

## Supplemental\_Fig\_S9

**Comparison of sequences of T7 and SP6 polymerases.** Tyr639 indicated by an arrow and the nearby fragment by a black box.

|     |                                                                |
|-----|----------------------------------------------------------------|
| T7  | MNTINIAKNDFSDIELAAIPFNTLADHYGERLAREQLALEHESYEMGEARFRKMFERQLK   |
| SP6 | -----MQDLHAIQLQLEEMFNGGIRRFADQQRQIA                            |
| T7  | AGEVADNAAAKPLITTLTPKMIARINDWFEEVKAKRGKRPTAFQFLQEIKEAVAYITIK    |
| SP6 | AGSESDTAWNRRLLSELIAAPMAEGIQAYKEEYEGKKGRAPRALAFLQCVENEAAYITMK   |
| T7  | TTLACLTSA DNTTVQAVASAI GRAIEDARFGRIRDLEAKHFKKNVEEQLNKRVGHVYKK  |
| SP6 | VVMDMLN--TDATLQAIA MSVAERIEDQVRF SKLEGHAAKYFEKVKKSLKASRTKSYRHA |
| T7  | AFMQVVEADMLSKGLLGGEAWSWHKEDSIHGVRCIEMLIES--TGMVSLHRQNAGVV      |
| SP6 | HNVAVVAEKSVAEKDADFDRWEAWPKETQLQIGTTLLEILEGVSFYNGEPVFMAMRTYG    |
| T7  | GQDSEITELAPYAEATATRAGALAGISPMFQPCVVPPKPWTGITGGGYWANGRRPLALV    |
| SP6 | GKTIYYLQTSESVGQWISAFKEHVAQLSPAYAPCVIPPRPWRTPFNGGFHTEKVASRIRL   |
| T7  | RTHSKKALMRYEDVYMPYVYKAINIAQNTAWKINKKVLAVANVITKWKHCP--VEDIPAI   |
| SP6 | VKGNREHVRKLTQKQMPKVYKAINALQNTQWQINKDVLAVIEEVIRLDLGYGVPSFKPLI   |
| T7  | EREELPMKPEDIDMN-----PEALTAWKRAAAVYRKDKARKSRRISLEF              |
| SP6 | DKENKPANPVPVEFQHLRGRELKEMLSPEQWQQFINWKGE CARLYTAETKRGSKSAVVVR  |
| T7  | MLEQANKFANHKAIFPYNMDWRGRVYAVS-MFNPQGNMTKGLLTLAKGKPIG-KEGY      |
| SP6 | MVGQARKYSAFESIFYVYAMDSRSRVYQSSTLSPQSNDLGKALLRFTEGRPVNGVEALK    |
| T7  | WLKIHGANCAGVDKVPFPERIK--FIEENHENIMACAKSPLENTWAEQDSPFCFLAFCF    |
| SP6 | WFCINGANLWGWDKKTFDVRVSNVLD EEFQDMCRDIAADPLTFTQWAKADAPYEFLAWCF  |
| T7  | EYAG----VQHHGLSYNCSLPLAFDGSCSGIQHFSAMLRDEVGGRVNLPSSETVQDIY     |
| SP6 | EYAQYLDLVDEGRADEFRTHLPVHQDGSCSGIQHYSAMLRDEVGAKAVNLKPSDAPQDIY   |
| T7  | GIVAKKVNEILQADAINGTDNEVVTVDENTGEISEKVKLGTKALAGQWLAYGVTRSVTK    |
| SP6 | GAVAQVV--IKKNALYMDADDATFTSG----SVTLSGTEL RAMASAWDSIGITRSLTK    |
| T7  | RSVMTLAYGSKEFGFRQQVLEDTIQPAID-----SGKGLMFTQPNQA                |
| SP6 | KPVMTLPYGSTRLTCRESVIDYIVDLEEKEAQKAVAEGRTANKVHPFEDDRQDYLT PGAA  |
| T7  | AGYMAKLIWESVSVTVVA AVEAMNWLKSAAKLLAEVKDKKTGEILRKRCVHWVTPDGF    |
| SP6 | YNYMTALIWPSISEVVKAPIVAMKMIRQLARFAAKRNE-----G-----LMTLPTGF      |
| T7  | PVWQEYKKPIQTRLNLMFLGQFRLQPTINTNKDSEIDAHKQESGIAPNFVHSQDGSHLRK   |
| SP6 | ILEQKIMATEMLRVRTCLMGDIKMSLQVETD--IVDEAAMMGAAPNFVHGH DASHLIL    |
| T7  | TVVWAHEKYGIESFALIHDSFGTIPADAANLFKAVRETMDTYESC DVLADFYDQFADQL   |
| SP6 | TVCELVDKG-VTSIAVIHDSFGTHADNTLTLRVALKGQMVAMYIDGNALQK LLEEHEVR-  |
| T7  | HESQLDKMPALPAKGNLNLRDILESDFafa                                 |
| SP6 | --WMVDTGIEVPEQGEFDLNEIMDSEYVFA                                 |