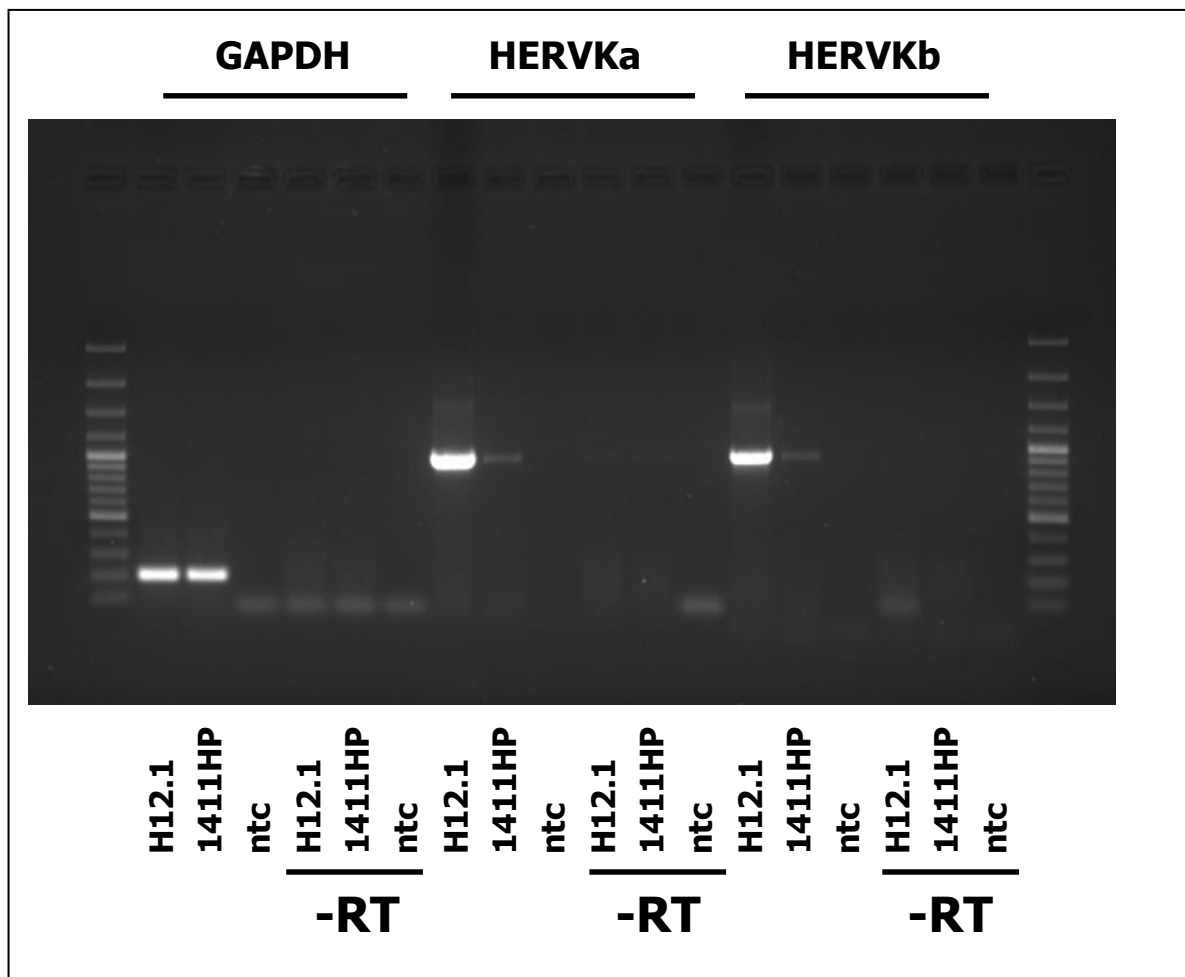


Differentiation-dependent regulation of human endogenous retrovirus K sequences
and neighbouring genes in germ cell tumour cells

