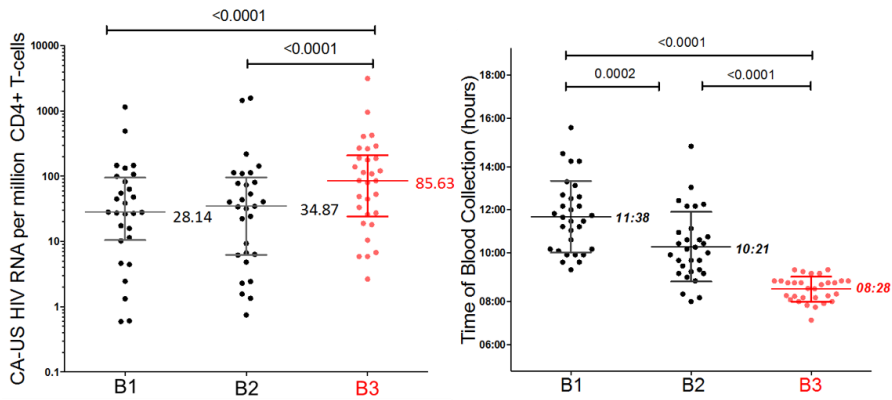


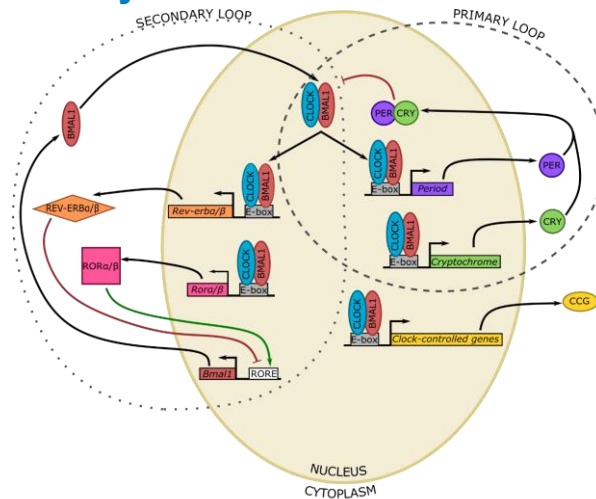
Preliminary Findings – Disulfiram Clinical Trial



N=30 participants in a randomised clinical trial. B1 = screening; B2 = between screening and enrolment; B3 = day of administration of disulfiram

Elliott et al., Lancet HIV 2015

Circadian Cycles

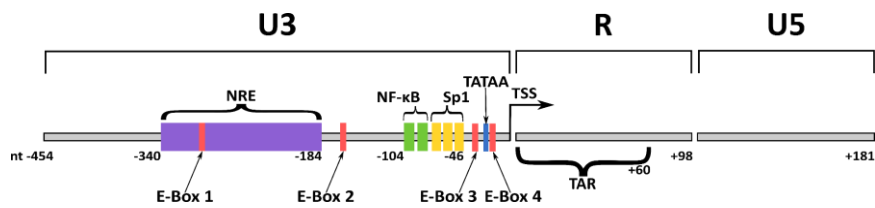


- CLOCK and BMAL1 heterodimers bind to an E-box to drive transcription
- Control is mediated through *Per* and *Cry* genes

Gekakis et al., Science 1998; Hogenesch et al., PNAS 1998; Kume et al., Cell 1999; Jin et al., Cell 1999

