Supplemental Data

Autosomal recessive retinitis pigmentosa E150K opsin mice exhibit photoreceptor disorganization

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Tadao Maeda, Christel Genoud, Andreas Engel, Vladimir J. Kefalov, and Krzysztof Palczewski

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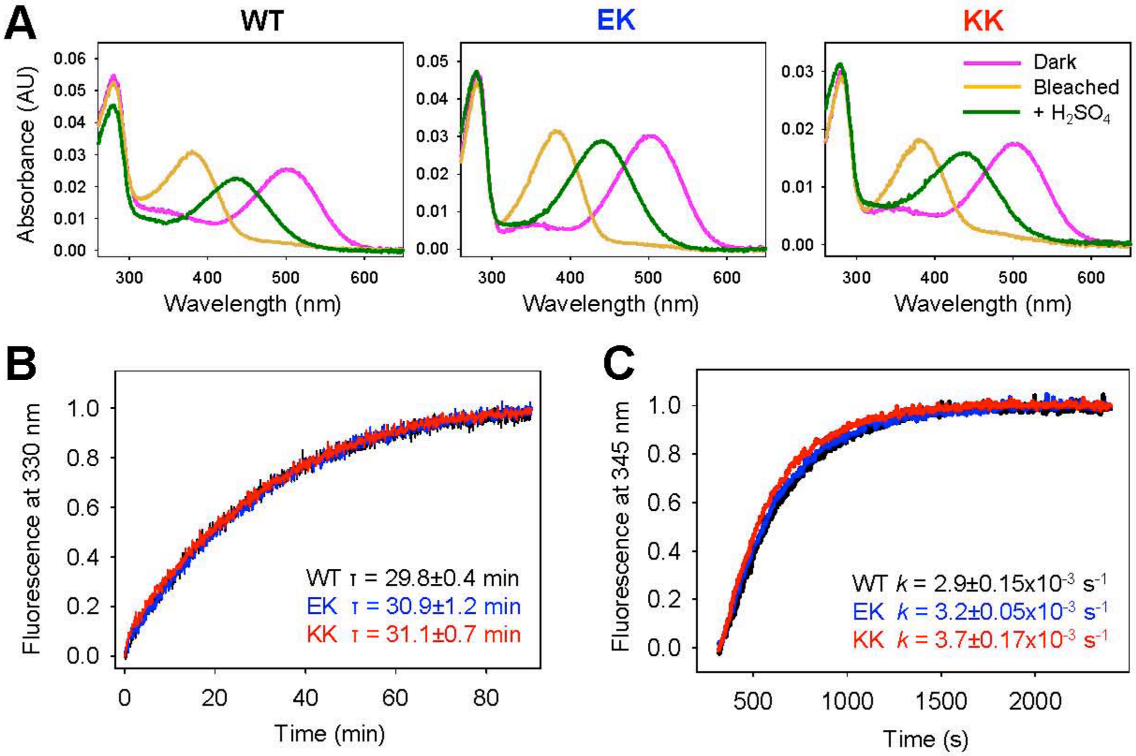

FIGURE S1. **Spectra, Meta II decay and G_t activation of immunoaffinity-purified rhodopsin from P30 WT, EK and KK mice.** **A**, UV-visible absorbance spectra of dark-adapted, photo-bleached and acidified rhodopsin purified from WT, EK or KK mouse eyes. No notable differences were seen in the spectra among WT, EK and KK rhodopsin under these different conditions, but lower amounts of rhodopsin were isolated from KK mouse eyes. **B**, Meta II decay of WT, EK and KK rhodopsin measured by fluorescence emission at 330 nm. Relaxation times (τ) for WT, EK and KK rhodopsin were 29.8, 30.9 and 31.1 min, respectively. **C**, G_t activation by WT, EK and KK rhodopsin monitored by the increase in fluorescence at 345 nm. Calculated initial pseudo first order reaction rates for WT, EK and KK rhodopsin were 2.9, 3.2 and 3.7 $\times 10^{-3}$ s^{-1}, respectively. Measurements were performed twice in triplicate. Data are presented as means ± SDs. The difference observed in transducin activation rates between WT and KK rhodopsin was statistically significant ($p<0.005$).

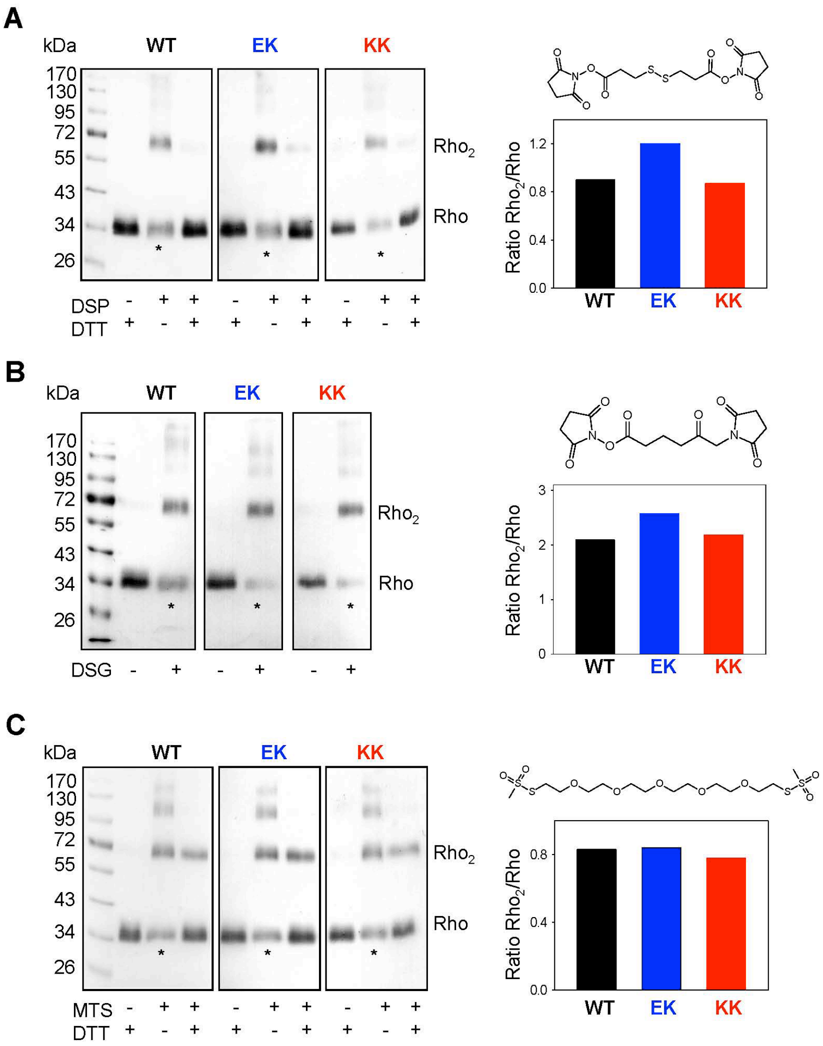

FIGURE S2. **Chemical crosslinking of rhodopsin in retinas isolated from P30 WT, EK and KK mice.** Crosslinking of rhodopsin with reducible DSP (**A**), non-reducible DSG (**B**), or reducible MTS (**C**). Left panels, immunoblots detecting rhodopsin before and after the crosslinking reaction. Covalent linkages formed by either DSP or MTS crosslinkers can be cleaved by reducing agents such as DTT. Right panels, shown are chemical structures of the crosslinkers (*top*) and quantification of the rhodopsin dimer/monomer ratios formed as a result of each crosslinking reaction (*bottom*). Dimer/monomer stoichiometry was calculated based on the relative protein band intensities in lanes on the left marked with (*).

