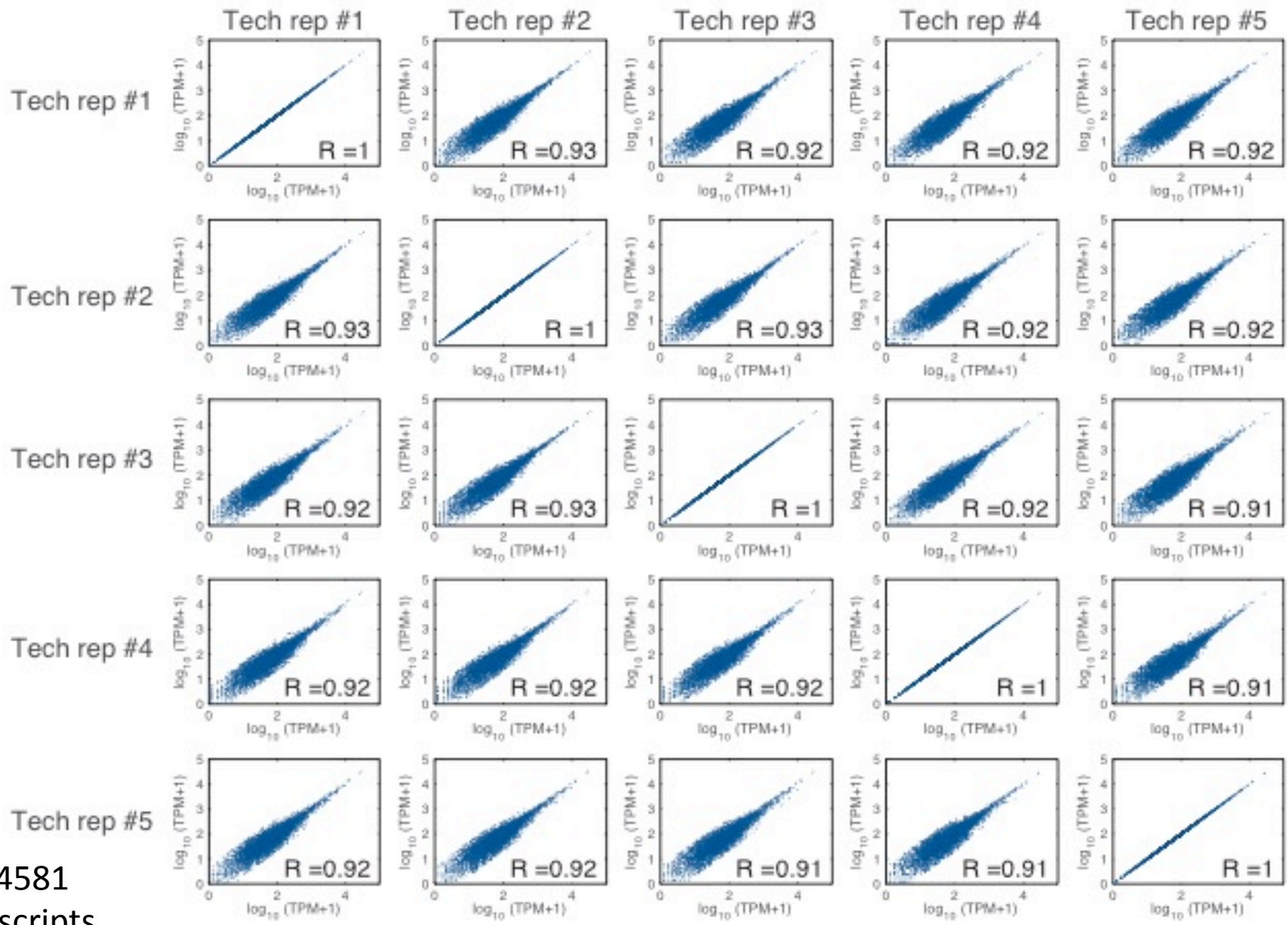
Tagmentation by Nextera XT



Sequencing calculations for NextSeq 500

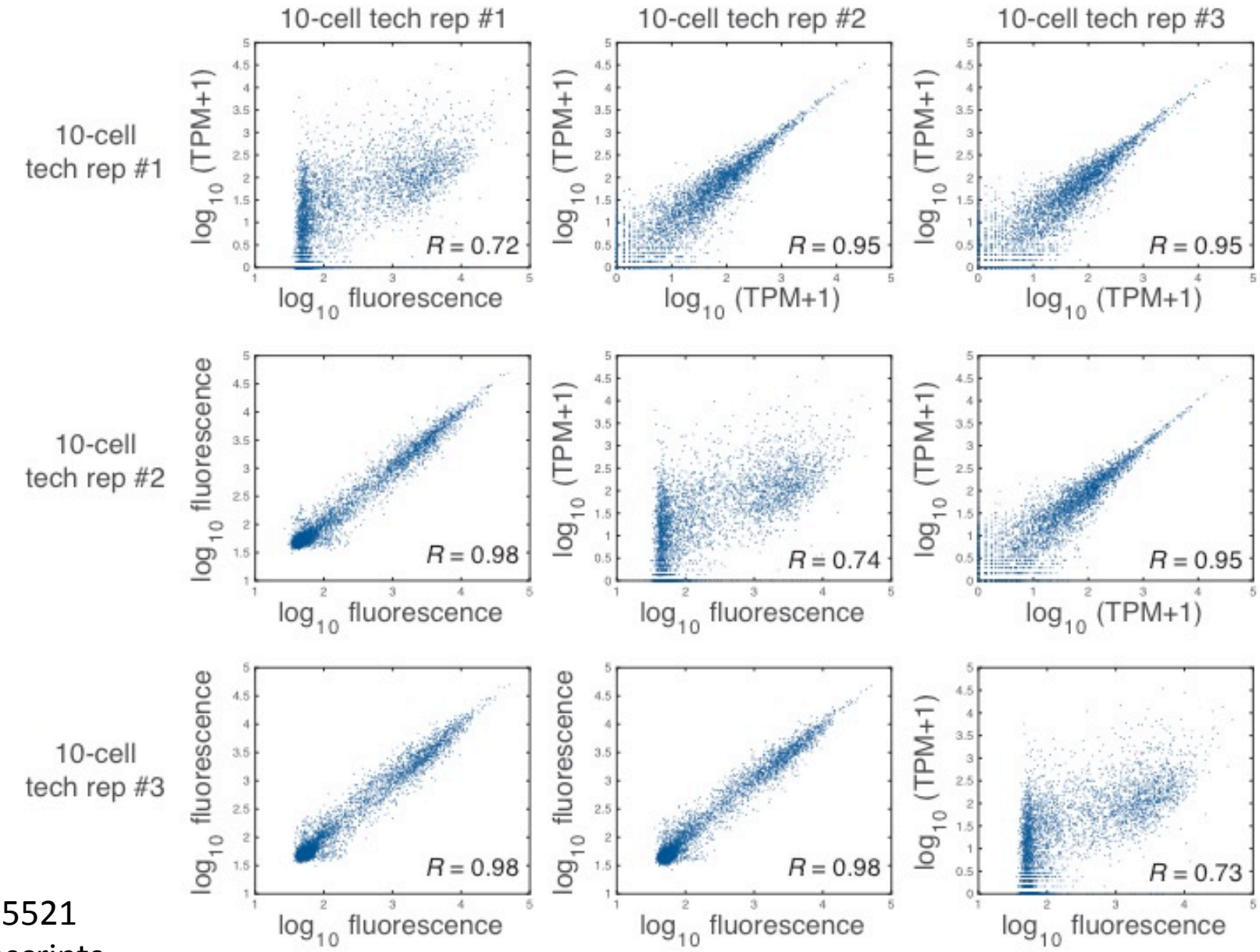
- 180M reads / 24 samples = 7.5M reads per sample (in practice 5 ± 1.9M reads per samples)
  - Mid-output cartridge (150 cycles, 75 bp pe): $1000
  - Nextera XT kit (24 samples): $775
  - Nextera XT library kit (24 indices for 96 samples): $250 / 4
  - TrueSeq dual indexing pe sequencing primers: $95
  - $1932.50 for 24 samples = $80.52 per sample
- 400M reads / 96 samples = 4.2M reads per sample
  - High-output cartridge (150 cycles, 75 bp pe): $2575
  - Nextera XT kit (96 samples): $2900
  - Nextera XT library kit (96 indices for 384 samples): $950 / 4
  - TrueSeq dual indexing pe sequencing primers: $95
  - $5807.50 for 96 samples = $60.50 per sample

Technical reproducibility



N = 4581 transcripts

Platform reproducibility



N = 5521 transcripts

Questions?