

New Phytologist Supporting Information

Article title: A blue-print for gene function analysis through Base Editing in the model plant *Physcomitrium* (*Physcomitrella*) *patens*

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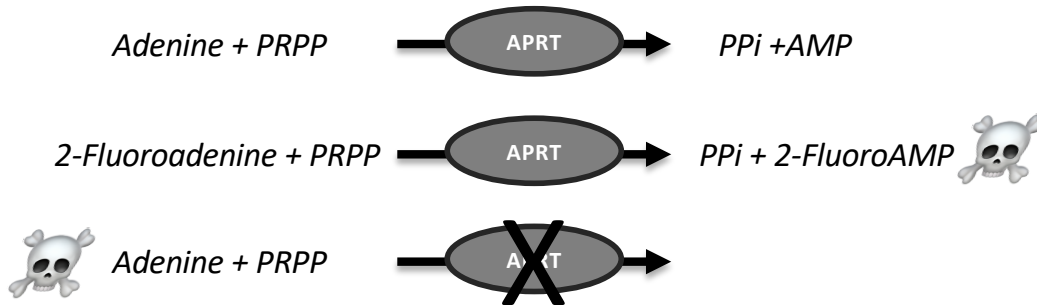
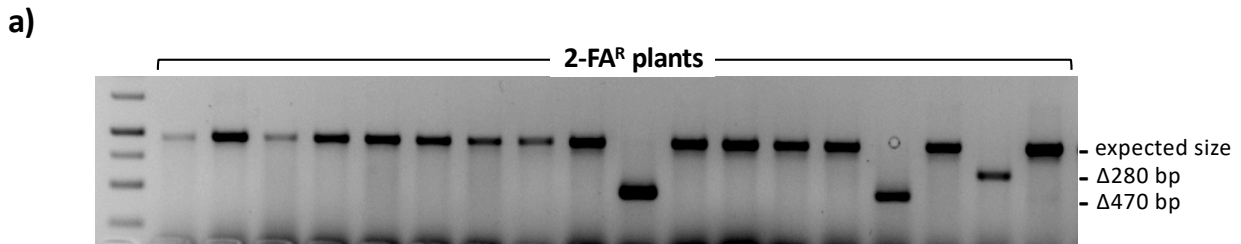


Figure S1. Schematic description of the *APT* reporter gene and APRT function.

APRT catalyses a phosphoribosyl transfer from Phosphoribosyl Pyrophosphate (PRPP) to adenine, forming AMP and releasing pyrophosphate (PPi). In presence of 2-Fluoroadenine APRT will form 2-FluoroAMP, a toxic compound for the cell. In absence of a functional APRT high concentration of adenine are toxic for the cell.



b)

WT **CCAGTATATCTCGGACAGCATCC**GTACCATTCC**TGATTTTCCTCACA**AAGGTACTATGCTCCACGCCATAGTCGTC**ACTCTC**
GGTTTTGTTTTGCAGTGTTCGTGACATGTGGTTTTTTTCTTTTTT**CACCTTTAGCGA**AGGTTTTGTATGGAATTCTCTTT**CG**
TAGCGCGAGTTTACGTGATGAATTTGGTGCAGGCATTATGTTCCGAGATGTGACGACGTTGCTGTTGGATCATAAGGCTTT**C**
AAAGACACGATCGACATCTTTGTT**GAGCGTTACCGGGACCAGAAGG**TGGACGTCATTGTGGGTGCGATGCCCTTGACTCCCT
TGACGTACCATAACGGTTTTTGTAATAAATTTGAAGGCAACCGTTACGTTTATAAATTTGGGAAGATTGCTGGCTGTTGTT
GATTACTTTTCGTGCTACTTTTTTTCAGGAATTGAAGCTCGAGGTTTTATCTTTGGG**CCACCCATTGCTCTTGCCATCGG**

CBE-d#12 **CCAGTATATCTCGGACAGCATCC**GTACCATTCC**TGATTTTCCTCACA**AAGGTACTATGCTCCACGCCATAGTCGTC**ACTCTC**
GGTTTTGTTTTGCAGTGTTCGTGACATGTGGTTTTTTTCTTTTTT**CACCTTTAGCGA**AGGTTTTGTATGGAATTCTCTTT**CG**
TAGCGCGAGTTTACGTGATGAATTTGGTGCAGGCATTATGTTCCGAGATGTGACGACGTTGCTGTTGGATCATAAGGCTTT**C**
AAAGACACGATCGACATCTTTGTT**GAGCGTTACCGGGACCAGAAGG**TGGACGTCATTGTGGGTGCGATGCCCTTGACTCCCT
TGACGTACCATAACGGTTTTTGTAATAAATTTGAAGGCAACCGTTACGTTTATAAATTTGGGAAGATTGCTGGCTGTTGTT
GATTACTTTTCGTGCTACTTTTTTTCAGGAATTGAAGCTCGAGGTTTTATCTTTGGG**. .ACTTATTGCTCTTGCCATCGG**

CBE-d#10 **CCAGTATATCTCG** . . . Δ268bp . . . **GACCAGAAGG**TGGACGTCATTGTGGGTGCGATGCCCTTGACTCCCTTGACTCCCTTGACTCCCT
AACGGTTTTTGTAATAAATTTGAAGGCAACCGTTACGTTTATAAATTTGGGAAGATTGCTGGCTGTTGTTGATTACTTT**CG**
TGCTACTTTTTTTCAGGAATTGAAGCTCGAGGTTTTATCTTTGGG**ATACCCATTGCTCTTGCCATCGG**

ABE-d#1 **CCAGTATATCTCGGACAGCATCC**GTACCATTCC**TGATTTTCCTCACA**AAGGTACTATGCTCCACGCCATAGTCGTC**ACTCTC**
GGTTTTGTTTTGCAGTGTTCGTGACATGTGGTTTTTTTCTTTTTT**CACCTTTAGCGA**AGGTTTTGTATGGAATTCTCTTT**CG**
TAGCGCGAGTTTACGTGATGAATTTGGTGCAGGCATTATGTTCCGAGATGTGACGACGTTGCTGTTGGATCATAAGGCTTT**C**
AAAGACACGATCGACATCTTTGTT**GAGCGTTACCGGGACCAGAAGG**TGGACGTCATTGTGGGTGCGATGCCCTTGACTCCCT
TGACGTACCATAACGGTTTTTGTAATAAATTTGAAGGCAACCGTTACGTTTATAAATTTGGGAAGATTGCTGGCTGTTGTT
GATTACTTTTCGTGCTACTTTTTTTCAGGAATTGAAGCTCGAGGTTTTATCTTTGGG**CCACC CATCGG**

Figure S2. Examples of deletions observed during BE multiplexing.
(a) PCR genotyping of plants selected on 2-Fluoroadenine. **(b)** Examples of mutations in the *APT* gene, obtained after multiplex BE. Sequence for sgRNA#5 is in green, in orange for sgRNA#2 and in blue for sgRNA#21 (PAMs in bold). Mutations, editions or deletions are indicated in red.

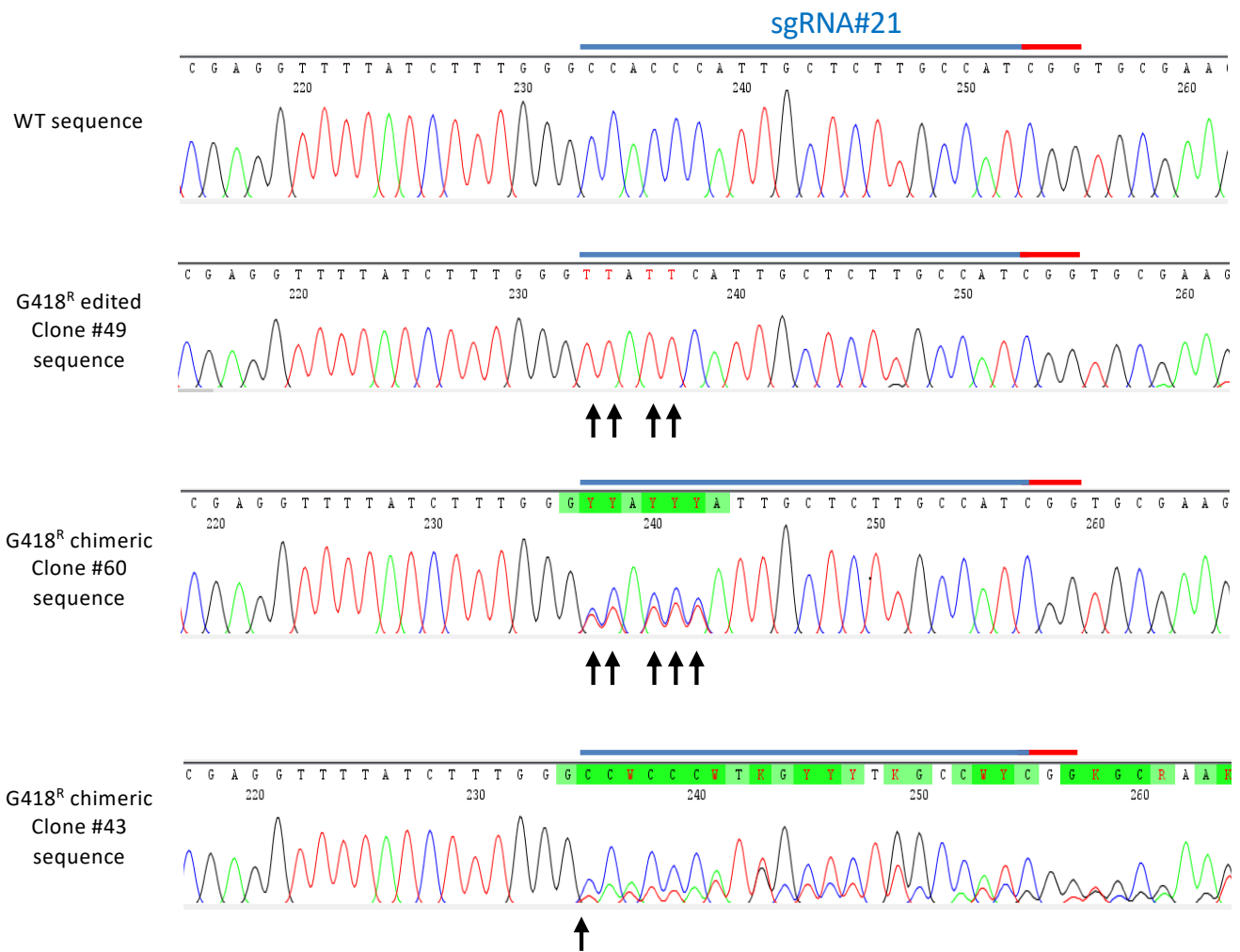
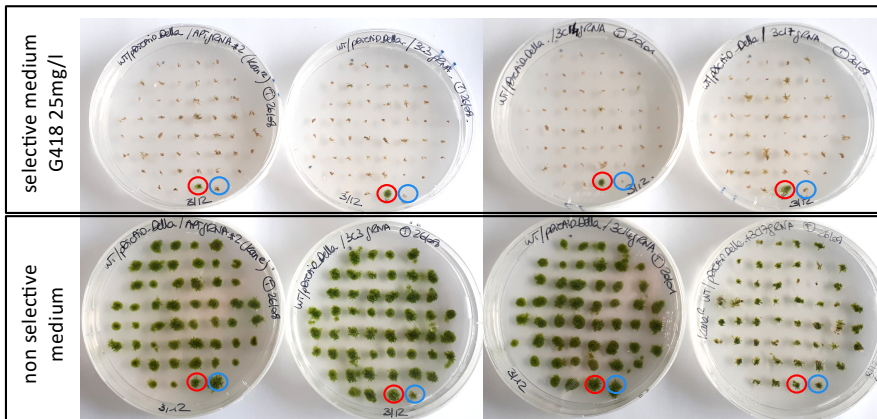


