AccuGenomics

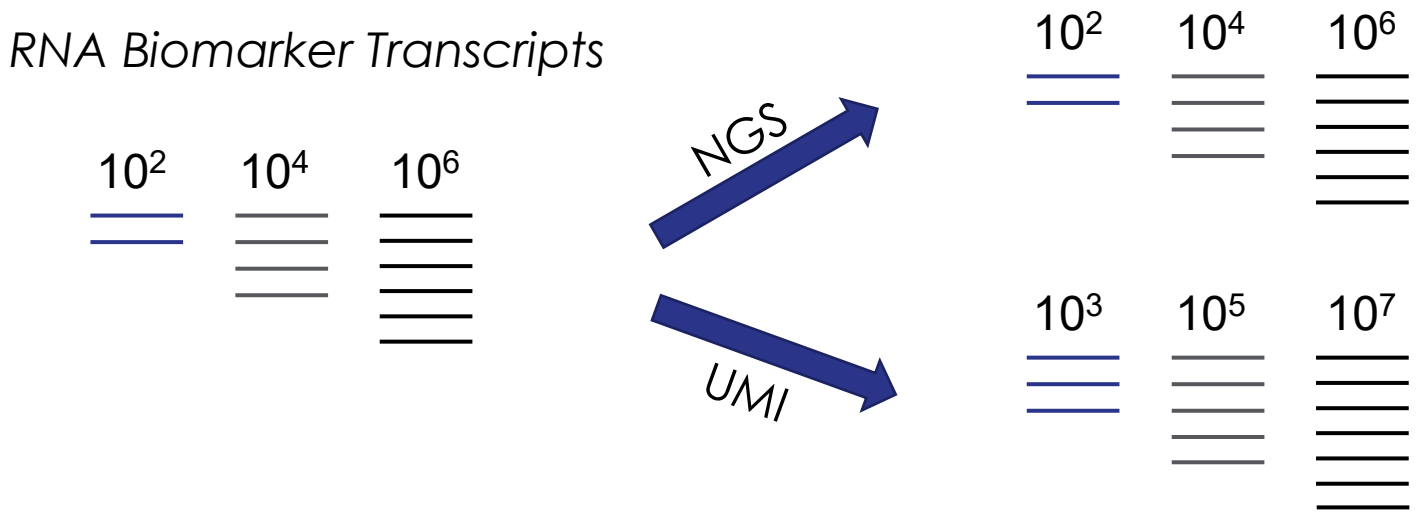
A higher standard of accuracy

SNAQ-SEQ AND RNA TRANSCRIPT ABUNDANCE

Method

Tom Morrison, CSO, AccuGenomics

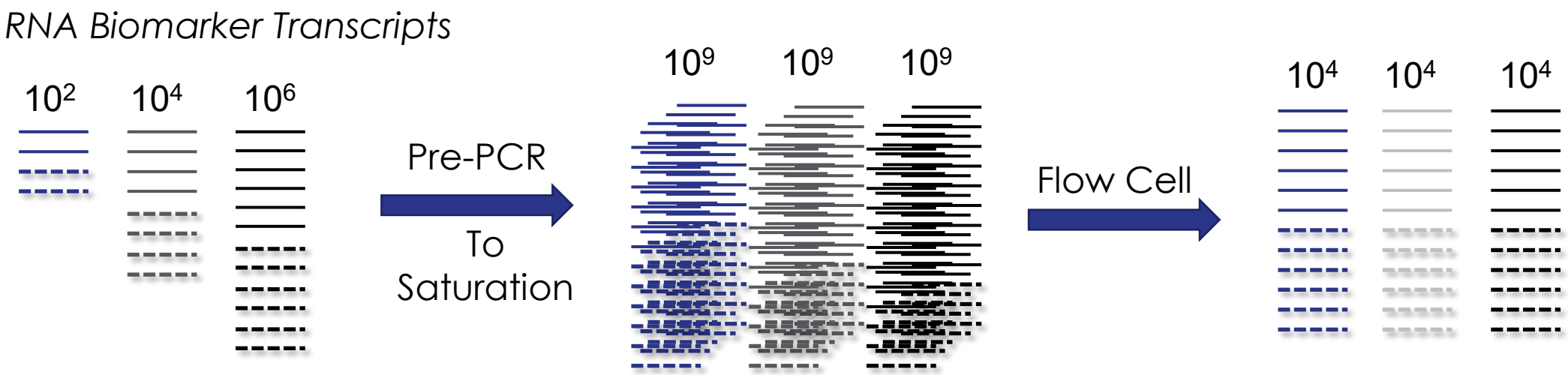
NGS BIAS AND SAMPLE READ DEPTH



- **Traditional transcript NGS measurement is like digital PCR as it counts target reads.**
  - **NGS example above requires 1,010,100 reads.**
- **Measurement accuracy of expression level affected by yield biases between targets**
  - **Use of Unique Molecular Tags can reduce bias but increase read depth >8-fold**
- **SNAQ-SEQ will reduce read depth requirements while eliminating yield bias**

AccuGenomics

ELIMINATE IMPACT OF HIGH AND LOW TRANSCRIPT LEVELS



- **SNAQ-SEQ uses ratio between native template (solid lines) and internal standard (dashed lines) to calculate target abundance.**
