

Table 1. Sequences and locations of probes and primers, and cycling conditions used for *TP53* splice form quantification by multiplex long amplicon ddPCR.

Assay	Oligonucleotide (T _m)	Location	Sequence (5'- 3')
Detection of <i>TP53</i> transcripts t1/t3/4 (encoding FL/Δ40p53α/β/γ)	Forward primer (62.0°C)	Exon 2	CTGGATTGGCAGCCAGACT
	Reverse primer (60.1°C)	Exon 10	CTGGGCATCCTTGAGTTCC
(LRG_321t1/t3 and t4 transcripts. NCBI reference sequences for these are- NM_000546.5/ NM_001126114.2/ NM_001126113.2 respectively) Amplicon sizes = 1099, 1232 and 1,159bp respectively	α probe_HEX (64.9°C)	Exons 9/10	5HEX_CGGATCTGAAGGGTGAATATTCTCCA_3IABkFQ
	β probe_FAM_1 (62.2°C)	Exon 9β	56-FAM_ACTTTGCCTGATAACAGATGCTACT_3IABkFQ
	β probe_FAM_2* (64.6°C)	Exon 9β	56-FAM_TCTGTATCAGGCAAAGTCATAGAACCAT_3IABkFQ
Cycling conditions:	γ probe_FAM (64.6°C)	Exons 9/9γ	56-FAM_AGCATCTGAAGGGTGAATATTCTCCA_3IABkFQ
	94°C for 10 min, then 50 cycles of 94°C for 30 sec, 64°C for 1 min, 72°C for 6 min, followed by 98°C for 10 min and then hold temperature of 12°C.		
Detection of <i>TP53</i> transcripts t5/t6/t7 (encoding Δ133/Δ160p53α/β/γ)	Forward primer (60.6°C)	Intron 4	CCTGACTTTCAACTCTGTCCTCC
	Reverse primer (60.1°C)	Exon 10	CTGGGCATCCTTGAGTTCC
(LRG_321t5/t6 and t7 transcripts. NCBI reference sequences for these are- NM_001126115.1/ NM_001126116.1 NM_0011261137.1) Amplicon sizes = 858, 991 and 918bp respectively	α probe_HEX (64.9°C)	Exons 9/10	5HEX_CGGATCTGAAGGGTGAATATTCTCCA_3IABkFQ
	β probe_FAM_1 (62.2°C)	Exon 9β	56-FAM_ACTTTGCCTGATAACAGATGCTACT_3IABkFQ
	β probe_FAM_2* (64.6°C)	Exon 9β	56-FAM_TCTGTATCAGGCAAAGTCATAGAACCAT_3IABkFQ
Cycling conditions:	γ probe_FAM (64.6°C)	Exons 9/9γ	56-FAM_AGCATCTGAAGGGTGAATATTCTCCA_3IABkFQ
	94°C for 10 min, then 50 cycles of 94°C for 30 sec, 64°C for 1 min, 72°C for 6 min, followed by 98°C for 10 min and then hold temperature of 12°C.		
Detection of <i>TP53</i> transcripts t1 and t2 (both transcripts predicted to encode FL/Δ40p53α)	Forward primer (62.5°C)	Exon 1	TGGGAGCGTGCTTCCAC
(LRG_321t1 and t2 transcripts. NCBI reference sequences for these are- NM_000546.5/ NM_001126112.2)	Reverse primer (62.0°C)	Exons 9/10	CCCACGGATCTGAAGGGTG
	t1 probe_HEX (65.2°C)	Exon 1/2	5HEX_CCGGAAGGCAGTCTGGCTG_3IABkFQ

Assay	Oligonucleotide (T _m)	Location	Sequence (5'- 3')
Cycling conditions:	t2 probe_FAM (64.3°C)	Exon 1/2	56-FAM_CGGAAGGCAGTCTGGCCA_3IABkFQ
Amplicon sizes = 1075 and 1072bp respectively.			94°C for 10 min, then 50 cycles of 94°C for 30 sec, 62°C for 1 min, 72°C for 6 min, followed by 98°C for 10 min and then hold temperature of 12°C.
	Oligonucleotide	Location	Sequence (5'- 3')
cDNA synthesis	TP53 gene-specific primer	Exon 11	AAAGACCCAAAACCCAAAATG

*Reactions can be run as and , and , or , and , with concurrent detection and quantitation of and isoforms possible in the FAM channel by using two probes.