Figure S3. Examples of multiple cytosines editing or chimerism observed in some clones. G418 resistant clones obtained after transfection via CBE using sgRNA#21, were analysed by Sanger sequencing using the PpAPT#25/PpAPT#5 primers. Clone #49 shows co-editing of four cytosines. Clones #60 and #43 are chimeric clones with a mix of WT cells and cytosines edited or in/del cells. Target sequence is indicated with blue line, PAM sequence with red line and black arrows indicate chimerism or edited C inside the target sequence.

a)



b)

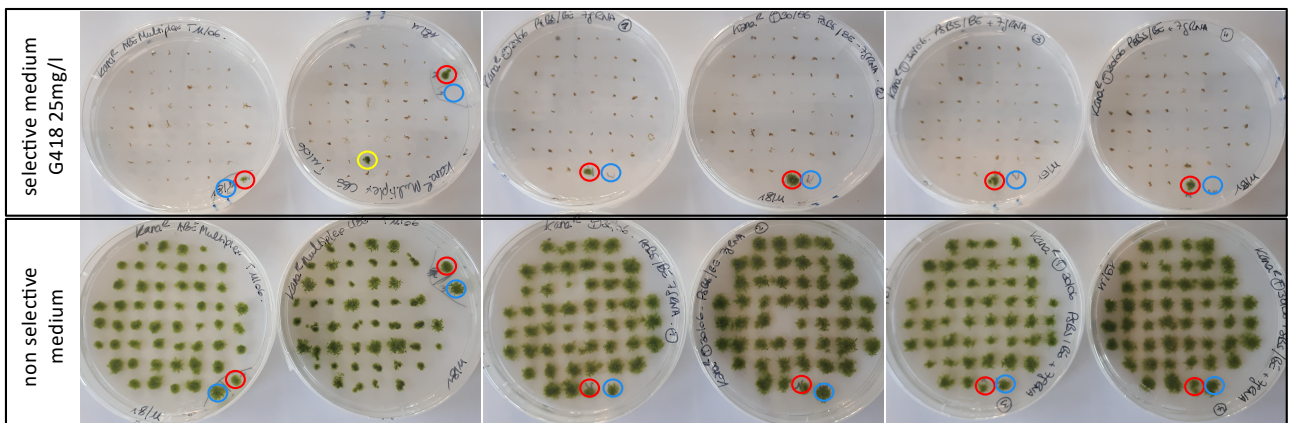
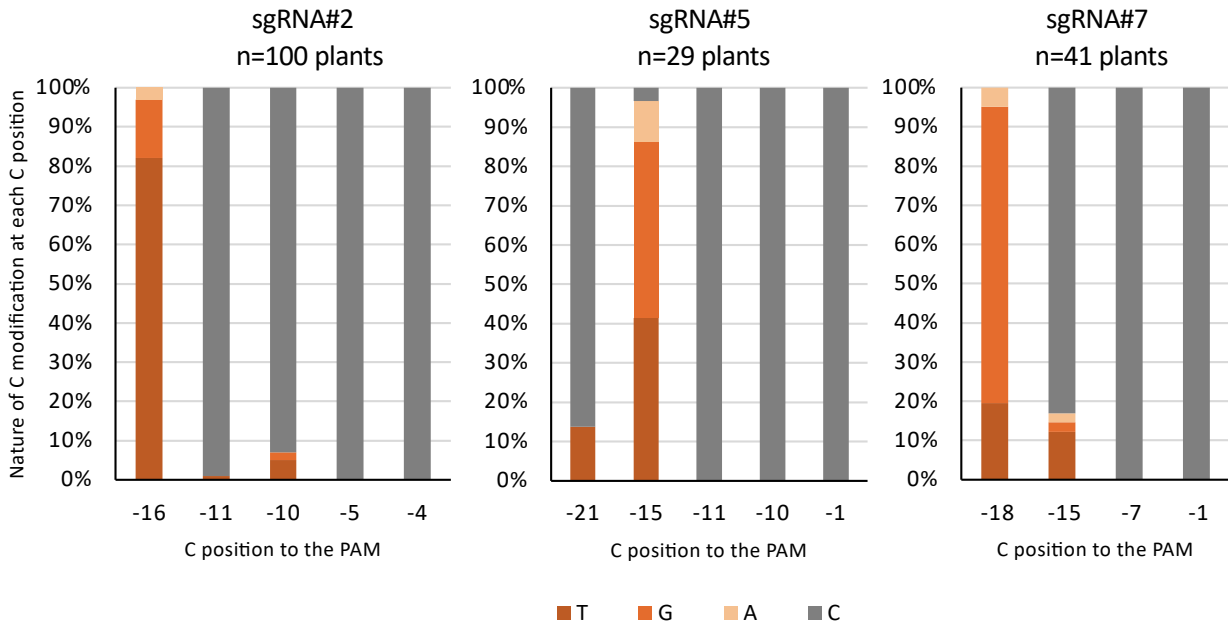


Figure S4. G418 sensitivity of ABE and CBE single and multiplex edited clones after relaxing of the antibiotic selection pressure.

A total of 480 clones, 192 from single editing strategy (a) plus 288 from multiplex editing strategies (b), that were first selected for transient expression of the ABE or CBE vectors were picked individually and grown for two weeks on non selective medium and then picked again in parallel on non selective or selective medium containing G418 (50mg/l). Sensitivity of the clones was estimated after two weeks of growth on selective medium. Circled in blue is the wild-type, in red is a control corresponding to a stable G418-resistant clone, in yellow is the unique clone that was still resistant to G418 after the step of relaxing of the antibiotic selection pressure.

a)



b)

