

Supplementary Table 1

PCR primers and probes for droplet digital PCR

Fluorescent reference probes have a 5' HEX label and a 3' Iowa Black quencher

Fluorescent mutant probes have a 5' FAM label and a 3' Iowa Black quencher

Variant nucleic acids within the probes are indicated in lower case.

p.C420R mutation

PCR forward primer: AGATACTAGAGTGTCTGTGTAATCA

PCR reverse primer: CCTTTTGGGGAAGAAAAGTGTT

Fluorescent reference probe: CCATGCCAATGGACaGTGTTCC

Fluorescent mutant probe: CCCATGCCAATGGACgGTGT

p.E542K mutation

PCR forward primer: CAGCTCAAAGCAATTTCTAC

PCR reverse primer: CACTTACCTGTGACTCCAT

Fluorescent reference probe: CTgAAATCACTGAGCAGGAGA

Fluorescent mutant probe: CTaAAATCACTGAGCAGGAGA

p.E545K mutation

PCR forward primer: CAGCTCAAAGCAATTTCTAC

PCR reverse primer: CACTTACCTGTGACTCCAT

Fluorescent reference probe: CTGAAATCACTgAGCAGGAGA

Fluorescent mutant probe: CTGAAATCACTaAGCAGGAGA

p.H1047R mutation

PCR forward primer: AACTGAGCAAGAGGCTTTGG

PCR reverse primer: TGTGTGGAAGATCCAATCCA

Fluorescent reference probe: TGCACaTCATGGTGGCTGGA

Fluorescent mutant probe: TGATGCACgTCATGGTGGCT

p.H1047L mutation

PCR forward primer: AACTGAGCAAGAGGCTTTGG

PCR reverse primer: TGTGTGGAAGATCCAATCCA

Fluorescent reference probe: TGCACaTCATGGTGGCTGGA

Fluorescent mutant probe: TGCACtTCATGGTGGCTGGA

Supplementary Table 2:

Sequences of the smMIP probes provided by Drs. Joe Hiatt and Jay Shendure to cover the *PIK3CA* coding sequence

There are 51 separate probes. The string of “N” nucleotide within each individual probe represents the random bar code site.

1. GAATGCCTCCGTGAGGCTACCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNTATATGTAAAACCTTGCAAAG
2. GTCACATCATTCATTTGGCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNGCTTCTTGAGTAACACTTAC
3. GGCAACCGTGAAGAAAAGATCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNCTTCAAGATGAATCTTCTTA
4. GGTTGAAAAAGCCGAAGGTCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNATATTTTAGAAAGGGACAAC
5. ACAGGACTTCCGAAGAAATATTCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNCCCTGTTTGCAAAAAAAA
6. GATCTTTAACCATATCAAATTCCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNAATTCTGGTGAAGATTCT
7. GCAGTCTTCTACCTGTGTCTCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNATTCACCTCATAGTAGAGCA
8. GTAATTGCTGAAGCAATCAGGACTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNAGATGGTGAATGCATCTA
9. ACACAGTCATGGTTGATTTTCACTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNTTCATCACATCCACACAC
10. ACATTGAGGATGAGTATCTGTACTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNTAGAATATCAGGGCAAGT
11. ACTGTTTTACAATGCCATCTTACTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNTGAAAAACCTTACAGGAA
12. CAACATCAAATTGGGCATCCTCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNTTTATAACCCAAAGGGATT
13. GTAAAGTCAAATGCTGATGCTTCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNCATTTCCACAGCTACACC
14. CACGTAGGTTGCACAAAGAACTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNACCATCTGAAAACATACTAC
15. AGTATATAGATTTATATTTCCCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNAGTGTATACATTAGTATAT
16. GTCACATAAGGGTTCTCCTCCCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNCCAGGCTGGTCTAAAAAAA
17. CGACTTTGCCTTTCCATTTGCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNTGATTTGTAGGAGTCATTTA
18. GCACGAGGAAGATCAGGAATCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNTTGTGAAAAAATTCAATCAG
19. GCTTTGAATCTTTGGCCAGTCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNNAAGTGTTTTGAAATGTGTTT
20. CCAGATACTAGAGTGTCTGTGTCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNCTGCCATAAAAAATATCT
21. ATTGAAGAGCATGCCAATTGGTCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNTTTTATGGCAGTCAAACC
22. GGGAACTTTACCACACTGCTCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNCCTAAATCCACAGATTATAA
23. CAAAGCAATTTCTACACGAGACTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNTTTCTGTAAATCATCTGTG
24. GCTAGTCTGTTACTCTGTAAAATACTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNTCCATTTTAGCACTTA
25. GAATTTACATGATTTTTAAACTACTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNTTTTCTATGGAGTCACA
26. CCCAGGTAAATGTATGTTTGAGCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNCCTTTTGAAACAGCATGCA
27. ATTTGGGTAGAATTCGGGGACTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNATTTATGTGGACTTTCTGA
28. AACTTCTGGACTGTAATTACCCCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNTTAAGGCAGTGTTTTAGA
29. ATCTTTTACCAAGCAATACACTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNNAATTAATACTGAGAAAGTT
30. CCTTTTAAAAACCTACTGCACTCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNGTGCTTGGAAAAATATT
31. ACTTTTCTTTTGGCATTAAAGTCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNNGGCCATGCAGAACTG
32. CCAATCCTTTGATTAGTCAATCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNCCAGCCAAACATAAAACA
33. GTCGTGCATGTGGGATGTATCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNGTTTAGCAAAGATTATTTGT
34. ACCTCTGGCTAACTGTTTTATTCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNGTTTCATCCTTCTTCTCC
35. GGTCCTTGGAAATCAAGCAGATCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNACTTAACTGACATTCTCAA
36. CCTCAGGTACTTCTTGGGGCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNCTTACTGTGACTATCCTTTT
37. CCTAGTTGATGAGCAGGGTTCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNATGAGAGAGAGAAGCATAA

38. CATCTTTAAAAATGGGGATGCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNTACCTAGTAAAGTTTTTAAC
39. CAACCACAGTGGCCTTTTTGCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNCTGTGTATAAAAAGATAGCT
40. GGCAACCTGTATGTTGAAAGTCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNTGGCATTAAATGGTGATAC
41. GTATCACCATTTAATGCCACAGCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNTTCAACATACAGGTTGCC
42. GCTTGAAAGGTGCACTGCAGCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNTCATTTAATTGTAAACGTGT
43. GCCTTTGCACTGAATTTGCACTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNACAGACTCATCTAACAAAA
44. GTGAAAGACGATGGACAAGTACTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNATACTACTCATGAGGTGTT
45. GCTACACAGTATCCAGCACATCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNATGCAGATACAAAATGTCT
46. GGTTATAAACGAGAACGTGTGCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNTGTTTTGGTGTCTTAATT
47. ATCCAAAAGTGTCCAAAATCTATCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNCGAGCTCACCTCTCAA
48. ATGTTGGCTGGGTGTGGTGGTTCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNAGGAGCCCAAGAATGCAC
49. GCCAGAACTACAATCTTTTGATGCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNGCTCCAAACTGACCAA
50. CCAGAGCCAAGCATCATTGAGACTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNTGTCCAGCCACCATGATG
51. GGATTCCACACTGCACTGTTCTTCAGCTTCCCGATCCGACGGTAGTGTNNNNNNNNNNNTGGAGTATTCATGAAACAA

