

FIGURE 1

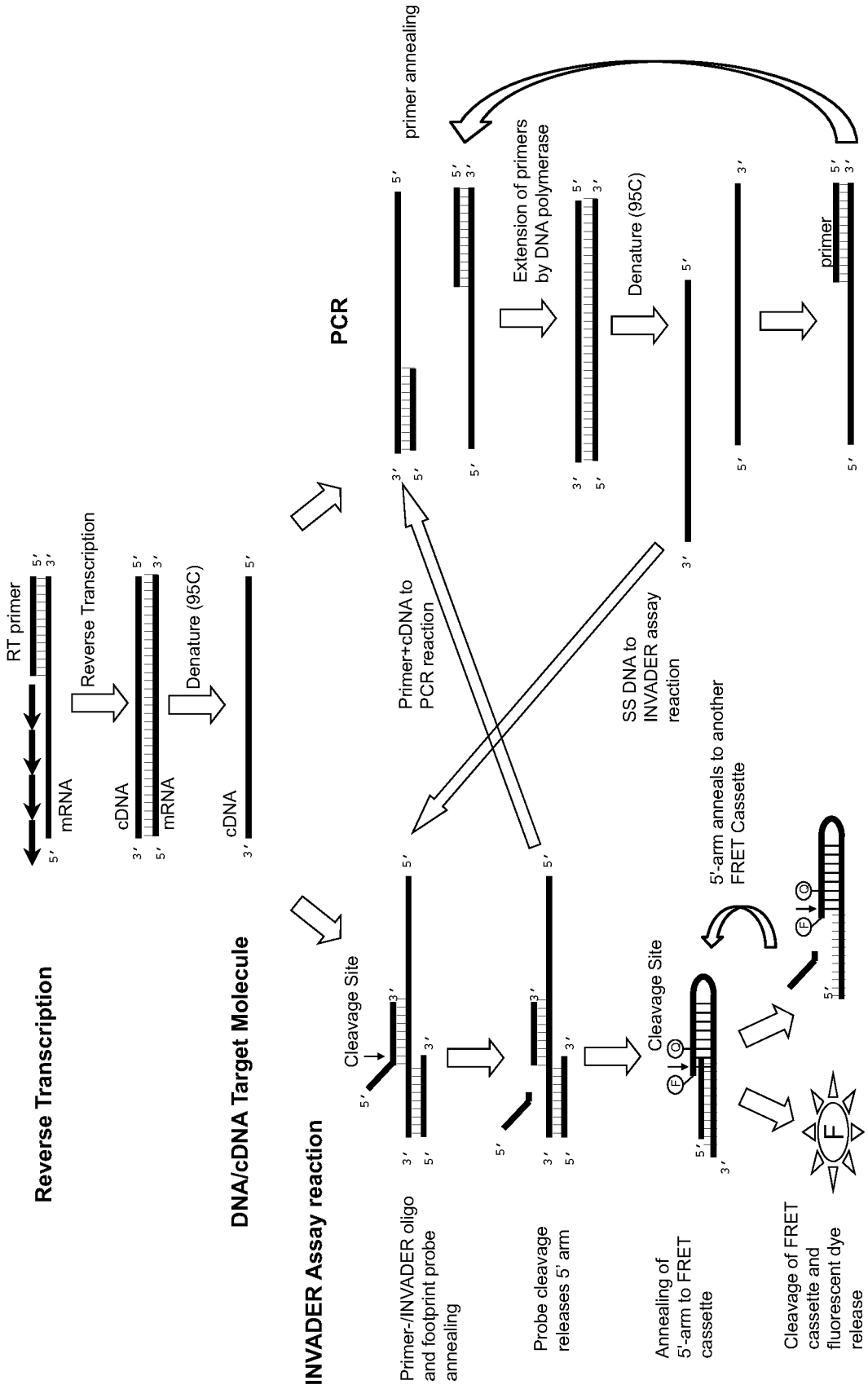


FIGURE 2A

(SEQ ID NO:144)

10-nt probe 3'-HEX-CATCCAACAT

3'GAGGCGCAG

T_m of 11-nt analyte-specific portion: 34.2°C

(SEQ ID NO:141)

11-nt probe 3'-HEX-CATCCAACATA

3'GAGGCGCAG

T_m of 11-nt analyte-specific portion: 36.2°C

5'-CCAGTGGCGATGAGGTAGTAGGTTGTATATAGTTCGCTGGACCGTG-3'

Let-7a Target

(SEQ ID NO:142) let-7a RT-Product

(SEQ ID NO:143) 3'-ATCAAGCGACCTGGCAC

INVADER oligonucleotide for 11-nt probe

(SEQ ID NO:145) 3'-TATCAAGCGACCTGGCAC

INVADER oligonucleotide for 10-nt probe

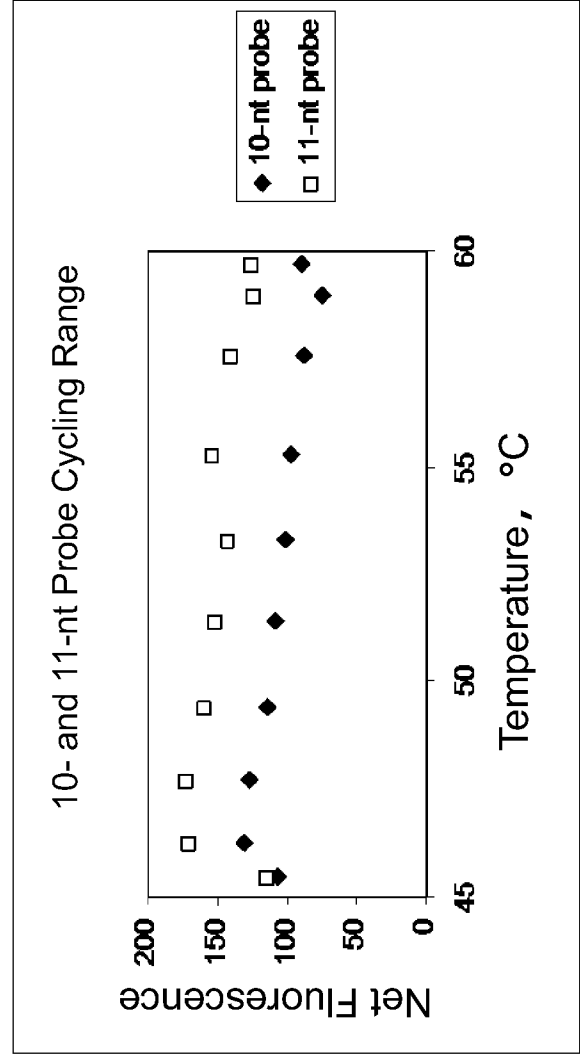


FIGURE 2B

5'-GACGCGGAG (SEQ ID NO:146) 9-nt probe
Tm of 9-nt analyte-specific portion: 45.6°C
(SEQ ID NO:147)
INVADER oligonucleotide 5'-AGACARAGCGGTCCATCCY
(SEQ ID NO:148)
Vzv Target 5'-ACGTACACGTGATACTGAGACAAAGCGGTCCATCCCTGGGCCACCTCTCGAGGGGCCACCGCGTCCACACACAGCA