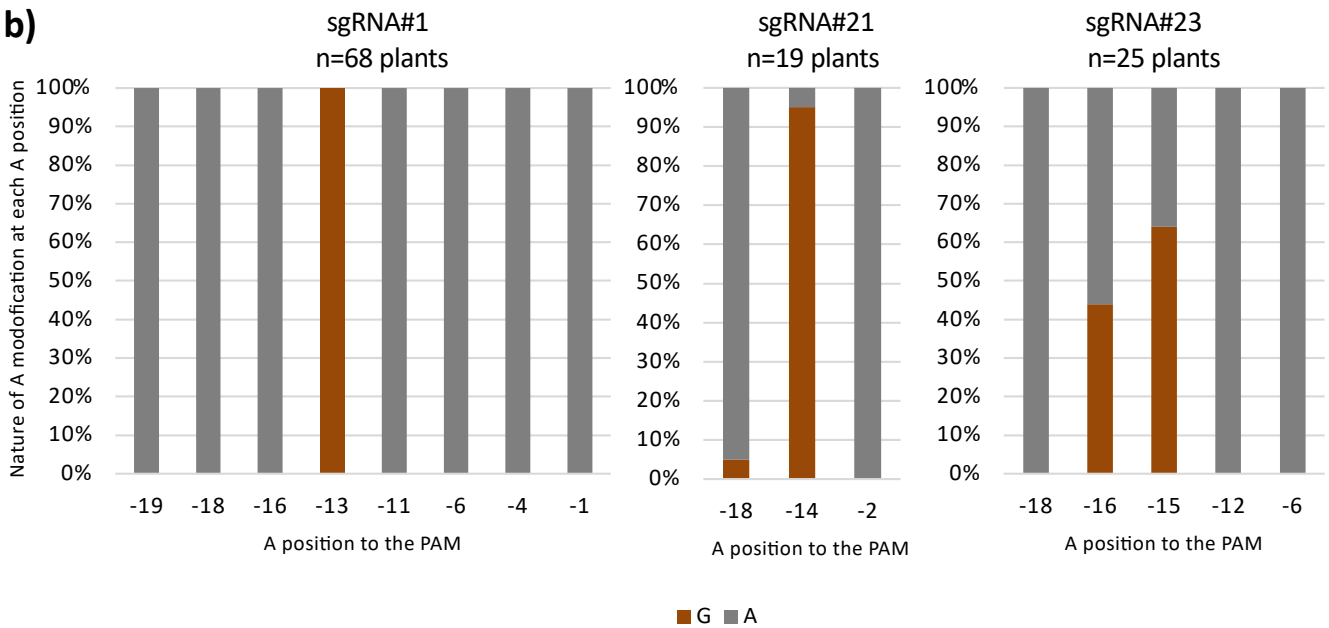


Figure S5. Nature of editing using CBE or ABE for each cytosine or adenine present in the target locus. (a) Nature of C editing using CBE with sgRNA#2, sgRNA#5 and sgRNA#7 for cytosines present in the target locus. (b) Nature of A editing using ABE with sgRNA#1, sgRNA#21 and sgRNA#23 for adenines present in the target locus. Primers used for amplification and Sanger sequencing can be found in Experimental procedures. Number of analysed plants is indicated.

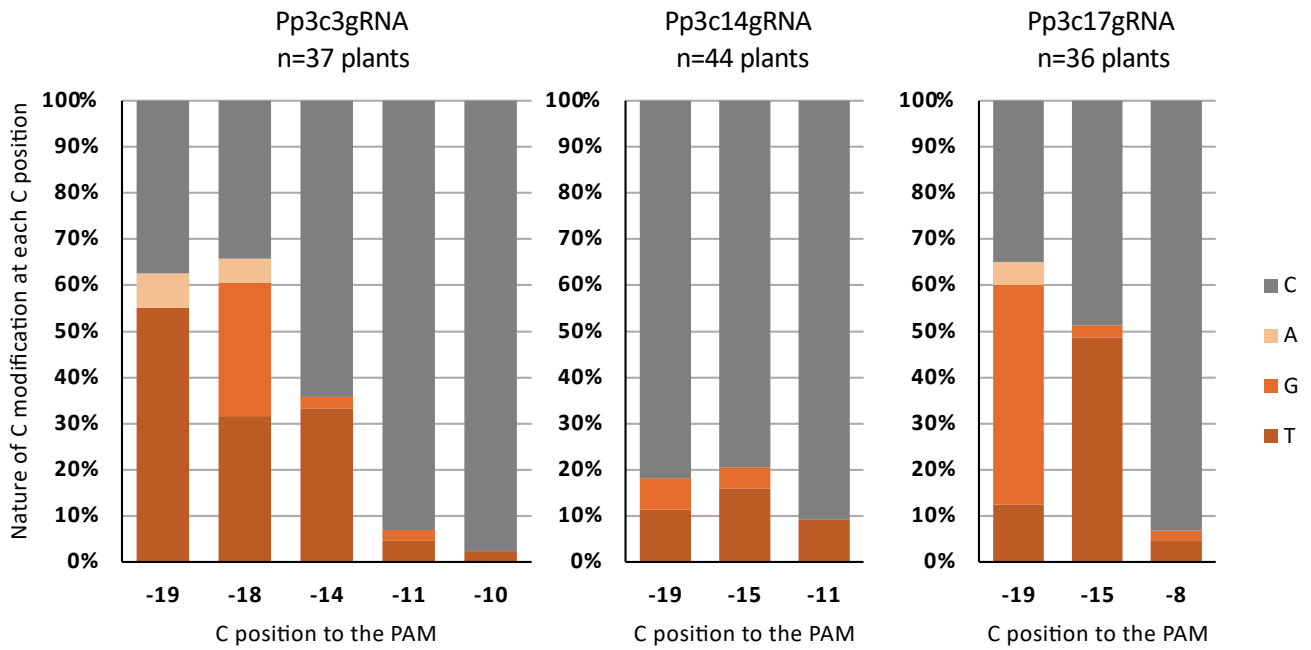


Figure S6. Nature of editing using CBE on genes of interest for each cytosine in the target locus. Nature of C editing using CBE with Pp3c3gRNA, Pp3c14gRNA and Pp3c17gRNA for each cytosines present in the target locus. Primers used for amplification and Sanger sequencing can be found in Experimental procedures. Number of analysed plants is indicated.

a)

sgRNA#24

5' -ATGTCTTAGGCCCTGTGATTAGG-3'

sgRNA#25

3' -GGACACTAATCCCTTTCATATC-5'

b)

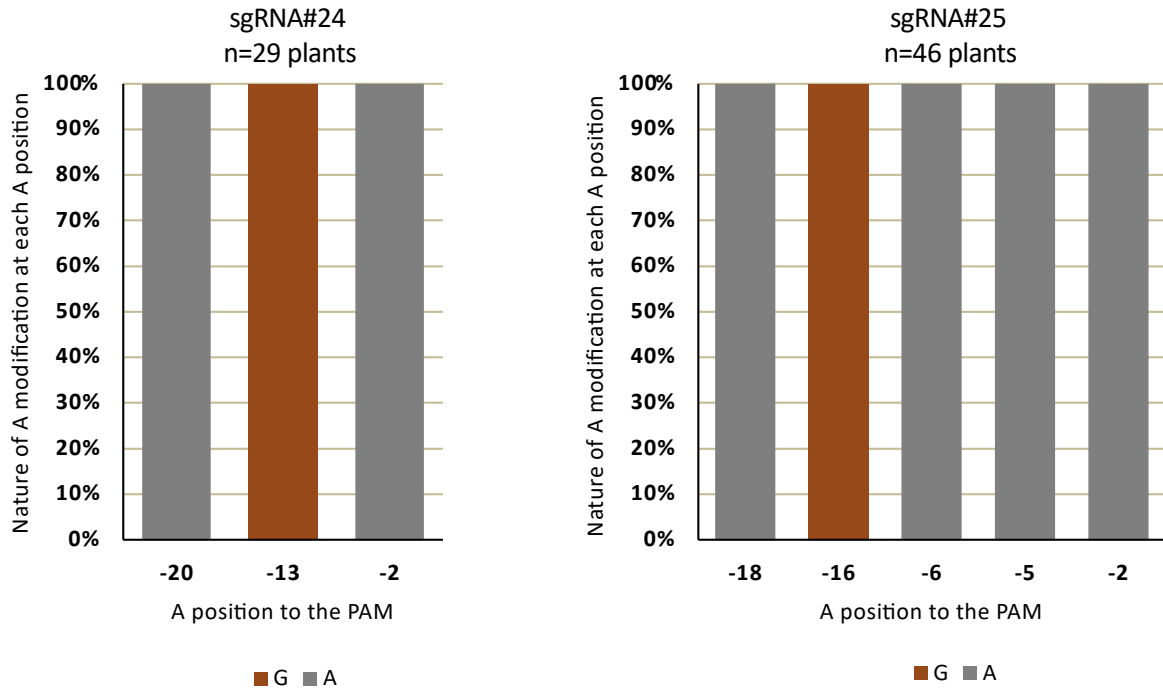
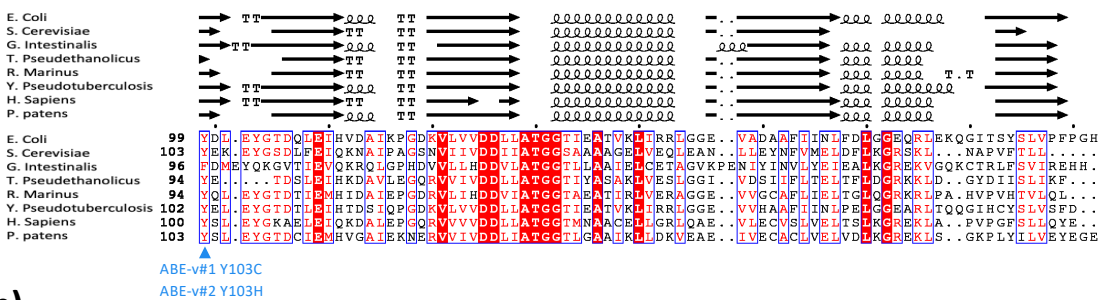
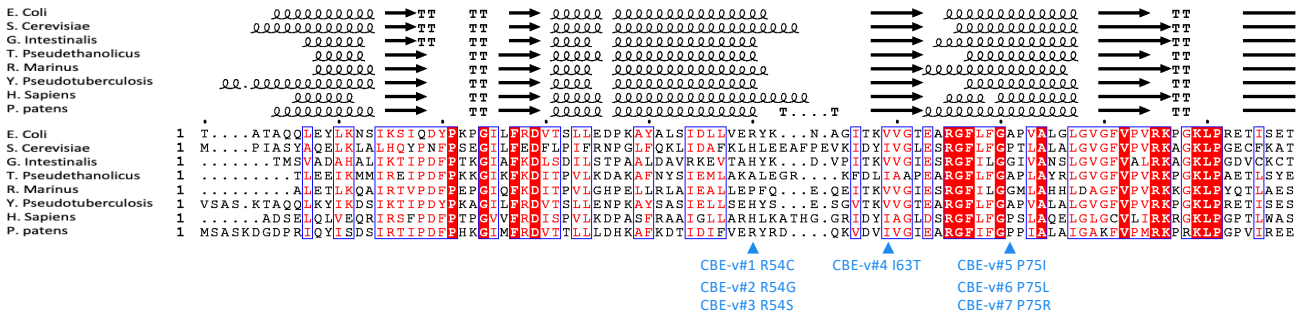


Figure S7. Sequence of two sgRNAs containing cytosines potentially target of ABE activity and nature of ABE editing using these sgRNAs.

