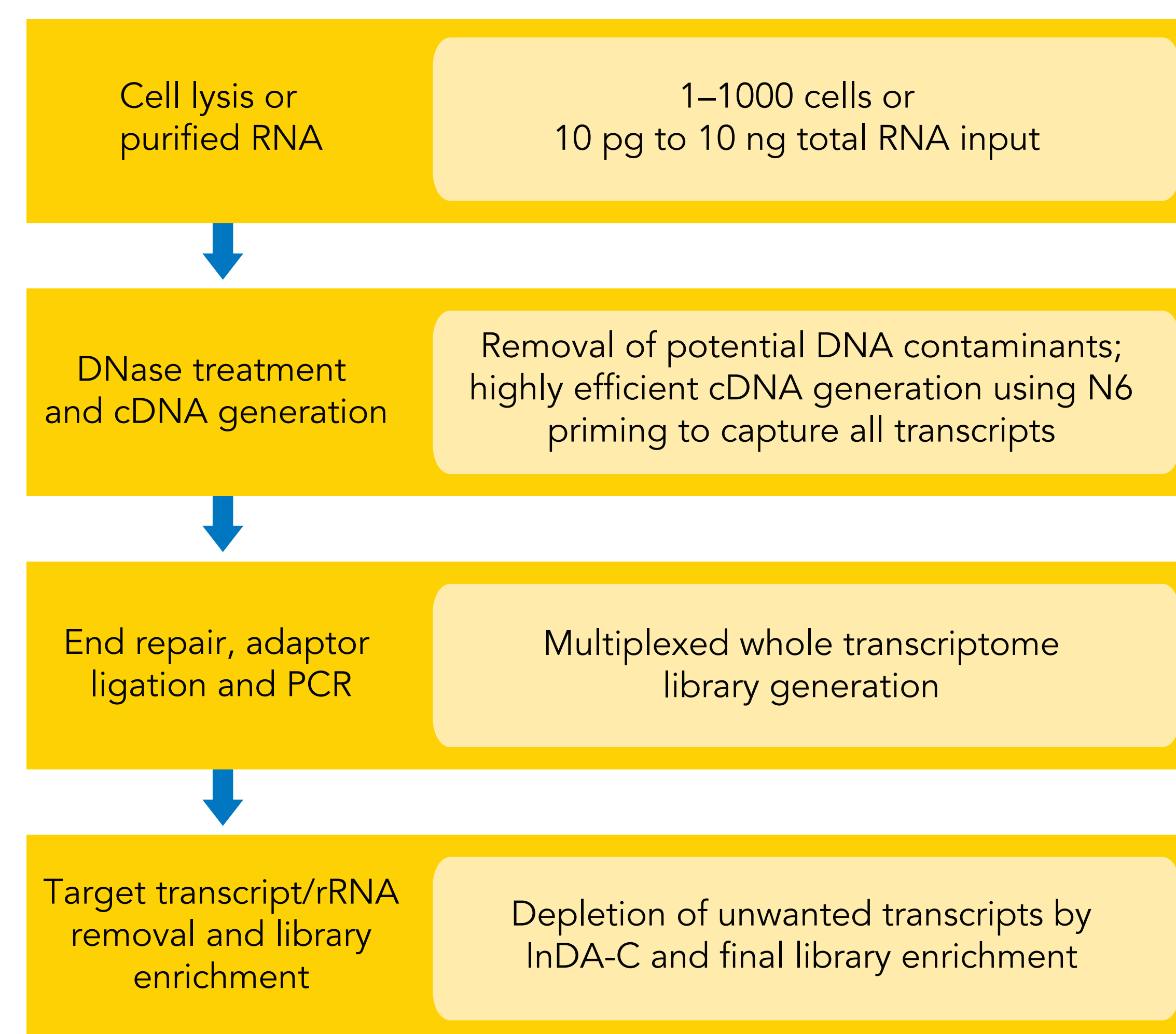
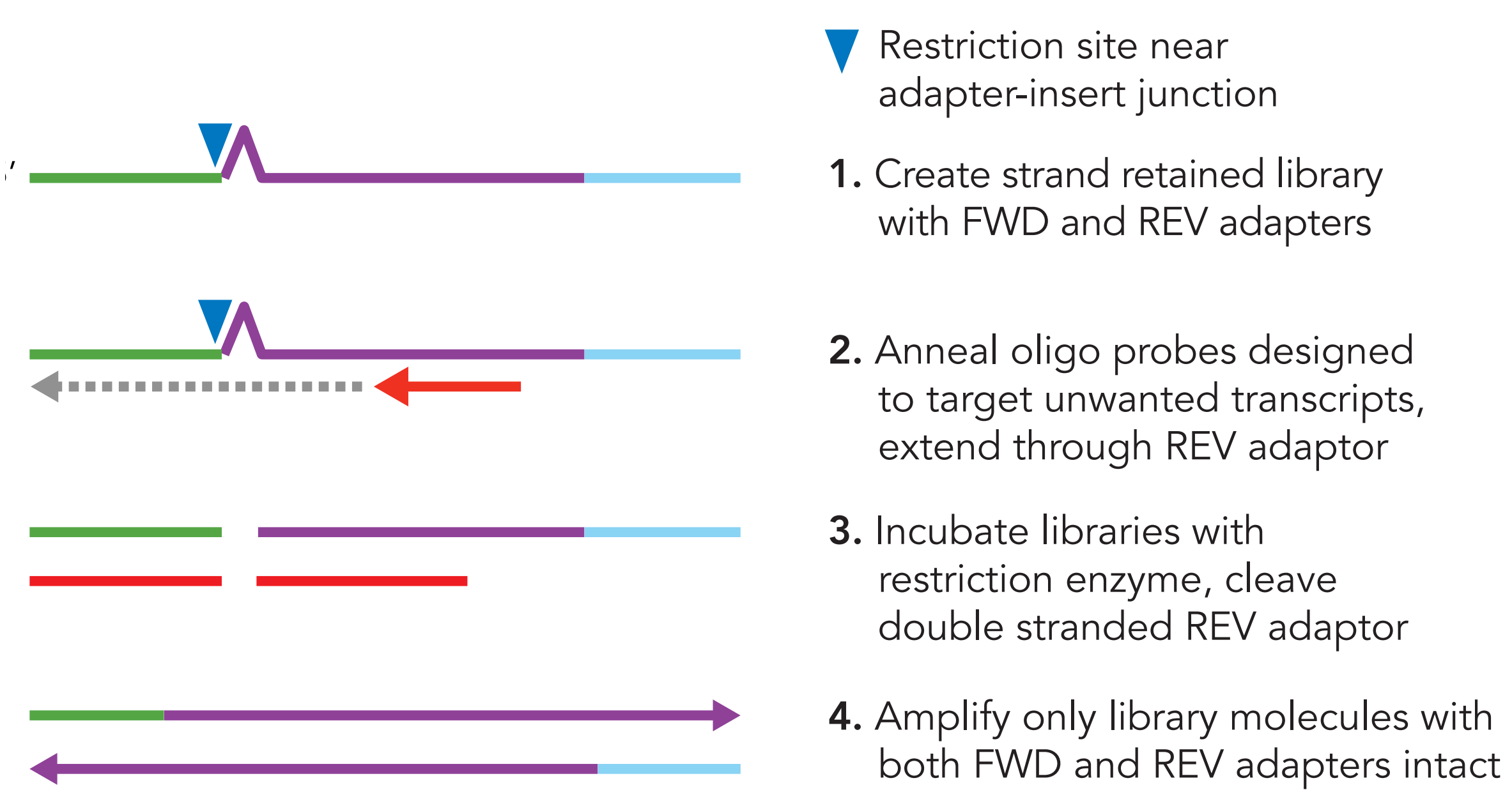
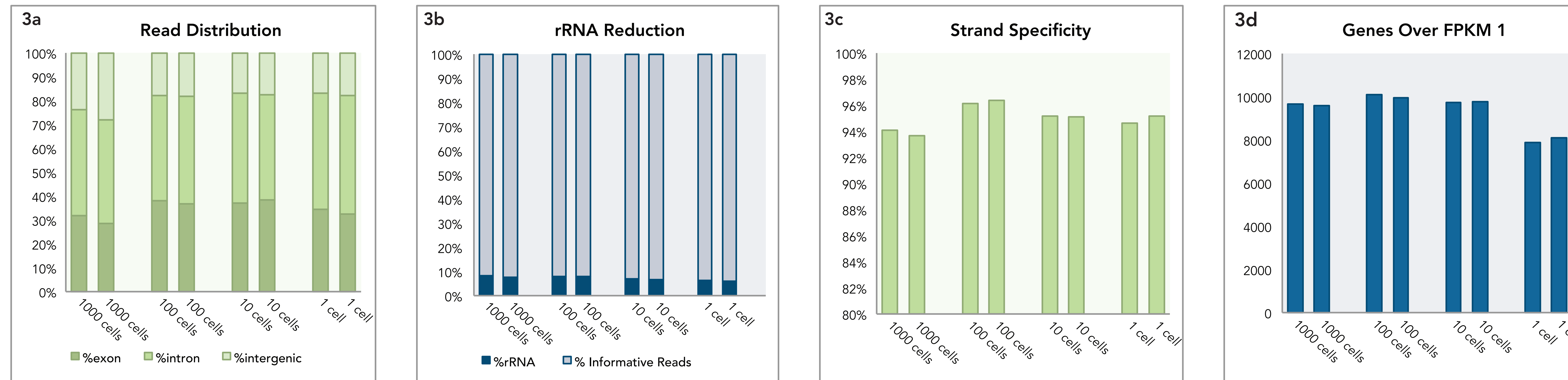
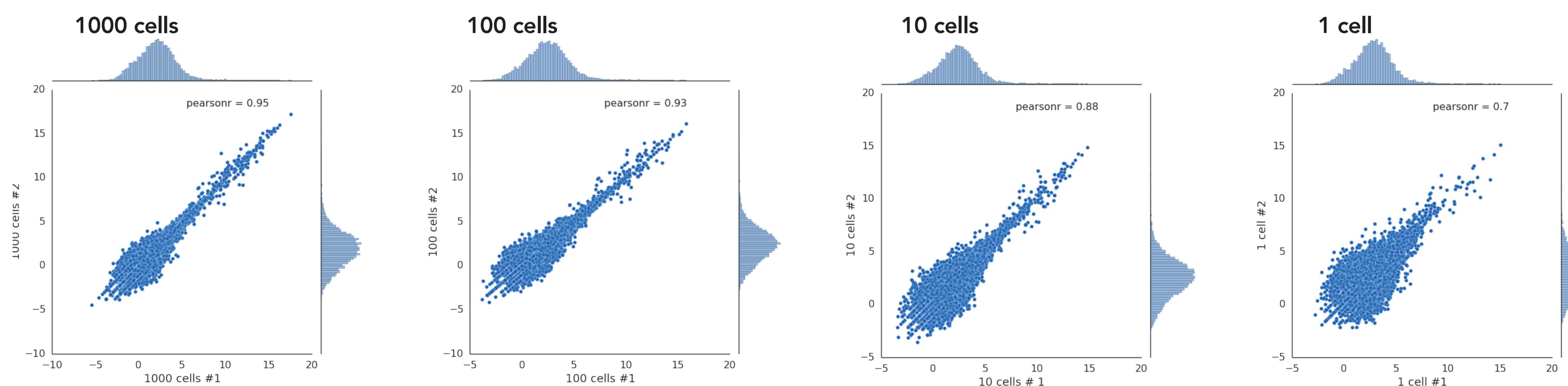
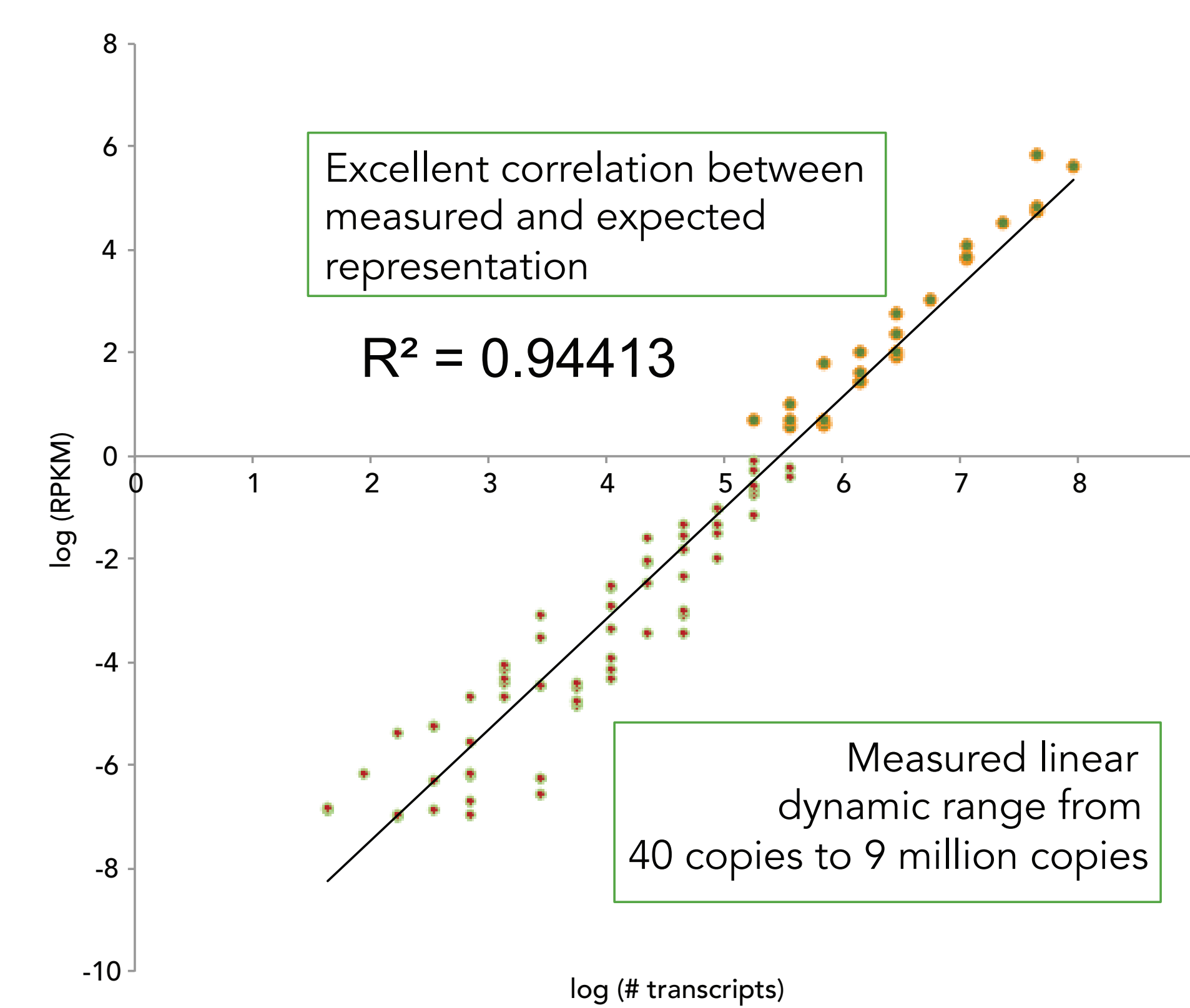
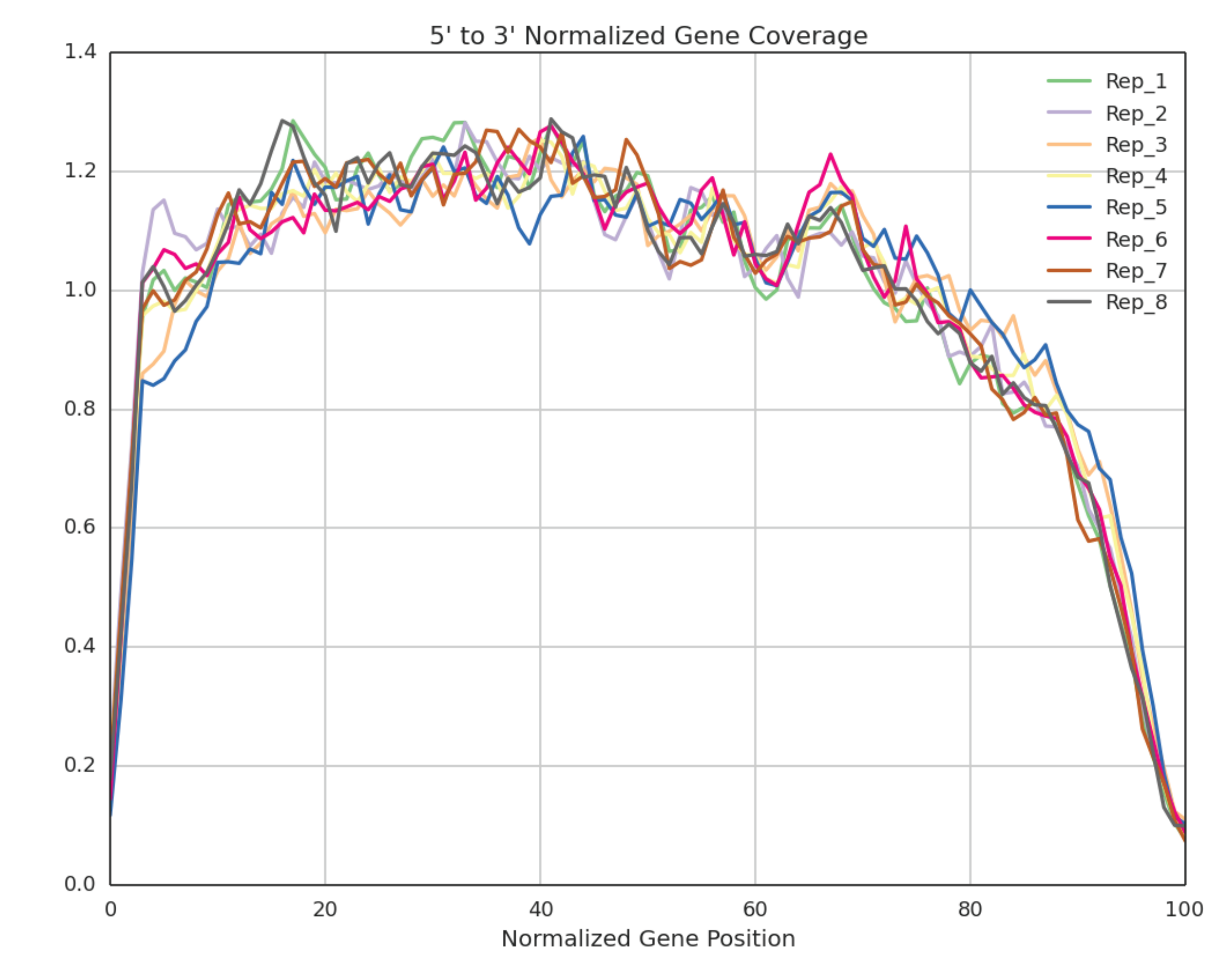