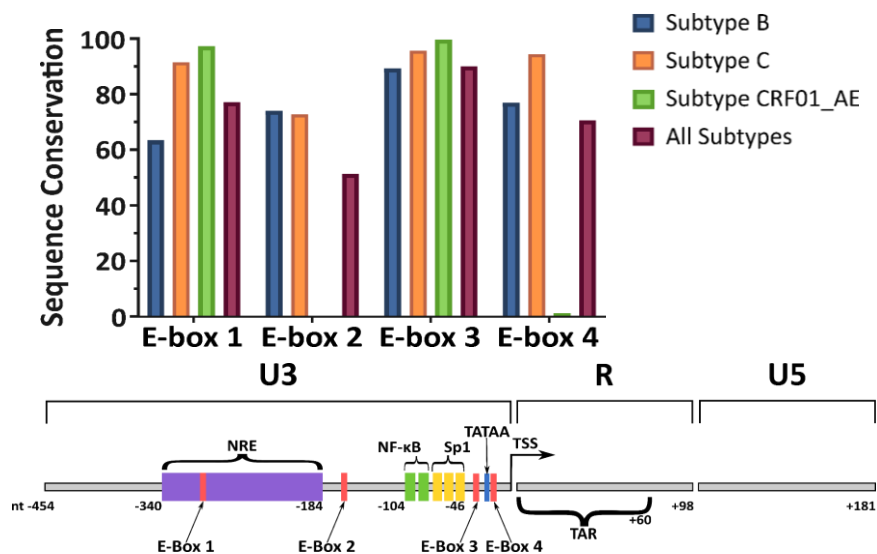
HIV and the Circadian Cycle

- CLOCK/BMAL1 has been found to bind to LTR
- Knockdown of CLOCK led to a log decrease in HIV infectivity



Terme et al., *Retrovirology* 2009; Tacheny et al., *Nucleic Acids Res.* 2012; Konig et al., *Cell* 2008

Sequence Conservation in the Long Terminal Repeat



Hypothesis

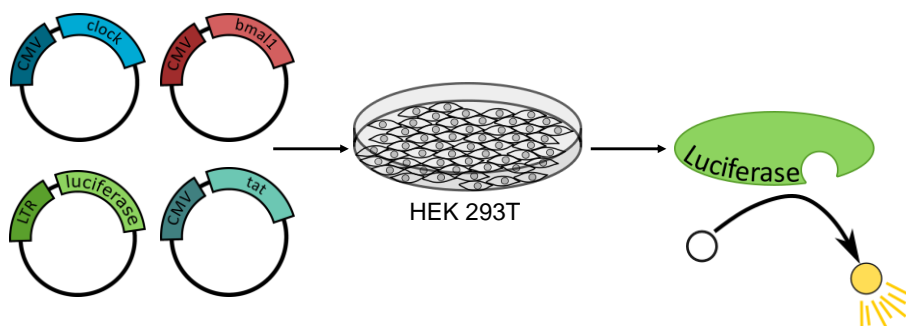
- In latently infected cells, HIV is under transcriptional control by the cell-autonomous circadian cycle.

Aims

- To determine whether the circadian proteins CLOCK and BMAL1 bind to the the HIV LTR to activate latent infection
- To exploit the circadian cycle of latently infected cells to identify new drug targets and optimise potency of other latency reversing agents

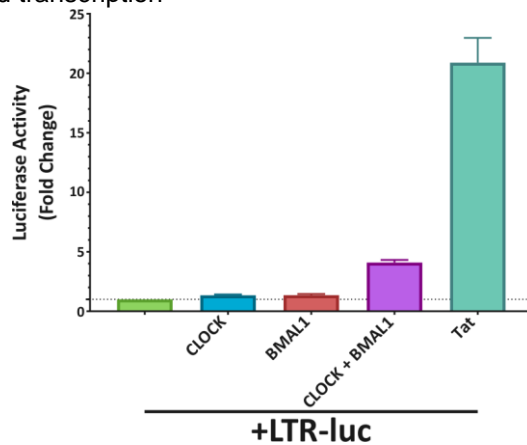
Effect of CLOCK/BMAL1 on HIV Transcription

- Genes encoding the human circadian transcription factors, CLOCK and BMAL1, were cloned into a pcDNA3.1 expression vector
- These proteins' effect on HIV transcription were assessed using an LTR-driven luciferase reporter plasmid (LTR-luc)