(a) Sequence of sgRNA#24 and sgRNA#25 : target sequence is indicated in blue, PAM sequence in red. Cytosines in “TCN” context are indicated in green. **(b)** Nature of A editing using ABE with sgRNA#24 and sgRNA#25 for each adenine present in the 20bp target. Primers used for amplification and Sanger sequencing can be found in Experimental procedures. Number of analysed plants is indicated.

a)



b)

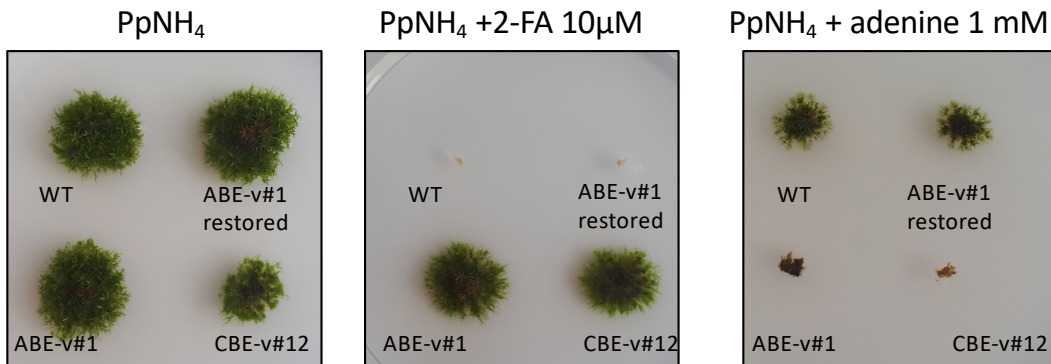


Figure S8. Alignment of APRT sequences from different species and phenotype of the *apt P. patens* mutants.

(a) Alignment using Modeller9.18 of APRT proteins from 8 species including *P. patens*. Black loops represent α -helix and black arrows represent β -strands. Single amino acids substitutions obtained in the base editing experiments are indicated by blue arrows. Accession numbers for the APRT used in this analysis are as follows: *Escherichia coli* (PDB:2DY0), *Saccharomyces cerevisiae* (PDB:1G2Q), *Giardia intestinalis* (PDB:1L1Q), *Thermoanaerobacter pseudethanolicus* (PDB:4LZA), *Rhodothermus marinus* (PDB:4M0K), *Yersinia pseudotuberculosis* (PDB:4MB6), *Homo sapiens* (PDB:4X45) and *P. patens* (Q45RT2). (b) Phenotypes on 2FA and adenine of the wild-type, ABE-v#1, CBE-v#12 single mutants and a restored ABE-v#1 mutant.

Figure S9. View of the *P. patens* APRT 3D model with amino acids (in blue) that could be modified as single substitutions using CBE or ABE.

The figure is supplied as a separate file

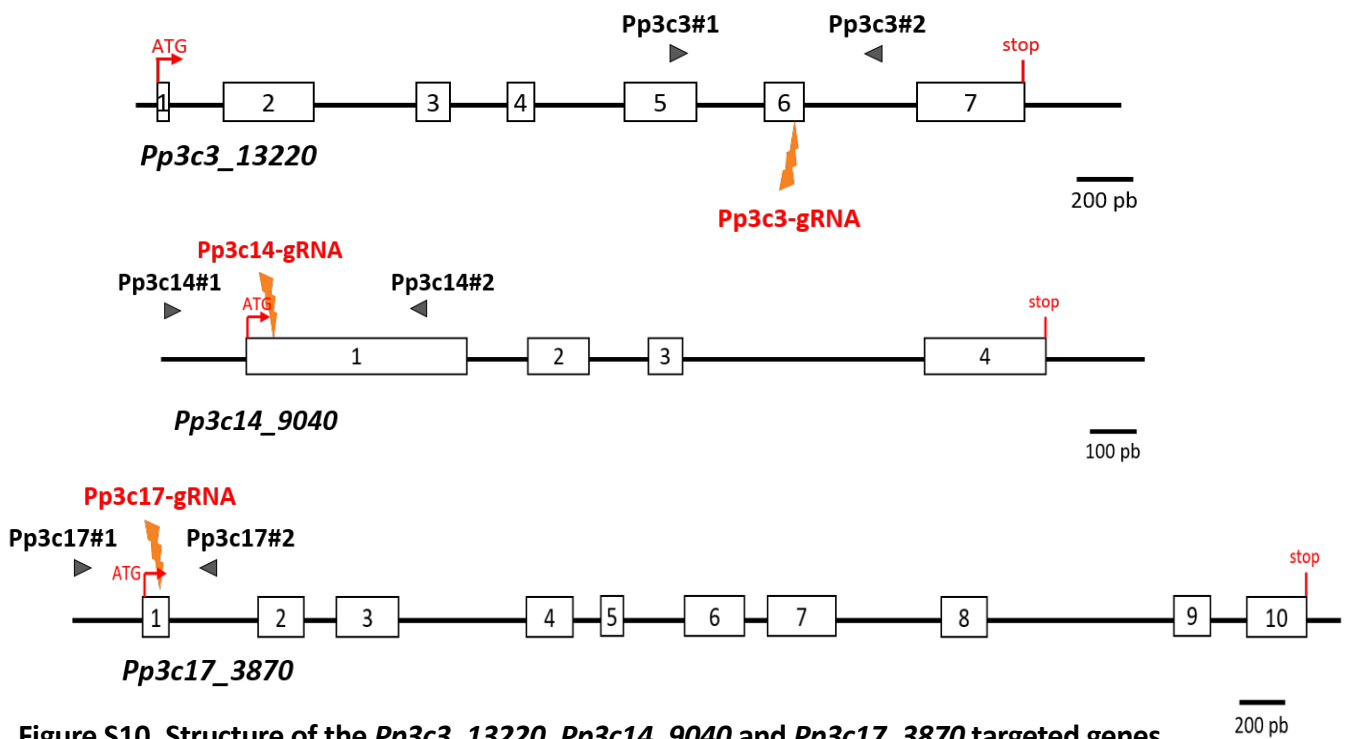


Figure S10. Structure of the *Pp3c3_13220*, *Pp3c14_9040* and *Pp3c17_3870* targeted genes.
 Structure of the *Pp3c3_13220*, *Pp3c14_9040* and *Pp3c17_3870* genes with their respective sgRNAs positions. Boxes represent the exons and black lines represent the introns. The 3 sgRNAs positions are indicated (in red) at the top for sgRNAs that target forward strand and at the bottom for sgRNA that target reverse strand. Grey arrows represent the primers used for PCR and sequencing.

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AtVDE      EFPAPDPSVLVQNFNISDFNGKWIYITSGLNPTFADFDCQLHEFHTEGDNKLVGNISWRIK 140
PpVDE      RFPVPAPSSLVQDFDTRFTGTWYITSGLNKTFDFTDCQKHEFTAE-PAKLSGNLSWRIA 139
      .**.* ** ***: **: *.*.***** ***:*** ***:** ** ***:***

AtVDE      TLDSGFFTRSAVQKFFVQDPNQPGVLYNHDNEYLHYQDDWYILSKIENKPEDYIFVYYRG 200
PpVDE      TPDGGFFTRSTVQNFVQDEKQPGILLNHGNEFLHYQDDWYILASRIENKPDYVFIYYRG 199
      * *.*****:***:*** ***:** ***.**:* ***:*** ***:** *:***:***:***:***

AtVDE      RNDAWDGYGGAVVYTRSSVLPNSI IPELEKAAKSIGRDFSTFIRTDNTCGP 251
PpVDE      KNDAWDGYGGAVVYTKSSTLPTS IPELGAAAKVNLDFKKFTTDNTCGP 250
      :***** ***:**.*.***** ***:.. **..* *****

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Figure S11. Sequence alignment of VDE from Arabidopsis and Physcomitrella.

Clustal alignment of VDE sequences from *A. thaliana* and *P. patens*. Only region corresponding to the lipocalin domain is shown (see Arnoux et al., 2009). Numbers are from Arabidopsis mature protein. Residues identified to be within 6 Å of violaxanthin ring (see Saga et al., 2010) are boxed in red. Amino acids that could be modified using the CBE strategy are in blue.

Arnoux P, Morosinotto T, Saga G, Bassi R, Pignol D. 2009. A structural basis for the ph-dependent xanthophyll cycle in *Arabidopsis thaliana*. *Plant Cell* 21: 2036–2044.

Saga G, Giorgetti A, Fufezan C, Giacometti GM, Bassi R, Morosinotto T. 2010. Mutation analysis of violaxanthin de-epoxidase identifies substrate-binding sites and residues involved in catalysis. *Journal of Biological Chemistry* 285: 23763–23770.

