Web Figure 1: Sequence alignment of Hbr DME in *D. melanogaster* and *D. virilis*. Verified and predicted TF binding sites are shown in color. Basepair numbering corresponds to that shown in Figure 3.

```
                 10        20        30        40        50        60
D. mel   aaagtcacaggtttatggtttatgagtcgctcaggactcctttgcacgatcattgtgtat

D. vir   ------------------------------------------------------------


                 70        80        90       100       110       120
D. mel   tcatcataagcgttttcaaggtttcgctttcatgacatctgatgatttaagttgatttaa

D. vir   ------------------------------------------------------------


                130       140       150       160       170       180
D. mel   ggcacttcgtgtgacgtttaaatccagcgcaattttcgagtctcaaaagagatgagaaat
         |||||        ||                | || |
D. vir   ggcac--------ac----------------anttnc-----------------------
                                         10

                190       200       210       220       230       240
D. mel   aagtagtagatgagatactgaggagtgtttcatacatagaaattcagagcatgtagatct
                                                                    |
D. vir   ------------------------------------------------------------cg


                250       260       270       280       290       300
D. mel   gctttagaagtgccgcaatttattagaagtgtgccattaaagtttttactattataaaaa
         ||   | ||| |||       | | | ||| |||        ||||| || ||
D. vir   gc---aaaagggcc-------agtngcagt-tgc-------ntttttgctntt-------
               20                30                 40        50

                310       320       330       340       350       360
D. mel   caatagattctctcctttattgttattattatcaattataataacggttgcccacagcgc
            ||||  ||||||                |||||  |     |||
D. vir   --ttaga--ctctcc----------------caattgt-----cgg-------------
                     60                              70
```

```
                370       380       390       400       410       420
D. mel   cttattctaaaaatgatcacccaccatattcgacacacttatcgctatggtcacttgcaa
                 ||| ||| | || |       |  ||| ||  || ||   ||
D. vir   --------aaanatgttgactc-------tgtaca-ac--atggc---gg----------
                        80                90             100

                430       440 twi   450       460       470       480
D. mel   tccattcgaatctttcgccatatgcaggaaactaggcaattcgcctcaatcggcaattaa
            || || | | | |||     |||  |||  ||||||
D. vir   --natgcgcaacctgcgc-----gca--aaa--aggcaa--------------------
                  110              120

                490       500       510       520       530       540
D. mel   cccacaacacccacgaacaattcatttctaacacctaaatttatggcaaagtaaaaacaa
             |||| |    ||| |                                ||||||||
D. vir   -----aacatc----aacna-------------------------------caaaaacaa
             130                                        140

                550       560       570       580       590       600
D. mel   accgaaccgaatatctctgccatatttgcgagtgtcccactgcagttgccagttcgattg

D. vir   ------------------------------------------------------------


                610       620       630       640       650       660
D. mel   gcataagttatacgcatttccgctctttcaataacaatggccattgcattttgttattat
                                   ||| |||||
D. vir   ----------------------------caacaacaa----------------------
                                   150

                670       680       690       700       710       720
D. mel   gttgctggcaacggggcggatgggcaacatgggcgatattggatgtgcggatggtggagt
                            |||    |||                           ||||
D. vir   ------------------aatg----aca--------------------------agagt
                            160

                730            740          750       760
D. mel   caacaaacgaatcg-----cttgacgtg---ggcggtgggtgtggccgc-------atga
         ||| |||||||||      |   | ||     | |||||||||| ||       |  |
D. vir   caataaacgaatcgtagcgatgcgcctgccctccagtgggtgtggcagccgtgacaagca
       170       180       190       200       210       220
