Comparison of methods to purify 5’ Capped microRNAs

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Adenovirus is one of large virus families that have double-strand DNA and can infect mammals, birds, and amphibians. Since Rowe and colleagues found adenovirus and tried to isolate from cultured human tonsil and adenoid cells in 1953, several types of adenovirus have been isolated from various animal species. Among those, human adenoviruses appeared to be a common virus type that causes respiratory, ocular, and gastrointestinal disease. Adenovirus produces short non-coding small RNAs that are called virus-associated RNAs (VA RNAs). Dicer enzyme cuts end of the VA RNA resulting around 22 nucleotides long small RNA called Virus-Associated RNA derived Small RNA (mivaRNA). In previous studies, it has been found that VA-RNAs and mivaRNAs have important function as regulators of cellular processes such as proliferation, differentiation and immune responses. During the investigation of adenoviral small RNAs, surprisingly, a new family of miRNAs was found. This miRNA family is named MLP-TSS-miRNA and it has unique features such as a longer size, different transcription start site and, moreover, the cap structure at 5’ end. In order to investigate this MLP-TSS-miRNAs, genome sequencing is required, and before that, purification of capped miRNAs is necessary. In this study the double immunopurification techniques has been used in order to enrich for capped miRNAs, including an immune-purification of miRNA-Ago2 protein complexes using an anti-Flag antibody and further purification of the capped miRNAs by an anti-cap monoclonal antibody. As an alternative strategy, GST pull-down assay has been performed using cap-binding protein, GST-EIF4E fusion protein. We tried to optimize the condition of these two methods for efficient purifying of MLP-TSS-miRNA and compared several strategies in this study.