  - **Controls and target level covary, so their molar ratio is maintained from sample through sequence reads.**
- **SNAQ-SEQ**
  - **First PCR step is 'over-cycled' to drive all the products to same level**
  - **NGS flow cell loaded to support 10^4 reads per target.**
  - **Target abundance is calculated from the ratio of target and control reads.**
- **In this example, 30,000 reads required to measure the three targets (traditional NGS required >10^6 reads)**
- **SNAQ-SEQ will increase throughput (# samples per flow cell) while ensuring accurate measurements**

AccuGenomics

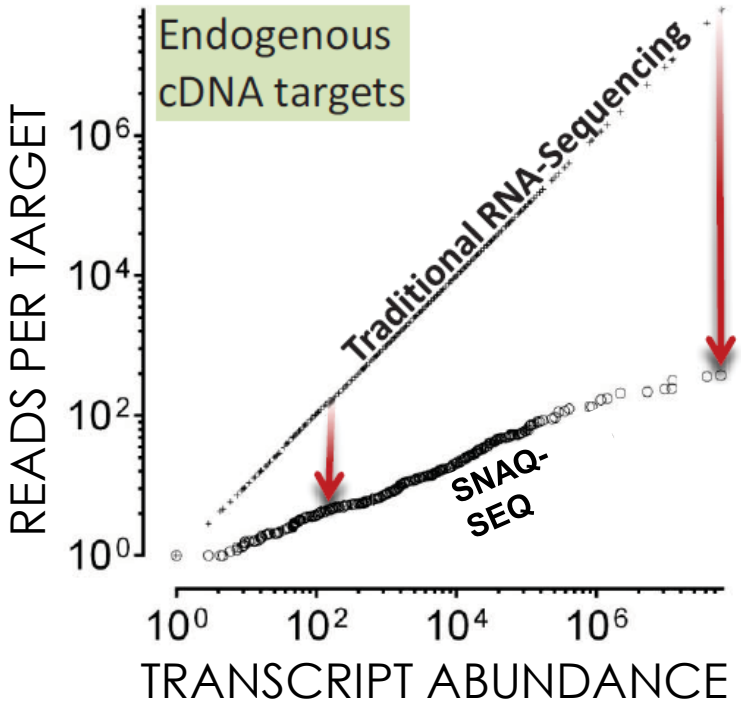
QUANTIFICATION BY SNAQ-SEQ RATIO PROVIDES LARGER DYNAMIC RANGE

NT	IS	NT/IS	[NT]
100	9900	0.010	101
200	9800	0.020	204
400	9600	0.042	417
800	9200	0.087	870
1600	8400	0.19	1905
3200	6800	0.47	4706
6800	3200	2.1	21250
8400	1600	5.3	52500
9200	800	12	115000
9600	400	24	240000
9800	200	49	490000
9900	100	99	990000