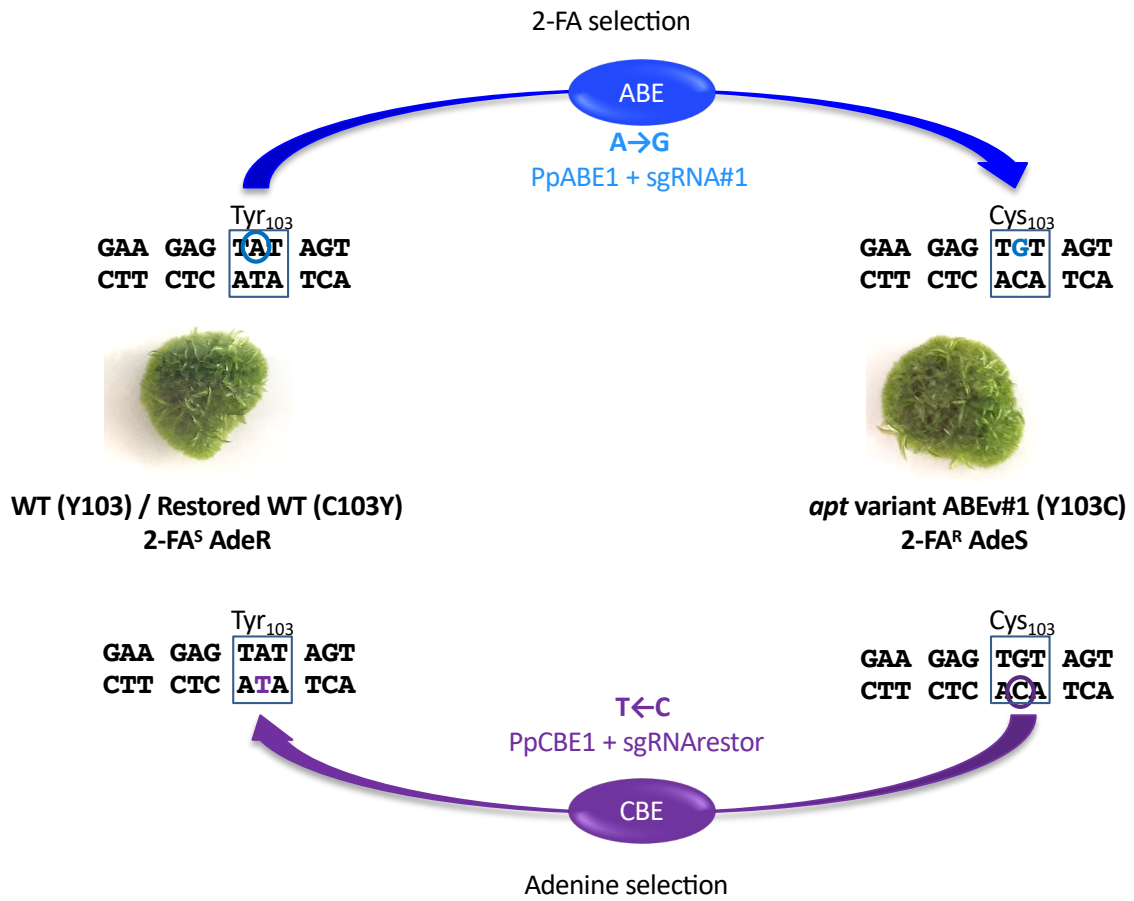


Figure S12. Use of the *APT* gene as a marker of base editing efficiency.
Schematic representation of the SMART strategy.

Table S1. List of sgRNAs expression cassettes used in this study.

Promoter sequences are in blue, target sequences in red and tracrRNA sequences in green.