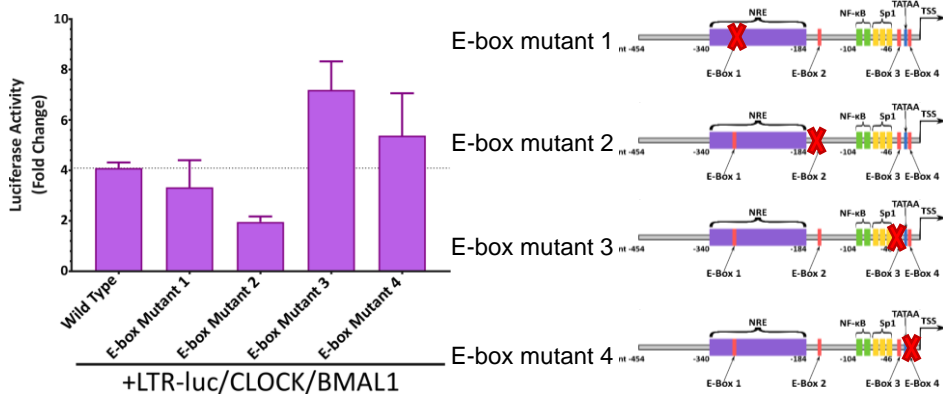


CLOCK/BMAL1 Together Increase LTR Activity

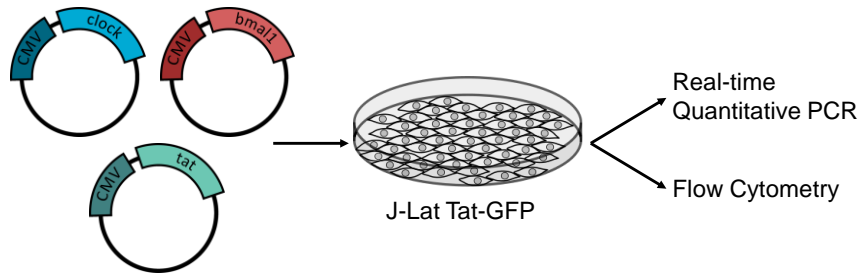
- CLOCK and BMAL1 alone had little effect on LTR-driven transcription
- CLOCK and BMAL1 together resulted in a 4.09-fold increase in LTR-mediated transcription



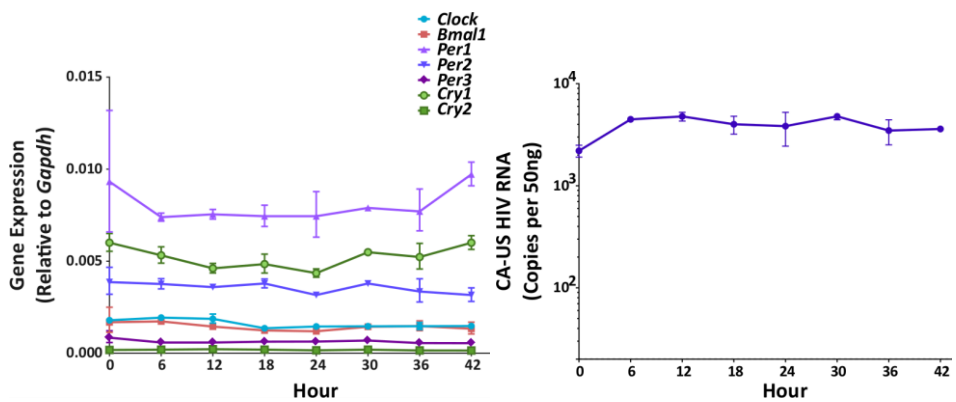
CLOCK/BMAL1 interact with at least one E-box



