

Table S1. Optimal primer sequences for RT-RPA amplification

Primer name	Sequence (5'-3')
F1-CD74(E6)	TGAAATGAGCAGGCACTCCTTGGAGCAAAAGC
F2-SDC4(E4)	GCAGCAACATCTTTGAGAGAACGGAGGTCCTG
F3-SLC34A2(13del2046)	CCTGAGACCTTTGATAACATAACCATTAGCAG
F4-SLC34A2(E4)	CGTGTGCTCCCTGGATATTCTTAGTAGCGCCT
F5-EZR(E10)	CTCTCGGAGCAGATTCAGAGGGGCCCTGCAGCT
F6-SDC4(E2)	CCCTGAAGTTGTCCATCCCTTGCTGGAGTC
F7-TPM3(E8)	CGTGCTGAGTTTACTGAGAGATCGGTAACC
F8-GOPC(E8)	ACCCTGGTGCTAGTTGCAAAGACACAAGTGGG
F9-LRIG3(E16)	GATTTTTCTTACCACAACATGACAGTAGTG
F10-GOPC(E4)	GCGAGACTAGCTGCCAAGTACTTGGATAAGGA
R1-ROS1(E34)	CGTTTATAAGCACTGTCACCCCTTCCTTGGCA
R2-ROS1(E32)	ATTATCTTCAGCTTTCTCCCACTGTATTGAAT
R3-ROS1(E35)	GCCAGACCTCGCAGCTCAGCCAACCTTTTGTC
R4-ROS1(E36)	CTCCAAAGGCTCCACTTCCCAGCAAGAGACGC

Table S2. All RT-RPA Primers Used for Screening

Primer name	Sequence (5'-3')
F1-CD74(E6)	TGAAATGAGCAGGCACTCCTTGGAGCAAAAGC
F1b-CD74(E6)	CTGTTTGAAATGAGCAGGCACTCCTTGGAGCA
F1c-CD74(E6)	ACAACCAGAAATATTCCAACATAATAGTAAG
F2-SDC4(E4)	GCAGCAACATCTTTGAGAGAACGGAGGTCCTG
F3-SLC34A2(13del2046)	CCTGAGACCTTTGATAACATAACCATTAGCAG
F3b-SLC34A2(13del2046)	TTGATAACATAACCATTAGCAGAGAGGCTCAG
F3c-SLC34A2(13del2046)	CAGGATGTCCCTGTCAAGGCTCCTGAGACCTT
F4-SLC34A2(E4)	CGTGTGCTCCCTGGATATTCTTAGTAGCGCCT
F4b-SLC34A2(E4)	GGAGATTGATTTTACTTCTCGGATTTCTCTAC
F4c-SLC34A2(E4)	ATATTCTTAGTAGCGCCTTCCAGCTGGTTGGA
F5-EZR(E10)	CTCTCGGAGCAGATTCAGAGGGCCCTGCAGCT
F5b-EZR(E10)	TGCACTGCGGGCTAAGGAGGAGCTGGAGAGAC
F5c-EZR(E10)	AGGCGGTGGATCAGATAAAGAGCCAGGAGCAG
F5d-EZR(E10)	AGGAGGCCGAGCGCCTAGAGGCTGACCGTATG
F5e-EZR(E10)	TGCACTGCGGGCTAAGGAGGAGCTGGAGAGAC
F5f-EZR(E10)	AGGCGGTGGATCAGATAAAGAGCCAGGAGCAG
F5g-EZR(E10)	AGCGCCTAGAGGCTGACCGTATGGCTGCACTG
F5h-EZR(E10)	GAGGAGGAGAGGAAGCGGGCACAGGAGGAGGC
F5i-EZR(E10)	AAGAGCCAGGAGCAGCTGATGATTTTTGGA
F6-SDC4(E2)	CCCTGAAGTTGTCCATCCCTTGCTGGAGTC
F6b-SDC4(E2)	TGACTTGGAAGACTCCATGATCGGCCCTGAAG
F6c-SDC4(E2)	CTCTGGAGATCTGGATGACTTGGAAGACTCCATG
F6d-SDC4(E2)	GACTTTGAGCTGTCTGGCTCTGGAGATCTGGA
F6e-SDC4(E2)	CATGATCGGCCCTGAAGTTGTCCATCCCTTG
F6f-SDC4(E2)	ACTCCATGATCGGCCCTGAAGTTGTCCATC
F7-TPM3(E8)	CGTGCTGAGTTTACTGAGAGATCGGTAACC
F7b-TPM3(E8)	CTGAGTTTGCTGAGAGATCGGTAGCCAAGCTG
F7c-TPM3(E8)	TTTATAAGCACTGTCACCCCTTCCTTGGCACT
F7d-TPM3(E8)	CGTGCTGAGTTTGCTGAGAGATCGGTAGCCA
F7e-TPM3(E8)	GCCAAGCTGGAAAAGACAATTGATGACCTGGA
F7f-TPM3(E8)	GCTGAGAGATCGGTAGCCAAGCTGGAAAAGAC
F7g-TPM3(E8)	AAGACAATTGATGACCTGGAAGTCTGGCATAG
F7h-TPM3(E8)	CGTGCTGAGTTTGCTGAGAGATCGGTA
F7i-TPM3(E8)	CGTGCTGAGTTTGCTGAGAGATCGGTAACC
F8-GOPC(E8)	ACCCTGGTGCTAGTTGCAAAGACACAAGTGGG
F9-LRIG3(E16)	GATTTTCTTACCACAACATGACAGTAGTG
F10-GOPC(E4)	GCGAGACTAGCTGCCAAGTACTTGATAAGGA
R1-ROS1(E34)	CGTTTATAAGCACTGTCACCCCTTCCTTGGCA
R1b-ROS1(E34)	CTTTGAGAGCTGGATGCACCATTGGCTCCTGT