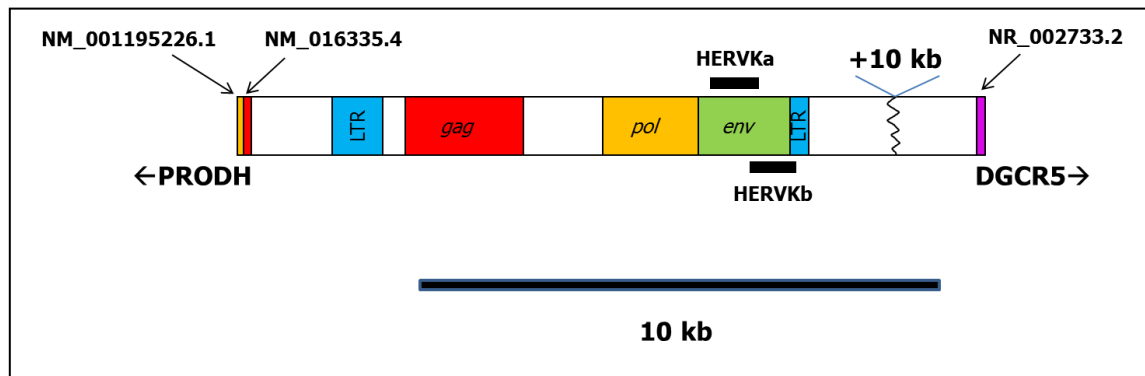
Thomas Mueller, Claudia Hantsch, Ines Volkmer, Martin S. Staeger

Supplementary Material

1. Supplementary Figure 1. Prove of absence of relevant DNA contamination in RNA isolated from tumour cells. Page 2
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Supplementary Figure 1. Prove of absence of relevant DNA contamination in RNA isolated from tumour cells. RNA was isolated from indicated tumour cells, transcribed into cDNA and used as template for PCR with indicated primer combinations. In addition, RNA was processed without addition of reverse transcriptase (-RT) and used as target for PCR; ntc: no template control. GAPDH serves as housekeeping control and showed similar expression in both cell lines. HERVK signals were stronger in H12.1 cells than in 1411HP cells. Without reverse transcription, amplification was nearly undetectable.



Supplementary Figure 2. Schematic presentation of the PRODH-DGCR5 region on human chromosome 22. For identification of ERV open reading frames, the intergenic region between PRODH and DGCR5 (accession number NC_000022.11:18936553-18970498) was scanned with getorf (<http://www.hpa-bioinfotools.org.uk/pise/getorf.html>) for large (>1 kb) open reading frames. The predicted protein sequences were analysed with BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) in order to assign the open reading frames to the typical viral gene products. LTR were identified with RepeatMasker (<http://www.repeatmasker.org/>). Only the positions of the two LTR sequences flanking the HERV are shown. According to RepeatMasker, Both LTRs are from the same family LTR5. The position of the PCR amplicons generated with primer combinations HERVKa and HERVKb as well as the start positions of transcripts from PRODH and DGCR5 are shown. For PRODH, two different start positions of the mRNA have been described.

Sequence of PCR product from H12.1 cells with primer combination HERVKa (without primer sequences):

GCACCCGTAGCTGTGACTGCAATTAATCCCATAATCACTGCAATTAAGTAAAAATGAATCTTTTGGATCTATTTAAACACC
 TTTTAATACTTCAGTCAAATATGGACGGATGGTGAGGCCCTCCACGGTCCGATGGACACAAGGATCCACACGCCCTCTC
 TTGCTCTCACCAGCAGAATACGGTGTGCCAATTAAGGTTGAATCAATGCAAGTAAGCAATCTACAGTTTTCACAGGTATA
 GTCTGGGAGTCTGGTTAATAACTATATTTTCTTACACTAGCATATAAGGGGGCTTTACGCAACTTTGTAAAGGAAGTGTAG
 ACTGGAATTTAGGTGACAGTATAAAATGGCTTACGATCTCTTGTCTTAAAGTTTGAATTTCCAGACCAAATCTAATGTGGT
 GTGAGGCCACAGTAAGCCTCCATAATCTGGATGTTTACAGACCAGAAACAGGACTTATATTTTTGGTCTTGGGGTAGAGATT
 CCTTTTTCTCCCCATTTCCAAGGTTAGAAAGACTGCAATTTTTTATGCTTATGTTTGTCTAAACTTTCTGTTAAGTTCGTATC
 AACAGCTGGACTCACTTGTGCACCTGGACATGACTGAGTTTGTCTGAGCAATTTGTGGTAGAATTGACCTCGAGGTGCCCAAT
 CTATAATAGTTCCGAATTCATTGTTTTGTAATATCACCGCACTATTGGCCACACATTTCTCCAAACTAAAACCTTCTGTATTT
 TTTGATTCTTTGGGAATTTCTTGGGGCAAGGTTTCCCTTTAGGTCTAAATTTAATGATCTTTGATAAGGAAAGTCTTGTA
 ATAATTTACCCGTGGCCTGAGTGACATCCCGCTTACCATGTGATAAGTGAATCTACTGATGGGACTGACAAATAGGTACTTCTA
 CC