NT Native sequence reads(target)
IS Internal standard reads
NT/IS ratio created from IS & NT reads
[NT] = NT/IS * [IS spike-in level]
IS spike-in level = 10,000

- The ratio between target and control is maintained during library prep and detection.
- Driving all targets to saturation in first library prep PCR eliminates need for high read depths.
- Example: 10^4 reads per target
  - Traditional NGS of 100 to 10^4 reads supports a 100-fold reporting range.
  - SNAQ-SEQ ratio approach supports a 9800-fold reporting range, approaching dPCR-like ranges

SNAQ-SEQ INCREASES SAMPLE THROUGHPUT

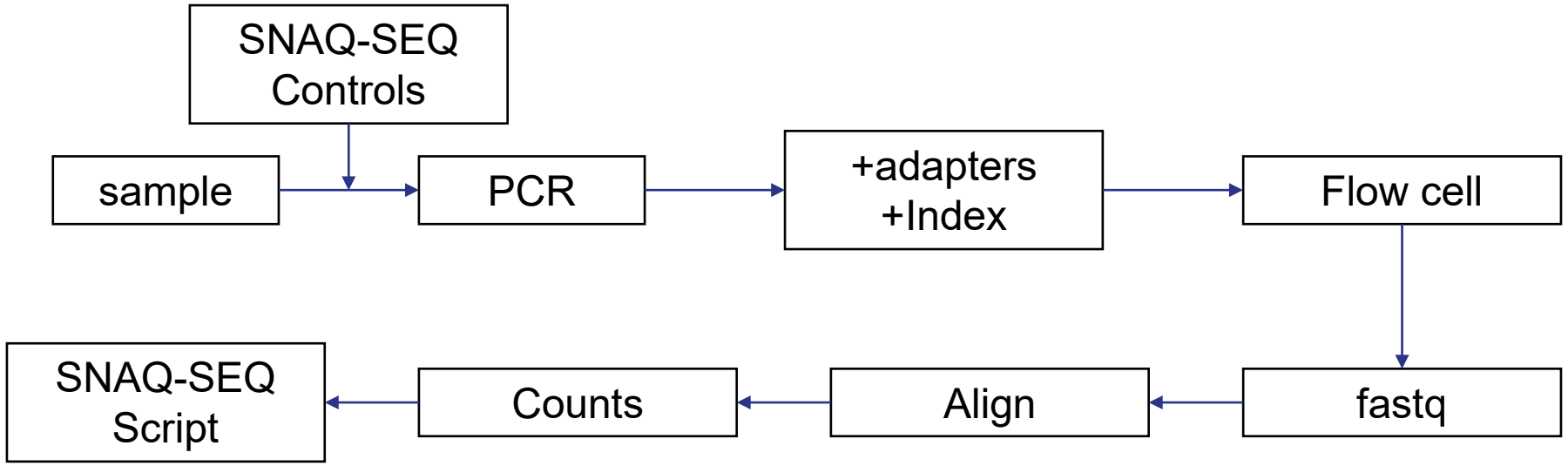


- **SNAQ-SEQ ratio measurements eliminate the existing requirement for abundance reporting range based on total count.**
- **Targets separated over 5 log concentration will be accurately measured with a 500-fold reduction in coverage**

PLoS ONE (2013) 8(11)

AccuGenomics

PROPOSED TESTING PIPELINE



- SNAQ-SEQ controls could be RNA or (c)DNA
  - RNA would allow control for RT step (if a concern)
  - DNA would allow for control of most of the variable testing steps
- Will require some custom integration into customized library prep and bioinformatic analysis. (e.g. Ampliseq RNA Seq)