Table S2. Sequences of gBlocks used for cloning as positive controls for *TP53* transcripts.
Lowercase font indicates sequence for restriction sites appended to *TP53* sequences

TP53 transcript	Sequence of gBlocks
<i>LRG321_t1</i> (encoding FL/Δ40p53 α) (NCBI Reference Sequence NM_000546.5)	ccggattcGCCTCGGGCTGGAGCGTGCTTCCACGACGGTGACACGCCCTGGATTG GCAGCCAGACTGCCCTCCGGGTCACTGCCATGGAGGAGCCGAGTCAGATCCTAGCGTC GAGCCCCCTCTGAGTCAGGAACATTTCAGACCTATGAAACTACTTCCCTGAAAACAAGC TTCTGTCCCCCTGCCGCTCCAAAGCAATGGATGATTGATGCTGTCCCCGGACGATATTGA ACAATGGTTCACTGAAGACCCAGGTCCAGATGAAGCTCCAGAATGCCAGAGGCTGCTCC CCCCGTGGCCCTGACCAGCAGCTCTACACCCGGCCCTGCACCAGCCCCCTCCT GGCCCTGTGATCTCTGTCCCTCCAGAAAACCTACCAGGGCAGCTACGGTTCTCGT GGGCTCTTGCACTCTGGAGAGACCCGGCGACAGAGGAAGAGAATCTCCGCAAGAAA AAGATGTTTGCAACTGGCAAGACCTGCCCTGTGAGCTGCTGAGTGGAGTATTGGATGACAGAAA CGCCCGGCACCCCGTCCGCGCATGGCCATCTACAAGCAGTCACAGCACATGACGGAG GTTGTAGGGCGCTGCCCTCCAGATGAGCTGCTCAGATAAGCGATGGCTGGCCCTCCT CAGATCTTACCGAGTGAAGGAATTTCGCTGTGGAGTATTGGATGACAGAAA TTGACATAGTGTGGTGGCCCTATGAGCCGCTGAGGTTGGCTCTGACTGTACCCACAC TCCACTACAACATGTGTAACAGTCTCTGCATGGCGGCATGAACCCGGAGCCATCCT CACCATCATCACACTGGAAAGACTCCAGTGGAAATCTACTGGGACGGAACAGCTTGGAG CGTGGTGTGCTGCTGGAGAGACCCGGCGACAGAGGAAGAGAATCTCCGCAAGAAA GGGAGCCTCACCACGAGCTGCCCTCAGGGAGACTAACAGGAGACTAACGGAGACTGCC CAGCTCTCTCCCAGCAAAGAAAGAAACACTGGATGGAGAATATTCACTTCAAGGAC CAGACAGCTTCAAAAGAAAATTGTAAGAGAGCATGAAAATGGTTATGACTTGC TGATACAGATGCTACTTGACTTACGATGGTGTACTTCTGTATAAACCTCGCTGAAGTGA AATATTATCCGTGGCGTGAGCGCTCGAGATGGCTGAGCTGAATGAGGCTTGGAA CTCAAGGATGCCAGGCTGGAGAGGAGCCAGGGGGGAGCAGGGCTACTCCAGCACCT GAAGTCAAAAGatccgcg
<i>LRG321_t3</i> (encoding FL/Δ40p53 β) (NCBI Reference Sequence NM_001126114.2)	ccggattcGCCTCGGGCTGGAGCGTGCTTCCACGACGGTGACACGCCCTGGATTG GCAGCCAGACTGCCCTCCGGGTCACTGCCATGGAGGAGCCGAGTCAGATCCTAGCGTC GAGCCCCCTCTGAGTCAGGAACATTTCAGACCTATGAAACTACTTCCCTGAAAACAAGC TTCTGTCCCCCTGCCGCTCCAAAGCAATGGATGATTGATGCTGTCCCCGGACGATATTGA ACAATGGTTCACTGAAGACCCAGGTCCAGATGAAGCTCCAGAATGCCAGAGGCTGCTCC CCCCGTGGCCCTGACCAGCAGCTCTACACCCGGCCCTGCACCAGCCCCCTCCT GGCCCTGTGATCTCTGTCCCTCCAGAAAACCTACCAGGGCAGCTACGGTTCCGT GGGCTCTTGCACTCTGGAGAGCCAGTGTGACTTGCACGTACTCCCTGCCCTCAAC AAGATGTTTGCAACTGGCAAGACCTGCCCTGTGAGCTGTGGTTGATTCCACAC CGCCCGGCACCCCGTCCGCGCATGGCCATCTACAAGCAGTCACAGCACATGACGGAG GTTGTAGGGCGCTGCCCTCAGATGAGCGCTGCTCAGATAAGCGATGGCTGGCCCTCCT