

Supplementary Figure 1. APOBEC3H N15Δ genotyping assay.

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

Forward primer: 60.6°C CCGAACATTCCGCTTACAG

Reverse primer: 59.5°C GCGTCAGCTGGTAACACAAG

exon 2 (first coding exon)

CCGAAACATTCCGCTTACAG ---

ATGGCTCTGTTAACAGCCGAAACATTCCGCTTACAGTTAACACAAGCGCCGCCTCAGA

M A L L T A E T F R L Q F N N K R R L R
1 (-) 20

3'-GAACACAATGGTCGACTGCG-5'

AGGCCTTACTACCCGAGGAAGGCCCTCTGTGTTACCAGCTGACGCCGCAGAATGGCTCC

R P Y Y P R K A L L C Y Q L T P Q N G S
21 40

Expected sizes for the APOBEC3H ΔN15 polymorphism.

| 15N/15N | 15N/15Δ | 15Δ/15Δ |
|---------|---------|---------|
| 90 | 90 | |
| | 87 | 87 |

Assay note: Run the amplification products on high-resolution gels.

Supplemental Figure 2. APOBEC3H R18L genotyping assay.

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

Forward primer: 61.0 C TGGGTTTGA~~AAAAGTGGCTTG~~

Reverse primer: 60.0 C AACTGGGCCACTCAGATCC

TGGGTTTGA~~AAAAGTGGCTTG~~

tgggtt~~gaaaagtggcttgagcctgggtgactcaagaggacgtccctcatcttggtttccccttctgttg~~

exon 2 (first coding exon)

cacagAAACACGATGGCTCTGTTAACAGCGAACATTCCGCTTACAGTTAACAAACAAGCGCCGCCTCAGAAGGCC

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|----|---|
| M | A | L | L | T | A | E | T | F | R | L | Q | F | N | N | K | R | R | L | R | R | P |
| 1 | | | | | | | | | | | | | | | | | | | L | 20 | |

TTACTACCCGAGGAAGGCCCTTGTGTTACCAGCTGACGCCGCAGAATGGCTCCACGCCACGAGAGGCTACTTTG

| | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|----|----|---|
| Y | Y | P | R | K | A | L | L | C | Y | Q | L | T | P | Q | N | G | S | T | P | T | R | G | Y | F |
| | | | | | | | | | | | | | | | | | | | | | | 30 | 40 | |

AAAAACAAGgtgccacacggctctctggcacagggccggcagggcataacccatctgatgtgcagaaaataca

| | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|----|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|
| E | N | K | | | | | | | | | | | | | | | | | | | | | |
| | | | 50 | | | | | | | | | | | | | | | | | | | | |

tgaaatgcctgcctataaattctcaattttgatgtaaacaccctctgtgtttcgtaacccttggtgcctgcctgg

3'-CCTAGACTCACCGGGTCAA-5'
gttttggcttagctggatctgagtgcccagttccctaggacactcccattttccgtccccggacctctgaag

Assay is based upon the Fnu4HI restriction enzyme (recognition sequence; GCNGC). The internal digestion control site, common to both alleles, cuts faster than the diagnostic site, and so additional care must be taken for this assay. Underlined letters in the sequence above show the location of the recognition sites.

Expected sizes for the R18L genotyping assay.

| Uncut | 18L/18L | 18L/18R | 18R/18R |
|-------|---------|---------|---------|
| 418 | | | |
| | 222 | 222 | 222 |
| | 196 | 196 | |
| | | 141 | 141 |
| | | 55 | 55 |

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)