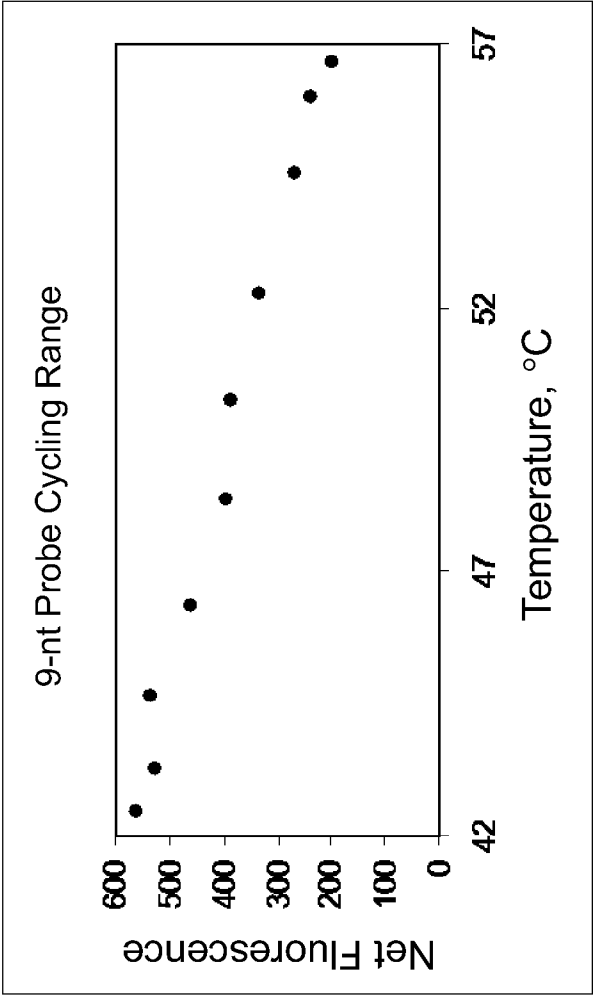


FIGURE 3

TARGET	COMPLETE PROBE SEQUENCE (5' to 3'; All Probe Have 3' HEX)	SEQ ID NO:	5'-flap name	ANALYTE-SPECIFIC REGION (5' to 3')	SEQ ID NO:	LENGTH OF ASR	Tm
microRNA							
let-7a	GACGGGAGTACACCTACT	52	Arm 3	TACACCTACT	53	11	36.8
let-7b	CCAGGACGACACCTACT	54	Arm 4	CACACCTACT	55	11	40.5
miR-9	GACGGAGTATCTAGCTGA	56	Arm 3	TATCTAGCTGA	57	12	38.4
miR-15a	CCAGGAGGACCAATATGTC	58	Arm 4	ACCAATATGTC	59	12	44.9
miR-15b	GACGGAGACCAATATGTC	60	Arm 3	ACCAATATGTC	61	12	49.2
miR-17-5p	CCAGGAGGTCACCTGTAAG	62	Arm 4	CTGCACTGTAAG	63	12	45.7
miR-18a	GACGGAGGCACTAGATGA	64	Arm 3	TCCATGCAAAAC	65	12	48.2
miR-19b	GACGGAGGCACTAGATGA	66	Arm 3	TCCATGCAAAAC	67	12	46.3
miR-21	GACGGAGATCACTCTGAT	68	Arm 3	ATCAGTCTGAT	69	11	37.7
miR-28	GACGGAGTAGACTGTGAGC	70	Arm 3	TAGACTGTGAGC	71	12	46.3
miR-92	CCGGGACGGGACAAAGTG	72	Arm 4	CGGACAAAGTG	73	11	48.2
miR-126	CCAGGAGGTGAGTAATAAG	74	Arm 4	TGAGTAATAAG	75	12	34.7
miR-223	CCGGGACGATTTGACAAAC	76	Arm 4	ATTTGACAAAC	77	11	35.5
miR-29a	GACGGAGTTTCAGATGGT	78	Arm 3	TTTCAGATGGT	79	11	39.3
miR-22	CCAGGACGAGTTGAAGAACT	80	Arm 4	AGTTGAAGAACT	81	12	41.7
miR-16	GACGGAGATATTTAGGTGC	82	Arm 3	ATATTTAGGTGC	83	12	41.2
miR-20a	CCAGGACGGCATTATAAGCA	84	Arm 4	GCACTATAAGCA	85	12	44.0
miR-155	GACGGAGATCAGGATTAGC	7	Arm 3	ATCAGGATTAGC	87	12	44.3
miR-342	GACGGAGTGCGATTCTGT	88	Arm 3	TGCGATTCTGT	89	12	47.9
mRNA							
NM_001823-[670]-5 (CKB)	GACGGGAGCCAGGAGTCT	90	Arm 3	CCAGGAGGTC	91	12	49.7
NM_016139-[1005]-3 (CHCHD2)	GACGGGAGGCTAATGAAGA	92	Arm 3	GCCTAATGAAGA	93	12	43.2
NM_006908-[8084]-3 (RAC1)	GACGGGAGCAAGGAAAGC	94	Arm 3	CAAGGAAAGC	95	12	47.0
NM_001791-[4644]-3 (CDC42)	GACGGGAGGAGTATTAGCA	96	Arm 3	AGGATTATTAGCA	97	12	40.3
NM_002086-[4070]-4 (GRB2)	GACGGGAGGAAGCAATGTC	98	Arm 3	GAAAGCAATGTC	99	12	48.2
NM_002654-[487]-4 (PRM2)	GCGGTCCCACTGCGAGAGT	100	Arm 7	CACCTGCGAGAGT	101	12	51.5
NM_030662-[6763]-2 (MAP2K2)	GCGGTCCCACTGCTGAGAT	102	Arm 7	CCACTCTGAGAT	103	12	46.6
NM_000584-[819]-1 (IL18)	GACGGGAGATCTCTGGCAA	104	Arm 3	ACTCTCTGGCAA	105	12	50.5
NM_000858-[774]-4 (GUK1)	GACGGGAGCTCCCTGTTTAC	106	Arm 3	CTCCCTGTTTAC	107	12	47.4
NM_001110-[9479]-11 (ADAM10)	GACGGGAGGTCAAGTCCCT	108	Arm 3	GTCAAGTCCCT	109	12	47.0
NM_004396-[81]-6 (DDX5)	GACGGGAGGTGCAATACAG	110	Arm 3	GTTCGATACAG	111	12	43.3
NM_002634-[2204]-3 (PHB)	GACGGGAGCAGAAATGCAAC	112	Arm 3	CAGAAATGCAAC	113	12	42.5
NM_004323-[2011]-4 (BAG1)	GACGGGAGGCAATCTCTGG	114	Arm 3	SCAATCTCTGG	115	12	47.2
NM_014740-[2548]-1 (EIF4A3)	GACGGGAGATGTGATGGT	116	Arm 3	ATTGCTGATGGT	117	12	47.1
NM_002046-[193]-7 (GAPDH)	GCGGAGGATCCATGSCAT	118	Arm 1	AGTCCATGSCAT	119	12	50.3
NM_005030-[183]-9 (PLK1)	GCGGTCCCACTCAAGTCAAT	120	Arm 7	CACCAAGTCAAT	121	12	48.4
NM_002709-[2064]-4 (PPP1CB)	GCGGTCCATTTGAGGCTG	122	Arm 7	ATTGAGGCTG	123	12	48.4
NM_015646-[2062]-7 (RAP1B)	GACGGGAGGAAGCACTGTTGC	124	Arm 3	AAGAAGCTGTTGC	125	12	45.7
NM_002512-[1285]-2 (NME1)	GACGGGAGTCAAGAACCT	126	Arm 3	TGAGAACACCT	127	12	45.5
NM_000125-[34232]-1 (ESR1)	GACGGGAGGCTGATATTC	128	Arm 3	GCGTGGATATTC	129	12	46.7
DNA							
FV (mutant)	CCAGGACGAAGGAATACAGG	46	Arm 4	AAGGAATACAGG	131	12	42.3
FV (wildtype)	CGAGCCGGAGGAATACAGG	28	Arm 6	GAGGAATACAGG	133	12	43.4
FTI (wildtype)	GACGGGAGGAGCTCAATGC	34	Arm 3	GAGCTCAATGC	135	12	50.6
FTI (mutant)	GCGGAGGAAGCCATCAATGC	136	Arm 1	AAGCCATCAATGC	137	12	49.7
CT	GACGGGAGGCTTTAAATCA	138	Arm 3	CGTTTAAATCA	139	12	38.2
NG	CCAGGACGGTTCCTCAG	140	Arm 4	CTGTTCCTCAG	134	12	49.4