Blast result; best hit: Homo sapiens chromosome 22, GRCh38.p12 Primary Assembly (NC_00022.11); Identities 100%, Gaps 0%:

Query	1	GCACCCGTAGCTGTGACTGCAATTAATCCCATAATCACTGCAATTAAGTAAAAATGAAT	60
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Query	61	CTTTTGGATCTATTTAAACACCTTTTAATACTTCAGTCAAATATGGACGGATGGTGAG	120
Sbjct	18946224	CTTTTGGATCTATTTAAACACCTTTTAATACTTCAGTCAAATATGGACGGATGGTGAG	18946165
Query	121	GCCTCCACGGTCCGATGGACACAAGGATCCACACGCCCTCTCTTGTCTCTCACCAGC	180
Sbjct	18946164	GCCTCCACGGTCCGATGGACACAAGGATCCACACGCCCTCTCTTGTCTCTCACCAGC	18946105
Query	181	AGAATACGGTGTGCCAATTAAGTGAATCAATGCAAGTAAGCAATCTACAGTTTTCA	240
Sbjct	18946104	AGAATACGGTGTGCCAATTAAGTGAATCAATGCAAGTAAGCAATCTACAGTTTTCA	18946045
Query	241	CAGGTTATAGTCTGGGAGTCTGGTTAATAACTATATTTCTTACAACCTAGCATATAAGG	300
Sbjct	18946044	CAGGTTATAGTCTGGGAGTCTGGTTAATAACTATATTTCTTACAACCTAGCATATAAGG	18945985
Query	301	GGCTTTACGCAACTTTGTAAAGGAAGTGTAGACTGGAATTTAGGTCGACAGTATAAAAT	360
Sbjct	18945984	GGCTTTACGCAACTTTGTAAAGGAAGTGTAGACTGGAATTTAGGTCGACAGTATAAAAT	18945925
Query	361	GGCTTACGATCTCTTGTCTTAAAGTTTGAATTTCCAGACCAAATCTAATGTGGTGTGAG	420
Sbjct	18945924	GGCTTACGATCTCTTGTCTTAAAGTTTGAATTTCCAGACCAAATCTAATGTGGTGTGAG	18945865
Query	421	GCCACAGTAAGCCTCCATAATCTGGATGTTTACAGACCAGAAACAGGACTTATATTTTT	480
Sbjct	18945864	GCCACAGTAAGCCTCCATAATCTGGATGTTTACAGACCAGAAACAGGACTTATATTTTT	18945805
Query	481	GGTCTTGGGGTAGAGATTCTTTTTCTCCCCATTCCCAAGGGTAGAAAGACTGCAATTTT	540
Sbjct	18945804	GGTCTTGGGGTAGAGATTCTTTTTCTCCCCATTCCCAAGGGTAGAAAGACTGCAATTTT	18945745
Query	541	TTATGCTTATGTTTGTCTAAACTTTCGTGTTAAGTCGCTATCAACAGCTGGACTCACTTGT	600
Sbjct	18945744	TTATGCTTATGTTTGTCTAAACTTTCGTGTTAAGTCGCTATCAACAGCTGGACTCACTTGT	18945685
Query	601	GCACCTGGACATGACTGAGTTTGTCTGAGCAATTTGGGTAGAATTGACCTCGAGGTGCC	660
Sbjct	18945684	GCACCTGGACATGACTGAGTTTGTCTGAGCAATTTGGGTAGAATTGACCTCGAGGTGCC	18945625
Query	661	CAATCTATAATAGTTCCGAATTCATTGTTTTGTAATATCACCGCACTATTGGCCACACAT	720
Sbjct	18945624	CAATCTATAATAGTTCCGAATTCATTGTTTTGTAATATCACCGCACTATTGGCCACACAT	18945565
Query	721	TCTTCCCAAATAAACTTCTGTATTTTTGATTCTTTGGGAATTTCTTGGGGCAAGGT	780
Sbjct	18945564	TCTTCCCAAATAAACTTCTGTATTTTTGATTCTTTGGGAATTTCTTGGGGCAAGGT	18945505
Query	781	TTCCCTTTAGGTCTAAATTTAATGATCTTTGATAAGGAAAGTCTTGTAATAATTTACC	840
Sbjct	18945504	TTCCCTTTAGGTCTAAATTTAATGATCTTTGATAAGGAAAGTCTTGTAATAATTTACC	18945445
Query	841	CGTGGCCTGAGTGACATCCCGCTTACCATGTGATAAGTGAATCTACTGATGGGACTGACA	900
Sbjct	18945444	CGTGGCCTGAGTGACATCCCGCTTACCATGTGATAAGTGAATCTACTGATGGGACTGACA	18945385
Query	901	ATAGGTACTTCTACC 915	
Sbjct	18945384	ATAGGTACTTCTACC 18945370	