CATGGGACTGGACGAAGCGCA

AACGAGATCAAGTCCATGGGACTGGACGAACGCAGTGCTACCAAGTCACCTGTTACCTC
N E I K S M G L D E T Q C Y Q V T C Y L
61 80

ACGTGGAGCCCCTGCTCCTCCTGTGCCTGGGAGCTGGTTGACTTCATCAAGGCTCACGAC
T W S P C S S C A W E L V D F I K A H D
81 100
C
CATCTGAACCTGGGCATCTCGCCTCCGCCTGTACTACCAC^TGCTGCAAGCCCCAGCAG
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'
AAGGGGCTCGGGCTTCTGTGTGGATCCCAGGTCCCGGTGGAGGT^CATGGGCTTCCCAGgt
K G L R L L C G S Q V P V E V M G F P E
121 140

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 CCACGCACTAGAAAGTTCACCC

Reverse primer: 60.5°C GGTTTGCACCTTATAACTGCAAAG

CCACGCACTAGAAAGTTCACCC

ctgtataaaaccagggaaggcaggaagcggtgcttgcctgcactaggccaggcacgcactagaaagtccacggac
cagacccctctgccccccatccccgccccgtcccggcccccccccagtcacatgactcctggctctcttc

exon 3 (second coding exon)

tccccccttctctgtttggaccctccccagAAAAAGTGCATGCAGAAATTGCTTATTAAACGAGATCAAG
K K C H A E I C F I N E I K
51

TCCATGGGACTGGACGAAACGCAGTGCTACCAAGTCACCTGTTACCTCACGTGGAGCCCCCTGCTCCTCTGTGCCTG
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

GGAGCTGGTTGACTTCATCAAGGCTCACGACCATCTGAACCTGGCATCTTCGCCTCCGCCTGTACTACCAGTGGT
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

GCAAGCCCCAGCAGAAAGGGCTGCGGCTTCTGTGTGGATCCCAGGTCCGGTGGAGGTATGGGCTTCCCAAgtagg
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'-GAAACGTCAATATTCTCACGTTGG-5'
aaagaggcttgcagttataagagtgc aaaccccccggggcacaggcttggca

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 GCATCTTCGCCTCCGCCTGT
Reverse primer: 70.7°C CCCTGCCAAGCCTGTGCC

exon 3 (second coding exon)

GCATCTTCGCCTCCGCCTGT
GACTTCATCAAGGCTCACGACCATCTGAACCTGGCATCTCGCCTCCGCCTGTACTACCACTGGTGCAAGCCCCA
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
GCAGAAGGGGCTGCGGCTTCTGTGTGGATCCAGGTCCGGTGGAGGTCATGGCTTCCAGgtaggaaagaggctt
Q K G L R L L C G S Q V P V E V M G F P E
120
A
K
140
3'-CCCGTGTCCGAACCGTCCC-5'
tgcaagtataagagtgcaaaccggggcacaggcttgcagggctggggttggggttggggttggaggag

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 and 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
caagagctcgtggcactgcagctgctgccccctggggcctggctggttcccttccttcagAGTTTGCTGACTG
E F A D C
140

CTGGGAAAACCTTGTGGACCACGAGAAACCGCTTCCTCAACCCCTATAAGATGTTAGAGGAGCTAGATAAAAACA
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
GT CGAGCCATAAAGCGACGGCTTGAGAGGATAAAGGTGAGGCACTGTCCTGCCTGCTGCCCGACCTCCTCACCGC
S R A I K R R L E R I K
D 181

ctgccgcagccccatacccagtcacacctctgcccactgcccattaccctacccctaccttttttttttttttttttct
tttttgagacagggtctcaactctgtcacccaggcaggaggcagtggtgtatctcggtcagtgcacccctcgct
cccggttcaagtgattctcctgcctcagcctcccagtagctggagtagacaggcactcgccaccatgcccagctaa
ttttgcatttttagtagagatggggttaccatgttgccaggtctggcttagaactcttggccaagtgacct
gccttcctggcctccaaagtgtggattacagggtgtgagccaccgtgccccgccccactacctgtttttttttttt

CACAAGAGGAGAGCGTGGTC-5'

gtgttctcctctcgaccagggtcttc

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.