FIGURE 4

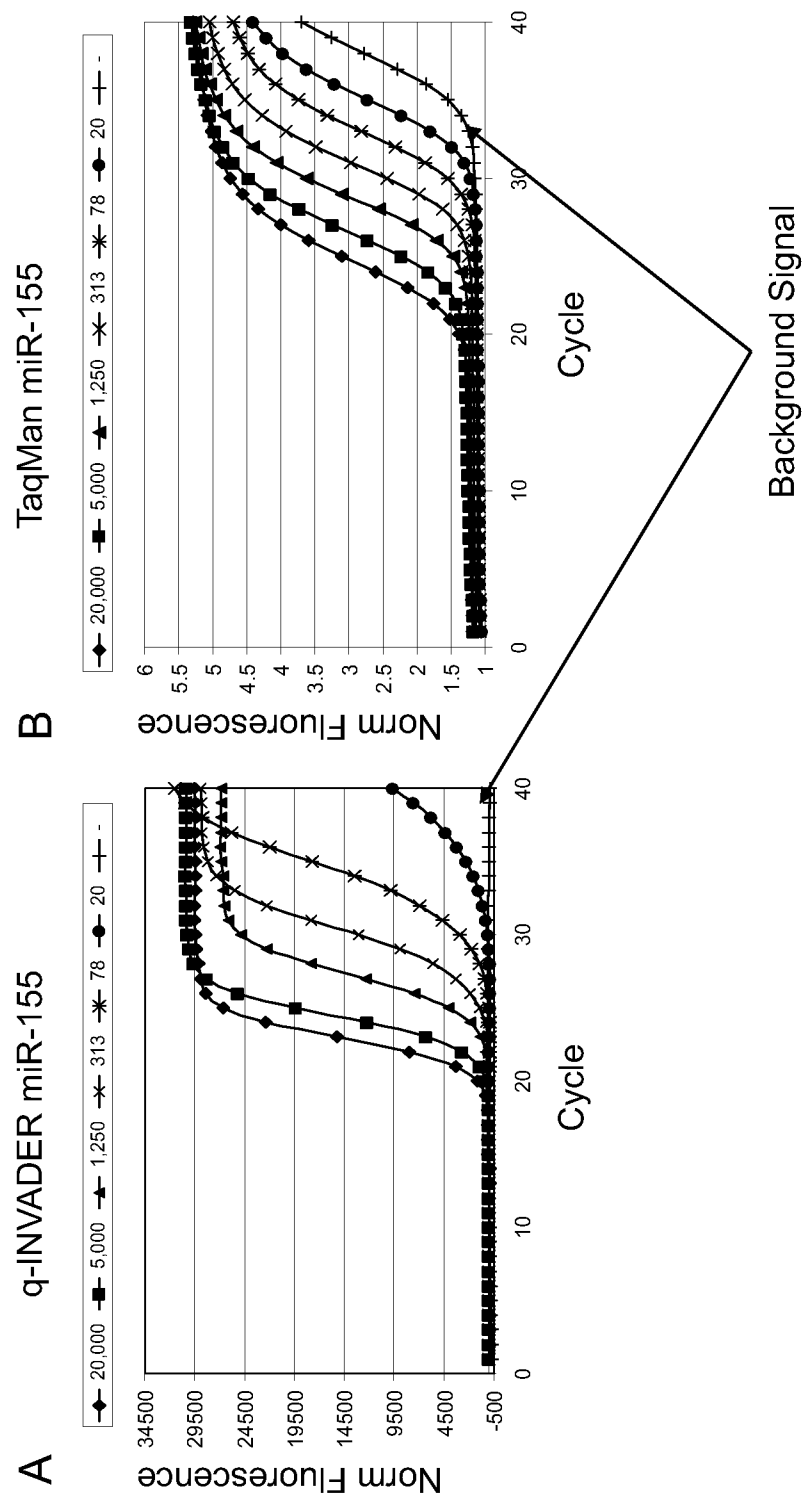


FIGURE 5

FV genotyping design

2749-70-03, Arm 4-Yellow (Mutant) 5'-CCACGGACG
2749-70-04, Arm 6-FAM (Wildtype) 5'-CGAGGCCG
2749-70-07 (SEQ ID NO:48)
AGGACTACTTCTAATCTGTAAGC->
|||||
5'-TAATAGGACTACTTCTAATCTGTAAGACGAGATCCCTGGACAGGCAGGAATACAGGTATTTGTCTTGAAGTAACCTTTCAGAAATT-3'
|||||x
GATCCCTGGACAGGCC
2749-70-05 (SEQ ID NO:49)
2749-70-06 (SEQ ID NO:50)
2749-70-06 (SEQ ID NO:51)
Arm 6 FAM FRET = 5'-FAM-TCT-Z28-AGCCGGTTTTCCGGCTGAGACGGCCTCGCG-HEX-3' (SEQ ID NO:6)
Arm 4 Yellow FRET = 5'-Yellow-TCT-Z28-AGCCGGTTTTCCGGCTGAGACGTCCTGGCCT-HEX-3' (SEQ ID NO:51)

FIGURE 6

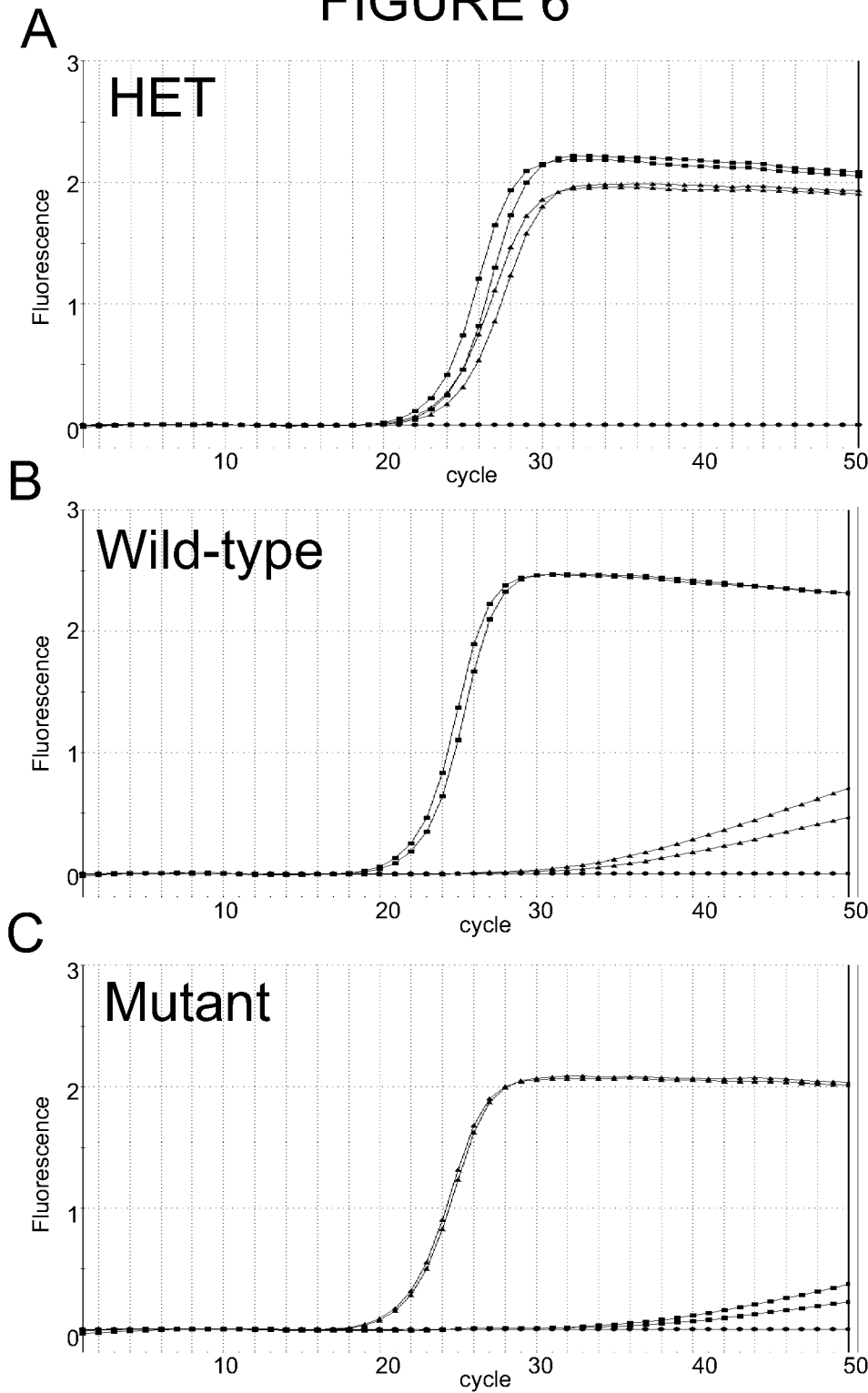


FIGURE 6 (cont.)