Sequence of PCR product from H12.1 cells with primer combination HERVKb (without primer sequences):

GAGAGCACGGGGTTGGGGTAAGGTTATAGATTAACAGAAaTCTCAAGGCAGAAGAATTTTCTTAGCACATAACAAAATGGAG
TCTCCTATGTCTACTTCTTTCTACACAGACACAGTAACAATCTGATCTCTCTTGGCTTTTCCCCACATTTCCCCCTTTTCTTTT
CGACAAAACCGCCATCGTCATCATGCCCCGTTCTCGATGGTCGCTGTCTCTTCGGAGCTGTTGGGTACACCTGCAGACTAACA
ACAGACAAAACAGGCACACAAGGATTAATATGAGATTATAAATCGTAGTACTTCCAATGGTCTTAACCCAAGTGACAGGGTTA
AGATTTGCGAGGCCATCAGCAACTCCTGCAATTGCCAGTTCCTGGCACCAAATTTAAATGGGCTTTTGATGCTTCGAAAAT
TTGTTCTTTTAAATTTGGAAATGTCTAAAGTGAGATTATCTTCTCTTCCCTGTAGATGGCGTCTAACCATGTCCCAGTGATGCT
CAGACTCATTATAAAATTTGGGGTGTAAATACAAAATCTGACGATTCAGTTCACATTGTAACCTGAAACGATGTTCTAAGCTC
ATGAGTCTGTCTCCATCCAAATGACAGTTTGTCTAAGATCATTAAATTTGATTTGCCAATTTTGGATCAATACTAGATTGTGA
ATTCACAATCTTGTAAGAATTTTTTTGCCAATCATTAACAAAAGTTTACTGACTGAACAGAAAGAGTGCAATGCAACTCCTGCTA
CAGCACCCGTAGCTGTGACTGCAATTAATCCATAATCACTGCAATTAAGTAAAAATGAATCTTTTGGATCTATtTAAAAACA
CCTTTTAATACTTCAGTCAAAATATGGACGGATGGTGAGGCCTCCCACGGTCCGTCCATGGACACAAGGATCCACACGCCCTC

Blast result; best hit: Homo sapiens chromosome 22, GRCh38.p12 Primary Assembly (NC_000022.11); Identities 100%, Gaps 0%:

Query	1	GAGAGCACGGGGTTGGGGTAAGGTTATAGATTAACAGAAATCTCAAGGCAGAAGAATTTT	60
Sbjct	18947033	GAGAGCACGGGGTTGGGGTAAGGTTATAGATTAACAGAAATCTCAAGGCAGAAGAATTTT	18946974
Query	61	TCTTAGCACATAACAAAATGGAGTCTCCTATGTCTACTTCTTTCTACACAGACACAGTAA	120
Sbjct	18946973	TCTTAGCACATAACAAAATGGAGTCTCCTATGTCTACTTCTTTCTACACAGACACAGTAA	18946914
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Sbjct	18946913	CAATCTGATCTCTCTTGCTTTTCCCCACATTTCCCCCTTTTCTTTTTCGACAAAACCGCCA	18946854
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Sbjct	18946853	TCGTATCATGCCCCGTTCTCGATGGTCGCTGTCTCTTCGGAGCTGTTGGGTACACCTGC	18946794
Query	241	AGACTAACAACAGACAAAACAGGCACACAAGGATTAATATGAGATTATAATCGTAGTAC	300
Sbjct	18946793	AGACTAACAACAGACAAAACAGGCACACAAGGATTAATATGAGATTATAATCGTAGTAC	18946734
Query	301	TTCCAATGGTCTTAACCCAAGTGACAGGGTTAAGATTTGCGAGGCCATCAGCAACTCCTG	360
Sbjct	18946733	TTCCAATGGTCTTAACCCAAGTGACAGGGTTAAGATTTGCGAGGCCATCAGCAACTCCTG	18946674
Query	361	CAATTGCCCTCAGTTCCCTGGCACCAAATTTAAATGGGCTTTTGATGCTTCGAAAATTTGTT	420
Sbjct	18946673	CAATTGCCCTCAGTTCCCTGGCACCAAATTTAAATGGGCTTTTGATGCTTCGAAAATTTGTT	18946614
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Sbjct	18946613	CTTTTAAATTTGGAAATGTCTAAAGTGAGATTATCTTCTCTTCCCTGTAGATGGCGTCTAA	18946554
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Sbjct	18946493	TATTCCAGTCACATTGTAACCTGGAACGATGTTCTAAGCTCATGAGTCTGTCTCCCATCC	18946434
Query	601	AAATGACAGTTTGTCTAAGATCATTAAATTTGATTTGCCAATTTTGTCAATACTAGATT	660
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Sbjct	18946313	CAGAAGAGTGCAATGCAACTCCTGCTACAGCACCCGTAGCTGTGACTGCAATTAATCCCA	18946254
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Sbjct	18946253	TAATCACTGCAATTAAGTAAAAATGAATCTTTGGATCTATTTAAAAACACCTTTTAAATA	18946194
Query	841	CTTCAGTCAAAAATATGACCGGATGGTGAGGCCTCCCACGGTCCGTCCATGGACACAAGGA	900
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Blast result; top hits:

Chromosome	Start	Stop	identities
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NC_000007.14	4583245	4584157	908/913
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NC_000005.10	156658525	156659437	906/913
NC_000011.10	101703709	101702797	904/913
NC_000008.11	139460725	139461637	903/913

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Chr7 : GAGGCAACGGGGTGGGGTAAGGTTATAGATTAAACGAAATCTCAAGGCAGAGAAATTTTCTTAGCACATAACAAAATGGAGCTCCCTATGCTACTTCTTTCTACACAGACAGTAAACA : 123
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Chr11(r.c) : GAGGCAACGGGGTGGGGTAAGGTTATAGATTAAACGAAATCTCAAGGCAGAGAAATTTTCTTAGCACATAACAAAATGGAGCTCCCTATGCTACTTCTTTCTACACAGACAGTAAACA : 123
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Chr1(r.c) : TCGATCTCTCTGCTTTTCCCCAGATTTCCCTCTTTCTTTTTCGACAAAACCGCCATCGCTCATGCCCCCTCTGATGGTGGCTGTCTCTTGGAGCTGTGGGACACCTGCAGACTA : 246
Chr5 : TCGATCTCTCTGCTTTTCCCCAGATTTCCCTCTTTCTTTTTCGACAAAACCGCCATCGCTCATGCCCCCTCTGATGGTGGCTGTCTCTTGGAGCTGTGGGACACCTGCAGACTA : 246
Chr11(r.c) : TCGATCTCTCTGCTTTTCCCCAGATTTCCCTCTTTCTTTTTCGACAAAACCGCCATCGCTCATGCCCCCTCTGATGGTGGCTGTCTCTTGGAGCTGTGGGACACCTGCAGACTA : 246
Chr8 : TCGATCTCTCTGCTTTTCCCCAGATTTCCCTCTTTCTTTTTCGACAAAACCGCCATCGCTCATGCCCCCTCTGATGGTGGCTGTCTCTTGGAGCTGTGGGACACCTGCAGACTA : 246
TCGTACTCTCTTGGCTTTTCCCCAGATTTCCCTCTTTCTTTTTCGACAAAACCGCCATCGCTCATGCCCCCTCTGATGGTGGCTGTCTCTTGGAGCTGTGGGACACCTGCAGACTA
    