Name	Sequence (5'-3')
sgRNA#1	ATTGAATGCCATTGAAGCAGACGTTGTCGACAGGTTAGCGACGATGGGTGTAGATGTGATGTGATGGTGTGGTTCTCCACG GCGGCGTCTTGGCGTGGCGGAGAAGGGGATATCCCGAAGGAGCGGCAGCGGGAGAGCACAAGCAGAAAGGGTGCAGTGAGTGAGT GGGTCCAGCTGGGTGGCTGGCCGAGTGGACGCGACCGGGTTTCGAGGGGGcGGGGGAGAAAAGGGATGGAGCGAGGGATATAACCC ACATGGAATGGAGGTGGGTGTGAAGCGGGTATATAGGAAGGTGGAGGACTTACAACCgaagagtatagctagagtaGTTTTAGAGCTAGA AATAGCAAGTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGTCTTTTTCTAGACCCAGCTTCTTGTAC AAAGTTGGCATTAA
sgRNA#2	ATTGAATGCCATTGAAGCAGACGTTGTCGACAGGTTAGCGACGATGGGTGTAGATGTGATGTGATGGTGTGGTTCTCCACG GCGGCGTCTTGGCGTGGCGGAGAAGGGGATATCCCGAAGGAGCGGCAGCGGGAGAGCACAAGCAGAAAGGGTGCAGTGAGTGAGT GGGTCCAGCTGGGTGGCTGGCCGAGTGGACGCGACCGGGTTTCGAGGGGGcGGGGGAGAAAAGGGATGGAGCGAGGGATATAACCC ACATGGAATGGAGGTGGGTGTGAAGCGGGTATATAGGAAGGTGGAGGACTTACAACCggagcgtaccgggaccagaGTTTTAGAGCTAG AAATAGCAAGTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGTCTTTTTCTAGACCCAGCTTCTTGTGTA CAAAGTTGGCATTAA
sgRNA#5	GTCCATTGAAGCAGACGTTGTCGACAGGTTAGCGACGATGGGTGTAGATGTGATGTGATGGTGTGGTTCTCCACGCGCGCG TCCTTGGCGTGGCGGAGAAGGGGATATCCCGAAGGAGCGGCAGCGGGAGAGCACAAGCAGAAAGGGTGCAGTGAGTGAGTGGGTCC AGCTGGGTGGCTGGCCGAGTGGACGCGACCGGGTTTCGAGGGGGGGGGGAGAAAAGGGATGGAGCGAGGGATATAACCCACATG GAATGGAGGTGGGTGTGAAGCGGGTATATAGGAAGGTGGAGGACTTACAACCCATggatgctgtccgagatatacGTTTTAGAGCTAGAAA TAGCAAGTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGTCTTTTTGAGCTCGTC
sgRNA#7	GATCTAGAGTATTGGATAAAATAATACGTATAGATGTATAGATGAGCAAGTAGTAAAAGATGACTTACTCTAGTTAAATAAAGCATGG AAAGAAAGTGTAGCATGTACAAAAGAGAAATAAAAAATAAACACAAAACCTCTGTATCGATAGATTCTTAGAAGGGGAAACGAAAC ATAAACTTCAAGTGAATGCAAGTGTAGTACTATTGAGTGTAGTGGCGGGCAGGGGAGGTGGAGTTGACCATAGCCGTTGC GGTGAAGGGGAAAGCGGGTATATGGAGGTGGGGTGGAGCCGGTCTGGcatcaacaaatttcggaagcGTTTTAGAGCTAGAAAATAGC AAGTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGTCTTTTTGAGCTCGTC
sgRNA#21	GAAGCAGACGTTGTCGACAGGTTAGCGACGATGGGTGTAGATGTGATGTGATGGTGTGGTTCTCCACGCGCGCGTCTTTCG GGTGGCGGAGAAGGGGATATCCCGAAGGAGCGGCAGCGGGAGAGCACAAGCAGAAAGGGTGCAGTGAGTGAGTGGGTGCAGCTGG GTGGCTGGCCGAGTGGACGCGACCGGGTTTCGAGGGGGCGGGGAGAAAAGGGATGGAGCGAGGGATATAACCCACATGGAATGG AGTGGGTGTGAAGCGGGTATATAGGAAGGTGGAGGACTTACAACCCATgccaccattgcttccatGTTTAAAGACTATGCTGGAAC AGCATAGCAAGTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGTCTTTTTACTAGTGACC
sgRNA#23	GAAGCAGACGTTGTCGACAGGTTAGCGACGATGGGTGTAGATGTGATGTGATGGTGTGGTTCTCCACGCGCGCGTCTTTCG GGTGGCGGAGAAGGGGATATCCCGAAGGAGCGGCAGCGGGAGAGCACAAGCAGAAAGGGTGCAGTGAGTGAGTGGGTGCAGCTGG GTGGCTGGCCGAGTGGACGCGACCGGGTTTCGAGGGGGCGGGGAGAAAAGGGATGGAGCGAGGGATATAACCCACATGGAATGG AGTGGGTGTGAAGCGGGTATATAGGAAGGTGGAGGACTTACAACCCATccaatgacgtccacttcGTTTAAAGACTATGCTGGAAC AGCATAGCAAGTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGTCTTTTTACTAGT
sgRNA#24	GAAGCAGACGTTGTCGACAGGTTAGCGACGATGGGTGTAGATGTGATGTGATGGTGTGGTTCTCCACGCGCGCGTCTTTCG GGTGGCGGAGAAGGGGATATCCCGAAGGAGCGGCAGCGGGAGAGCACAAGCAGAAAGGGTGCAGTGAGTGAGTGGGTGCAGCTGG GTGGCTGGCCGAGTGGACGCGACCGGGTTTCGAGGGGGCGGGGAGAAAAGGGATGGAGCGAGGGATATAACCCACATGGAATGG AGTGGGTGTGAAGCGGGTATATAGGAAGGTGGAGGACTTACAACCCATatgtcttagccctgtattGTTTAAAGACTATGCTGGAAC GCATAGCAAGTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGTCTTTTTACTAGT
sgRNA#25	GAAGCAGACGTTGTCGACAGGTTAGCGACGATGGGTGTAGATGTGATGTGATGGTGTGGTTCTCCACGCGCGCGTCTTTCG GGTGGCGGAGAAGGGGATATCCCGAAGGAGCGGCAGCGGGAGAGCACAAGCAGAAAGGGTGCAGTGAGTGAGTGGGTGCAGCTGG GTGGCTGGCCGAGTGGACGCGACCGGGTTTCGAGGGGGCGGGGAGAAAAGGGATGGAGCGAGGGATATAACCCACATGGAATGG AGTGGGTGTGAAGCGGGTATATAGGAAGGTGGAGGACTTACAACCCATctatacttccctaatcacGTTTAAAGACTATGCTGGAAC GCATAGCAAGTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGTCTTTTTACTAG
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CCAGGCTGCTTCTGCTTTCGCGCATGGCTCGCGCGGAGAACTTGTAGT
ACGTTCCGCAACATGGGAGGAGCAACCGCGCGGCTGCTGCTGCTGCT
TTCCGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
CCAGCTGCTAATCCACACTTGGCATTGGCATTGGCATTGGCATTGG
TCTACGCTGCTGGAAGCTGCTAGCGGATCACTGCGCAGCTGCTG
GTACGCTTCCAGCAGCAAGAAACGCGCGCTTCCATGATGCTGCGAT
GCGGGTGCACGCTATAGGACATCGGAACGAAAAATCTGTTGCTG
TCGCTTGGCGGCTTCTTATCAGCGCGCAGCGCTGCGCGGCTT
CGGGATTTTGGGATTCGATCAGCGCGCTTGGCAGATTCACCG
GGCTGCTTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTT
AACTTCTCCACAGGTCATCCAGCGCGCGGCTTGTGACCGGGC
GGATGTTTGGCAGCTCAAGCGGATCTTCTGGGTTGGGGTTCCAGTG
CCTTGGAGCGCGGAGCAACCGCGCTTACGCTGGCAACCGCGCT
CGTTCTCCACACATGGGAGGAGCAACCGCGCGGCTGCTGCTGCTGCT
GATTTTCAACCGCTTCTTACCGCTTAAATCAGTCACTGCTGCTGCT
TCATTTGCTCAATTTACTCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
CGTAAATGCTTTCCTTGGCTACCGCTTACCTTCTGCTTGGTGTGA
TCTTCCGCGGCACTGAAAGTTCAGCGCTTACGCTGGGCTGCTGCT
CAGGCTGGCAAGCTTTCAGCTTCTGCTGCTGCTGCTGCTGCTGCTG
CACTTAGCGTGTGCTGCTTCTGCTCAATTTCTTACCTCAATTAAC
AAATGATTTTGAATTAATTCAGCGCGCAGCGCTGGACCTCGCGGCA
CGCTGCGCTTGGGTTCTGATTAAGAAGGTTGCTGCGCGCGGCGAGT
CGCTGGGATCTACCGCTGCTGATACGGGCAAGAAATGGGAGCT
CGTACCGCGCAGCGCTTGGCAACTCAGCGGATGCGGCTGCTTGT
ATCGCGGACAGCAAAAGCGCTTGTAGCTTCCATCGTGGACCTC
AATGGCTGCTTAAACAGCTTCCACAGGTCGCGGCTGGCCATATGCTG
AAGGCTTGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG
GACAGCGCAAGCTCGCGCTTGGGCGCTGCTGCTGCTGCTGCTGCTG
GCGCGCGGATGGCTTCACTGCTGCTGCTGCTGCTGCTGCTGCTGCT
CGCAACGGTGTAGCGTGTCTTCCCGCAGCGCGCCAAATCGCGGCA
CTGCGCTGGGATCGAATCGACTAACAGAACTCGCGCGCGGAGTTG
CAGGCGCGGCTGATGGTGTGATGCTGCTTTCGCTGACCGCTT
TCTGGTTAAGTACAGGATAACCTTCAAGCTTCCCTTGGGATTTGTT
TATTTACTCATCGATATATACGAGCGCAGCTGACGCAAGCTGTTT
TACTCAAAATACATCACTTTTGTAGCGCGCGCTGCTTCTTCAAG
GGCAAGCTGGCGCGCAGCGCTTGGCACTGACGAAACCGGCA
GGATTTCAAGCGCGCAGGTTGAGAGCTGCGCGGCGCTGCAACAG
TACCGGCGGATCATCTCGCTGCTGCTGCTGCTGCTGCTGCTGCTG
TTGCTTGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG
ATCTCGCGCGCGCGCGCGCTGCGCTGCTGCTGCTGCTGCTGCTG
AGGACCGCGCGCTGCGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
AGTGGCGGTCAGGCTGAGGATGACCGCAAGCACTGAGCGCTGCTG
TTTACGCTGCGGCTTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG
GTGCGGGGTGAGGTTAGGCGGGGCGCAACTTACGCTTGGGCTT
CGGCTTCCGCGCGCTCGGCTGCTGCTGCTGCTGCTGCTGCTGCTG
CTGCGCAATGCGCGCAACAGGTTCAACCACTGCGCGCGCGGCTG
TGCTGCTGCGCGCAGCTTTCAGGCTGACGAGCGCGCGCGCTTCC
TGGATGCTGCGCAATGCTCAGTGGTGGCGGCTGCTGCGCGCAGG
GTCTAGCCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
TCTGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG
AACAGCTTGGTGAAGCGCGCGCGCGCTGCTGCTGCTGCTGCTGCT
GTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG
CGCTTCTTCTAGGCTAATGCTGCTGCTGCTGCTGCTGCTGCTGCT
TTTATGCGCAATAAACCGCGCAAGAAACCGGAAAGGCGAGGCG
GGCAGCTGCTGCGGTAATTTAGGACTTGTGCGCAATGCTGCTTCA
ACGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
TGGATCAAAGTACTTTGATCCGAGGGAAACCTTGGTTGGCATGACA
TACAAATGGAGCAACGGATAAACCTTTTACCGCTTTTAAATACCGTT
ATTCTAA

Table S3. List of PCR primers used in this study.

Name	Sequence (5'-3')
Rad51.1#6	TGAGGAGGAAGTTCATCATGG
Rad51.1#7	ACCGCCAATGGGTTTATGC
PpAPT#5	ACAAGGTGGTGTCAACTTCA
PpAPT#8	AACGCGAGGATGACCCAAGCC
PpAPT#10	GCGGCGCGTAACGAAGCAA
PpAPT#25	GTCGTCACTCTCGGTTTTG
PpAPT#60	ATGGTCAATGTGGCAGCAAG
PpAPT#61	CCTGTCAACCCCTACCTGGA
Pp3c3#1	GTCAAAAGCATGAGTTCACAG
Pp3c3#2	GTTTCCATTTGCATTAGGTAG
Pp3c14#1	ATGGAGGTTTTAGAGCTTTGA
Pp3c14#2	CTACCGAAGCCTCAGCCGACT
Pp3c17#1	AAAACCTCCGAAAGACACCAGT
Pp3c17#2	TAGTTTCAACCCATTCAACAC
gRNA1OT1#1	GCTGATAAGCGTGGTGACAGA
gRNA1OT1#2	TGGGGAGGGTGGATACAGAA
gRNA1OT2#1	AGAAGTTGGCATATTGAGGTCGT
gRNA1OT2#2	GAGAATGCACCGAGCGACTA
gRNA1OT3#1	GCAGCAAGTGCTGGTTCTTC
gRNA1OT3#2	AGCGCGCAGTCTCTGTTTAT
gRNA1OT4#1	AGCTCCTTTGAAGCCTTACCA
gRNA1OT4#2	ACGAGCCTAGACTATGTGAGA
gRNA1OT5#3	GGCAAGTTGCAAGTGGTCTG
gRNA1OT5#4	CGCAGGTGAGATTTGGGACT
gRNA1OT6#1	GTCGGGGTTGATCCGATTGA
gRNA1OT6#2	CTCTCTTTCCCTGCCTCCG
gRNA1OT7#1	CTTGCAGGCCGTGATAATGC
gRNA1OT7#2	TCCGCATCTTGGTGTGTGAA
gRNA1OT8#2	TCTTTCGTAGAGCTCGGAGT
gRNA1OT8#3	TTGTTTGAAAGATCTTATCATAG
gRNA1OT9#2	TCATGTTTGTCCATGTTTTTGA
gRNA1OT9#3	TGTTGGCAAAATGCCTACCT
gRNA2OT1#1	GCAGAGGAACAGAAGGAACC
gRNA2OT1#2	GAGCGTCATCTGCTTGCTTG
gRNA2OT2#1	TCGGTTTTGCAGCTGCTTTC
gRNA2OT2#2	GAGCAAAGCACAGTATGCGG
gRNA2OT3#1	CTCTTTCCCGCATTCTGT
gRNA2OT3#2	CTACGGAGCCGTTACCACTT
gRNA2OT4#1	TCTTACAGCAGGACGAACC
gRNA2OT4#2	AGCAGAACAAGCTCCTCGAC
ABE7.10-AttB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTACGCCACCATGTCCGAAGT
ABE7.10-AttB2	GGGGACCCTTTGTACAAGAAAGCTGGGTATTAGACTTTCCTTCTTCTTGGG

Table S4. Mutation rates of the CBE and ABE systems tested (2-FA direct selection).

editing strategy		sgRNAs used	Regenerant clones	2-FA ^R clones	Relative mutation efficiency (%) ^a
Native Cas9	simplex	sgRNA#1	23475	604	2.7 ± 0.9 ^b
	simplex	sgRNA#2	23615	672	2.9 ± 0.4
	simplex	sgRNA#21	23625	798	3.5 ± 0.5
	simplex	sgRNA#23	22425	522	2.3 ± 0.8
ABE	simplex	gRNA#1	57600	493	0.8 ± 0.3
	multiplex	sgRNAs#1/21/23	21000	149	0.7 ± 0.1
CBE	simplex	sgRNA#2	64425	1025	1.6 ± 0.6
	simplex	gRNA#21	55050	813	1.5 ± 0.2
	multiplex	sgRNAs#2/5/7/21	54150	1448	2.8 ± 0.6

^a Relative mutation efficiency expresses the frequency of 2-FA resistant clones among the population of regenerants.