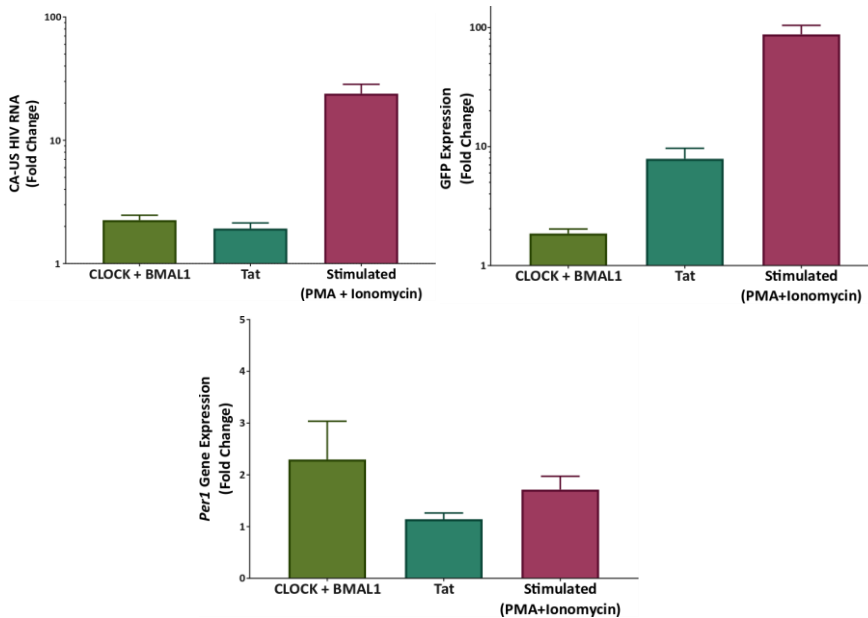
Method: Nucleofection of CLOCK and BMAL1



J-Lat Tat-GFP Cell Lines Lack Circadian Cycling in vitro



CLOCK:BMAL1 Activate Proviral Transcription in vitro



Summary

- We observed a relationship between time and cell associated unspliced HIV RNA in individuals living with HIV on ART
- The circadian transcription factors, CLOCK and BMAL1, upregulate HIV LTR-mediated transcription 2-7 times above basal transcription using reporter cell lines
- This upregulation required at least one E-box motif
- These results suggest that latent HIV transcription is controlled by circadian rhythms and this presents a novel pathway to exploit for latency reversal

Future directions

- Knock out multiple E-boxes simultaneously to determine if redundancy exists
- Demonstrate an effect in primary T-cells from HIV-positive individuals on ART
- Determine the effects of circadian-altering drugs on activation of latent HIV infection
- Determine the interaction of CLOCK/BMAL1 on the effects of other latency reversing agents (LRA) which would have implications for the best time of day to administer an LRA

Acknowledgements

The Peter Doherty
Institute for Infection
and Immunity

Sharon Lewin
Paul Cameron
Michael Roche
Carolin Tumpach
Ajantha Rhodes
Talia Mota
Youry Kim
Renee van der Sluis
Judy Chang
Paula Cevaal

RMIT University

Melissa Churchill
Wan-jung Chen



With thanks

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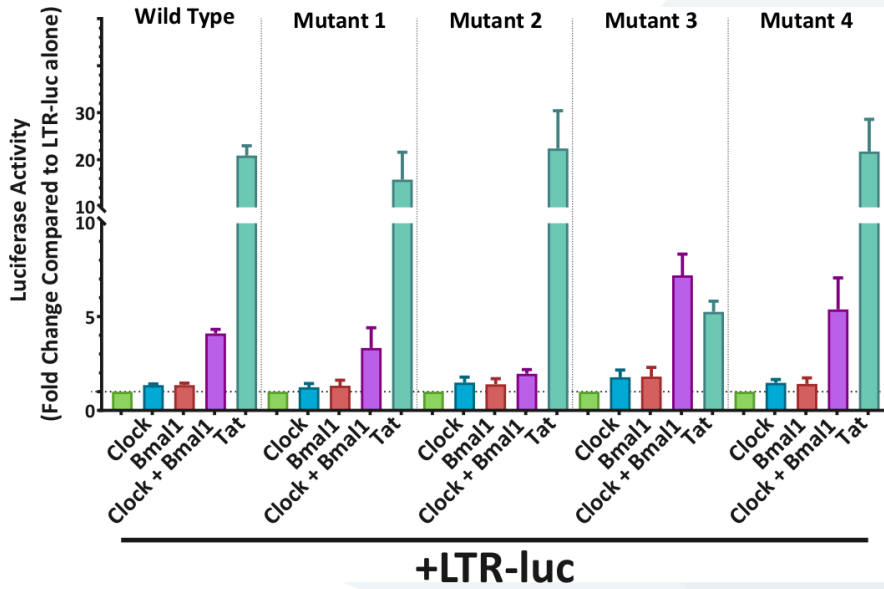
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A joint venture between The University of Melbourne and The Royal Melbourne Hospital

