**Virologica Sinica**

**Supplementary Data**

**Semen Extracellular Vesicles Mediate Vertical Transmission of Subgroup J Avian Leukosis Virus**

**Liqin Liao a,b,e,1, Weiguo Chen a,b,e,6,1, Xiangyu Zhang a,e, Huanmin Zhang c, Aijun Li d, Yiming Yana,b,e, Zi Xiea,b,e, Hongxing Li a,b,e, Wencheng Lin a,b,e,f, Jingyun Ma a,b,e,f,** **Xinheng Zhang a,b,e\*, Qingmei Xie a,b,e,f\***

*aCollege of Animal Science, South China Agricultural University & Guangdong Provincial Key Lab of Agro Animal Genomics and Molecular Breeding, Guangzhou 510642, China*

*bLingnan Guangdong Laboratory of Modern Agriculture, Guangzhou, 510642, China*

*cUSDA, Agriculture Research Service, Avian Disease and Oncology Laboratory, East Lansing, MI, 48823, USA*

*dCollege of science and engineering, Jinan University, Guangzhou, 510632, China*

*eKey Laboratory of Animal Health Aquaculture and Environmental Control, Guangdong, Guangzhou, 510642, China*

*fGuangdong Engineering Research Center for Vector Vaccine of Animal Virus, Guangzhou, 510642, China*

\*Corresponding authors,

Email: xhzhang@scau.edu.cn (X. Zhang), ORCID: 0000-0001-6409-3160

Email: qmx@scau.edu.cn (Q. Xie), ORCID: 0000-0001-6537-7798

1Contributed equally to this work.

Supplementary Table S1 PCR Primers designed and used in this study

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Fragment | Primers | Sequence (5'-3') | Genome position | Size(bp) |
| A | Forward | GCCATTTTACCTCCCACCAC | ALV-J 225-2778 | 2553 |
| Reverse | ATTTGAGCGGAATAGCCAG |
| B | Forward | GACCCCTGCTCCTCTTCCCC | ALV-J 2631-5274 | 2643 |
| Reverse | TTGTGTTCTCACCACGCTCA |
| C | Forward | TGTGCTTGCGGAGGGGGACG | ALV-J 4964-7328 | 2364 |
| Reverse | TCCCCCTCCCTATGCAAAAG |
| J | Forward | GAAAGACCCGGAGAAGACACC | ALV-J 5390-5538 | 148 |
| Reverse | CCAGGTGACCCACACGTTTC |
| *gp85* | Forward | TTTCAAATGATACTTGTGTG | ALV-J 5440-6363 | 924 |
| Reverse | AAAGTTAGGAGAGAGCATAG |

Primers were designed based on ALV-J strain GD1109 genomic sequences (GenBank accession no. JX254901.1).A,B,andC = primer pairs for amplifying overlapping fragments of the ALV-J genome; J = primers for cloning a portion of *gp85* cDNA used as standard plasmid for qRT-PCR analysis; *gp85* = primers for cloning ALV-J gp85 coding sequence used for analysis of the homology of ALV-J strain GD1109, ALV-J isolated from SE-ALV-J and the progeny chicks, respectively.

Supplementary Table S2 Total reads, mapped reads, and mapped fractions of SE-ALV-J and SE-Mock to chicken reference genome (galGal6)

|  |  |  |  |
| --- | --- | --- | --- |
| SE fraction | Total reads | Mapped reads | Mapped fraction (%) |
| SE-ALV-J | 43,074,934 | 23,316,345 | 54.13 |
| SE-Mock | 22,983,798 | 9,283,195 | 40.39 |

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| SE fraction | ALV-J genomic RNA | | Gag\_polyprotein | | Pol\_polyprotein | | Envelope\_protein | |
| Total mapped  reads | Mapped  fraction (%) | Mapped  reads | Mapped  percentage (%) | Mapped  reads | Mapped  percentage (%) | Mapped  reads | Mapped  percentage (%) |
| SE-ALV-J | 9,731,520 | 22.59 | 3,222,748 | 7.48 | 3,435,557 | 7.98 | 3,073,215 | 7.13 |
| SE-Mock | 102,937 | 0.45 | 62,736 | 0.27 | 36,482 | 0.16 | 3,719 | 0.02 |

Supplementary Table S3 Total mapped reads, and mapped fractions of SE-ALV-J and SE-Mock to ALV-J strain GD1109 genomic RNA sequences as well as *Gag*, *Pol*, and *Env* coding region sequences.