

Supplementary Figure 3. APOBEC3H G105R genotyping assay.

Primers for the assay (melting temperature followed by sequence)

Forward primer: 71.0°C CATGGGACTGGACGAAGCGCA

Reverse primer: 72.3°C TGGGATCCACACAGAAGCCGCA

Underlined base in the forward primer creates a HhaI internal digestion control in the amplicon (no naturally occurring site exists nearby).

exon 3 (second coding exon)

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                CATGGGACTGGACGAAGCGCA
AACGAGATCAAGTCCATGGGACTGGACGAAACGCAGTGCTACCAAGTCACCTGTTACCTC
 N E I K S M G L D E T Q C Y Q V T C Y L
61
ACGTGGAGCCCCTGCTCCTCCTGTGCCTGGGAGCTGGTTGACTTCATCAAGGCTCACGAC
 T W S P C S S C A W E L V D F I K A H D
81
                C
CATCTGAACCTGGGCATCTTCGCCTCCCGCCTGTACTACCACTGGTGCAAGCCCCAGCAG
 H L N L G I F A S R L Y Y H W C K P Q Q
101                R                120

        3-ACGCCGAAGACACACCTAGGGTCC-5'
AAGGGGCTGCGGCTTCTGTGTGGATCCCAGGTCCCGGTGGAGGTCATGGGCTTCCCAGgt
 K G L R L L C G S Q V P V E V M G F P E
121                140
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Assay is based upon the HhaI restriction enzyme (recognition sequence GCGC).

Expected sizes for the G105R genotyping assay.

| Uncut | 105G/105G | 105G/105R | 105R/105R |
|-------|-----------|-----------|-----------|
| 197 | | | |
| | 180 | 180 | |
| | | 102 | 102 |
| | | 78 | 78 |
| | 17 | 17 | 17 |

Assay notes: The amplified region is GC-rich (60.9%), so PCRs are conducted with 1 M betaine included. The primers have relatively high Tm's, so annealing and extension can be done at the same temperature (that is, 72°C).

Supplemental Figure 4. APOBEC3H K121D genotyping assay.

Primers for the assay (melting temperature followed by the sequence)

Forward primer: 59.8°C CCACGCACTAGAAAGTTCACC

Reverse primer: 60.5°C GGTTGCACTCTTATAACTGCAAAG

CCACGCACTAGAAAGTTCACC

ctgtataaaccagggaaggcaggaagcggggtgcttgccctgcactaggccaggccacgcactagaaagtccaccgga

cagaccctctgccccccatccccgccccgctcccgccccgccccagtcacatgactcctggcctctctcttc

exon 3 (second coding exon)

tcccctcccttctctctgtttgggaccctccccagAAAAAGTGCCATGCAGAAATTTGCTTTATTAACGAGATCAAG

K K C H A E I C F I N E I K

51

TCCATGGGACTGGACGAAACGCAGTGCTACCAAGTCACCTGTTACCTCACGTGGAGCCCCTGCTCCTCCTGTGCCTG

S M G L D E T Q C Y Q V T C Y L T W S P C S S C A W

GGAGCTGGTTGACTTCATCAAGGCTCACGACCATCTGAACCTGGGCATCTTCGCCTCCCGCCTGTACTACCACTGGT

E L V D F I K A H D H L N L G I F A S R L Y Y H W

G C

GCAAGCCCCAGCAGAAGGGGCTGCGGCTTCTGTGTGGATCCCAGGTCCCGGTGGAGGTCATGGGCTTCCCAAgtagg

C K P Q Q K G L R L L C G S Q V P V E V M G F P

D

139

3' -GAAACGTCAATATTCTCACGTTTGG-5'

aaagaggctttgcagttataagagtgcaaaccggggggcacaggcttggca

Assay is based upon the HpyAV restriction enzyme (recognition sequences; [CCTTC]N6 and N6[GAAGG]). Underlined letters in the sequence above show the location of the recognition sites.