E-box Mutants



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      100      140      180      220      260      300      340      380      420      460      500      540      580      620      660      700      740      780      820      860      900
      U3      -40      -80      -120      -160      -200      -240      -280      -320      -360      -400      -440      -480      -520      -560      -600      -640      -680      -720      -760      -800
      HXB2      GGAAGGGCTA      ATTCACTCCC      AAAGAAGACA      AGATATCCIT      GATCTGTGGA      TCTACCACAC      ACAAGGCTAC      TTCCTGATT      AGCAGAACTA      90
      Wild-Type LTR-luc      GGAAGGGCTA      ATTTGGTCC-      AAAGAAGACA      AGATATCCIT      GATCTGTGGA      TCTACCACAC      ACAAGGCTAC      TTCCTGATT      AGCAGAACTA      89
      E-box mutant 1 LTR-luc      GGAAGGGCTA      ATTTGGTCCC      AAAGAAGACA      AGATATCCIT      GATCTGTGGA      TCTACCACAC      ACAAGGCTAC      TTCCTGATT      AGCAGAACTA      90
      E-box mutant 2 LTR-luc      GGAAGGGCTA      ATTTGGTCCC      AAAGAAGACA      AGATATCCIT      GATCTGTGGA      TCTACCACAC      ACAAGGCTAC      TTCCTGATT      AGCAGAACTA      90
      E-box mutant 3 LTR-luc      GGAAGGGCTA      ATTTGGTCCC      AAAGAAGACA      AGATATCCIT      GATCTGTGGA      TCTACCACAC      ACAAGGCTAC      TTCCTGATT      AGCAGAACTA      90
      E-box mutant 4 LTR-luc      GGAAGGGCTA      ATTTGGTCCC      AAAGAAGACA      AGATATCCIT      GATCTGTGGA      TCTACCACAC      ACAAGGCTAC      TTCCTGATT      AGCAGAACTA      90

      HXB2      CACACACAGG      CCAGGGGTCA      GATATCCAAT      GACCTTTGGA      TGGTGCTACA      AGCTAGTACC      AGTTGAGCCA      GATAAGTAG      AAGAGGCCAA      179
      Wild-Type LTR-luc      CACACACAGG      CCAGGGGTCA      GATATCCAAT      GACCTTTGGA      TGGTGCTACA      AGCTAGTACC      AGTTGAGCCA      GATAAGTAG      AAGAGGCCAA      180
      E-box mutant 1 LTR-luc      CACACACAGG      CCAGGGGTCA      GATATCCAAT      GACCTTTGGA      TGGTGCTACA      AGCTAGTACC      AGTTGAGCCA      GATAAGTAG      AAGAGGCCAA      180
      E-box mutant 2 LTR-luc      CACACACAGG      CCAGGGGTCA      GATATCCAAT      GACCTTTGGA      TGGTGCTACA      AGCTAGTACC      AGTTGAGCCA      GATAAGTAG      AAGAGGCCAA      180
      E-box mutant 3 LTR-luc      CACACACAGG      CCAGGGGTCA      GATATCCAAT      GACCTTTGGA      TGGTGCTACA      AGCTAGTACC      AGTTGAGCCA      GATAAGTAG      AAGAGGCCAA      180
      E-box mutant 4 LTR-luc      CACACACAGG      CCAGGGGTCA      GATATCCAAT      GACCTTTGGA      TGGTGCTACA      AGCTAGTACC      AGTTGAGCCA      GATAAGTAG      AAGAGGCCAA      180

