




## VCP-201



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## VCP-201





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## VCP-20



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## VCP-201






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## VCP-201




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## VCP-201










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## $\frac{\text { PCR Reverse }}{\text { TCAGGTITGTCACTGCTCT }}$

atatggcagangtcagancttgatgggcttctgacatgtcaggttttgttcactgacctcttgtcagaggactcttcacagtttacctttctcatcttgcctgctgcttattangacaggtgggitgagttgg
 tatacggtcttcagtcttganctaccggangactgtacagtccaanacangtgactggagancagtctccgtgagangtgtcanatgganagagtaganggacgacgantanttctgtccaccceacctcancc

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## VCP-20






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## VCP-201










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acgtatcctttagtttacctanggctctanattcattagagctgatggtctananccagagtaggctantcanattgictgitg tgigcgigtgcgcacananacacacacacatatatatatgggttttctitta tg cataggantcanatg VCP
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## VCP-201






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## VCP-201






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tg ggccttgagccagagtancccatcagcactgcggganccgtggtagaggtgccacaggtancctggganacatcggggcctagaggatgtcanacgtgagctacaggagctgetccaggtag







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## VCP-201



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## VCP-201


 CTGTACAACATTTTCCTGTTATTTGCAACCCAGTTTTACCTCGGACTCAGGACCCGGGACACGGACGAAGAAAAGGACCCTTGTCGGAACCCGATGGGTGGTGAGGGTTCCGTAAGAAGGTTTACACTTTAGGAC

## VCP-201

gangtangattgcaccttcttcctctcctgatcancatcggtatgatgtctcctgttgcctcaccctttgtctgcagtatcactggataggactggtgganagggagcagcctgacagagctccanatgtggaga
 CTTCATTCTAACGTGGAAGAAGGAGAGGACTAGTTGTAGCCATACTACAGAGGACAACGGAGTGGGAAACAGACGTCATAGTGACCTATCCTGACCACCTTTCCCTCGTCGGACTGTCTCGAGGTTTACACCTCT $\square>\mathrm{VCP} \quad \mathrm{VCP-201}>$




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GTGGATATTCTCTATACTCTCTTTTAATGCATCTAAAAATCCCAAACATCCCCTGGTTGGTGATCACTTACAGTTGTGTCCACCTTTATTTTATGTACTTTGATTAAAAAAAAAAACTTTTGGTAATATAAAA










































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- 16 segments $=2202 \mathrm{bp}$
/note $\quad=$ coding sequence ENSP00000506216



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VCP-231 316 .. 15,552 15,237 bp a l CDS
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- 16 segments $=2265 \mathrm{bp}$
／note $\quad=$ coding sequence ENSP000000505230
 YRPIRK，，GDIFLVRGGMRAVEFKVVETDPSPYCIVAPDTVIHCEGEPIKRE，，DEEESLNEVGYDDIGGCRKQLAQIKEMVELPLRHPALFKAIGVK，，PPRGILLYGPPGTGKTLIARAVANETGAFFFLIN ，，GPEIMSKLAGESES NLRKAFEEAEKNAPAIIFIDELDAIAPKREK，，THGEVERRIVSQLLTLMDGLKQRAHVIVMAATNRPNSIDPALRRF，，GRFDREVDIGIPDATGRLEILQIHTKNMKLADDVDLEQ，，VANETHGHVGADLAALCSEAALQAIRKK MDLIDLEDETIDAEVMNSLAVTMDDFR，，WA LSQSNPSALRETVVEVPQVTWEDIGGLEDVKRELQELVQ，，YPVEHPDKFLKFGMTPSKGVLFYGPPGCGKTLLAKAIANECQANFISIKGPELLTM WFGESEANVREIFDK，，A RQAAPCVLFFDELDSIAKARGGNIGDGGGAADRV INQILTEMDGMSTKKNVFIIGATNRPDIIDPAILRPGRLDQLIYIPLPDEKSRVAILKANLRKSPVAK，，EVEEDDPVPEIRRDHFEEAMRFARRSVSDNDIRKYEMFAQTL Q 44 S路所

－ 15 segments $=2286 \mathrm{bp}$
／note $\quad=$ coding sequence ENSP00000399456
 IKRE，，DEEESLNEVGYDDIGGCRKQLAQIKEMVELPLRHPALFKAIGVK，，PPRGILLYGPPGTGKTLIARAVANETGAFFFLIN，，GPEIMSKLAGESESNLRKAFEEAEKNAPAIIFIDELDAIAPKREK，，THGEVERRIVSQLLTLM GLKQRAHVIVMAATNRPNSIDPALRRF，，GRFDREVDIGIPDATGRLEILQIHTKNMKLADDVDLEQ，，VANETHGHVGADLAALCSEAALQAIRKKMDLIDLEDETIDAEVMNSLAVTMDDFR，，WALSQSNPSALRETVVEVP QVTWEDIGGLEDVKRELQELVQ，，YPVEHPDKFLKFGMTPSKGVLFYGPPGCGKTLLAKAIANECQANFISIKGPELLTMWFGESEANVREF KKNV FIIGATNRPDIIDPAILRPGRLDQLIYIPLPDEKSRVAILKANLRKSPVAK，，DVDLEFLAKMTNGFSGADLTEICQRACKLAIRESIESEIRRERERQTNPSAM，，EVEEDDPVPEIRRDHFEEAMRFARRSVSDNDIRKYEM