R1c-ROS1(E34)	GGTCAGTGGGATTGTAACAACCAGAAATATTC
R2-ROS1(E32)	ATTATCTTCAGCTTTCTCCCACTGTATTGAAT
R2b-ROS1(E32)	TACTCCCTTCTAGTAATTTGGGAATGCCTGGT
R2c-ROS1(E32)	CAAGGATATAGTATGTAATTCTACATCCATTA
R3-ROS1(E35)	GCCAGACCTCGCAGCTCAGCCAACCTTTGTC
R4-ROS1(E36)	CTCCAAAGGCTCCACTTCCCAGCAAGAGACGC

Table S3. ROS1 gene fusions and their RT-RPA product size

ROS1 fusion partner (splicing exon)	ROS1 (splicing exon)	Gene fusion	Label	RT-RPA product size
SDC4 (E2)	ROS1 (E32)	S2R32	1	129 bp
SDC4 (E4)		S4R32	2	119 bp
SLC34A2 (13del2046)		S13R32	3	126 bp
SLC34A2 (E4)		SL4R32	4	167 bp
CD74 (E6)		C6R32	5	136 bp
CD74 (E6)	ROS1 (E34)	C6R34	6	200 bp
SDC4 (E4)		S4R34	7	183 bp
SLC34A2 (13del2046)		S13R34	8	190 bp
SLC34A2 (E4)		SL4R34	9	194 bp
EZR (E10)		E10R34	10	248 bp
TPM3 (E8)	ROS1 (E35)	T8R35	11	158 bp
GOPC (E8)		G8R35	12	148 bp
LRIG3 (E16)		L16R35	13	127 bp
GOPC (E4)	ROS1 (E36)	G4R36	14	139 bp

Table S4. Optimal crRNA Sequence and Probe Sequence

Name	Sequence (5'-3')
crRNA1-ROS1 E34	UAAUUUCUACUAAGUGUAGAUUGGUUGUUACAA UCCCACUGA
crRNA2-ROS1 E32	UAAUUUCUACUAAGUGUAGAUCCCUUCUAGUA AUUUGGGA
crRNA3-ROS1 E35	UAAUUUCUACUAAGUGUAGAUAUUCUUUAAUCU UCUAUGCCA
crRNA4-ROS1 E36	UAAUUUCUACUAAGUGUAGAUCCGAGGGAAGGC AGGAAGAUU
Probe	FAM-TTATTATT-BHQ1

Supplementary Sequences

>Plasmid S2R32

gactttgagctgtctggctctggagatctggatgacttgaagactccatgatcgccctgaagttgtccatcccttgcctga
gtcccaataaaccaggcattcccaattactagaagggagtaaaattcaatacagtgggagaaagctgaagataatg

>Plasmid S4R32

gcagcaacatctttgagagaacggaggtcctggcagctggagtcccaataaaccaggcattcccaattactagaaggg
agtaaaattcaatacagtgggagaaagctgaagataatg