      HXB2      TAAAGGAGAG      AAACCCAGCT      TGTACACCC      TGTGAGCCTG      CATGGGATGG      ATGACCCGGA      GAGAGAAGTG      TTAGAGTGA      GGTTTGACAG      270
      Wild-Type LTR-luc      TAAAGGAGAG      AAACCCAGCT      TGTACACCC      TGTGAGCCTG      CATGGGATGG      ATGACCCGGA      GAGAGAAGTG      TTAGAGTGA      GGTTTGACAG      269
      E-box mutant 1 LTR-luc      TAAAGGAGAG      AAACCCAGCT      TGTACACCC      TGTGAGCCTG      CATGGGATGG      ATGACCCGGA      GAGAGAAGTG      TTAGAGTGA      GGTTTGACAG      270
      E-box mutant 2 LTR-luc      TAAAGGAGAG      AAACCCAGCT      TGTACACCC      TGTGAGCCTG      CATGGGATGG      ATGACCCGGA      GAGAGAAGTG      TTAGAGTGA      GGTTTGACAG      270
      E-box mutant 3 LTR-luc      TAAAGGAGAG      AAACCCAGCT      TGTACACCC      TGTGAGCCTG      CATGGGATGG      ATGACCCGGA      GAGAGAAGTG      TTAGAGTGA      GGTTTGACAG      270
      E-box mutant 4 LTR-luc      TAAAGGAGAG      AAACCCAGCT      TGTACACCC      TGTGAGCCTG      CATGGGATGG      ATGACCCGGA      GAGAGAAGTG      TTAGAGTGA      GGTTTGACAG      270

      HXB2      CCGCCTAGCA      TTTTCATCAG      TGGCCGAGA      GCTGCATCCG      GAGTACTTCA      AGAACTGCTG      ACATCGAGCT      TGCTACAAGG      GACTTTCCGG      359
      Wild-Type LTR-luc      CCGCCTAGCA      TTTTCATCAG      TGGCCGAGA      GCTGCATCCG      GAGTACTTCA      AGAACTGCTG      ACATCGAGCT      TGCTACAAGG      GACTTTCCGG      359
      E-box mutant 1 LTR-luc      CCGCCTAGCA      TTTTCATCAG      TGGCCGAGA      GCTGCATCCG      GAGTACTTCA      AGAACTGCTG      ACATCGAGCT      TGCTACAAGG      GACTTTCCGG      360
      E-box mutant 2 LTR-luc      CCGCCTAGCA      TTTTCATCAG      TGGCCGAGA      GCTGCATCCG      GAGTACTTCA      AGAACTGCTG      ACATCGAGCT      TGCTACAAGG      GACTTTCCGG      360
      E-box mutant 3 LTR-luc      CCGCCTAGCA      TTTTCATCAG      TGGCCGAGA      GCTGCATCCG      GAGTACTTCA      AGAACTGCTG      ACATCGAGCT      TGCTACAAGG      GACTTTCCGG      360
      E-box mutant 4 LTR-luc      CCGCCTAGCA      TTTTCATCAG      TGGCCGAGA      GCTGCATCCG      GAGTACTTCA      AGAACTGCTG      ACATCGAGCT      TGCTACAAGG      GACTTTCCGG      360

      HXB2      TGGGACATTT      CGAGGGAGGC      GTGGCCTGGG      CGGGAATCGG      GAGTGGCGAG      CCCTCAGATC      CTGCATATAA      GCAGCTGCTT      TTTGCCGTGA      450
      Wild-Type LTR-luc      TGGGACATTT      CGAGGGAGGC      GTGGCCTGGG      CGGGAATCGG      GAGTGGCGAG      CCCTCAGATC      CTGCATATAA      GCAGCTGCTT      TTTGCCGTGA      449
      E-box mutant 1 LTR-luc      TGGGACATTT      CGAGGGAGGC      GTGGCCTGGG      CGGGAATCGG      GAGTGGCGAG      CCCTCAGATC      CTGCATATAA      GCAGCTGCTT      TTTGCCGTGA      450
      E-box mutant 2 LTR-luc      TGGGACATTT      CGAGGGAGGC      GTGGCCTGGG      CGGGAATCGG      GAGTGGCGAG      CCCTCAGATC      CTGCATATAA      GCAGCTGCTT      TTTGCCGTGA      450
      E-box mutant 3 LTR-luc      TGGGACATTT      CGAGGGAGGC      GTGGCCTGGG      CGGGAATCGG      GAGTGGCGAG      CCCTCAGATC      CTGCATATAA      GCAGCTGCTT      TTTGCCGTGA      450
      E-box mutant 4 LTR-luc      TGGGACATTT      CGAGGGAGGC      GTGGCCTGGG      CGGGAATCGG      GAGTGGCGAG      CCCTCAGATC      CTGCATATAA      GCAGCTGCTT      TTTGCCGTGA      450