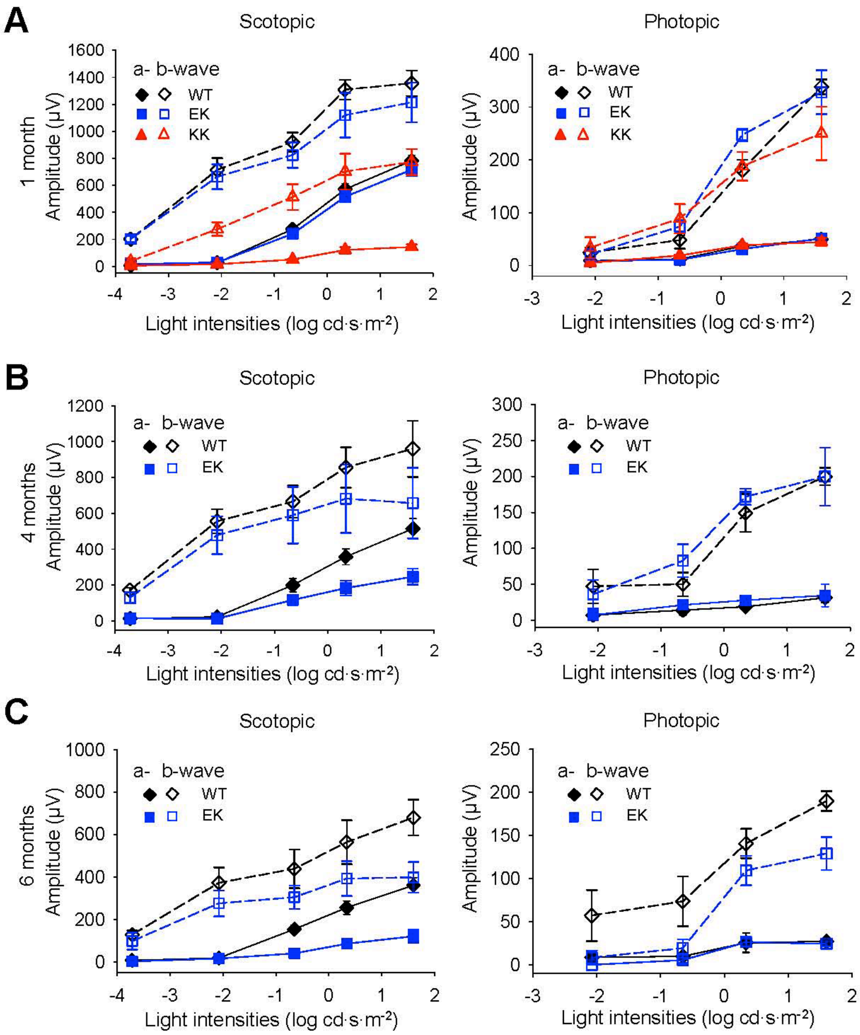

FIGURE S3. **ERG responses of WT, EK and KK mice.** Amplitudes of a- and b-waves under scotopic (*left*) and photopic (*right*) conditions in WT, EK and KK mice at 1-month (**A**), 4-months (**B**) and 6-months (**C**) of age. Amplitudes of rod photoreceptor cell-evoked scotopic a- and b-waves were reduced in KK mice at 1 month of age whereas cone photoreceptor cell-dependent photopic a- and b-wave amplitudes were normal at this age. Because neither scotopic nor photopic ERG responses were detected in KK mice by 4 months of age, ERG data from 4-month- and 6-month-old KK mice are not shown in **B** and **C**. For EK mice relative to WT mice, scotopic a- and b-wave amplitudes started to decrease around 4 months of age and photopic b-wave amplitudes were also decreased by 6-months of age (n > 4).

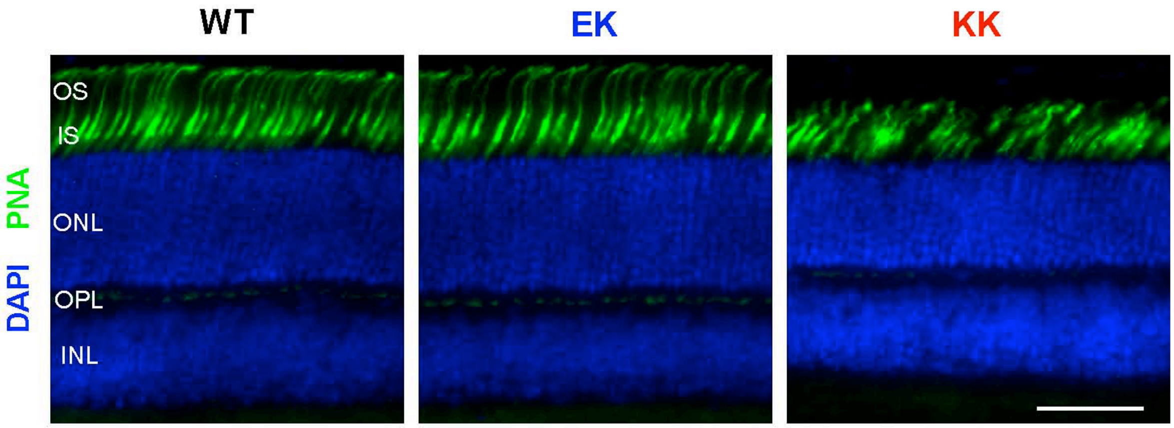

FIGURE S4. **Stained cryo-sections of P30 WT, EK and KK mouse retinas.** Staining of retinas with lectin PNA and DAPI revealed reduced thickness of rod photoreceptor cell layers in KK mouse retina, but the number of cone photoreceptor cells labeled by PNA was not reduced at this age. OS, photoreceptor outer segment; IS, photoreceptor inner segment; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer. Scale bar, 40 μm.