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Chr22(r.c) : ACAACAGACAAAACAGGCACACAGGGTTAATAGGATTTAATGAGATTTAATGTAAGTCTACTTCCAAATGGCTTAAACCCAAAGGACAGGGGTAAAGATTGCGAGCCATCAGCAACTCCGCAATGGCT : 369
Chr7 : ACAACAGACAAAACAGGCACACAGGGTTAATAGGATTTAATGAGATTTAATGTAAGTCTACTTCCAAATGGCTTAAACCCAAAGGACAGGGGTAAAGATTGCGAGCCATCAGCAACTCCGCAATGGCT : 369
Chr1(r.c) : ACAACAGACAAAACAGGCACACAGGGTTAATAGGATTTAATGAGATTTAATGTAAGTCTACTTCCAAATGGCTTAAACCCAAAGGACAGGGGTAAAGATTGCGAGCCATCAGCAACTCCGCAATGGCT : 369
Chr5 : ACAACAGACAAAACAGGCACACAGGGTTAATAGGATTTAATGAGATTTAATGTAAGTCTACTTCCAAATGGCTTAAACCCAAAGGACAGGGGTAAAGATTGCGAGCCATCAGCAACTCCGCAATGGCT : 369
Chr11(r.c) : ACAACAGACAAAACAGGCACACAGGGTTAATAGGATTTAATGAGATTTAATGTAAGTCTACTTCCAAATGGCTTAAACCCAAAGGACAGGGGTAAAGATTGCGAGCCATCAGCAACTCCGCAATGGCT : 369
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Chr7 : CAGTCCGGCCCAAAATTTAAAAGGGCTTTTGAAGCTTGGAAAATTTGCTTTTAAATTTGGAAAATGCTAAAGGAGGATTTATCTCTCTCCCTGAGATGGGGCTAAACCACTGCCAGT : 492
Chr1(r.c) : CAGTCCGGCCCAAAATTTAAAAGGGCTTTTGAAGCTTGGAAAATTTGCTTTTAAATTTGGAAAATGCTAAAGGAGGATTTATCTCTCTCCCTGAGATGGGGCTAAACCACTGCCAGT : 492
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Chr7 : GATGCTCAGACTCATTATAAATTTGGGGTGAATACAAAATCTGACGTATTCAGTCCACATGTAAGTCCGAAACGATGTTCTAAGCTCATGAGTCTGCTCCCATCCAAATGACAGTTTGTG : 615
Chr1(r.c) : GATGCTCAGACTCATTATAAATTTGGGGTGAATACAAAATCTGACGTATTCAGTCCACATGTAAGTCCGAAACGATGTTCTAAGCTCATGAGTCTGCTCCCATCCAAATGACAGTTTGTG : 615
Chr5 : GATGCTCAGACTCATTATAAATTTGGGGTGAATACAAAATCTGACGTATTCAGTCCACATGTAAGTCCGAAACGATGTTCTAAGCTCATGAGTCTGCTCCCATCCAAATGACAGTTTGTG : 615
Chr11(r.c) : GATGCTCAGACTCATTATAAATTTGGGGTGAATACAAAATCTGACGTATTCAGTCCACATGTAAGTCCGAAACGATGTTCTAAGCTCATGAGTCTGCTCCCATCCAAATGACAGTTTGTG : 615
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Chr7 : TAAGTCAATTAATTTGATTTGCCAATTTTTGATCAACTACTAGATTGGAATTCACCAATCTTGTAGAATT TTTTGGCAATCATTAAACAAGTTTACTGACTGAACAGAGAGTGCATGCAA : 738
Chr1(r.c) : TAAGTCAATTAATTTGATTTGCCAATTTTTGATCAACTACTAGATTGGAATTCACCAATCTTGTAGAATT TTTTGGCAATCATTAAACAAGTTTACTGACTGAACAGAGAGTGCATGCAA : 738
Chr5 : TAAGTCAATTAATTTGATTTGCCAATTTTTGATCAACTACTAGATTGGAATTCACCAATCTTGTAGAATT TTTTGGCAATCATTAAACAAGTTTACTGACTGAACAGAGAGTGCATGCAA : 738
Chr11(r.c) : TAAGTCAATTAATTTGATTTGCCAATTTTTGATCAACTACTAGATTGGAATTCACCAATCTTGTAGAATT TTTTGGCAATCATTAAACAAGTTTACTGACTGAACAGAGAGTGCATGCAA : 738
Chr8 : TAAGTCAATTAATTTGATTTGCCAATTTTTGATCAACTACTAGATTGGAATTCACCAATCTTGTAGAATT TTTTGGCAATCATTAAACAAGTTTACTGACTGAACAGAGAGTGCATGCAA : 738
TAAGTCAATTAATTTGATTTGCCAATTTTTGATCAACTACTAGATTGGAATTCACCAATCTTGTAGAATT TTTTGGCAATCATTAAACAAGTTTACTGACTGAACAGAGAGTGCATGCAA
    