^b Average and standard deviations (± SD) were determined from at least three independent experiments.

Table S5. Transfection efficiency of the CBE and ABE systems.

editing strategy		sgRNAs used	Regenerant clones	G418 ^R clones	Transfection efficiency (%)
ABE	multiplex	sgRNAs#1/21/23	28950	427	1.5 ± 0.4 ^a
	simplex	sgRNA#2	60000	1281	2.1 ± 0.6 ^a
CBE	simplex	sgRNA#21	58725	929	1.6 ± 0.1
	multiplex	sgRNAs#2/5/7/21	31125	617	2.0 ± 0.5

^a Average and standard deviations (± SD) were determined from at least three independent experiments

Table S6. Mutation rates of the CBE system after pre-selection on G418.

editing strategy	sgRNAs used	Number of G418 ^R clones analyzed	Number of mutated clones ^a	Number of 2FA ^R clones (from mutated ones)	Knock-out efficiency (%) ^b	
CBE	simplex	sgRNA#2	46	17	17	100%
	simplex	sgRNA#21	86	60	60	100%

^a The *APT* gene has been sequenced for a number of G418^R clones obtained after CBE transfection (see Table S2).

^b Knock-out efficiency (in %) expresses the frequency of 2-FA resistant clones among the population of G418^R clones mutated at the *APT* locus.

Table S7. Frequency of substitution for cytosines at each position of the 8 sgRNAs used in this study.

		C position to the PAM																				
		-21	-20	-19	-18	-17	-16	-15	-14	-13	-12	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1
sgRNA#2	number of C of edited C						100					100	100					100	100			
							100					1	7					0	0			
sgRNA#5	number of C of edited C	29						29				29	29									29
		4						28				0	0									0
sgRNA#7	number of C of edited C				42			42									42					42
					42			7								0						0
sgRNA#21	number of C of edited C		86	86		86	86	86					86		86					86	86	
			69	58		86	82	36					0		2					0	0	
sgRNApp3c3	number of C of edited C			31	31				31			31	31									
				25	24				15			3	1									
sgRNApp3c14	number of C of edited C			10				10				10				10					10	
				9				9				4				0					0	
sgRNApp3c17	number of C of edited C			29				29								29						
				26				20								3						
sgRNArestor	number of C of edited C	14			14		14		14			14	14	14						14		14
		2			14		10		4			0	1	1						0		0
Total	number of C of edited C	43	86	156	87	86	200	196	45	^a nd	^a nd	184	260	14	115	52	^a nd	100	200	96	14	71
		6	69	118	80	86	192	100	19			8	9	1	5	0		0	0	0	0	0
editing (in %)		14	80	76	92	100	96	51	42			4,3	3,5	7,1	5,8	0		0	0	0	0	0

^a No data, there was no C in positions -13, -12 and -6 for any of the 8 sgRNAs.

The predicted editing window (from Shimatani et al., 2017 and Eid et al., 2018), is highlighted in yellow.

Shimatani Z, Kashojiya S, Takayama M, Terada R, Arazoe T, Ishii H, Teramura H, Yamamoto T, Komatsu H, Miura K, et al. 2017. Targeted base editing in rice and tomato using a CRISPR-Cas9 cytidine deaminase fusion. *Nature Biotechnology* 35: 441–443.

Eid A, Alshareef S, Mahfouz MM. 2018. CRISPR base editors: genome editing without double-stranded breaks. *The Biochemical journal* 475: 1955–1964.

Table S8. Sequences and positions of possible off target sites for sgRNA1 and sgRNA2.

Mismatches with the target sites are indicated in red). Physcomitrella genomic off target sequences were identified using the CRISPOR v2.0 tool (<http://crispor.tefor.net>).

Locus name	Target sequence + PAM*	Position on <i>P. patens</i> reference genome from Phytozome ^a
sgRNA1 Target	gaagagtatagctagagtaTGG	Chr08:10812103 to 10812081
sgRNA1 Off-Target#1	gaagagt g tagagtagagtaGGG	Chr07:4705222 to 4705244
sgRNA1 Off-Target#2	gaag ctt attgtctagagttAGG	Chr05:17438841 to 17438819
sgRNA1 Off-Target#3	g taaagtatagctagagttGGG	Chr24:6867383 to 6867405
sgRNA1 Off-Target#4	caagc gtata ctct tgagtaTGG	Chr14:2675353 to 2675375
sgRNA1 Off-Target#5	gaat attata atctagagttGGG	Chr22:3453377 to 3453399
sgRNA1 Off-Target#6	gca aagtatag tata aagtaGGG	Chr06:582960 to 582982
sgRNA1 Off-Target#7	gaag a ctatagtttagag gg CGG	Chr02:13705150 to 13705128
sgRNA1 Off-Target#8	gaa a agtatt gtca gactaAGG	Chr05:6043528 to 6043550
sgRNA1 Off-Target#9	gaag a ctata ttctata cttaCGG	Chr17:5887705 to 5887683
sgRNA2 Target	tgagcgttaccgggaccagaAGG	Chr08:10812681 to 10812659
sgRNA2 Off-Target#1	tga gg ag tcca agggaccagaAGG	Chr16:7631517 to 7631494
sgRNA2 Off-Target#2	tga agg ttact gg accag=AGG	Chr4:12028646 to 12028667
sgRNA2 Off-Target#3	tgg gc gt gac ggggagcagaAGG	Chr17:4446370 to 4446348
sgRNA2 Off-Target#4	tgagcgt ttcag gt act gaAGG	Chr09:9115552 to 9115574

* Coordinates of the off-target sequences were identified using Basic Local Alignment Search Tool (<http://phytozome.jgi.doe.gov/pz/portal.html#!search>).

Table S9. List of amino acids modified in the *APT* gene using the CBE or ABE strategy.

In blue, clones where 1 amino acid has been modified, in green clones where 2 amino acids have been modified, in yellow clones where 3 amino acids have been modified, in orange clones where 4 amino acids have been modified.

clones	sgRNAs used and amino acid modified in the WT sequence								
	sgRNA#5	sgRNA#2		sgRNA#23	sgRNA#21				sgRNA#1
WT	S17	R54	R56	I63	P75	P76	I77	L79	Y103
CBE-v#1*		C							
CBE-v#2		G							
CBE-v#3		S							
ABE-v#1*									C
ABE-v#2									H
ABE-v#3				T					
CBE-v#4					I				
CBE-v#5					L				
CBE-v#6					R				
CBE-v#8	N	C							
CBE-v#9		C	G						
CBE-v#10		C	W						
ABE-v#4				T					C
ABE-v#5				T			V		
CBE-v#11					I	S			
CBE-v#12					L	C			
CBE-v#13					L	F			
CBE-v#14					L	I			
CBE-v#15					L	S			
CBE-v#16					V	S			
ABE-v#6							V		C
CBE-v#17	N	C			R				
CBE-v#18	N	C				F			
CBE-v#19	T	C				F			
CBE-v#20		C			L	F			
CBE-v#21		C			L	C			
CBE-v#22		C				F		F	
ABE-v#7				T			V		C
CBE-v#23	I	C			R	Y			
CBE-v#24	N	C	W			L			
CBE-v#25	N	C			L	F			
CBE-v#26	N	G			L	I			
CBE-v#27	T	C			L	F			
CBE-v#28	T	C			L	C			
CBE-v#29	T	C			L	I			
CBE-v#30	T	C			L	Y			
CBE-v#31	T	C			V	C			
CBE-v#32		C	W		L	C			

* Correspond to mutants that are 2FA^R but show a wild-type developmental phenotype (one example in figure S6).

Table S10. Consequence of CBE editing in the different edited clones for the 3 genes of interest.
 In blue nucleotide substitutions, in green amino acid changes, in red stop codons.