>Plasmid S13R32

caggatgtccctgtcaaggctcctgagacctttgataacataaccattagcagagaggctcaggctggagtcccaataaa
ccaggcattcccaattactagaagggagtaaaattcaatacagtgggagaaagctgaagataatg

>Plasmid SL4R32

caagggattgggagattgattttacttctcggatttctactttttcgtgtgctccctggatattcttagtagcgcttccagctgg
ttggagctggagtcccaataaaccaggcattcccaattactagaagggagtaaaattcaatacagtgggagaaagctg
aagataatg

>Plasmid C6R32

tgaatgagcaggcactccttggagcaaaagcccactgacgctccaccgaaagctggagtcccaataaaccaggcattc
ccaattactagaagggagtaaaattcaatacagtgggagaaagctgaagataatg

>Plasmid C6R34

tgaatgagcaggcactccttggagcaaaagcccactgacgctccaccgaaagatgattttggataccagaacaagtttcatacttac
tattatagttggaatatttctggtgttacaatcccactgacctttgtctggcatagaagattaaagaatcaaaaaagtgccaaggaagggg
tgacagtgttataaacgaagacaaagagttggctgagctgcgaggtctggcagccggagta

>Plasmid S4R34

caagagaatctcaccgtgaagagagtgaggatgtgtccaacaaggtgtcaatgtccagcactgtgcagggcagcaaca
tctttgagagaacggaggtcctggcagatgattttggataccagaacaagtttcatacttactattatagttggaatatttctg
gtgttacaatcccactgacctttgtctggcatagaagattaaagaatcaaaaaagtgccaaggaaggggtgacagtgttat
aaacgaagacaaagagttggctgagctgcgaggtctggcagccggagta

>Plasmid S13R34

caggatgtccctgtcaaggctcctgagacctttgataacataaccattagcagagaggctcaggatgattttggataccaga
aacaagtttcatacttactattatagttggaatatttctggtgttacaatcccactgacctttgtctggcatagaagattaaagaat
caaaaaagtgccaaggaaggggtgacagtgttataaacgaagacaaagagttggctgagctgcgaggtctggcagccg
gagta

>Plasmid SL4R34

tcgtgtgctccctggatattcttagtagcgcttccagctggttggagatgattttggataccagaacaagtttcatacttact
attatagttggaatatttctggtgttacaatcccactgacctttgtctggcatagaagattaaagaatcaaaaaagtgccaagg
aaggggtgacagtgttataaacgaagacaaagagttggctgagctgcgaggtctggcagccggagta

>Plasmid E10R34

tcgggctgcaggactatgaggagaagacaaagaaggcagagagagagctctcggagcagattcagagggccctgcag
ctggaggaggagaggaagcgggcacaggaggaggccgagcgcctagaggctgaccgtatggctgcactgcgggcta
aggaggagctggagagacaggcgggtggatcagataaagagccaggagcagctatgatttttgataaccagaaacaagt
ttcacttactattatagttggaatatttctggtgttacaatcccactgacctttgtctggcatagaagattaaagaatcaaaaa
agtccaaggaaggggtgacagtgcctataaacgaagacaaagagttggctgagctgcgaggtctggcagccggagta

>Plasmid T8R35

cgtgctgagtttctgagagatcggtagccaagctggaaaagacaattgatgacctggaagtctggcatagaagattaaag
aatcaaaaaagtgccaaggaaggggtgacagtgcctataaacgaagacaaagagttggctgagctgcgaggtctggc

>Plasmid G8R35

accctggctgtagttgcaaagacacaagtggggaaatcaaagtattacaagtctggcatagaagattaaagaatcaaaaa
gtgccaaggaaggggtgacagtgcctataaacgaagacaaagagttggctgagctgcgaggtctggca

>Plasmid L16R35

gattttcttaccacaacatgacagtagtgctggcatagaagattaaagaatcaaaaaagtgccaaggaaggggtgacagt
gcctataaacgaagacaaagagttggctgagctgcgaggtctggc

>Plasmid G4R36

gagaatgaagccctcgtagacatatagctgttctccaggctgaagtatatggggcgagactagctgccaagtacttgata
aggaactggcaggaagtactcttccaaccaagaggagattgaaaatcttctgccttcctcgggaaaaactgactctgc
gtctcttgctgggaagtggagcctttggagaagtgtatgaaggaacagcagtgagaca