CAGATCTTACCGAGTGAAGGAATTTCGCTGTGGAGTATTGGATGACAGAAA TTGACATAGTGTGGTGGCCCTATGAGCCGCTGAGGTTGGCTCTGACTGTACCCACAC TCCACTACAACATGTGTAACAGTCTCTGCATGGCGGCATGAACCCGGAGCCATCCT CACCATCATCACACTGGAAAGACTCCAGTGGAAATCTACTGGGACGGAACAGCTTGGAG CGTGGTGTGCTGCTGGAGAGACCCGGCGACAGAGGAAGAGAATCTCCGCAAGAAA GGGAGCCTCACCACGAGCTGCCCTCAGGGAGACTAACAGGAGACTAACGGAGACTGCC CAGCTCTCTCCCAGCAAAGAAAGAAACACTGGATGGAGAATATTCACTTCAAGGAC CAGACAGCTTCAAAAGAAAATTGTAAGAGAGCATGAAAATGGTTATGACTTGC TGATACAGATGCTACTTGACTTACGATGGTGTACTTCTGTATAAACCTCGCTGAAGTGA AATATTATCCGTGGCGTGAGCGCTCGAGATGGCTGAGCTGAATGAGGCTTGGAA CTCAAGGATGCCAGGCTGGAGAGGAGCCAGGGGGGAGCAGGGCTACTCCAGCACCT GAAGTCAAAAGatccgcg
<i>LRG321_t4</i> (encoding FL/Δ40p53 γ) (NCBI Reference Sequence NM_001126113.2)	ccggattcGCCTCGGGCTGGAGCGTGCTTCCACGACGGTGACACGCCCTGGATTG GCAGCCAGACTGCCCTCCGGGTCACTGCCatGAGGAGCCGAGTCAGATCCTAGCGTC AGCCCCCTCTGAGTCAGGAACATTTCAGACCTATGAAACTACTTCCCTGAAAACAACGTT CTGCCCCCTGCCGCTCCAAAGCAatGATGATTGATGCTGTCCCCGGACGATATTGAACA ATGGTTCACTGAAGACCCAGGTCCAGATGAAGCTCCAGAATGCCAGAGGCTGCTCC CGTGGCCCTGACCAGCAGCTCTACACCCGGGGCCCTGCACCAGCCCCCTCTGGC CCCTGTGATCTCTGTCCCTCCAGAAAACCTACCAGGGCAGCTACGGTTCCGT CTTCTGGCATTCTGGAGACCCAAGCTGTGACTTGCACGTACTCCCTGCCCTCAACAG ATGTTTGCCAAGTGGCAAGACCTGCCCTGTGAGCTGTGGTTGATTCCACACCCCG CCCGGCACCCCGTCCGCGCATGGCCATCTACAAGCAGTCACAGCACATGACGGAGGT TGTAGGGCGCTGCCCTCAGATGAGCGCTGCTCAGATAAGCGATGGCTGGCCCTCCTCA GCATCTTACCGAGTGGAGGAATTTCGCTGTGGAGTATTGGATGACAGAAA CGACATAGTGTGGTGGCCCTATGAGCCGCTGAGGTTGGCTCTGACTGTACCA CACTACAACATGTGTAACAGTCTCTGCATGGCGGCATGAACCCGGAGGCCATCCT ACCATCATCACACTGGAAAGACTCCAGTGGAAATCTACTGGGACGGAACAGCTTGGAG GTGTTTGCCGCTCTGGAGAGACCCGGCGACAGAGGAAGAGAATCTCGCAAGAAA GGGAGCCTCACCACGAGCTGCCCTCAGGGAGACTAACAGCAGACTGCCCAACAC AGCTCCTCTCCCAGCAAAGAAGAAACCACTGGATGGAGAATATTCACTTCAAGGAC TACTTGACTTACGATGGTGTACTTCTGTATAAACCTCGCTGAAGTGAAGTGA GGCGTGAGCGCTCGAGATGGCTGAGCTGAATGAGGCTTGGAACTCAAGGATGCC CAGGCTGGAGAGGAGCCAGGGGGAGCAGGGCTACTCCAGCACCTGAAGTCA GAAGTCAAAAGatccgcg
<i>LRG321_t2</i> (encoding FL/Δ40p53 α)	ccggattcGCCTCGGGCTGGAGCGTGCTTCCACGACGGTGACACGCCCTGGATTG GCCAGACTGCCCTCCGGGTCACTGCCatGAGGAGCCGAGTCAGATCCTAGCGTC CCCCCTCTGAGTCAGGAACATTTCAGACCTATGAAACTACTTCCCTGAAAACA GAAGTCAAAAGatccgcg

(NCBI Reference
Sequence
NM_001126112.2)

TCCCCCTGCCGTCCAAGCAatgGATGATTGATGCTGTCGGGACGATATTGAACAATG
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