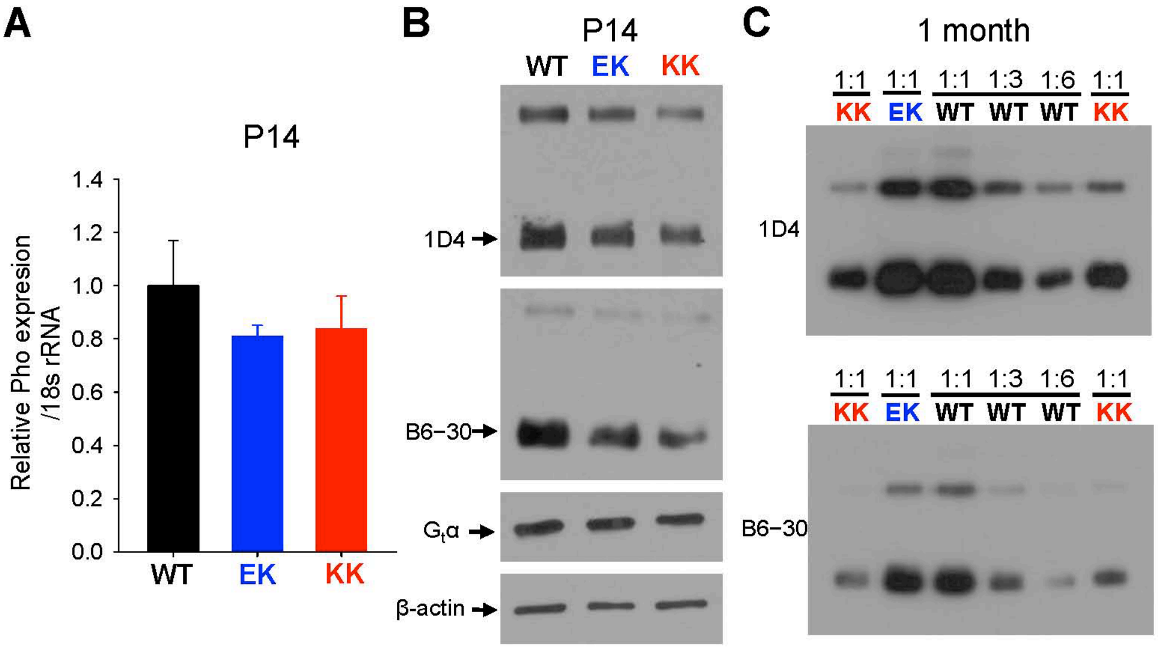

FIGURE S5. **Rhodopsin expression in E150K knock-in mouse retina. A**, Normalized mRNA levels of rhodopsin. Real-time PCR was performed to quantify mRNA levels of rhodopsin relative to 18s rRNA in P14 WT (n = 4), EK (n = 5) and KK (n = 4) mouse retinas. Values, normalized to WT levels for mRNA comparison, represent means ± SEMs analyzed by the Student's *t*-test. No statistically significant differences were noted. **B**, Immunoblot analyses of rhodopsin, probed with either 1D4 or B6-30 antibody, and G_t α-subunit in retinas of P14 WT, EK and KK mice. Equal gel loading is indicated by the β-actin bands. The level of mutant rhodopsin was reduced in the KK mouse, but G_t α-subunit expression remained the same as that in the WT mouse. **C**, Immunoblot analyses of serially diluted retinal extracts from WT mice were compared to those of their EK and KK littermates at 1-month of age. Rhodopsin was probed with either 1D4 or B6-30 antibody. Amounts of rhodopsin in both WT and EK retina were similar but were 3-fold higher than in KK retina.

E150K knock-in mouse generation- sequencing data

RHEK Knock-in Allele – Neo-deleted

1) The short homology arm (~2.1kb) is underlined**.**
2) The location of the G>**A** mutation is indicated in exon 2.
3) The long homology arm (~5.3 kb) is in **bold**.
4) Exons 1-5 are **INDICATED**
5) The sequence (44 bp) remaining after Cre-mediated removal of the Neo cassette is indicated in red text and the remaining loxP site is shown as: ATAACTTCGTATAGCATACATTATACGAAGTTAT
6) Primers used for cloning the whole knock-in locus are indicated:
    a. A4: 5’- GTCTCCATAGCCCATGGTCATCCCTC-3’ (forward primer)
    b. B4: 5’- AGGCAGGAGTAAAGATGACACCCCAC -3’ (reverse primer)
7) Primers used for sequencing the whole knock-in locus are shown below:
    2F: CAAGCCAATTAGGCCCCGGTGG
    3F: AAGGGGCCACGAAAGGGCCT
    4F: ACCACTATGCTACGCCAGGTGT
    5F: AGGGTGTGGCCCTAAGCCCC
    9F: GCCGAGTCCCTAATCCTCGGCT
    10F: TCCCATGCACAAAGTGCAGCA
    11F: AGCAGCAGGAGTCAGCCACCA
    12F: TGTGTCCCCCTCTCCCCAAAG
    14F: CCTCAGGTGCCCAACCTGGC
    15F: GGCCTGTGGAGATCCAGCCCT
    16F: ACAGTCCAGAGTCCCGGGGA
    17F: CAGACCTGTGACCCCTTGTGTCT

>RHEK KI
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CLUSTAL 2.1 multiple sequence alignment


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CLUSTAL 2.1 multiple sequence alignment


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RHEKKI          CTGTCTACGAAGAGCCCGTGGGGCAGCCTCGAGAGCCGCAGCCATGAACGGCACAGAGGG 240
                ************************************************************

2F              CCCCAATTTTTATGTGCCCTTCTCCAACGTCACAGGCGTGGTGCGGAGCCCCTTCGAGCA 251
RHEKKI          CCCCAATTTTTATGTGCCCTTCTCCAACGTCACAGGCGTGGTGCGGAGCCCCTTCGAGCA 300
                ************************************************************

2F              GCCGCAGTACTACCTGGCGGAACCATGGCAGTTCTCCATGCTGGCAGCGTACATGTTCCT 311
RHEKKI          GCCGCAGTACTACCTGGCGGAACCATGGCAGTTCTCCATGCTGGCAGCGTACATGTTCCT 360
                ************************************************************

2F              GCTCATCGTGCTGGGCTTCCCCATCAACTTCCTCACGCTCTACGTCACCGTACAGCACAA 371
RHEKKI          GCTCATCGTGCTGGGCTTCCCCATCAACTTCCTCACGCTCTACGTCACCGTACAGCACAA 420
                ************************************************************

2F              GAAGCTGCGCACACCCCTCAACTACATCCTGCTCAACTTGGCCGTGGCTGACCTCTTCAT 431
RHEKKI          GAAGCTGCGCACACCCCTCAACTACATCCTGCTCAACTTGGCCGTGGCTGACCTCTTCAT 480
                ************************************************************

2F              GGTCTTCGGAGGATTCACCACCACCCTCTACACATCACTCCATGGCTACTTCGTCTTTGG 491
RHEKKI          GGTCTTCGGAGGATTCACCACCACCCTCTACACATCACTCCATGGCTACTTCGTCTTTGG 540
                ************************************************************

2F              GCCCACAGGCTGTAATCTCGAGGGCTTCTTTGCCACACTTGGAGGTATGAGCAGAGAGAC 551
RHEKKI          GCCCACAGGCTGTAATCTCGAGGGCTTCTTTGCCACACTTGGAGGTATGAGCAGAGAGAC 600
                ************************************************************

2F              TGGGGCGGGGGGGTGTAGCATGGGAGCCAAGGGGCCACGAAAGGGCCTGGGAGGGTCTGC 611
RHEKKI          TGGGGCGGGGGGGTGTAGCATGGGAGCCAAGGGGCCACGAAAGGGCCTGGGAGGGTCTGC 660
                ************************************************************

2F              AGCTTACTTGAGTCTCTTTAATTGGTCTCATCTAAAGG---------------------- 649
RHEKKI          AGCTTACTTGAGTCTCTTTAATTGGTCTCATCTAAAGGCCCAGCTTATTCATTGGCAAAC 720
                **************************************



CLUSTAL 2.1 multiple sequence alignment


3F              -----------------------------------TTACTTGAGTCTCTTTAATTGGTCT 25
RHEKKI          AAGGGGCCACGAAAGGGCCTGGGAGGGTCTGCAGCTTACTTGAGTCTCTTTAATTGGTCT 60
                                                   *************************

3F              CATCTAAAGGCCCAGCTTATTCATTGGCAAACACTGTGACCCTGAGCTAGGCTGCTGTTG 85
RHEKKI          CATCTAAAGGCCCAGCTTATTCATTGGCAAACACTGTGACCCTGAGCTAGGCTGCTGTTG 120
                ************************************************************

3F              AGAGCAGGCACGGAACATTCATCTATCTCATCTTGAGCAATGCAAGAAACATGGGTTCAG 145
RHEKKI          AGAGCAGGCACGGAACATTCATCTATCTCATCTTGAGCAATGCAAGAAACATGGGTTCAG 180
                ************************************************************

3F              AGAGGCCAAGGACTCACCGAGGAGTCACAGAGTGTGGGGGTGTCCTCTGAGGCAGCTGAG 205
RHEKKI          AGAGGCCAAGGACTCACCGAGGAGTCACAGAGTGTGGGGGTGTCCTCTGAGGCAGCTGAG 240
                ************************************************************