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VCP-203
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- 15 segments $=2286 \mathrm{bp}$
／note $\quad=$ coding sequence ENSP00000392088KRE，，DEEESLNEVGYDDIGGCRKQLAQIKEMVELPLRHPALFKAIGVK，，PPRGILLYGPPGTGKTLIARAVANETGAFFFLIN，，GPEIMSKLAGESESNLRKAFEEAEKNAPAIIFIDELDAIAPKREK，THGEVERRIVSQLLTLM位QVTWEDIGGLEDVKRELQELVQ，，YPVEHPDKFLKFGMTPSKGVLFYGPPGC GKTLLA KAIANECQANFISIKGPELLTM WFGESEANVREIFDK，，＇ARQAAPCVLFFDELDSIAKARGGNIGDGGGAADRVINQILTEMDGMSTKKNV FIIGATNRPDIIDPAILRPGRLDQLIYIPLPDEKSRVAILKANLRKSPVAK，DVDLEFLAKMTNGFSGADLTEICORACKLAIRESIESEIRRERERQTNPSAM，EVVEDDPVVPEIRRDHFEEAMRFARRSVSDNDIRKYEMF

VCP－212 $4612 \ldots 15,552 \quad 10,941 \mathrm{bp} \quad \square \quad \rightarrow \quad$ CDS
＊ 15 segments $=2286 \mathrm{bp}$
－coding sequence ENSP00000503426
 IKRE，，DEEESLNEVGYDDIGGCRKQLAQIKEMVELPLRHPALFKAIGVK，，PPRGILLYGPPGTGKTLIARAVANETGAFFFLIN，，GPEIMSKLAGESESNLRKAFEEAEKNAPAIIFIDELDAIAPKREK，，THGEVERRIVSQLLTLM DGLKQRAHVIVMAATNRPNSIDPALRRF，，GRFDREVDIGIPDATGRLEILQIHTKNMKLADDVDLEQ，，VANETHGHVGADLAALCSEAALQAIRKKMDLIDLEDETIDAEVMNSLAVTMDDFR，，WALSQSNPSALRETVVEVP QVTWEDIGGLEDVKRELQELVQ，，YPVEHPDKFLKFGMTPSKGVLFYGPPGCGKTLLA KAIANECQANFISIKGPELLTM WFGESEANVREIFDK，，ARQAAPCVLFFDELDSIAKARGGNIGDGGGAADRVINQILTEMDGMST KKNVFIIGATNRPDIIDPAILRPGRLDQLIYIPLPDEKSRVAILKANLRKSPVAK，，DVDLEFLAKMTNGFSGADLTEICQRACKLAIRESIESEIRRERERQTNPSAM，，EVEEDDPVPEIRRDHFEEAMRFARRSVSDNDIRKYEMF


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VCP-219
4612 ．．15，552 10，941 bp ■ \(\rightarrow \quad\) CDS
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－ 15 segments $=2283 \mathrm{bp}$
note $\quad=$ coding sequence ENSP0000050499
 KRE，，DEEESLNEVGYDDIGGCRKQLAQIKEMVELPLRHPALFKAIGVK，，PPRGILLYGPPGTGKTLIARAVANETGAFFFLIN，，GPEIMSKLAGESESNLRKAFEEAEKNAPAIIFIDELDAIAPKREK，，THGEVERRIVSQLLTLM DGLKQRAHVIVMAATNRPNSIDPALRRF，，GRFDREVDIGIPDATGRLEILQIHTKNMKLADDVDLEQ，VANETHGHVGADLAALCSEAALQAIRKKMDLIDLEDETIDAEVMNSLAVTMDDFR，，WALSQSNPSALRETVVEVP QVTWEDIGGLEDVKRELQELVQ，，YPVEHPDKFLKFGMTPSKGVLFYGPPGCGKTLLAKAIANECQANFISIKGPELLTM WFGESEANVREIFDK，，ARQAAPCVLFFDELDSIAKARGGNIGDGGGAADRVINQILTEMDGMST KKNVFIIGATNRPDIIDPAILRPGRLDQLIYIPLPDEKSRVAILKANLRKSPVAK，，DVDLEFLAKMTNGFSGADLTEICQRACKLAIRESIESEIRRERERQTNPSAM，，VEEDDPVPEIRRDHFEEAMRFARRSVSDNDIRKYEMFA

$=$ coding sequence ENSP00000505509
/translation $\quad=$ MDELQLFRGDTVLLKGKKRREAVCIVLSDDTCSDEKIRMNRVVRNNLRVRLGDVI
55 amino acids $=6.4 \mathrm{kDa}$

