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Functional Gene Expression in Human Cells Using Linear Vectors Derived from Bacteriophage N15 Processing

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Abstract : This paper adapts the bacteriophage N15 protelomerase enzyme to assemble linear chromosomes as vectors for gene expression in human cells. Phage N15 has the unique ability to replicate as a linear plasmid with telomeres in E. coli during its prophage stage of life-cycle. The virus-encoded protelomerase enzyme cuts its circular genome and caps its ends to form hairpin telomeres, resulting in a linear human-chromosome-like structure in E. coli. In mammalian cells, however, no enzyme with TelN-like activities has been found. In this work, we show for the first-time transfer of the protelomerase from phage into human and mouse cells and demonstrate recapitulation of its activity in these hosts. The function of this enzyme is assayed by demonstrating cleavage of its target DNA, followed by detecting telomere formation based on its resistance to recBCD enzyme digestion. We show protelomerase expression persists for at least 60 days, which indicates limited silencing of its expression. Next, we show that an intact human β-globin gene delivered on this linear chromosome accurately retains its expression in the human cellular environment for at least 60 hours, demonstrating its stability and potential as a vector. These results demonstrate that the N15 protelomerse is able to function in mammalian cells to cut and heal DNA to create telomeres, which provides a new tool for creating novel structures by DNA resolution in these hosts.

Keywords : chromosome, beta-globin, DNA, gene expression, linear vector
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