

## Adeno-Associated Virus (AAV)-2 Genome in Arthrobacter sp. LS16?

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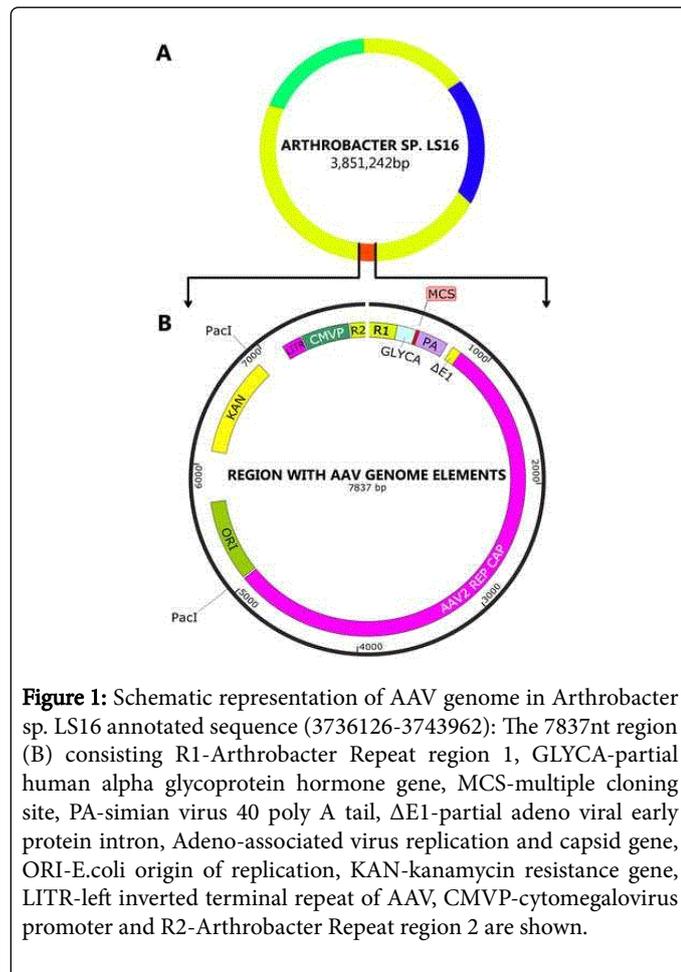
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### Introduction

In our BLAST analysis against Adeno-associated virus (AAV)-2 genome (GenBank accession no. AF043303.1), we found a 90% homologous sequence of AAV2 in Arthrobacter sp. LS 16 (GenBank accession no. CP012171) published recently [1]. AAV2 is a non-pathogenic single-stranded DNA virus of ~ 4.7 kb in length.



**Figure 1:** Schematic representation of AAV genome in Arthrobacter sp. LS16 annotated sequence (3736126-3743962): The 7837nt region (B) consisting R1-Arthrobacter Repeat region 1, GLYCA-partial human alpha glycoprotein hormone gene, MCS-multiple cloning site, PA-simian virus 40 poly A tail, ΔE1-partial adeno viral early protein intron, Adeno-associated virus replication and capsid gene, ORI-E.coli origin of replication, KAN-kanamycin resistance gene, LITR-left inverted terminal repeat of AAV, CMVP-cytomegalovirus promoter and R2-Arthrobacter Repeat region 2 are shown.

The genome of AAV contains a short 145nt of inverted terminal repeats (ITR) flanking the coding region of replication (Rep) and capsid (Cap) genes [2]. The nucleotides in and around the AAV2 integrated sequence of Arthrobacter sp. LS16 was carefully analyzed, annotated and distinguished by their nature and function using standard bioinformatics tools. A region of 4238 bp (3736925-3741162)

encoding Rep and Cap genes of AAV2 was found in the Arthrobacter sp. LS16 sequence (Figure 1 and supplementary data file 1). In addition, the upstream region from AAV2 sequence contained partial coding sequence of human alpha subunit of glycoprotein hormone (GLYCA) (154bp, 3736375-3736528), short polyclonal/multiple cloning site (26bp, 3736529-3736554), simian virus 40 polyadenylation signal (242bp, 3736555-3736796), incomplete adenovirus early protein (E1) intron sequence (78bp, 3736847-3736924) and the downstream regions comprised E.coli origin of replication (652bp, 3741177-3741828), neomycin/kanamycin resistance gene (795bp, 3742240-3743034) followed by AAV left ITR (162bp, 3743268-3743429) and cytomegalovirus (CMV) promoter/enhancer (383bp, 3743440-3743822) (Supplementary Table 1).

All the foreign components identified (7461bp, 3736375-3743835) in Arthrobacter genome indicates the presence of AAV2 genome along with portions of a shuttle vector plasmid. The two PaclI restriction sites (3741176, 3743040) commonly used in shuttle vectors for linearization after recombination in competent E.coli cells were preserved exactly at their respective positions [3]. In addition, a non-plasmid GLYCA gene sequence (GenBank accession no. J00152.1) was also identified between a multiple cloning site. It must be noted that GLYCA is used as a quantitative serum expression marker for in vivo studies [4]. To further understand if the presence of foreign DNA sequence in Arthrobacter is mediated by integration elements we screened for the presence of repeat elements. Our analysis revealed two non-identical repeat regions of 249nt (R1) and 127 nt (R2) on both DNA strands in LS16 (Supplementary Table 2) flanking the AAV2 genome. Such a repeat element was also identified in a closely related Arthrobacter sp. YC-RL1 (GenBank accession no. LCYH00000000.1) demonstrating that this feature is a hotspot for integration in Arthrobacter sp. genome. BLASTX search against non-redundant protein databases also revealed the presence of putative conserved domains of Integration Host Factor (IHF) in LS16 (2857050-2857333).

There are many possibilities for the presence of AAV2 based plasmid sequence in Arthrobacter sp. LS16. This sequence may either have naturally integrated or laboratory-induced, both situation's that require further detailed analysis of the source samples used for characterization of Arthrobacter sp. LS16. This is important considering that vertical transmission of AAV/ antibiotic resistance gene in Arthrobacter has not been reported earlier. More importantly, if the natural integration of AAV genome in Arthrobacter sp. LS16 a common soil bacterium is proven, it may potentially explain the high levels of AAV2 specific neutralizing antibodies (~70%) seen in humans [5].

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