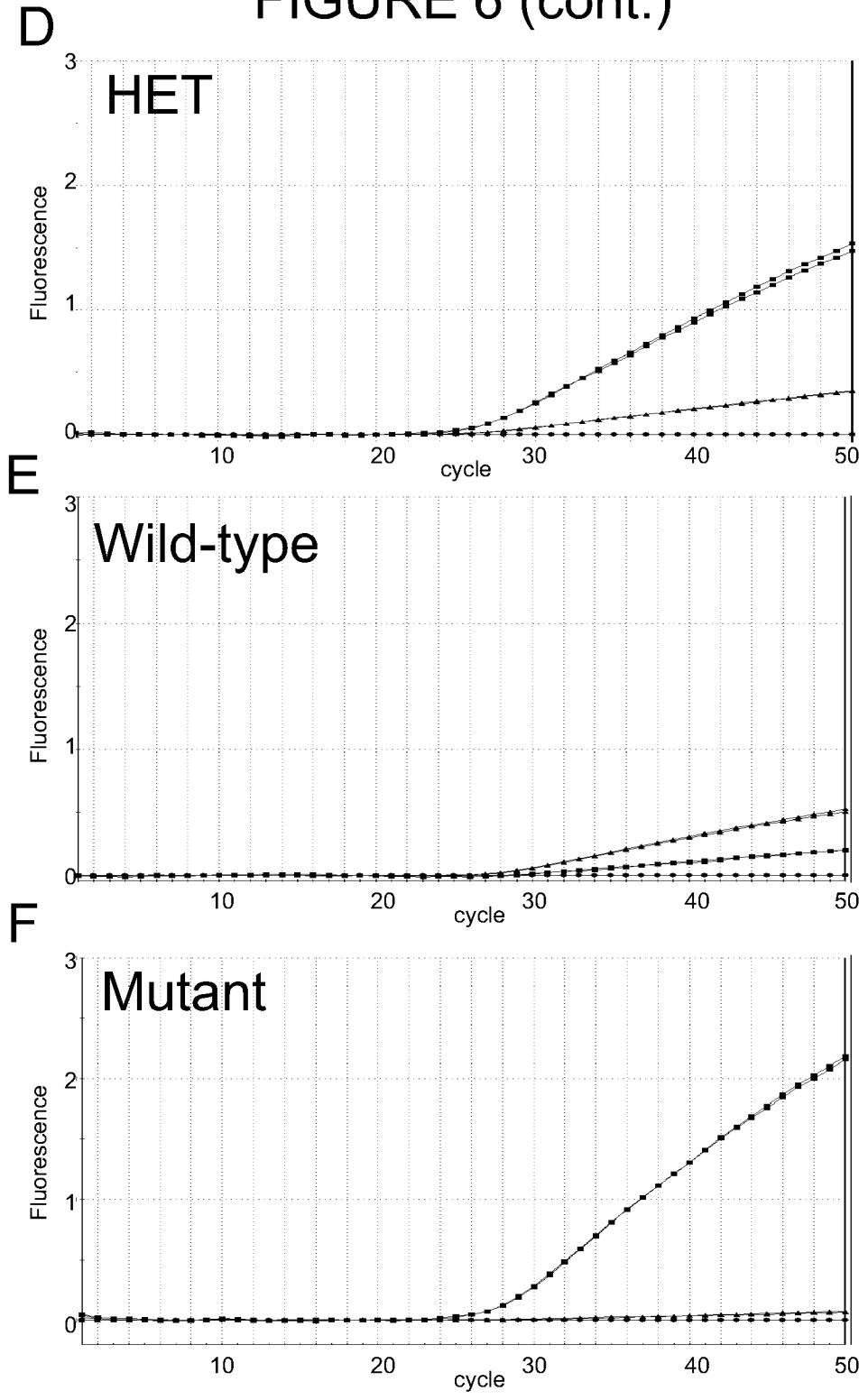
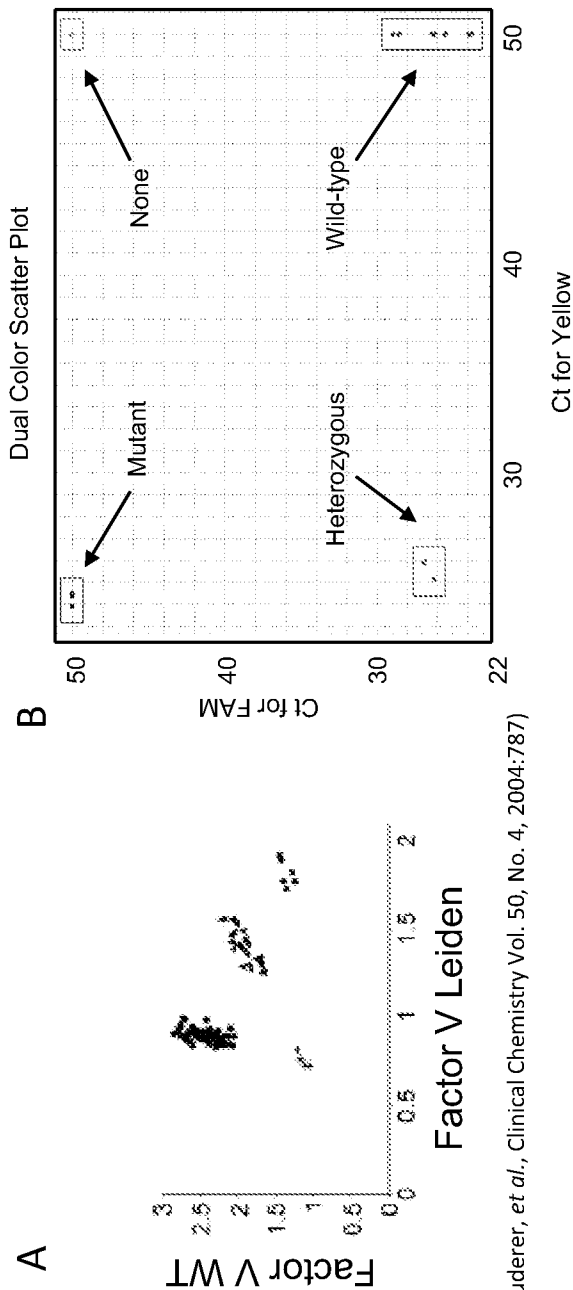


FIGURE 7



(From Luderer, *et al.*, Clinical Chemistry Vol. 50, No. 4, 2004:787)

FIGURE 8

A

Differentiation between wild-type and vaccine mutant VZV using the Invader Plus reagents^a

Sample type	Number tested	FAM average FOZ ^b	% CV ^c	Red average FOZ ^b	% CV ^c	FOZ ^b ratio FAM/Red
wild-type ^d	29	0.97	0.16	1.97	0.44	0.49
Vaccine mutant ^d	18	3.52	1.22	1.12	0.10	3.13
Negative	3	0.94	0.21	1	0.05	0.94
Webster (wild-type control)	1	1.14		2.61		0.44
Oka (Vaccine mutant control)	3	5.22	0.72	1.01	1.01	5.2
VTM (No target blank)	1					

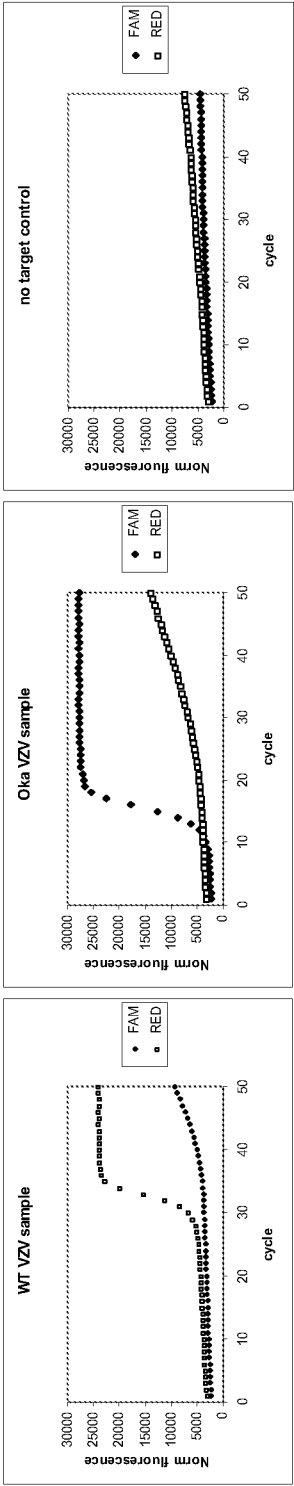
^a The Invader Plus differentiation reaction is configured to generate Red or FAM fluorescence signal for wild-type or Oka strains of VZV, respectively

^b FOZ = fold over zero.