3F              CTGGGGCACACACAGACTGAGCACCAGGAGTGAGCTCTAGCTTTTTTTTTTCTATGTGTC 265
RHEKKI          CTGGGGCACACACAGACTGAGCACCAGGAGTGAGCTCTAGCTTTTTTTTTTCTATGTGTC 300
                ************************************************************
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3F              TTTTCTAAAAAACACATAGGTTTAGGACTGTCCCTGGTCCAGGTAAAAACTGGTTCAGTA 325
RHEKKI          TTTTCTAAAAGACACATAGGTTTAGGACTGTCCCTGGTCCAGGTAAGAACTGGTTCAGTA 360
                ********** *********************************** *************

3F              AACTTGTACATCTCACTGCCTGGCCAGCCCTGTCAGCTTCCACCAAAGGGCGTGCACTAC 385
RHEKKI          AACTTGTACATCTCACTGCCTGGCCAGCCCTGTCAGCTTCCACCAGAGTGCGTGCACTAC 420
                ********************************************* ** ***********

3F              ACACCCGGCATCTCAAAGGATTCATTCCTATCTTTCCTATCTTTGGAGTGAGGCACAGTC 445
RHEKKI          ACACCCGGCATCTCAAAGGATTCATTCCTATCTTTCCTATCTTTGGAGTGAGGCACAGTC 480
                ************************************************************

3F              TCACGTAGTCCAGTCCAAACTGGCCTTAAATTCTGCAGCTGAGGATGTACTTAAACTTGT 505
RHEKKI          TCACGTAGTCCAGTCCAGACTGGCCTTAAATTCTGCAGCTGAGGATGTACTTAAACTTGT 540
                ***************** ******************************************

3F              CATCCTCCTGCCCCAGCCTCTCAAGTGCTGTGATCACAGGCACGGACCACTATGCTACGC 565
RHEKKI          CATCCTCCTGCCCCAGCCTCTCAAGTGCTGTGATCACAGGCACGGACCACTATGCTACGC 600
                ************************************************************

3F              CAGGGGTTTCCAAACATTTTCTCTCCCTTAACTGGAAGGTCAATGAGGCTCTTTCAAGAA 625
RHEKKI          CAGGTGTTTCCAAACATTTTCTCTCCCTTAACTGGAAGGTCAATGAGGCTCTTTCGAGAA 660
                **** ************************************************** ****

3F              GCAACAAAGCCTGTTTAGCTGA-------------------------------------- 647
RHEKKI          GCAACAGAGCCTGTTTAGCTGAGAAAACTGAGGCAGGGAGCAGGCAAAAATCACATCTAG 720
                ****** ***************


CLUSTAL 2.1 multiple sequence alignment


4F              ------------------------------------------------------------
RHEKKI          TCACAGGCACGGACCACTATGCTACGCCAGGTGTTTCCAAACATTTTCTCTCCCTTAACT 60


4F              -------CAATGAGGCTCTTTCGAGAAGCAACAGAGCCTGTTTAGCTGAGAAAACTGAGG 53
RHEKKI          GGAAGGTCAATGAGGCTCTTTCGAGAAGCAACAGAGCCTGTTTAGCTGAGAAAACTGAGG 120
                       *****************************************************

4F              CAGGGAGCAGGCAAAAATCACATCTAGAGATATGGGAGAGCCAGGACCAGAGCCAGGGTC 113
RHEKKI          CAGGGAGCAGGCAAAAATCACATCTAGAGATATGGGAGAGCCAGGACCAGAGCCAGGGTC 180
                ************************************************************

4F              TCCGGGCTGGGACCTAGAGATGTTTCCAGTGGATACAGGAGGAAACAGAAGTGGGTGTAG 173
RHEKKI          TCCGGGCTGGGACCTAGAGATGTTTCCAGTGGATACAGGAGGAAACAGAAGTGGGTGTAG 240
                ************************************************************

4F              CAAAGCCCAAAGCCAGGGTGATGGGTGGGTCGAGCTTGCTTATCTCCCCGTGTCCAGGGT 233
RHEKKI          CAAAGCCCAAAGCCAGGGTGATGGGTGGGTCGAGCTTGCTTATCTCCCCGTGTCCAGGGT 300
                ************************************************************

4F              ACTGCCTTGGTAGCACTGTTGGGCATCTCTGTTACCTTCTGTGGTCACACACACACACAC 293
RHEKKI          ACTGCCTTGGTAGCACTGTTGGGCATCTCTGTTACCTTCTGTGGTCACACACACACACAC 360
                ************************************************************

4F              ACACACACACACACACACACACACACACATACACACACACACACCCGGGTTCTGGATAAAAG 353
RHEKKI          ACACACACACACACACACACACACACACATACACACACACACACCCAGGTTCTGGATAGAAG 420
                ******************************************** *********** ***

4F              GTGGGGTACCGTGCC--------------------------------------------- 368
RHEKKI          CTGGGGTACCATGCCGGTGAGCTTGTCTCTGTGGGGTCAGACCCAGGCCACATCTACCAT 480
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CLUSTAL 2.1 multiple sequence alignment


5F              --------------------------------------GCAGTCTCTCATGAGCCTAAAG 22
RHEKKI          AGGGTGTGGCCCTAAGCCCCAGCTCCAGGCACTCCGAGGCAGTCTCTCATGAGCCTAAAG 60
                                                      **********************

5F              CTCTAACTGTACATAACTTCGTATAGCATACATTATACGAAGTTATGTGCACAAACAGAA 82
RHEKKI          CTCTAACTGTACATAACTTCGTATAGCATACATTATACGAAGTTATGTGCACAAACAGAA 120
                ************************************************************

5F              GAGCTTCTGTTTTGGCACACGGGTTCTTCACCCCATCCCTTTCTCCTCGCCAGCCCAAAC 142
RHEKKI          GAGCTTCTGTTTTGGCACACGGGTTCTTCACCCCATCCCTTTCTCCTCGCCAGCCCAAAC 180
                ************************************************************

5F              TCACTGCAGTCGCTAAGGCTTGGATCAAGCCTCAAACCAGAAGCTTGCATCCTAGCCTGC 202
RHEKKI          TCACTGCAGTCGCTAAGGCTTGGATCAAGCCTCAAACCAGAAGCTTGCATCCTAGCCTGC 240
                ************************************************************

5F              TCTCTCTGAGGTGAGGTTAGAGCTGGAGGACTGACGGCTACTAACTGCCTTACAGGTGAA 262
RHEKKI          TCTCTCTGAGGTGAGGTTAGAGCTGGAGGACTGACGGCTACTAACTGCCTTACAGGTGAA 300
                ************************************************************

5F              ATCGCCCTGTGGTCCCTGGTGGTCCTGGCCATTGAGCGCTACGTGGTGGTCTGCAAGCCG 322
RHEKKI          ATCGCCCTGTGGTCCCTGGTGGTCCTGGCCATTGAGCGCTACGTGGTGGTCTGCAAGCCG 360
                ************************************************************

5F              ATGAGCAACTTCCGCTTCGGGAAGAATCACGCTATCATGGGTGTGGTCTTCACCTGGATC 382
RHEKKI          ATGAGCAACTTCCGCTTCGGGAAGAATCACGCTATCATGGGTGTGGTCTTCACCTGGATC 420
                ************************************************************

5F              ATGGCGTTGGCCTGTGCTGCTCCCCCACTCGTTGGCTGGTCCAGGTAATGGCACTGAGTA 442
RHEKKI          ATGGCGTTGGCCTGTGCTGCTCCCCCACTCGTTGGCTGGTCCAGGTAATGGCACTGAGTA 480
                ************************************************************