Gene	PAM	Target	PAM	
Gene Pp3c3_13220 (VDE)	WT	5'- T T C C T C C A C T A T C A G G A T G A T T G G T A T A A A -3'	3'- A A G G A G G T G A T A G T C C T A C T A A C C A T A T T T -5'	F L H Y Q D D W Y I
	#12	5'- T T C C T C C A C T A T C A G G A T G A T T c a T A A A T T -3'	3'- A A G G A G G T G A T A G T C C T A C T A A C C A T A T T T -5'	F L H Y Q D D S Y I
	#15	5'- T T C C T C C A C T A T C A G G A T G A T T t a T A A A T T -3'	3'- A A G G A G G T G A T A G T C C T A C T A A C C A T A T T T -5'	F L H Y Q D D L Y I
	#20	5'- T T C C T C C A C T A T C A G G a A T G A T T a a T A A A T T -3'	3'- A A G G A G G T G A T A G T C C T A C T A A C C A T A T T T -5'	F L H Y Q N D stop Y I
	#22	5'- T T C C T C C A C T A T C A G G A T G A T T a t T A A A T T -3'	3'- A A G G A G G T G A T A G T C C T A C T A A C C A T A T T T -5'	F L H Y Q D D Y Y I
	#34	5'- T T C C T C C A C T A T C A G G A T a A T T c a T A A A T T -3'	3'- A A G G A G G T G A T A G T C C T A C T A A C C A T A T T T -5'	F L H Y Q D N S Y I
	#37	5'- T T C C T C C A C T A T C A G G A T G A T T a t a A A A T T -3'	3'- A A G G A G G T G A T A G T C C T A C T A A C C A T A T T T -5'	F L H Y Q D D Y T I
Gene Pp3c14_9040	WT	5'- A C C C A T G C T G T C G A T C A T G C G G A C A A T G G A -3'	3'- T G G G T A C G A C A G C T A G T A C G C C T G T T A C C T -5'	T H A V D H A D N G
	#3	5'- A C C C A T G g T G T C G A T C A T G C G G A C A A T G G A -3'	3'- T G G G T A C G A C A G C T A G T A C G C C T G T T A C C T -5'	T H G V D H A D N G
	#8	5'- A C C C A T G t T G T t G A T t A T G C G G A C A A T G G A -3'	3'- T G G G T A C G A C A G C T A G T A C G C C T G T T A C C T -5'	T H V V D Y A D N G
	#11	5'- A C C C A T G t T G T t G A T C A T G C G G A C A A T G G A -3'	3'- T G G G T A C G A C A G C T A G T A C G C C T G T T A C C T -5'	T H V V D H A D N G
	#16	5'- A C C C A T G g T G T t G A T t A T G C G G A C A A T G G A -3'	3'- T G G G T A C G A C A G C T A G T A C G C C T G T T A C C T -5'	T H G V D Y A D N G
	#25	5'- A C C C A T G C T G T g G A T t A T G C G G A C A A T G G A -3'	3'- T G G G T A C G A C A G C T A G T A C G C C T G T T A C C T -5'	T H A V D Y A D N G
Gene Pp3c17_3870	WT	5'- T C T A T G C A A A C G G A G T T C G A G G T T G A G G A G -3'	3'- A G A T A C G T T T G C C T C A A G C T C C A A C T C C T C -5'	S M Q T E F E V E E
	#7	5'- T C T A T G t A A A t G G A G T T C G A G G T T G A G G A G -3'	3'- A G A T A C G T T T G C C T C A A G C T C C A A C T C C T C -5'	S M stop E M E F E V E E
	#9	5'- T C T A T G g A A A t G G A G T T C G A G G T T G A G G A G -3'	3'- A G A T A C G T T T G C C T C A A G C T C C A A C T C C T C -5'	S M E M E F E V E E
	#26	5'- T C T A T G g A A A C G G A G T T C G A G G T T G A G G A G -3'	3'- A G A T A C G T T T G C C T C A A G C T C C A A C T C C T C -5'	S M E T E F E V E E
	#28	5'- T C T A T G a A A A t G G A G T T C G A G G T T G A G G A G -3'	3'- A G A T A C G T T T G C C T C A A G C T C C A A C T C C T C -5'	S M K M E F E V E E
	#40	5'- T C T A T G g A A A g G G A G T T C G A G G T T G A G G A G -3'	3'- A G A T A C G T T T G C C T C A A G C T C C A A C T C C T C -5'	S M E R E F E V E E

Table S11. Sequence analysis of the *APT* and *Pp3c3_13220* locus in adenine resistant clones obtained after co-transfection of the ABEv#1 mutant with the CBE system and the two sgRNAs, sgRNArestor and sgRNApp3c3. Clones where the APRT is reverted to the wild-type protein are highlighted in blue. One clone, highlighted in orange corresponds probably to an escape on adenine selection.

clone #	cytosines of sgRNApp3c3					AA sequence of VDE	cytosines of sgRNArestor								AA sequence of APRT
	C19	C18	C14	C11	C10		C18	C16	C14	C11	C10	C9	C4	C2	
WT	C	C	C	C	C	-D323-D324-W325-	T	C	C	C	C	C	C	C	-E102-Y103-
apt-ABEv#1	C	C	C	C	C	-D323-D324-W325-	C	C	C	C	C	C	C	C	-E102-C103-
vde #40	A	T	T	C	C	-D323-N324-Y325-	T	T	C	C	C	C	C	C	-E102-Y103-
vde #41	T	T	T	C	C	-D323-N324-stop	A	T	C	C	T	C	C	C	-E102-F103-
vde #42	T	T	T	C	C	-D323-N324-stop	A	T	C	C	C	C	C	C	-E102-F103-
vde #43	T	G	C	C	C	-D323-D324-S325-	T	T	C	C	C	C	C	C	-E102-Y103-
vde #44	?	?	C	C	C	chimeric	?	?	C	C	C	C	C	C	chimeric
vde #45	T	T	T	C	C	-D323-N324-stop	T	T	T	C	C	C	C	C	-K102-Y103-
vde #46	T	T	T	C	C	-D323-N324-stop	A	C	C	C	C	C	C	C	-E102-F103-
vde #47	T	T	T	C	C	-D323-N324-stop	T	G	C	C	C	C	C	C	-D102-Y103-
vde #48	T	T	T	C	C	-D323-N324-stop	A	T	C	C	C	C	C	C	-E102-F103-
vde #49	T	T	T	C	C	-D323-N324-stop	A	T	C	C	C	C	C	C	-E102-F103-
vde #50	A	A	C	C	C	-D323-D324-F325-	T	C	T	C	C	C	C	C	-K102-Y103-
vde #51	T	T	T	C	T	-D323-N324-stop	T	A	C	C	C	C	C	C	-D102-Y103-
vde #52	T	G	G	C	C	-D323-H324-S325-	T	G	C	C	C	C	C	C	-D102-Y103-
vde #53	T	T	T	C	C	-D323-N324-stop	T	T	C	C	C	C	C	C	-E102-Y103-
vde #54	T	T	T	C	C	-D323-N324-stop	T	T	C	C	C	C	C	C	-E102-Y103-
vde #55	G	T	T	C	C	-D323-N324-Y325-	T	C	G	C	C	C	C	C	-Q102-Y103-
vde #56	T	T	C	C	C	-D323-D324-stop	T	T	C	C	C	C	C	C	-E102-Y103-
vde #57	T	T	T	C	C	-D323-N324-stop	T	T	C	C	C	C	C	C	-E102-Y103-
vde #58	T	G	C	C	C	-D323-D324-S325-	T	T	T	C	C	C	C	C	-K102-Y103-
vde #59	T	T	C	C	C	-D323-D324-stop	T	C	C	C	C	C	C	C	-E102-Y103-
vde #60	T	T	C	C	C	-D323-D324-stop	T	G	C	C	C	C	C	C	-D102-Y103-
vde #61	C	C	C	C	C	-D323-D324-W325-	C	C	C	C	C	C	C	C	-E102-C103-
vde #62	T	G	T	C	C	-D323-N324-S325-	A	T	C	C	C	C	C	C	-E102-F103-
vde #63	T	T	T	T	C	-N323-N324-stop	T	C	G	C	C	C	C	C	-Q102-Y103-
vde #64	T	T	T	C	C	-D323-N324-stop	T	C	T	C	C	C	C	C	-K102-Y103-
vde #65	T	T	T	C	C	-D323-N324-stop	T	T	C	C	C	C	C	C	-E102-Y103-
vde #66	T	T	T	G	C	-H323-N324-stop	T	C	G	C	C	C	C	C	-Q102-Y103-
vde #67	A	T	T	C	C	-D323-N324-Y325-	A	G	C	C	C	C	C	C	-D102-F103-
vde #68	T	T	C	C	C	-D323-D324-stop	A	C	C	C	C	C	C	C	-E102-F103-
vde #69	T	T	T	C	C	-D323-N324-stop	T	T	C	C	C	C	C	C	-E102-Y103-
vde #70	10pb deletion					frameshift	A	T	C	C	C	C	C	C	-E102-F103-
vde #71	T	T	C	C	C	-D323-D324-stop	T	T	C	C	C	C	C	C	-E102-Y103-
vde #72	T	T	T	C	C	-D323-N324-stop	T	C	C	C	C	C	C	C	-E102-Y103-
vde #73	T	G	T	C	C	-D323-N324-S325-	T	T	T	C	C	C	C	C	-K102-Y103-
vde #74	T	T	T	T	C	-N323-N324-stop	T	T	C	C	C	C	C	C	-E102-Y103-
vde #75	T	T	C	C	C	-D323-D324-stop	A	C	C	C	C	C	C	C	-E102-F103-
vde #76	T	T	T	C	C	-D323-N324-stop	A	A	C	C	C	C	C	C	-D102-F103-
vde #77	T	T	T	C	C	-D323-N324-stop	T	T	C	C	C	C	C	C	-E102-Y103-
vde #78	C	T	C	T	C	-N323-D324-stop	A	A	C	C	C	C	C	C	-D102-F103-
vde #79	?	?	?	?	?	chimeric	T	?	C	C	C	C	C	C	chimeric
vde #80	T	T	T	C	C	-D323-N324-stop	T	T	C	C	C	C	C	C	-E102-Y103-
vde #81	T	T	T	C	C	-D323-N324-stop	T	T	C	C	C	C	C	C	-E102-Y103-