^c CV: coefficient of variation; VTM: viral transport medium.

^d The genotypes were determined by an allele-specific TaqMan real-time assay (Loparev et al., 2000).

(From: Y.-Wang, et al., Journal of Clinical Virology 40 (2007) 129-134)

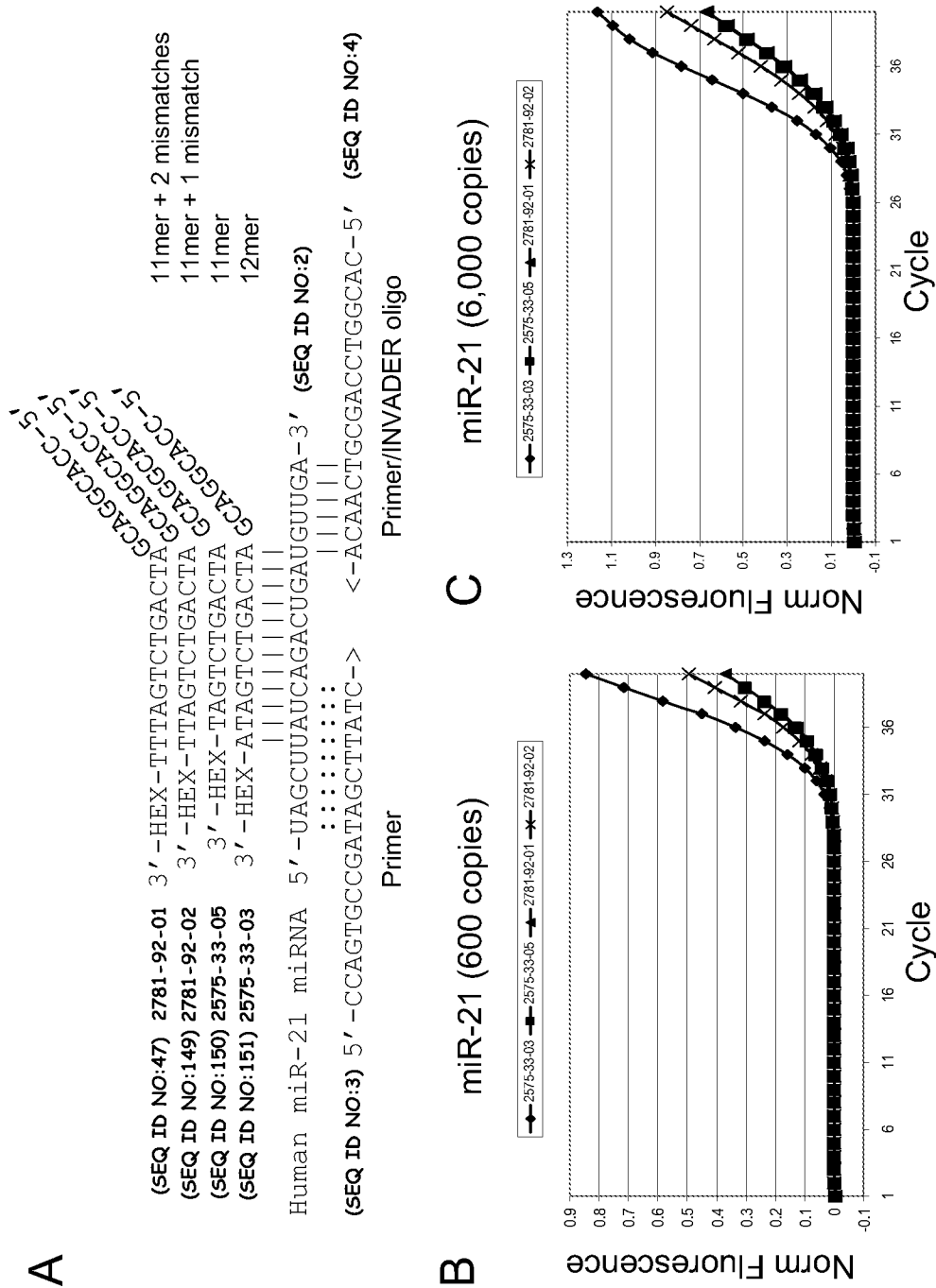


B

C

D

FIGURE 9



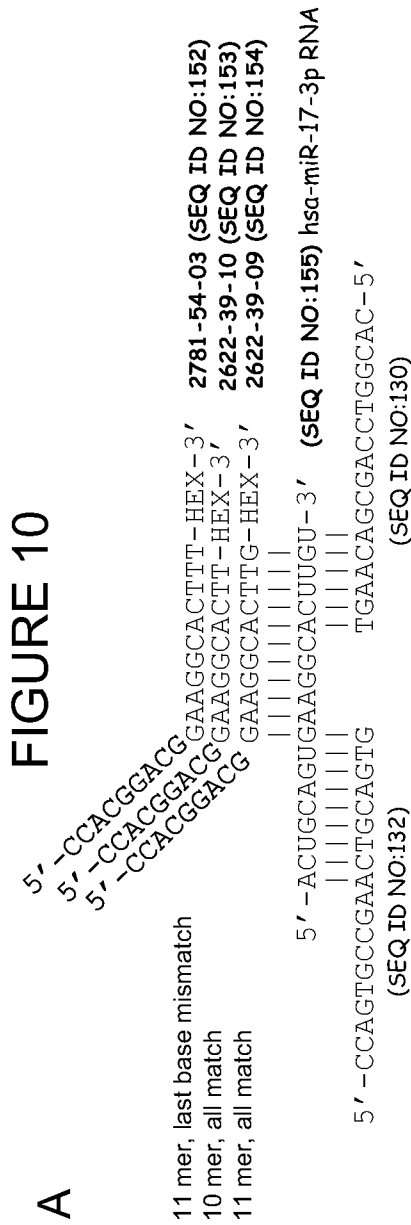
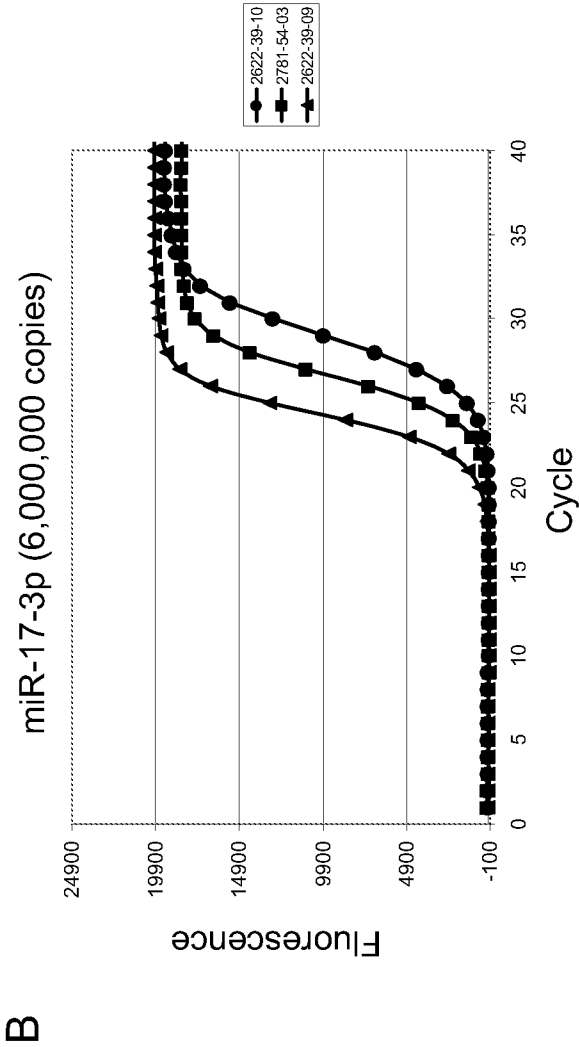
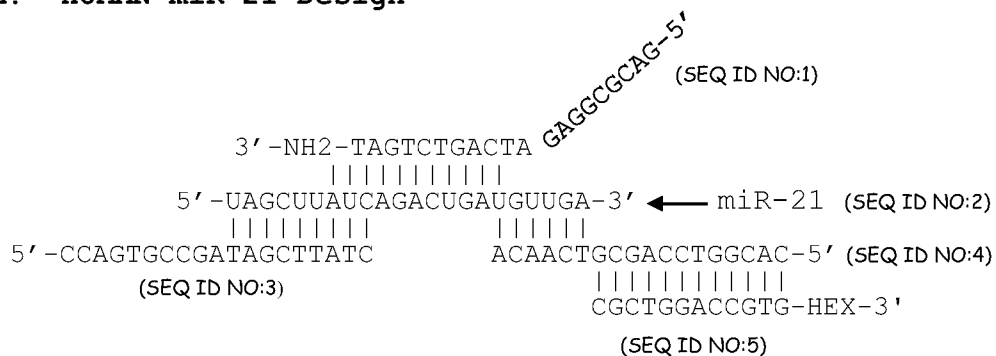
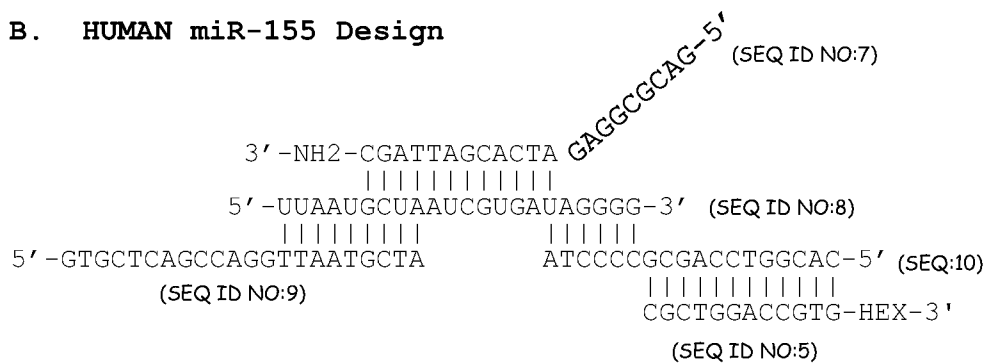