5F              TCGGGTCTGGCAAGGTCTTTGTGGGATTCCCTTTGAGGACACAGAGCCCTCGGATTGGTT 502
RHEKKI          TCGGGTCTGGCAAGGTCTTTGTGGGATTCCCTTTGAGGACACAGAGCCCTCGGATTGGTT 540
                ************************************************************

5F              CCAGGCATAATGTAACATGGTATTGCCCCCCGAAAACCATCCTGGTGACTTTCCCAGGCT 562
RHEKKI          CCAGGCATAATGTAACATGGTATTGCCCCCCGAAAACCATCCTGGTGACTTTCCCAGGCT 600
                ************************************************************

5F              AAGGTCTAAGGTAGGGGAGAAGAGAGGGACTGAATGGTCCAATCAGTCTTATTCCATGTC 622
RHEKKI          AAGGTCTAAGGTAGGGGAGAAGAGAGGGACTGAATGGTCCAATCAGTCTTATTCCATGTC 660
                ************************************************************

5F              TGAGACCCATAACAAGGAGAACCCTGGACATTCCAACCCTTCACCTTGGCCGAGTCCCTA 682
RHEKKI          TGAGACCCATAACAAGGAGAACCCTGGACATTCCAACCCTTCACCTTGGCCGAGTCCCTA 720
                ************************************************************

5F              ATCCTCGGCTAAGCCAAGGCCAAACCACAATCCTCTTTGGTTGAGTTCTGGCCGTGGGCC 742
RHEKKI          ATCCTCGGCTAAGCCAAGGCCAAACCACAATCCTCTTTGGTTGAGTTCTGGCCGTGGGCC 780
                ************************************************************

5F              TCTCTCTCTCTTCCTCTCTCTCTCTCACTCACCTTGGACCTTAGCCCCTGGAGAGGCTGA 802
RHEKKI          TCTCTCTCTCTTCCTCTCTCTCTCTCACTCACCTTGGACCTTAGCCCCTGGAGAGGCTGA 840
                ************************************************************

5F              ACCTTCCCAAAATGCATGGTGACATTGTAGCCCCAGGAACTGG----------------- 845
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RHEKKI          ACCTTCCCAAAATGCATGGTGACATTGTAGCCCCAGGAACTGGGTCCCATCCAGCCTCCA 900
                *******************************************
CLUSTAL 2.1 multiple sequence alignment


9F              ----------------------------------------------------TTGAGTTC 8
RHEKKI          GCCGAGTCCCTAATCCTCGGCTAAGCCAAGGCCAAACCACAATCCTCTTTGGTTGAGTTC 60
                                                                    ********

9F              TGGCCGNGGNCCTNTCTNTCTCTTCCTCTCTCTCTCTCTCACCTTGGACCTTAGCCCC 68
RHEKKI          TGGCCGTGGGCCTCTCTCTCTCTTCCTCTCTCTCTCACTCACCTTGGACCTTAGCCCC 120
                ****** ** *** *** ******************** *********************

9F              TGGAGAGGCTGAACCTTCCCAAAATGCATGGTGACATTGTAGCCCCAGGAACTGGGNCCC 128
RHEKKI          TGGAGAGGCTGAACCTTCCCAAAATGCATGGTGACATTGTAGCCCCAGGAACTGGGTCCC 180
                ******************************************************** ***

9F              ATCCAGCCTCCAGGCCACCATATCTAAATGAGACAATANAAGGTTGGGACAGTGGTTTGG 188
RHEKKI          ATCCAGCCTCCAGGCCACCATATCTAAATGAGACAAGAGAAGGTTGGGACAGTGGTTTGG 240
                ************************************ * *********************

9F              ACACCTANACAGGCTATGAGGTACACAGAGCCTCAGAGACTCTCCATTCTCTGTCCTCAT 248
RHEKKI          ACACCTAGACAGGCTATGAGGTACACAGAGCCTCAGAGACTCTCCATTCTCTGTCCTCAT 300
                ******* ****************************************************

9F              GTCCTCCCCACCCCGGGAGCCCACCCAGTGGCCCTCACTGAGTCAGACCCCCTCACTCCT 308
RHEKKI          GTCCTCCCCACCCCGGGAGCCCACCCAGTGGCCCTCACTGAGTCAGAGCCCCTCACTCCT 360
                *********************************************** ************

9F              CACTGGCCTCTTTCCTCATCCTCNCCCACCTGGCTTGGCAGGTCTCTACAGACACACTCA 368
RHEKKI          CACTGGCCTCTTTCCTCATCCTCACCCACCTGGCTTGGCAGGCCTCTACAGACACACTCA 420
                *********************** ****************** *****************

9F              GTGGACACTTGGGTTTCTGAGTGTGGCCCAGTGTCACCGTCCTCATATATCATCACAACA 428
RHEKKI          GTGGACACTTGGGTTTCTGAGTGTGGCCCAGTGTCACCGTCCTCAGATATCATCACAACA 480
                ********************************************* **************

9F              TCCTTGTTTCTAGAAGCTGCACACAGCCCTGATGCCCGCAG------------------- 469
RHEKKI          TCCTTGTTTCTAGAAGCTGCACACAGCCCTGATGCCAGCAGCGAGCCCACCTTTACTGTA 540
                ************************************ ****


CLUSTAL 2.0.12 multiple sequence alignment


10F             ---------------------------------------------GCTCCTCTCTCCAGC 15
RHEKKI          TCCCATGCACAAAGTGCAGCATTCCCAGGGAAGGCCTCAGAGAATGCTCCTCTCTCCAGC 60
                                                             ***************

10F             ATTCTCTGCCTACTCCCTTAACCACCGAAGGCAGGGCAGCAGGCTAGTGGAGCAGAGCTG 75
RHEKKI          ATTCTCTGCCTACTCCCTTAACCACCGAAGGCAGGGCAGCAGGCTAGTGGAGCAGAGCTG 120
                ************************************************************

10F             CGTGGTCAAGTGGCAGGGAGCTTAAGAATCGTCCAAGGGCGGAGACCAGTAAGTCTCATT 135
RHEKKI          CGTGGTCAAGTGGCAGGGAGCTTAAGAATCGTCCAAGGGCGGAGACCAGTAAGTCTCATT 180
                ************************************************************

10F             AGGTGATGGGGCCAGCAGGTAAAAGCCATTCATGCTTATGTCCAGCTGGGCGTGTGTTCT 195
RHEKKI          AGGTGATGGGGCCAGCAGGTAAAAGCCATTCATGCTTATGTCCAGCTGGGCGTGTGTTCT 240
                ************************************************************

10F             CTTCCTGTTTTATCATCCCTTGCGCTGACCATCAGGTACATCCCTGAGGGCATGCAATGT 255
RHEKKI          CTTCCTGTTTTATCATCCCTTGCGCTGACCATCAGGTACATCCCTGAGGGCATGCAATGT 300
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                ************************************************************

10F             TCATGCGGGATTGACTACTACACACTCAAGCCTGAGGTCAACAACGAATCCTTTGTCATC 315
RHEKKI          TCATGCGGGATTGACTACTACACACTCAAGCCTGAGGTCAACAACGAATCCTTTGTCATC 360
                ************************************************************

10F             TACATGTTCGTGGTCCACTTCACCATTCCTATGATCGTCATCTTCTTCTGCTATGGGCAG 375
RHEKKI          TACATGTTCGTGGTCCACTTCACCATTCCTATGATCGTCATCTTCTTCTGCTATGGGCAG 420
                ************************************************************

