

Kaposi's Sarcoma-associated herpesvirus encodes a mimic of cellular miR-23

Mark Manzano, Priscilla Shamulailatpam, Archana N. Raja and Eva Gottwein

Detailed Cloning Procedures

Amplification of the miR-K3 coding region and cloning of lentiviral miR-K3 expression vector pLCE/miR-K3

250bp centered on the miR-K3 stem-loop were amplified from BC-1 or BCBL-1 genomic DNA using primers 1087 and 1088 (see table below for sequences). Resulting fragment was cloned into pLCE using *XhoI* and *NotI*.

Cloning of lentiviral miR-23 expression vector pLCE/miR-23

A 250 bp fragment containing the miR-23a stem loop at a central location was amplified from BCBL-1 genomic DNA using primers 1445 and 1446 (see table below for sequences). Resulting fragment was cloned into pLCE using *XhoI* and *EcoRI* sites.

3'UTR reporter vectors and indicator assays. The 3'UTRs of putative target genes were amplified from PEL genomic DNA and cloned into the firefly luciferase reporter vector pLSG. Primers used are indicated by numbers and primer sequences are listed in the table below. Primers contain *XhoI* and *NotI* restriction sites unless otherwise indicated: CASP3 (1467/1515), CASP7 (1469/1470), JARID2 (1471/1525), MTUS1 (1476/1477), CARD8 (1484/1485), HMGB2 (1486/1487), CREBBP (1488, *XbaI*/1489, *EcoRI*), SMC1A (1490/1491), HMMR (1492F/1492R), KLDHC5 (1493/1494), TBL1XR1 (1495/1496), GATAD2B (1497/1498), PHF17 (1342/1518), PPP3CA (859/862), RAB4A (1499/1500), RBL2 (1501/1502), TGFBR2 (1516/ 1517), TNFRSF10B (1070/1071). The 3'UTR reporter constructs for WEE1, TPD52, RAD21 and SOS1

were described previously (1). To mutate seed sequences, overlap-extension PCRs were performed using the outer primers indicated above and the following mutated inner primer pairs: 1535 and 1536 for CASP3, 1537 and 1538 for CASP7, 1539 and 1540 for CREBBP, 1541 and 1542 for TNFRSF10B, 1543 and 1544 for HMGB2 site 2, 1545 and 1546 for HMGB2 site 1, 1547 and 1548 for HMMR, 1549 and 1550 for JARID2, 1551 and 1552 for MTUS1, 1553 and 1554 for RBL2, and 1704 and 1705 for RAB4A. In each case, the seed match was mutated from 5'-AATGTGA-3' to 5'-AAAGTCA-3'.

Primer sequences

Name	Sequence (5' to 3')
859	AGACTCGAGGAGCTGCGGGGCATGATGGG
862	AGAGGCGGCCGCCTATGCCATAGTTGCCTCAG
1070	AGACTCGAGGTGTGATTCTCTTCAGGA
1071	TTCGCGGCCGCAATCGCTTGAGCCTGAGA
1087	AGACTCGAGTTCCAGGGCTAGAGCTGC
1088	TTCGCGGCCGCCCTAGAGTACTGCGGTTT
1302	TAATACGACTCACTATAGGCGCAACAGCTACAATGCCTG
1303	CGGTTGTCGCTTGACC
1342	AGACTCGAGTGCAACAGAGATGATGCGGA
1445	AGTTACTCGAGGAGGGGAGGTGTCCCAAATCTC
1446	AGTTAGAATTCGCCAGGCACAGGCTTCGG
1467	AGTTACTCGAGAGAAATGGTTGGTTGGTGG
1469	AGTTACTCGAGCCATATCAGGGGTACATTCTAGC
1470	AGTTAGCGGCCGCCAATAAATATGAACATTTGTTTTTAACCAG
1471	AGTTACTCGAGAGATGCCAACGCCCGTGGTCCG
1476	AGTTACTCGAGTGCAGCCTCCTCTGCTGTG
1477	AGTTAGCGGCCGCGCAATTCTTGAGTATTCC
1484	AGTTACTCGAGACAGGCATGAGCCACCGTGC
1485	AGTTAGCGGCCGCCGCCAACCCTTGGAGCA
1486	AGTTACTCGAGCCTGGCAGGCCAACAGGCTC
1487	AGTTAGCGGCCGCTCCTACAAGTTTGCTGTGCTACCATAC
1488	AGTTATCTAGAACTCTGTGGGCGTCTCCAGT
1489	AGTTAGAATTCGGACCACCTTTTTGTCTGTTGCAC
1490	AGTTACTCGAGGGGAGGCTGGGCACCACAGT
1491	AGTTAGCGGCCGCGGGGCCAGAATGCCGTACC
1492F	AGTTACTCGAGACCGAGCTCCTATGGAGTGTCAC
1492R	AGTTAGCGGCCGCACAAGCCAAGGTGTTTTAGCCTAGC
1493	AGTTACTCGAGAACCTCTGAACCAAATCTTCCAGG