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PCR_E : CTCTGCTACAGCA CCGTAGCTGTGACTGCAATTAATCCCATAACTGCAATTAAGTAAAATGAATCTTTTGGATCTATTAAAACACCTTTTAAATCTTCTAGCAAAATATGGAGGG : 861
Chr22(r.c) : CTCTGCTACAGCA CCGTAGCTGTGACTGCAATTAATCCCATAACTGCAATTAAGTAAAATGAATCTTTTGGATCTATTAAAACACCTTTTAAATCTTCTAGCAAAATATGGAGGG : 861
Chr7 : CTCTGCTACAGCA CCGTAGCTGTGACTGCAATTAATCCCATAACTGCAATTAAGTAAAATGAATCTTTTGGATCTATTAAAACACCTTTTAAATCTTCTAGCAAAATATGGAGGG : 861
Chr1(r.c) : CTCTGCTACAGCA CCGTAGCTGTGACTGCAATTAATCCCATAACTGCAATTAAGTAAAATGAATCTTTTGGATCTATTAAAACACCTTTTAAATCTTCTAGCAAAATATGGAGGG : 861
Chr5 : CTCTGCTACAGCA CCGTAGCTGTGACTGCAATTAATCCCATAACTGCAATTAAGTAAAATGAATCTTTTGGATCTATTAAAACACCTTTTAAATCTTCTAGCAAAATATGGAGGG : 861
Chr11(r.c) : CTCTGCTACAGCA CCGTAGCTGTGACTGCAATTAATCCCATAACTGCAATTAAGTAAAATGAATCTTTTGGATCTATTAAAACACCTTTTAAATCTTCTAGCAAAATATGGAGGG : 861
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PCR_E : ATGGGAGGECCTCCCAAGGGTGGTCCATGGACACAGGGATCCACAGCCCTC : 913
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Chr7 : ATGGGAGGECCTCCCAAGGGTGGTCCATGGACACAGGGATCCACAGCCCTC : 913
Chr1(r.c) : ATGGGAGGECCTCCCAAGGGTGGTCCATGGACACAGGGATCCACAGCCCTC : 913
Chr5 : ATGGGAGGECCTCCCAAGGGTGGTCCATGGACACAGGGATCCACAGCCCTC : 913
Chr11(r.c) : ATGGGAGGECCTCCCAAGGGTGGTCCATGGACACAGGGATCCACAGCCCTC : 913
Chr8 : ATGGGAGGECCTCCCAAGGGTGGTCCATGGACACAGGGATCCACAGCCCTC : 913
ATGG GAGGCTCCCAAGGGTGGTCCATGGACACAGGGATCCACAGCCCTC
    
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r.c : reverse complement