10F             CTGGTCTTCACAGTCAAGGAGGTATGAGCAGGGGGGCCGCCCCAGCCTCGTGCCGGGTGG 435
RHEKKI          CTGGTCTTCACAGTCAAGGAGGTATGAGCAGGGGGGCCGCCCCAGCCTCGTGCCGGGTGG 480
                ************************************************************

10F             GTGGGCAGAGCCAGGTGGGCAGAGCTGGGTGCCAGGGTTCGTACAGACGCCATGGGCTAG 495
RHEKKI          GTGGGCAGAGCCAGGTGGGCAGAGCTGGGTGCCAGGGTTCGTACAGACGCCATGGGCTAG 540
                ************************************************************

10F             ACACAGGTCTGTGTCCCTGCAGGCGGCTGCCCAGCAGCAGGAGTCAGCCACCACTCAGAA 555
RHEKKI          ACACAGGTCTGTGTCCCTGCAGGCGGCTGCCCAGCAGCAGGAGTCAGCCACCACTCAGAA 600
                ************************************************************
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CLUSTAL 2.1 multiple sequence alignment


11F             -----------------------------------------------CGCATGGTTATCA 13
RHEKKI          AGCAGCAGGAGTCAGCCACCACTCAGAAGGCAGAGAAGGAAGTCACCCGCATGGTTATCA 60
                                                               *************

11F             TCATGGTCATCTTCTTCCTGATCTGCTGGCTTCCCTACGCCAGTGTGGCCTTCTACATCT 73
RHEKKI          TCATGGTCATCTTCTTCCTGATCTGCTGGCTTCCCTACGCCAGTGTGGCCTTCTACATCT 120
                ************************************************************

11F             TCACCCACCAGGGCTCCAACTTCGGCCCCATCTTCATGACTCTGCCAGCTTTCTTTGCTA 133
RHEKKI          TCACCCACCAGGGCTCCAACTTCGGCCCCATCTTCATGACTCTGCCAGCTTTCTTTGCTA 180
                ************************************************************

11F             AGAGCTCTTCCATCTATAACCCGGTCATCTACATCATGTTGAACAAGCAGGTGCCTGGGC 193
RHEKKI          AGAGCTCTTCCATCTATAACCCGGTCATCTACATCATGTTGAACAAGCAGGTGCCTGGGC 240
                ************************************************************

11F             TGAGGCAGGGCGTGTGGAGAGTGAGGGAGAGAAGGGGAGGGGGAGAATGGGACAGGGGAG 253
RHEKKI          TGAGGCAGGGCGTGTGGAGAGTGAGGGAGAGAAGGGGAGGGGGAGAATGGGACAGGGGAG 300
                ************************************************************

11F             GCATTGCACTCAGACTGCTGGCAGCCCGTGAGCTCTCAGGCTGGCGAACCCCCAAGCTCT 313
RHEKKI          GCATTGCACTCAGACTGCTGGCAGCCCGTGAGCTCTCAGGCTGGCGAACCCCCAAGCTCT 360
                ************************************************************

11F             TGGAGTAAGTCTTCCGGGTGACCTGCCGTCCACTCCTGTGAGATACAGCCGACCAAATGG 373
RHEKKI          TGGAGTAAGTCTTCCGGGTGACCTGCCGTCCACTCCTGTGAGATACAGCCGACCAAATGG 420
                ************************************************************

11F             TTCTGGAAGTCTGCTTTAGAAGTAGGCTAGAAGTCAGGTGCAATAGTGCAAATTTGTAAT 433
RHEKKI          TTCTGGAAGTCTGCTTTAGAAGTAGGCTAGAAGTCAGGTGCAATAGTGCAAATTTGTAAT 480
                ************************************************************

11F             CTTAATTCTGAGGCAGGAGGATCTTGAGCTCCAGGTCAGCCTGGGCTAAACAGTGGATGT 493
RHEKKI          CTTAATTCTGAGGCAGGAGGATCTTGAGCTCCAGGTCAGCCTGGGCTAAACAGTGGATGT 540
                ************************************************************
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11F             GTGTCCCCCTCTCCCCAAAGCAGGCTGTGAAGCACACTGCTATATCCTAGCATTCAGGAG 553
RHEKKI          GTGTCCCCCTCTCCCCAAAGCAGGCTGTGAAGCACACTGCTATATCCCAGCATTCAGGAG 600
                *********************************************** ************

11F             ACTGAAGTAGGTCGATTAGAAATTCAAAGCTATCTTTGGCCATAC---- 598
RHEKKI          ACTGAAGTAGGTCGATTAGAAATTCAAAGCTATCTTTGGCCATACAGTG 649
                *********************************************

CLUSTAL 2.1 multiple sequence alignment


12F             ------------------------------------------------------------
RHEKKI          TGTGTCCCCCTCTCCCCAAAGCAGGCTGTGAAGCACACTGCTATATCCCAGCATTCAGGA 60


12F             ---TGAAGTAGGTCGATTAGAAATTCAAAGCTATCTTTGGCCATACAGTGATTTGGGAGC 57
RHEKKI          GACTGAAGTAGGTCGATTAGAAATTCAAAGCTATCTTTGGCCATACAGTGATTTGGGAGC 120
                   *********************************************************

12F             CAACCTGGGCTACAATGAGAATCTGTCTCAAGAAGCAACCAGAAATATCAAGTAGTTCCA 117
RHEKKI          CAACCTGGGCTACAATGAGAATCTGTCTCAAGAAGCAACCAGAAATATCAAGTAGTTCCA 180
                ************************************************************

12F             AGTCTGGGAATAGAATGGTCTCACTGTAAAGCTGAAGAAATTGCTAGAAATGCTACTTGT 177
RHEKKI          AGTCTGGGAATAGAATGGTCTCACTGTAAAGCTGAAGAAATTGCTAGAAATGCTACTTGT 240
                ************************************************************

12F             AGGAAGTCAGGGATTACACCACTGTCAAACGCCACAGTCTCTCTGCAGAAAAGGATGTGG 237
RHEKKI          AGGAAGTCAGGGATTACACCACTGTCAAACGCCACAGTCTCTCTGCAGAAAAGGATGTGG 300
                ************************************************************

12F             TCTCTGGAACCTAGACTCTGGGTTCAAGTTCTAGTTCTGTCCCATTTAAGCTGTGTGACC 297
RHEKKI          TCTCTGGAACCTAGACTCTGGGTTCAAGTTCTAGTTCTGTCCCATTTAAGCTGTGTGACC 360
                ************************************************************

12F             TTGGGTGAGTCAGATATCTCCCCATGTGGACTTCGGTTTTCCTATTTATAGGAAGGGCCA 357
RHEKKI          TTGGGTGAGTCAGATATCTCCCCATGTGGACTTCGGTTTTCCTATTTATAGGAAGGGCCA 420
                ************************************************************

12F             AATCATAAACTCTCAAATCAATTGAGATCACTCACGGAAGGCCCTACGCATCCACAGGAC 417
RHEKKI          AATCATAAACTCTCAAATCAATTGAGATCACTCACGGAAGGCCCTACGCATCCACAGGAC 480
                ************************************************************

12F             ACATGGAATGCCACTTGACAATACCCCTTGTGCTATCTGTGTACCTGGCGTCTCTGGTTG 477
RHEKKI          ACATGGAATGCCACTTGACAATACCCCTTGTGCTATCTGTGTACCTGGCGTCTCTGGTTG 540
                ************************************************************

12F             AAGGGCAACATACATTTAGCAAGCTCCCCCAGGGAGCCAGGCTTAGTGAGGGGACATGCT 537
RHEKKI          AAGGGCAACATACATTTAGCAAGCTCCCCCAGGGAGCCAGGCTTAGTGAGGGGACATGCT 600
                ************************************************************