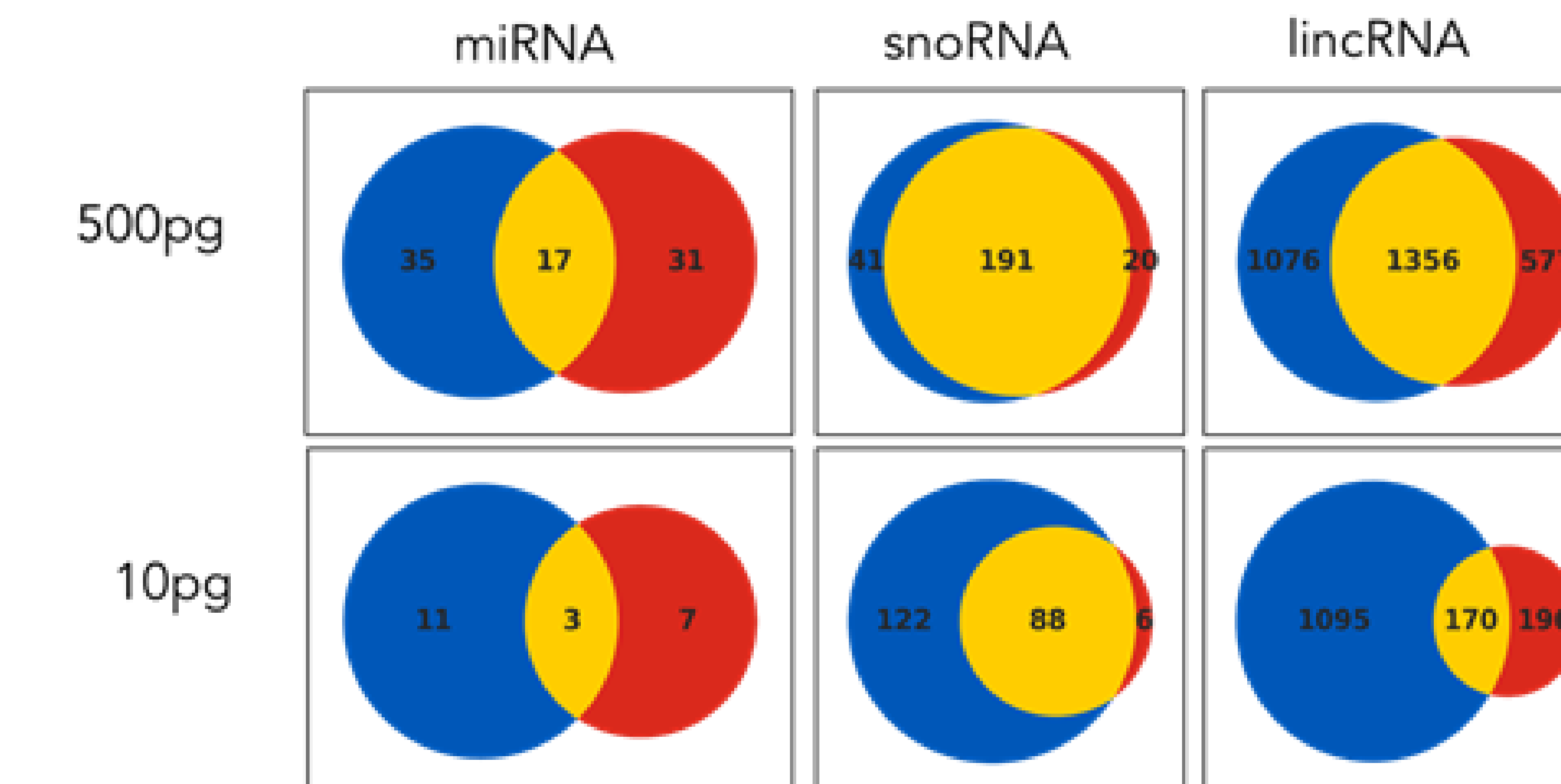


**ABSTRACT**

Understanding RNA expression in a single cell allows for greater understanding of developmental processes, disease progression and biological pathways. One of the challenges in studying whole transcriptome using RNA-Seq in single cells is the presence of abundant ribosomal RNA transcripts that decreases the overall information content of the data. Here we describe a novel approach for generating stranded total RNA-Seq libraries from single human cells or from single cell equivalents (10 pg of purified RNA). This method utilizes Insert Dependent Adaptor Cleavage (InDA-C) for effective removal of specific transcripts from RNA-Seq libraries without perturbing the original total RNA population. Here we demonstrate significant reduction in rRNA transcripts, high strand specificity, high FPKM correlation values even at minimal input levels, and effective capture of whole transcriptome including coding and non-coding RNA species. This novel approach provides a highly sensitive solution for researchers studying single cell transcriptomics and other applications with low amount of sample input such as from liquid biopsy, cell-free RNA or exosomes.