1494	AGTTAGCGGCCGCGCTGCCACCCCAGGTCCTCCA
1495	AGTTACTCGAGGTTTGTGTATTAGACCTTCG
1496	AGTTAGCGGCCGCGAGCATAATACCCTTTTACTG
1497	AGTTACTCGAGTCTTTTCTTTGCCCTCTGTGCTTTGA
1498	AGTTAGCGGCCGCGCGGTTGAACAAAGGGTCACCGA
1499	AGTTACTCGAGTGGCATTGTTGGGACACAATCGTTGG
1500	AGTTAGCGGCCGCTGGATGAGCGGCCCCAGCTT
1501	AGTTACTCGAGGGAAGATGGAAGTGAATCAC
1502	AGTTAGCGGCCGCGAAAAATACACTTATCTTC
1515	AGTTAGCGGCCGCAAGTTTGAATGTATATTTTG
1516	AGAGACTCGAGCTCTTCTGGGGCAGGCTGGG
1517	TCTCTGCGGCCGCGAGACTGTCAGTTGAGAAAG
1525	AGTTAGCGGCCGCATTAACCTTGTAGTACAAACC
1518	TCTCTGCGGCCGCTAAACAACCTGATTCAGGACT
1535	TGATTATTAGCCAGGTAAAGTCAATAAATTCTATAGGA
1536	TCCTATAGAATTTATTGACTTTACCTGGCTAATAATCA
1537	TGTAAGCCTGGCCCATAAAGTCAACATAAGTAATCACT
1538	AGTGATTACTTATGTTGACTTTATGGGCCAGGCTTACA
1539	TTCTTTTCTTTCTTCTATCTGTAACCTTGAATGAGGAA
1540	TTCCTCATTTC AAGTTACAGATAGAAGAAAGAAAAGAA
1541	TTATTTTTATAAGCTGAAAGTCATAATAAGGACACTAT
1542	ATAGTGTCTTATTATGACTTTT CAGCTTATAAAAATAA
1543	CAATTATTTTGCTAAGAAAGTCAATTCAAGTGCAGCTC
1544	GAGCTGCACCTTGAATTGACTTTCTTAGCAAATAATTG
1545	TTAATGATGCGTGTGGAAAGTCTGTGTGTGCTCAGGCA
1546	TGCCTGAGCACACACAGACTTTCCACACGCATCATTAA
1547	CTGCCAATCCTTAAATATCTGTAAGGAACATTTTTTAC
1548	GTA AAAAATGTTCCCTTACAGATATTTAAGGATTGGCAG
1549	CCTGCAGTATTTTTGAAAGTCAAATGCATTTGCGTT
1550	AACGCAAATGCATTTT GACTTTCAAATAACTGCAGG
1551	GCACGTCAA AAAATATGAAAGTCTAGACA ACTGTAGTTG
1552	CAACTACAGTTGTCTAGACTTTTCATATTTTTGACGTGC
1553	TGATAGCACTTTCTACAAAGTCAACTTTATTAATACA
1554	TGTATTTAATAAAGTTGACTTTGTAGAAAGTGCTATCA
1704	GATGTATGATATGATAGAAAGTCGCACTAAATGCAGTTTC
1705	GAAACTGCATTTAGTGCGACTTTCTATCATATCATA CATC

References

1. **Gottwein, E., D. L. Corcoran, N. Mukherjee, R. L. Skalsky, M. Hafner, J. D. Nusbaum, P. Shamulailatpam, C. L. Love, S. S. Dave, T. Tuschl, U. Ohler, and B. R. Cullen.** 2011. Viral microRNA targetome of KSHV-infected primary effusion lymphoma cell lines. *Cell Host Microbe* **10**:515-26.