12F             GGAGGTGAGGCTGAAGCTGGGCAGGTGGTAGGGCCTGTCCTGACTGGAGCCTCTT----- 592
RHEKKI          GGAGGTGAGGCTGAAGCTGGGCAGGTGGTAGGGCCTGTCCTGACTGGAGCCTCTTGCCTT 660
                *******************************************************



CLUSTAL 2.1 multiple sequence alignment


14F             --------------------------------------------TCTATAAGCAGTTTTA 16
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RHEKKI          CCTCAGGTGCCCAACCTGGCAGATCCAGTCATGTCTGGCTGGAATCTATAAGCAGTTTTA 60
                                                            ****************

14F             CATACCTGCCCTGGTTTTCTCTGCCCCCACCCCCACCCCAGTTGGATCTCCCAAATCCAG 76
RHEKKI          CATACCTGCCCTGGTTTTCTCTGCCCCCACCCCCACCCCAGTTGGATCTCCCAAATCCAG 120
                ************************************************************

14F             GGCCCTGATAGAATATGGCTGCTTCAAAGACAGAGAGATGAGGGGAGGGAGGGGGGAGGG 136
RHEKKI          GGCCCTGATAGAATATGGCTGCTTCAAAGACAGAGAGATGAGGGGAGGGAGGGGGGAGGG 180
                ************************************************************

14F             AGAGAGGGAGGGAGGGAGACACAGAGAGGGAATATGTGTGATGCGTGTGTATGTGTGTAT 196
RHEKKI          AGAGAGGGAGGGAGGGAGACACAGAGAGGGAATATGTGTGATGCGTGTGTATGTGTGTAT 240
                ************************************************************

14F             GTGTGTGTGTAAACACTTTGTATATAAAGAGTACAGCTGGTAGTTATGTTACAAGTAACA 256
RHEKKI          GTGTGTGTGTAAACACTTTGTATATAAAGAGTACAGCTGGTAGTTATGTTACAAGTAACA 300
                ************************************************************

14F             CCGACTAATATAATTAATTAACCATCCTAATGGTCTCTGCTTGTTAGTGACTGCTTGGGA 316
RHEKKI          CCGACTAATATAATTAATTAACCATCCTAATGGTCTCTGCTTGTTAGTGACTGCTTGGGA 360
                ************************************************************

14F             ATTAAGCAGGGCCCAAGCACTCAGATAAGGTATTTCCCTCAGCCTCAGTAGGCTTTTGCA 376
RHEKKI          ATTAGGCAGGGCCCAAGCACTCAGATAAGGTATTTCCCTCAGCCTCAGTAGGCTTTTGCA 420
                **** *******************************************************

14F             AATGACCCAGGCCTTCAGGCCTGTGCAGGGCTAGAGCTGGATTACAGAGATAAATGACAG 436
RHEKKI          AATGACCCAGGCCTTCAGGCCTGTGCAGGGCTAGAGCTGGATTACAGAGATAAATGACAG 480
                ************************************************************

14F             TGACAGCAACGTGAGCTGCAGCCCTTAGGACTGAAAAAGCATCGAGACCAGGGGTCTCCG 496
RHEKKI          TGACAGCAACGTGAGCTGCAGCCCTTAGGACTGAGAAAGCATCGAGACCAGGGGTCTCCG 540
                ********************************** *************************

14F             GCAAGGCCTAGGTCCTCCCTTCAGTATGGAAACCTTGCCTCATGTCTCTCAGCCTCCTTG 556
RHEKKI          GCAAGGCCTAGGTCCTCCCTTCAGTATGGAAACCTTGCCTCATGTCTCTCAGCCTCCTTG 600
                ************************************************************

14F             GCCTGTGGAGATCCAGCCCTTCCTCTTGGCTTCTGGATACATTTGCTCTTCTACACCAGC 616
RHEKKI          GCCTGTGGAGATCCAGCCCTTCCTCTTGGCTTCTGGATACATTTGCTCTTCTACACCAGC 660
                ************************************************************

14F             AACCAAGTGGCAACAGTTCCAGGCCAGTATGGAGTTTTAGAAGCCATGCCAATATGCCCA 676
RHEKKI          AACCAAGTGGCAACAGTTCCAGGCCAGTATGGAGTTTTAGAAGCCATGCCAATATGCCCA 720
                ************************************************************

14F             CCTTCAGGGAGCA----------------------------------------------- 689
RHEKKI          CCTTCAGGGAGCAGCTGAGTCCTTGATGCCACCCTTGTTCTGAAGAGTTCAGAAACACAG 780
                *************

CLUSTAL 2.1 multiple sequence alignment

15F             ------------AACAGTTCCAGGCCAGTATGGAGTTTTTTGAAGCCATGCCAATATGCC 48
RHEKKI          CAACCAAGTGGCAACAGTTCCAGGCCAGTATGGAGTTTTA-GAAGCCATGCCAATATGCC 119
                            ***************************  *******************

15F             CACCTTCAGGGAGCAGCTGAGTCCTTGATGCCACCCTTGTTCTGAAGACTTCCGAAACAC 108
RHEKKI          CACCTTCAGGGAGCAGCTGAGTCCTTGATGCCACCCTTGTTCTGAAGAGTTCAGAAACAC 179
                ************************************************ *** *******

15F             AGTGCAAGACATGACCAGGCCTCATCCTTAGGATGCTCATGGATCCAGTTCTTAGCTCCC 168
RHEKKI          AGTGCAAGACATGACCAGGCCTCATCCTTAGGATGCTCATGGATCCAGTTCTTAGCTCCC 239
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                ************************************************************

15F             TTGTTGGATATGCTGTTTTCCTTGGCCTTTGGNCTTTTCTTTATCCCATAGGGTTTTGGC 228
RHEKKI          TTGTTGGATATGCTGTTTTCCTTGGCCTTTGGTCTTTTCTTTATCCCAGAGGGTTTTGGC 299
                ******************************** *************** ***********

15F             TTTAAGGCCAACAGGAACTATGGGGTACCAGAATTGAGCAGCCTCAGTCTGCATCCCTCC 288
RHEKKI          TTTAAGGCCAACAGGAACTATGGGGTACCAGAATTGAGCAGCCTCAGTCTGCATCCCTCC 359
                ************************************************************

15F             TCTATAGAACCACAGCTGGGCCCTCATCAGGCCCAACTCTGCATGGGGACAGAGGCATTA 348
RHEKKI          TCTATAGAACCACAGCTGGGCCCTCAGCAGGCCCAACTCTGCATGGGGACAGAGGCATTA 419
                ************************** *********************************

15F             AAAGCTCAGCTCCTACACTTGGTGGCAGTGGTGGTCTGTTGCTCTCAAGCTCTTTCAAAA 408
RHEKKI          AAAGCTCAGCTCCTACACTTGGTGGCAGTGGTGGTCTGTTGCTCTCAAGCTCTTTCAAAA 479
                ************************************************************

15F             TGGATGGAAACTGGGACGCTTCCCTGACCCCTGGTTATGAAAGACTAGACTGTGTGGGGA 468
RHEKKI          TGGATGGAAACTGGGACGCTTCCCTGACCCCTGGTTATGAAAGACTAGACTGTGTGGGGA 539
                ************************************************************

15F             CAAACAGTCC-------------------------------------------------- 478
RHEKKI          CAAACAGTCCAGAGTCCCGGGGAATGTGATAGAGCAGCTCCATCATTTTTAGAAACCCAA 599
                **********