      HXB2      CTGGTCTCT      CTGGTAGAC      CAGATCTGAG      CCTGGGAGCT      CTCTGGCTAA      CTAGGGAACC      CACTGCTTAA      GCCTCAATAA      AGCTTGCCTT      540
      Wild-Type LTR-luc      CTGGTCTCT      CTGGTAGAC      CAGATCTGAG      CCTGGGAGCT      CTCTGGCTAA      CTAGGGAACC      CACTGCTTAA      GCCTCAATAA      AGCTTGCCTT      539
      E-box mutant 1 LTR-luc      CTGGTCTCT      CTGGTAGAC      CAGATCTGAG      CCTGGGAGCT      CTCTGGCTAA      CTAGGGAACC      CACTGCTTAA      GCCTCAATAA      AGCTTGCCTT      540
      E-box mutant 2 LTR-luc      CTGGTCTCT      CTGGTAGAC      CAGATCTGAG      CCTGGGAGCT      CTCTGGCTAA      CTAGGGAACC      CACTGCTTAA      GCCTCAATAA      AGCTTGCCTT      540
      E-box mutant 3 LTR-luc      CTGGTCTCT      CTGGTAGAC      CAGATCTGAG      CCTGGGAGCT      CTCTGGCTAA      CTAGGGAACC      CACTGCTTAA      GCCTCAATAA      AGCTTGCCTT      540
      E-box mutant 4 LTR-luc      CTGGTCTCT      CTGGTAGAC      CAGATCTGAG      CCTGGGAGCT      CTCTGGCTAA      CTAGGGAACC      CACTGCTTAA      GCCTCAATAA      AGCTTGCCTT      540

      HXB2      GAGTGCTTCA      AGTAGTGTGT      GCCCGTCTGT      TGTGTGACTC      TGGTAACTAG      AGATCCCTCA      GACCCCTTTA      GTGAGTGGG      AAAATCTCTA      630
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      E-box mutant 1 LTR-luc      GAGTGCTTCA      AGTAGTGTGT      GCCCGTCTGT      TGTGTGACTC      TGGTAACTAG      AGATCCCTCA      GACCCCTTTA      GTGAGTGGG      GCAATCTCTA      629
      E-box mutant 2 LTR-luc      GAGTGCTTCA      AGTAGTGTGT      GCCCGTCTGT      TGTGTGACTC      TGGTAACTAG      AGATCCCTCA      GACCCCTTTA      GTGAGTGGG      GCAATCTCTA      629
      E-box mutant 3 LTR-luc      GAGTGCTTCA      AGTAGTGTGT      GCCCGTCTGT      TGTGTGACTC      TGGTAACTAG      AGATCCCTCA      GACCCCTTTA      GTGAGTGGG      GCAATCTCTA      629
      E-box mutant 4 LTR-luc      GAGTGCTTCA      AGTAGTGTGT      GCCCGTCTGT      TGTGTGACTC      TGGTAACTAG      AGATCCCTCA      GACCCCTTTA      GTGAGTGGG      GCAATCTCTA      629

      HXB2      ATCACTAGTG      AATTGCGGGC      CGCCTGAGG      TCGAGATCTG      CGATCT 674      CA 638
      Wild-Type LTR-luc      ATCACTAGTG      AATTGCGGGC      CGCCTGAGG      TCGAGATCTG      CGATCT 675
      E-box mutant 1 LTR-luc      ATCACTAGTG      AATTGCGGGC      CGCCTGAGG      TCGAGATCTG      CGATCT 675
      E-box mutant 2 LTR-luc      ATCACTAGTG      AATTGCGGGC      CGCCTGAGG      TCGAGATCTG      CGATCT 675
      E-box mutant 3 LTR-luc      ATCACTAGTG      AATTGCGGGC      CGCCTGAGG      TCGAGATCTG      CGATCT 675
      E-box mutant 4 LTR-luc      ATCACTAGTG      AATTGCGGGC      CGCCTGAGG      TCGAGATCTG      CGATCT 675
  
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