```

```
            770       780       790          800         810
D. mel   ggagcataaattgaagaactgcaca---tccaataaggcac--aaccaactggc------
         | | |||||||||||| |  ||||||     ||||||| | |   ||| |  |||
D. vir   gaaacataaattgaaaatttgcacaattgtcaataagtcccgggaccgagaggcgagggg
       230       240       250       260       270       280

                        820        830       840
D. mel   ----------tgg--aac-ggaacacggcataattgatg---------ggc---ggact-
                   |||  ||| |||||   ||||||||| ||         |||    ||||
D. vir   cgtttgttgttggccaacaggaactttgcataattgttgttggctgttggccgttgactg
       290       300       310       320       330       340

                       850 twi     860       870       880       890
D. mel   ---------------catatgt-atag-gtatgtatatgtacatatacctaaacctatag
                        ||  |||   || || ||| | |||   | |   |     | |
D. vir   tttggcttttgtggccaagtgtgcgagtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgt
       350       360       370       380       390       400

              900       910       920 ets    930              940
D. mel   atatgtatgcttcaaacgtgttaatggcacggaagcgaga-----gaaaa--aagggga
          | ||| |  |||||||||||||||||||||||||| ||       | | | | | ||
D. vir   gtgtgtgttgttcaaacgtgttaatggcacggaagcgtgaactccgcacatgcactcga
       410       420       430       440       450       460

             950      960 mot.A twi970       980       990      1000
D. mel   gttgggtggccaactgctttgcatatggcgcgtgccaaccaaaacgattggccaaatttgg
                || |||||| |||||||||||||||||||  | ||||  |||||||||||||
D. vir   aacaaacgggcaactgttttgcatatggcgcgtgccaggc-aaacatttggccaaatttgg
       470       480       490       500        510       520

            1010      1020      1030                      1040      1050 tcf  1060
D. mel   ccctccttcgaccataccgagaaattggcagcacaa--------------actgtgaatacatcaaagctgcg
                                 |||||     ||||                      ||||||||||
D. vir   -----ctaaaatgcttttggccaattgtgcatacaaatacatacaacatacacagtcgcacatcaaagc----
             530       540       550               560       570

            1070      1080      1090  mad 1100      1110      1120
D. mel   ctgggctgtttacgctggccatgtttcctcttcggctttccaatcaaaagcgctacgga
                                                || || |||   ||   |
D. vir   ----------------------------------------aaccataag---taaaca
                       580              590              600
```

```
            1130      1140  mad 1150      1160      1170      1180
D. mel   gtcaataaaactggctgccgtggtggtggagtggaacaaaaattgggcagcaatgtgcca
         ||    ||||  | ||| || ||  | ||||             || |||||||||||||
D. vir   gttggcaaaatggtctgacg-ggcaggggag-------------ggccagcaatgtgcca
        610       620        630                     640       650

            1190       1200   tin 1210      1220 mad   1230      1240
D. mel   tttatcaattt-tacactg-tttaattgcaccagtcg-ctgcggaatgtagcaggtgatt
         | ||||| ||| |  |||| ||||||||||     ||| ||||  | ||| ||||||||||
D. vir   tatatcagtttatggactgtttttaattgc-----tcgactgc-aactgtggcaggtgatt
            660       670       680            690        700

              1250      1260       1270      1280      1290      1300
D. mel   tggggtcagcaatgcaagaacaga-agcaatgcattctgctcttttcccaatttccactg
         ||||||||    |   | |   ||  ||||||| ||   ||  |||   | |    | ||
D. vir   tggggtcaaacgtcagaaacgcgactgcaatgcgttgcacttgtttatgagt---gagtg
       710       720       730       740       750       760

              1310      1320      1330  mad 1340      1350      1360
D. mel   cacaaatgcacttgtttaccccttgttctgcccccaaaacgtctggcttctgttttcaa
         | || | ||    ||     | | ||                | | ||  |||  ||||
D. vir   c-cacaagc----gt---ggcatgtg-----------------cagcctgttgtactcaa
           770              780                      790       800

              1370 twi  1380                                  1390
D. mel   ttctcggaacatctgctcatcagattac-----------------------------gca
         ||| | |||||||||||||||||||||                             |||