CLUSTAL 2.1 multiple sequence alignment

16F             ------------------------------------TCATCATTTTTAGAA-CCCAATTT 23
RHEKKI          ACAGTCCAGAGTCCCGGGGAATGTGATAGAGCAGCTCCATCATTTTTAGAAACCCAATTT 60
                                                     ************** ********

16F             GAGGCAGTATAGAGAGATGGTGACCTCTATAAGCCTCTGTATCTGCAAAGAGGAGCTTAG 83
RHEKKI          GAGGCAGTATAGAGAGATGGTGACCTCTATAAGCCTCTGTATCTGCAAAGAGGAGCTTAG 120
                ************************************************************

16F             ACCTGCCCTTGAGGGGATTATATGAGATTTAAGGGACTTATGTGGCCAGCCTACTTCCTG 143
RHEKKI          ACCTGCCCTTGAGGGGATTATATGAGATTTAAGGGACTTATGTGGCCAGCCTACTTCCTG 180
                ************************************************************

16F             GCATGCTGAAGACATTGGCACACTCTGGTATTCTAGACCTTGGCTCAGAGCTGCCTTTAC 203
RHEKKI          GCATGCTGAAGACATTGGCACACTCTGGTATTCTAGACCTTGGCTCAGAGCTGCCTTTAC 240
                ************************************************************

16F             TAGGATACTGTCACTTAGCAAAAGAATGGGATGGAGCCTCAGATGTGGAGTGACACCATC 263
RHEKKI          TAGGATACTGTCACTTAGCAAAAGAATGGGATGGAGCCTCAGATGTGGAGTGACACCATC 300
                ************************************************************

16F             TTCCAAGAAGGAAAGGGTGCCAGGGTCTGGGATGAAAGCCCCTTTGGTGCTATGTTGGGCA 323
RHEKKI          TTCCAAGAAGGAAAGGGTGCCAGGGTCTGGGATGAAAGCCCCTTTGGTGCTATGTTGGGCA 360
                ************************************************************

16F             AGGGCGAGTGCCAGCAAGGGGTTATTTGCTTGCTCTCTCCATCAGTGATGAGGTTCCATT 383
RHEKKI          AGGGCGAGTGCCAGCAAGGGGTTATTTGCTTGCTCTCTCCATCAGTGATGAGGTTCCATT 420
                ************************************************************

16F             TGGTCACAAGAAATTCACCCCAATTGCTGAAACAGAGGCTGACTATTGGCTTATAGGCAT 443
RHEKKI          TGGTCACAAGAAATTCACCCCAATTGCTGAAACAGAGGCTGACTATTGGCTTATAGGCAT 480
                ************************************************************

16F             GAAACCCCACTCCCCTCCACTTCAGGCTGGCTAGATTAAAAGCTCAGACCTGTGACCCCT 503
RHEKKI          GAAACCCCACTCCCCTCCACTTCAGGCTGGCTAGATTAAAAGCTCAGACCTGTGACCCCT 540
                ************************************************************
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16F             TGTGTCTCTCTTCTGTGTTGACTTCACTCACAGGCCTAACCTCAGAGGTGTCTGTAGCAG 563
RHEKKI          TGTGTCTCTCTTCTGTGTTGACTTCACTCACAGGCCTAACCTCAGAGGTGTCTGTAGCAG 600
                ************************************************************

16F             TCCCAGGGCCAGACACCCTTCCAGAAAGGGGCTTTCCTTCCCTTTATAATCGTGGTGACA 623
RHEKKI          TCCCAGGGCCAGACACCCTTCCAGAAAGGGGCTTTCCTTCCCTTTATAATCGTGGTGACA 660
                ************************************************************

16F             GGTCAGATTCTTATTGGGCAGACCTAAGTTACCTGATTATCCTTAAGCCAATCATCATGT 683
RHEKKI          GGTCAGATTCTTATTGGGCAGACCTAAGTTACCTGATTATCCTTAAGCCAA--------- 711
                ***************************************************

CLUSTAL 2.1 multiple sequence alignment

17F             ----------------------------------------------CAGGCCTAACCTCA 14
RHEKKI          CAGACCTGTGACCCCTTGTGTCTCTCTTCTGTGTTGACTTCACTCACAGGCCTAACCTCA 60
                                                              **************

17F             GAGGTGTCTGTAGCAGTCCCAGGGCCAGACACCCTTCCAGAAAGGGGCTTTCCTTCCCTT 74
RHEKKI          GAGGTGTCTGTAGCAGTCCCAGGGCCAGACACCCTTCCAGAAAGGGGCTTTCCTTCCCTT 120
                ************************************************************

17F             TATAATCGTGGTGACAGGTCAGATTCTTATTGGGCAGACCTAAGTTACCTGATTATCCTT 134
RHEKKI          TATAATCGTGGTGACAGGTCAGATTCTTATTGGGCAGACCTAAGTTACCTGATTATCCTT 180
                ************************************************************

17F             AAGCCAATCATCATGTCTAAGACAGAGATTCATTTGATTGGCCAGGGCTGAGTCACTTGT 194
RHEKKI          AAGCCAATCATCATGTCTAAGACAGAGATTCATTTGATTGGCCAGGGCTGAGTCACTTGT 240
                ************************************************************

17F             TTACCTCTAAGTCAATCAACAGGCCTGAGATGGAGAGTCATCTTCTGATTAGCTAGACCT 254
RHEKKI          TTACCTCTAAGTCAATCAACAGGCCTGAGATGGAGAGTCATCTTCTGATTAGCTAGACCT 300
                ************************************************************

17F             GGGTCACCTGATCACCTCTAAGCCAACCACTATGTCTGATATAGGGCTCCCTCATTTGTA 314
RHEKKI          GGGTCACCTGATCACCTCTAAGCCAACCACTATGTCTGATATAGGGCTCCCTCATTTGTA 360
                ************************************************************

17F             ATGTGTTCCCACACTACAAGATAAAGTTGACTCCACATAGTTAAGGAGCCTGTAGGATGT 374
RHEKKI          ATGTGTTCCCACACTACAAGATAAAGTTGACTCCACATAGTTAAGGAGCCTGTAGGATGT 420
                ************************************************************

17F             GTTCCCCAAAAGCAAACAGGGGAACAGGATCAGGACCAGCCCTGAGGGGTGGGGACTGGG 434
RHEKKI          GTTCCCCAAAAGCAAACAGGGGAACAGGATCAGGACCAGCCCTGAGGGGTGGGGACTGGG 480
                ************************************************************

17F             ATGTGCTAGACAAGGCTCCGGGGTGTCATGGATGAAGGGGATTCCATGGCTGCCACACCT 494
RHEKKI          ATGTGCTAGACAAGGCTCCGGGGTGTCATGGATGAAGGGGATTCCATGGCTGCCACACCT 540
                ************************************************************

17F             CTGGTTGTCTCTCATGCTTTATGGGCCAGGAGTGCCTGTTGGAAGGCTACTTTAGCTGCC 554
RHEKKI          CTGGTTGTCTCTCATGCTTTATGGGCCAGGAGTGCCTGTTGGAAGGCTACTTTAGCTGCC 600
                ************************************************************

17F             TGGCCTTGACCTCCCTCACTCCTCCATGCCCAGTTCACTTGTCCTGTGGGGTGTCATCTT 614
RHEKKI          TGGCCTTGACCTCCCTCACTCCTCCATGCCCAGTTCACTTGTCCTGTGGGGTGTCATCTT 660
                ************************************************************

17F             TACTTCCTGC-- 624
RHEKKI          TACT-CCTGCCT 671
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