FIGURE 11 A-C

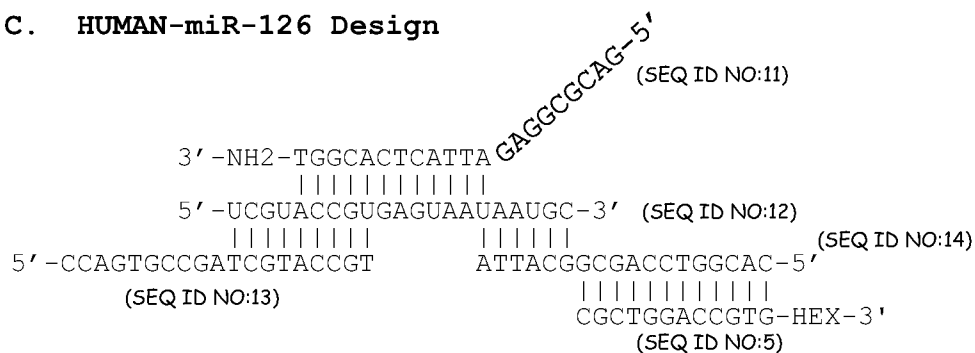
A. HUMAN miR-21 Design



B. HUMAN miR-155 Design



C. HUMAN-miR-126 Design



FRET: miR-21, 155, and 126

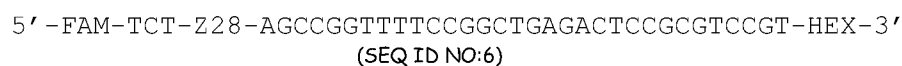
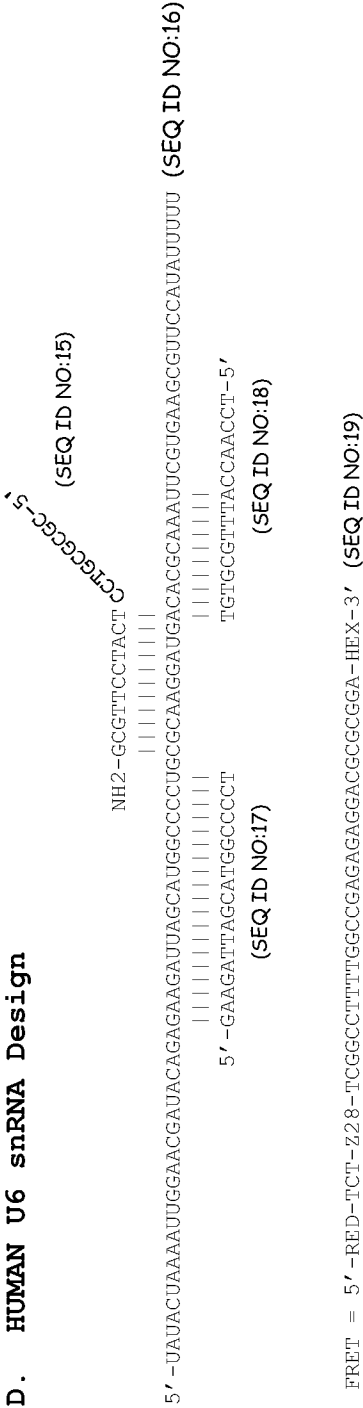