Expected sizes for the D121K genotyping assay.

| Uncut | 121D/121D | 121D/121K | 121K/121K |
|-------|-----------|-----------|-----------|
| 441 | 322 | 322 | |
| | | 220 | 220 |
| | 119 | 119 | 119 |
| | | 102 | 102 |

Supplementary Figure 5. APOBEC3H E140K genotyping assay

Forward primer: 72.0°C GCATCTTCGCCTCCCGCCTGT

Reverse primer: 70.7°C CCCTGCCAAGCCTGTGCC

exon 3 (second coding exon)

GCATCTTCGCCTCCCGCCTGT

GACTTCATCAAGGCTCACGACCATCTGAACCTGGGCATCTTCGCCTCCCGCCTGTACTACCACTGGTGCAAGCCCCA
 D F I K A H D H L N L G I F A S R L Y Y H W C K P Q
 94

A

GCAGAAGGGGCTGCGGCTTCTGTGTGGATCCCAGGTCCCGGTGGAGGTCATGGGCTTCCCAGgtaggaaagaggctt
 Q K G L R L L C G S Q V P V E V M G F P E
 120 K

140

3' -CCCGTGTCCGAACCGTCCC-5'

tgcagttataagagtgcaaacccccggggggcacaggccttggcaggggctggggggttggggggttggggggttggaggag

Assay is based on the BstNI restriction enzyme (recognition sequence = CCWGG, where W represents A or T).

Expected sizes for the E140K genotyping assay.

| Uncut | 140K/140K | 140K/140E | 140E/140E |
|-------|-----------|-----------|-----------|
| 166 | | | |
| | 90 | 90 | |
| | 76 | 76 | 76 |
| | | 62 | 62 |
| | | 28 | 28 |

Assay notes: The amplified region is GC-rich (60.9%), so PCRs are conducted with 1 M betaine included. The primers have relatively high Tm's, so annealing an extension can be done at the same temperature (that is, 72°C).

Supplemental Figure 6. APOBEC3H E178D genotyping assay.

Primers for the assay (melting temperature followed by the sequence)

Forward primer: 58.1°C CAAGAGCTCCTGGCACTG

Reverse primer: 61.0°C CTGGTGCGAGAGGAGAACAC

CAAGAGCTCCTGGCACTG exon 4
caagagctcctggcactgcagctgctgcccctggggcctggctgggtttccctcttccctctcagAGTTTGCTGACTG
E F A D C
140

CTGGGAAAACCTTTGTGGACCACGAGAAACCGCTTTCCTTCAACCCCTATAAGATGTTAGAGGAGCTAGATAAAAACA
W E N F V D H E K P L S F N P Y K M L E E L D K N

C

GTCGAGCCATAAAGCGACGGCTTGAGAGGATAAAGGTGAGGCACTGTCCTGCCTGCTGCCCCGACCTCCTCACCGC
S R A I K R R L E R I K
D 181

ctgccgcagccccataccagtcacacctctgccactgcccttaccctacctttttttctttttctttttctttttct
tttttgagacagggctcactctgtcaccagcaggagggcagtggtgtgatctcggctcagtgcaacctctgcct
cccgggttcaagtgattctcctgcctcagcctcccagtagctgggagtagcagggcactcgccaccatgccagctaa
tttttgatttttagtagagatggggtttcaccatggttcccagggctggtccttagaactcttggcctcaagtgacct
gccttccttggcctcccaaagtgctgggattacaggtgtgagccaccgtgccccgccccactacctgtttcttttt

CACAAGAGGAGAGCGTGGTC-5'
gtgttctcctctcgcaccaggggctccttc

Assay is based upon the SmlI restriction enzyme (recognition sequence; CTYRAG).

Expected sizes for the D178K genotyping assay.

| Uncut | 178D/178D | 178D/178E | 178E/178E |
|-------|-----------|-----------|-----------|
| 636 | 530 | 530 | |
| | 353 | 353 | |
| | | 177 | 177 |
| | 106 | 106 | 106 |

Assay note: Optimal digestion temperature for SmlI is 55°C.