PP029
A Methodological Comparison of Circular RNA Isolation Approaches

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Background/Aim: Circular RNAs (circRNAs) are promising biomolecules for transcriptomic and biomarker research, but their reliable analysis depends on robust isolation and enrichment strategies. This study aimed to systematically compare three methodological approaches for circRNA analysis from peripheral blood-derived total RNA in terms of technical performance, linear RNA depletion efficiency, and suitability for RT-qPCR.
Materials and Methods: Peripheral blood samples were collected from 20 individuals who provided written informed consent prior to sample collection. Total RNA was isolated and processed using three approaches: direct analysis without enrichment, conventional RNase R treatment, and an RPAD-like workflow involving polyadenylation, RNase R digestion, and magnetic bead-based purification. cDNA was synthesized using random primers, and circFBXW7, circERBB2, circCDR1as, circZNF609, ACTB, and GAPDH were analyzed by RT-qPCR.
Results: Conventional RNase R resulted in significantly greater circRNA recovery and higher relative expression levels than the RPAD-like method for circFBXW7, circCDR1as, and circZNF609 ($p < 0.01$). No statistically significant difference was observed for circERBB2 ($p = 0.114$). In contrast, the RPAD-like method achieved significantly more efficient depletion of residual linear RNA. Residual linear RNA levels were reduced to 13.84% for ACTB and 19.38% for GAPDH, compared with 55.46% and 73.04%, respectively, following conventional RNase R treatment ($p < 0.001$). Furthermore, the RPAD-like method exhibited markedly lower analytical variability and demonstrated good discriminatory performance in ROC analysis (ACTB: AUC = 0.837; GAPDH: AUC = 0.745).
Conclusion: Conventional RNase R appears more suitable when preservation and enrichment of target circRNAs are prioritized, whereas the RPAD-like method is advantageous when stricter linear RNA depletion is required. These findings support method selection according to analytical priorities in circRNA-focused RT-qPCR workflows.

Keywords: Circular RNA, RNase R, Polyadenylation