## VCP-234

 55 amino acids $=6.4 \mathrm{kDa}$- 3 segments $=328 \mathrm{bp}$
/note $\quad=$ coding sequence ENSP 00000505893
/translation $=$ HPAMP*CEVRQTYPCAAH**HSGRHYW*SLRGIP*AVLPGSVSTHPES,,RDGVSPCWSGSSRTLDLR,,RHFSCPVWDACCGVQSGGNRS*PLLHCCSRHSDPLRRGAYQTR 109 codons (6 internal stop codons)

| VCP-234 |  |
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| /note | $=$ primary transcript ENST00000681562 |

Donor Template WT -> SNV 7279 .. $7357 \quad 79 \mathrm{bp}$ ロ $\quad$ - misc_feature

| VCP-224 |  |
| :--- | :--- |
| /note | $=$ primary transcript ENST000000680520 |

note $\quad=\quad$ Nonsense mediated decay

| VCP-236 |  |
| :--- | :--- |
| /note | $=$primary transcript ENST000000681789 <br>  |
| Nonsense mediated decay |  |


| Protospacer Sequence |  | 7304 |  | 7323 | 20 bp | $\square$ | $\mapsto$ | misc_feature |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| SNV |  | 7318 | . | 7318 | 1 bp | $\square$ | $\mapsto$ | misc_feature |
| /note | $\begin{aligned} & =\quad \mathrm{WT}=\mathrm{G} \\ & \mathrm{SNV}=\mathrm{A} \end{aligned}$ |  |  |  |  |  |  |  |
| PAM |  | 7324 |  | 7326 | 3 bp | $\square$ | Н | misc_feature |
| VCP-227 |  | 7361 |  | 11,654 | 4294 bp | $\square$ | $\rightarrow$ | CDS |

- 6 segments $=709 \mathrm{bp}$

| /note | $=$ coding sequence ENSP00000506387 |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| /translation | $\begin{aligned}= & \text { LHCCSRHSDPLRRGAYQTR,,AS*RNPALRTSWNRKDPDCSSCSK*DWSLLLDQ }\end{aligned}$ |  |  |  |  |  |
| VCP-227 |  | 7361 .. 11,654 | 4294 bp | $\square$ | $\rightarrow$ | prim_transcript |
| /note | $=$ primary transcript ENST00000680834 |  |  |  |  |  |
| VCP-230 |  | 10,319 .. 11,654 | 1336 bp | $\square$ | $\rightarrow$ | prim_transcript |
| /note | $\begin{aligned} & =\text { primary transcript ENSTO0000681125 } \\ & \text { Nonsense mediated decay } \end{aligned}$ |  |  |  |  |  |
| VCP-205 |  | 12,645 .. 15,762 | 3118 bp | $\square$ | $\rightarrow$ | prim_transcript |
| /note | $\begin{aligned} &= \text { primary transcript ENSTO00000479300 } \\ & \text { Retained intron } \end{aligned}$ |  |  |  |  |  |
| VCP-204 |  | 14,836 .. 15,496 | 661 bp | $\square$ | $\rightarrow$ | prim_transcript |
| /note | $\begin{aligned} & =\begin{array}{l} \text { primary transcript ENST00000466100 } \\ \text { Retained intron } \end{array} \end{aligned}$ |  |  |  |  |  |
| VCP-233 |  | 15,139 .. 16,605 | 1467 bp | $\square$ | $\rightarrow$ | prim_transcript |
| /note | $=$ primary transcript ENST00000681537 |  |  |  |  |  |
| VCP-233 |  | 15,139 .. 15,533 | 395 bp | $\square$ | $\rightarrow$ | CDS |

- 2 segments $=231 \mathrm{bp}$
/note $\quad=$ coding sequence ENSP00000505847
/translation = EVEEDDPVPEIRRDHFEEAMRFARRSVSDNDIRKYEMFAQTLQQSRGFGSFR,,EPGWSWPQSGQWRRHRWQCIHRRQ* 76 amino acids $=9.4 \mathrm{kDa}$

Primer
PCR Reverse
Donor Template WT -> SNV $\quad 79-\mathrm{mer} \quad \bigcirc \quad 7279$.. $7357-76^{\circ} \mathrm{C} \quad \mathrm{Jun} 15,2022$
/sequence $\quad=$ GGGCTAGGATCTGTTTCCACCACTTTGAACTCCACAGCATGCATCCCACCACGGACAAGAAAAATGTCTCCTGCGAGAG
52\% GC / 24,232.8 Da
gRNA Protospacer
/sequence $\quad=$ CCGTGGTGGGATGCGTGCTG $70 \%$ GC / 6221.1 Da

## PCR Forward

/sequence
$=$ GCACCCAGTCCTGACAGTTA $55 \%$ GC / 6062.0 Da
Sanger Sequencing Primer $\quad 20-\mathrm{mer} \quad 7480$.. $7499 \quad 55^{\circ} \mathrm{C} \quad \mathrm{Jun} 15,2022$
$=$ GCACCCAGTCCTGACAGTTA $55 \%$ GC / 6062.0 Da

20-mer 7304 .. $7323 \rightarrow 67^{\circ} \mathrm{C}$ Jun 15,2022

20-mer 7480 .. $7499 \quad-\quad 55^{\circ} \mathrm{C}$ Jun 15, 2022

20-mer