FIGURE 11 D-E

D. HUMAN U6 snRNA Design



E. Human U24 snRNA Design

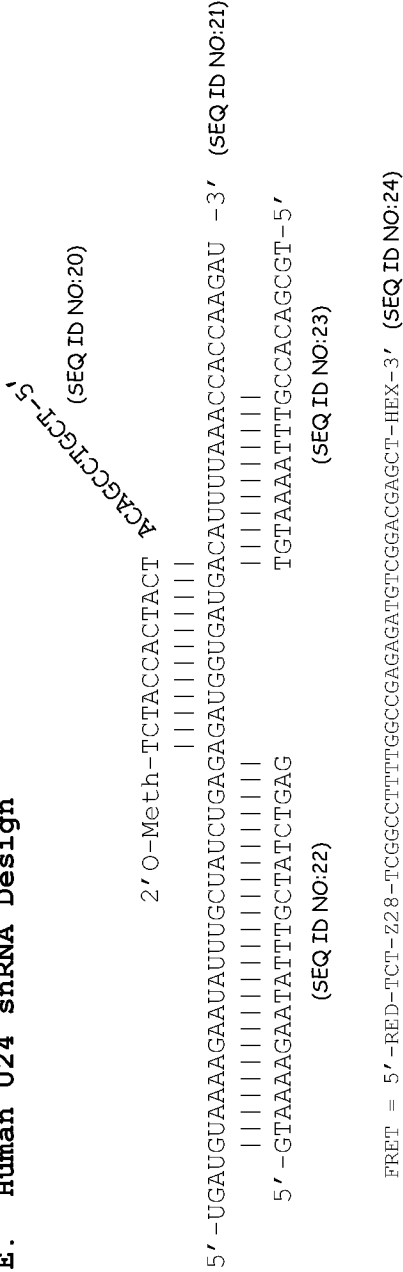


FIGURE 12A
miR-155

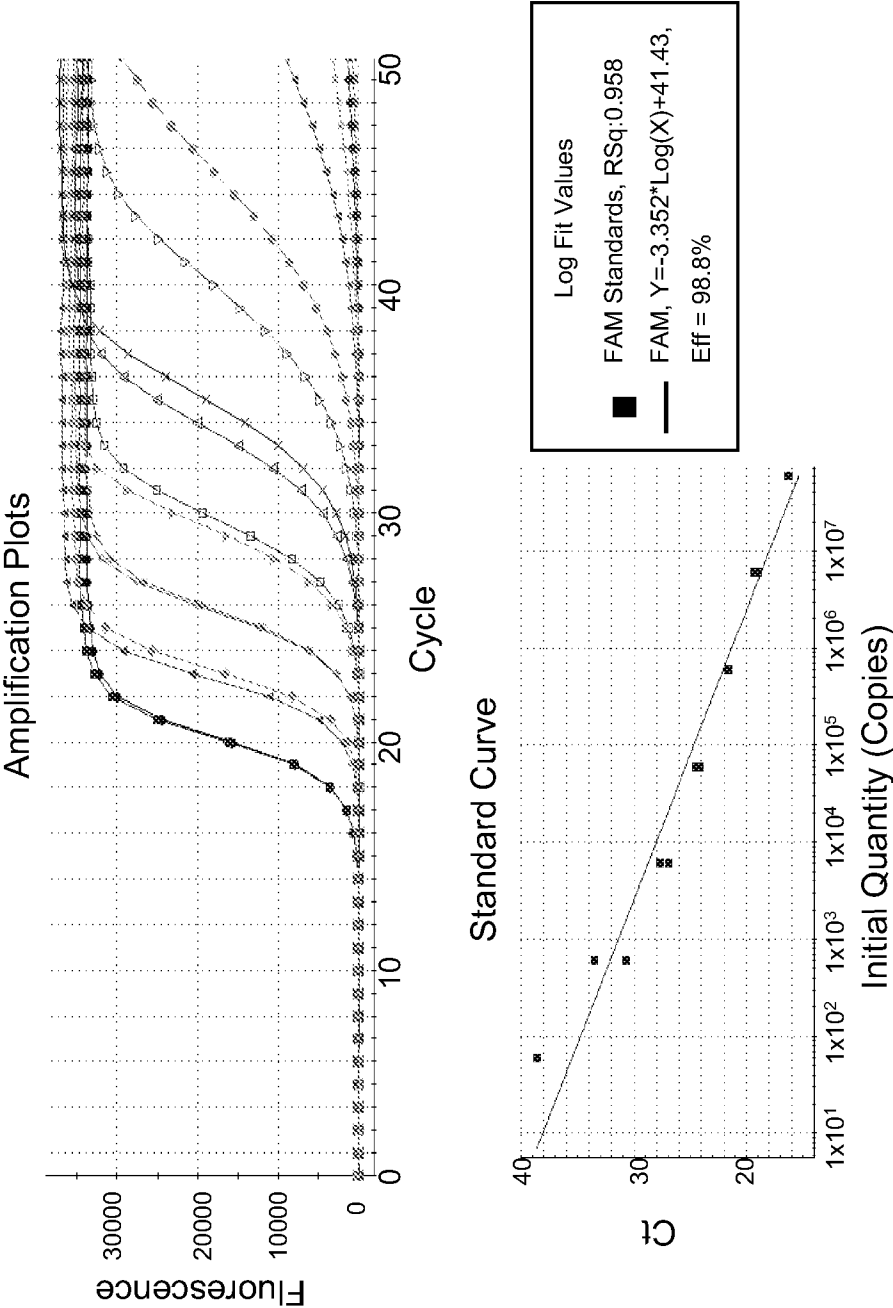


FIGURE 12B
miR-21

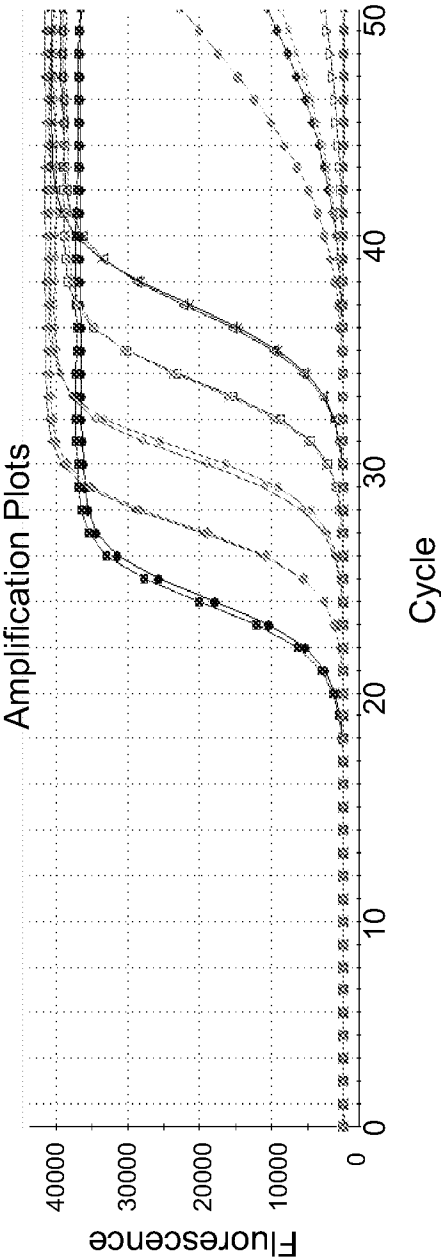
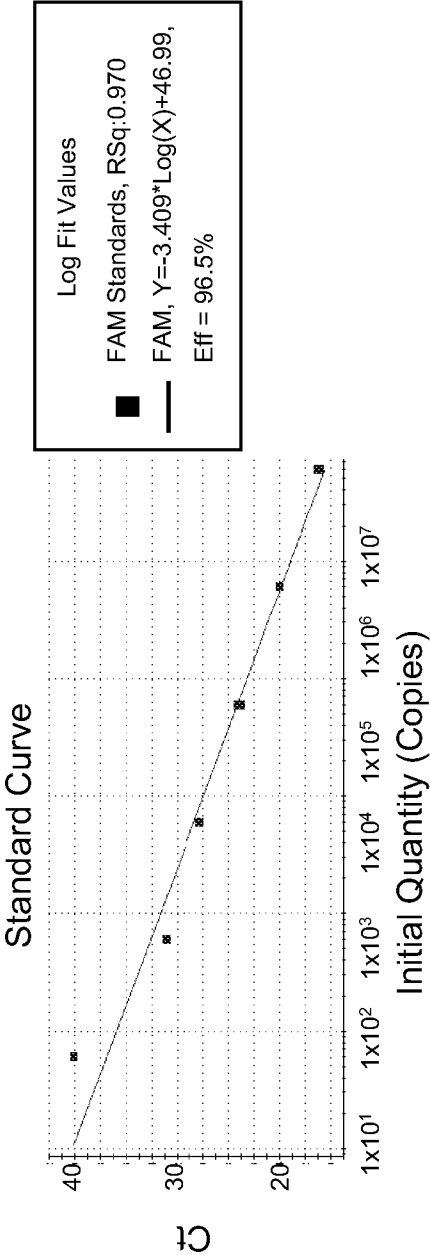


FIGURE 12C
miR-126

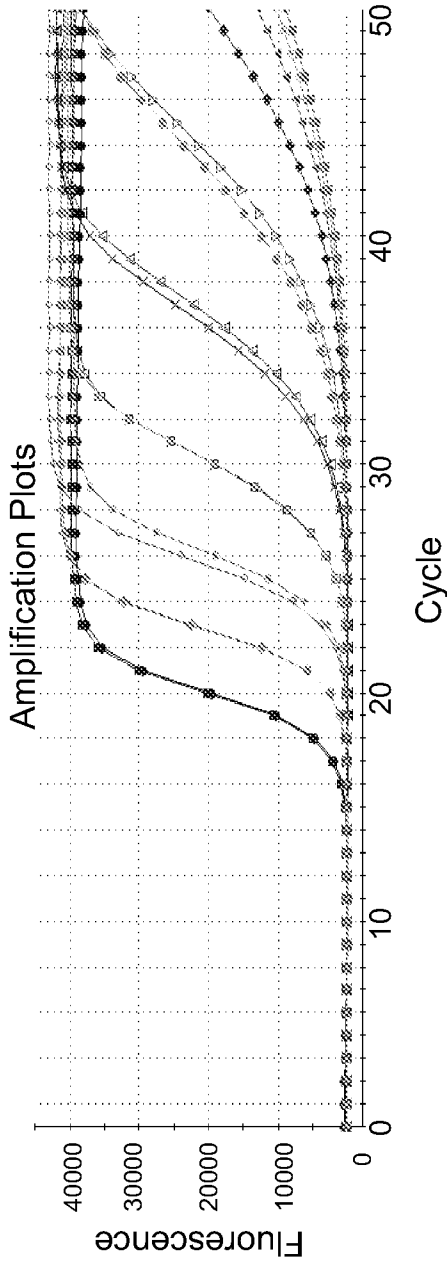
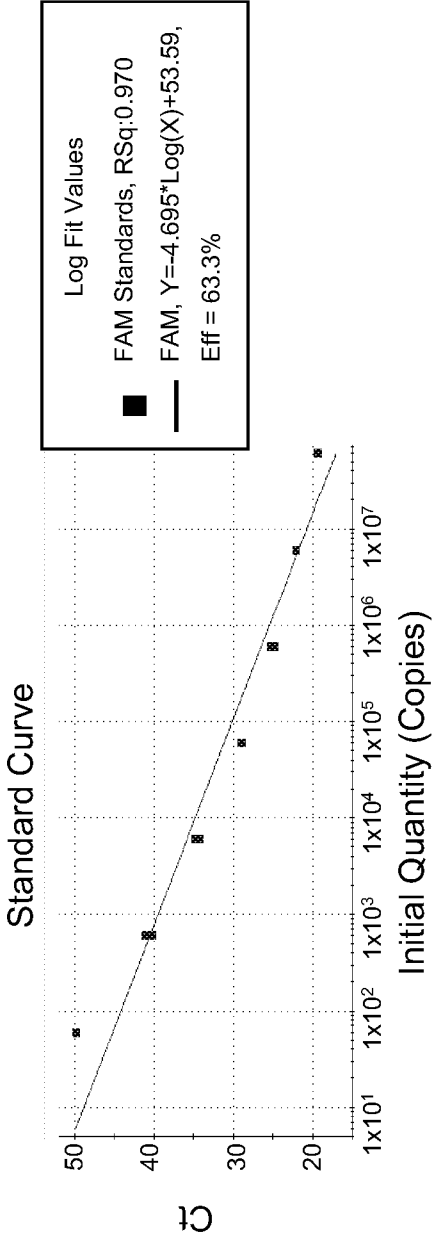