D. vir   ttcccagaacatctgctcatcagattacacacacacacacgcacacagagacacaaagca
              810       820       830       840       850       860

                          1400  ets         1410               1420
D. mel   gag------------tgt-actgcca--------catccaccat--------ct-cttcc
          ||            |||  | ||||        |  |||||||        || || |
D. vir   aagacaagccgcctttgtggcagccaatctttagctgccaccatttttgctgctgctgct
              870       880       890       900       910       920

              1430        1440  tin 1450      1460      1470
D. mel   tttgccattg--ggggttcaatacacttcacttctagactctatcttgggggtacgtttt
           |||   ||  |||||||||||||||      |||||      | |||   ||    |
D. vir   gctgctgctgctggggttcaatacact----ggctagaggtctgcatggccataaccctg
              930       940            950       960       970
```

```
      1480      1490      1500       1510  mad 1520      1530
D. mel   tcctttgctc-------------------------gccgcgcatatcaaacagcccgaa
         | |  |||                            ||||||   |    |  |    ||
D. vir   tgcactgcataacaagcagttaaaagaaatcctattgccgcggcgagtggaagggggaaa
         980       990      1000      1010      1020      1030

D. mel   aaaaaaaaaatgtggcaggcagccc

D. vir   -------------------------

       1540        1550      1560          1570 tin     1580    mad
D. mel   aaaat--tcaaaatcgaaaatcct--atcg--aggaacttaagtgtc---ttccttggcg
         |   |   |      |||  ||||  | ||  ||||  |    | ||   || |  |
D. vir   atgctggnctttgctgaatttccttgaccggcaggacatcgcatatccagttgcccgcac
        1040      1050      1060      1070      1080      1090

      1590      1600      1610      1620            1630        1640
D. mel   gctgagtccaacacctgttgcattcataaata------cgaagttgggatttgagc--ag
          |  || |||||||||||||||||||||||      | ||    | || | |||
D. vir   tcgtagcccaacacctgttgcattcataataataccaaaaaaagaatctcagcttta
        1100      1110      1120      1130      1140      1150

             1650      1660      1670      1680      1690      1700
D. mel   agtcggcgtaattaacataatttttggtaggataataatacctactccaccaaaaataaa
         ||   || ||||| |||||||||| ||   |     |        ||  || |  ||||
D. vir   agcatgcttaatttacataattttgggcccgcctgga------ggtctgccgaggataa-
        1160      1170      1180      1190            1200      1210

             1710      1720      1730      1740      1750      1760
D. mel   ttcaagtataagtaacatattaacaatattttgctcacgtttccattcggaggtttccct
               ||||  | |||  |    |||||||||||||  ||| || ||||||| |||
D. vir   ------cataacaagcatgct---tatattttgctcacgt-cccaatctgaggtttncct
                    1220      1230      1240       1250      1260

             1770      1780      1790      1800      1810      1820
D. mel   tttttcctgtctttgttgcaactattttataaaaggcgataataaggaggatcaaaatgc
         ||||        ||||||      ||||                            ||
D. vir   tttt-------gttgttg------tttt-----------------------------tg-
                     1270

             1830      1840      1850      1860      1870      1880
D. mel   taagataacacatcgttaatttatatgtgggtaaattctttattaattttccaagtttc
```

```
                          | ||||  ||                || |||||||     |||
D. vir   ----------------tcatttccat----------------tttatttttc-----ttc
                        1280                      1290           1300

             1890      1900      1910      1920      1930      1940
D. mel   aaaatatttactttttattactctcccagtactttcaagttaaggtttccatctcccct
         |           |||||               ||||  || | |              ||
D. vir   a-----------ttttt---------------cttt--agctga--------------ct
                                          1310

             1950
D. mel   ttaagatttat-----------------------------------------------
            |  |||||
D. vir   ---atttttatctgaattcgtcgacaagcttctcgagcctaggctagntctagaccacac
          1320      1330      1340      1350      1360      1370


D. mel   ---------------------------------------g

D. vir   gtgtgggggcccgagtcgcggccgctgtttctatagtgtc
          1380      1390      1400      1410
```