**Fig. 1** A novel workflow for whole transcriptome analysis of single cells or low input total RNA**Fig. 2** Insert dependent adaptor cleavage (InDA-C) is an efficient method to reduce unwanted transcripts prior to sequencing**Figure 2.** The InDA-C method efficiently removes unwanted transcripts with minimal manipulation of the RNA. This reduces the chances of RNA degradation and potential bias.**Fig. 3** Protocol performed consistently from 1000 cells down to single cells**Figure 3.** Protocol robustness was tested with HEK-293 cells at different input numbers from 1000 cells to single cells. We observed consistent performance at all cell input levels for:

- Even Read Distribution at all input levels (Figure 3a)
- Significant rRNA Reduction down to 10% or less (Figure 3b)
- Strand Specificity at over 90% (Figure 3c)
- Genes over FPKM 1 consistent with the input cell amount (figure 3d)

**Fig. 4** Consistent expression was observed at all input cell levels tested**Figure 4.** From libraries generated with different amounts of starting inputs (see Figure 3 legend for description), we ascertained the reproducibility in gene detection at each indicated input level. Using 10 cells or more, we observed correlations at 0.88 and above; at the single cell level, we observe correlations at 0.7.**Fig. 6** Broad dynamic range and sensitivity observed with ERCC spike-in controls**Figure 6.** Gene detection dynamic range and sensitivity was measured via ERCC spike-in Mix 1, a commonly used control of known transcripts of various expression levels and lengths. ERCC was spiked into 1 ng of purified HeLa RNA for library generation and results show a broad linear dynamic range that ensures accurate detection of high and low expressing transcripts.**Fig. 5** Even transcript coverage was observed with excellent representation of the entire transcript length**Figure 5.** Eight replicates of libraries with 10 HEK-293 cells as input were generated to display consistent and even 5' to 3' coverage of the entire transcript length.**Fig. 7** Multiple ncRNA classes were efficiently detected at the low 10 pg input level**Figure 7.** Comparisons at 10 pg and 500 pg input of purified HeLa RNA between NuGEN's SoLo kit and a commercially available whole transcriptome, low input kit from another vendor (AV) for various ncRNA classes: ■ Detected by NuGEN; ■ Detected by AV; ■ Detected by both. The Venn diagrams show that the NuGEN workflow is superior in generating whole transcriptome libraries from a minimal input level.**CONCLUSIONS**

- Whole transcriptome analysis of single cells is made possible by N6 priming, highly efficient library construction and Insert Dependent Adaptor Cleavage (InDA-C) technology to capture all transcripts while eliminating unwanted transcripts, leaving original RNA population unperturbed
- Significant reduction in rRNA transcripts, high strand specificity, and high FPKM correlation values are achieved at minimal input levels
- Novel and sensitive approach allows for analyses of whole transcriptome profiles from challenging samples such as single cells