FIGURE 12D
miR-U6

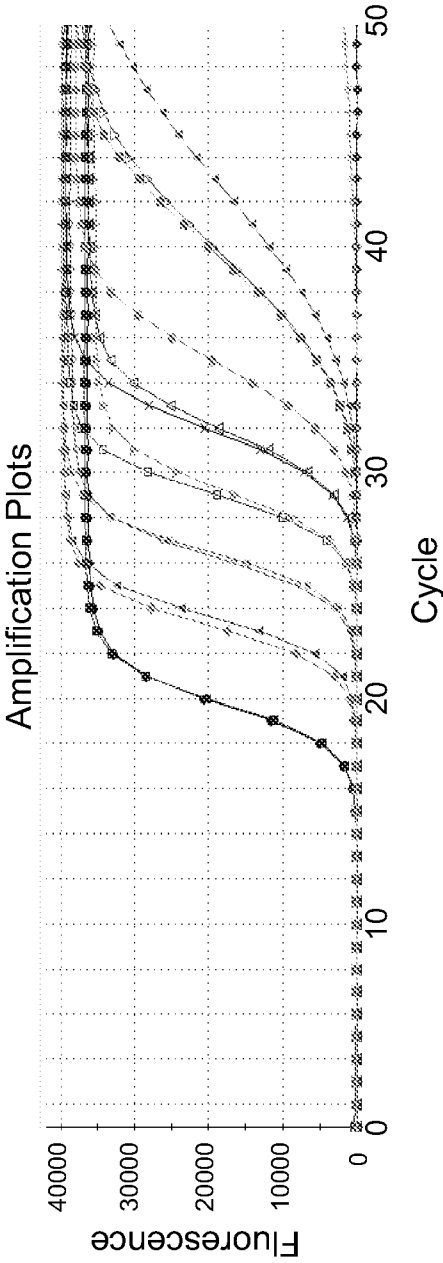
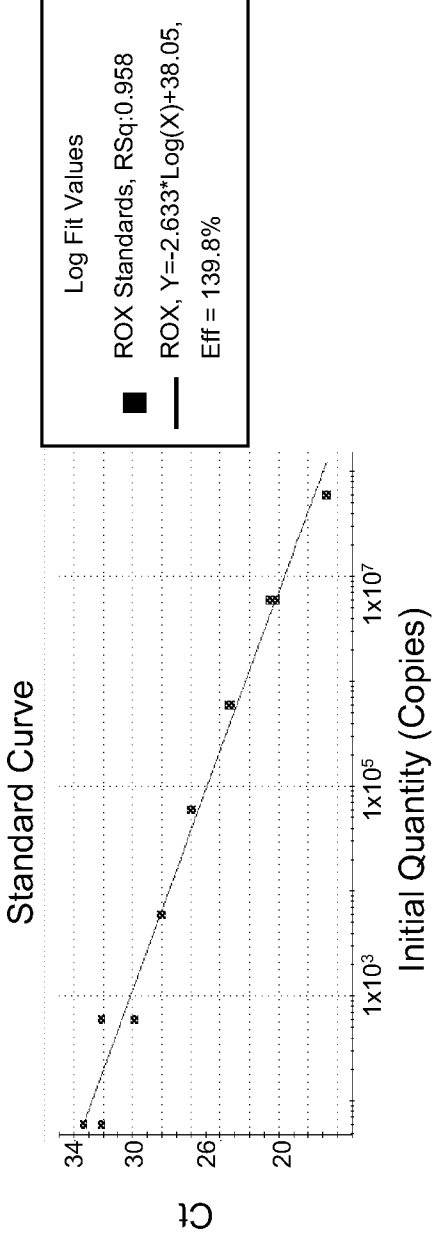


FIGURE 12E
U24

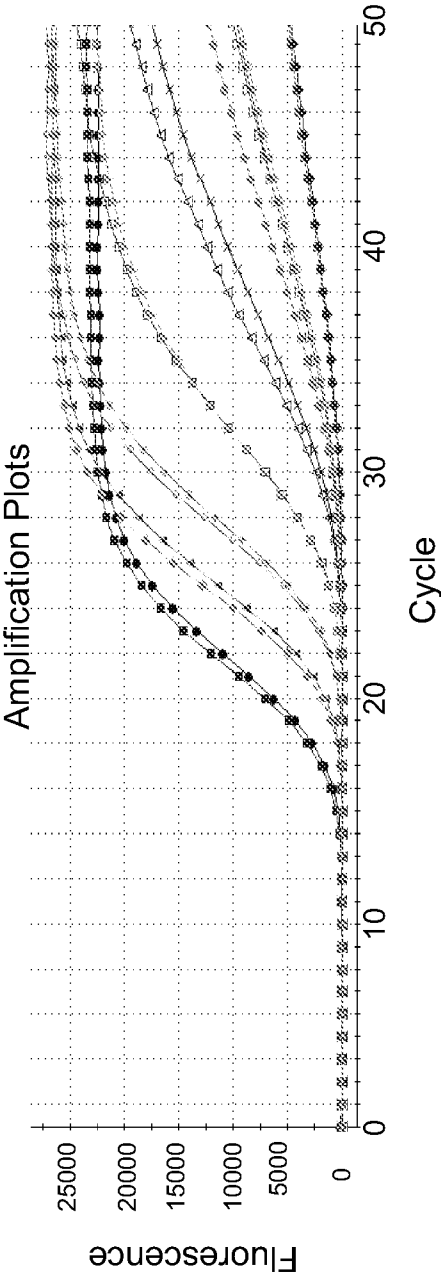
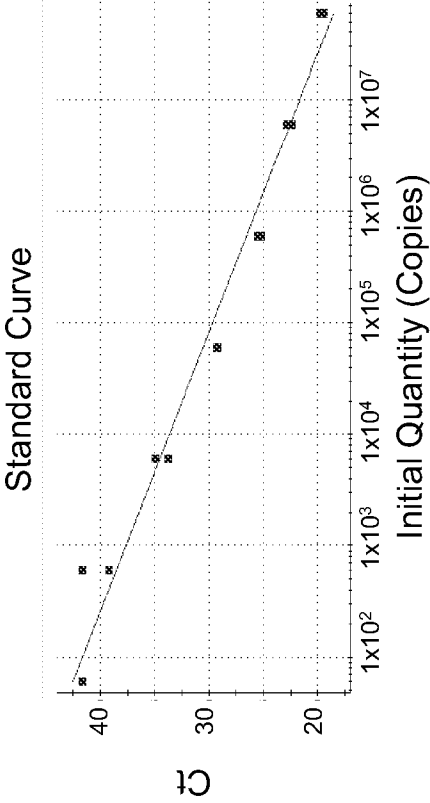


FIGURE 13 A-C

A. U6 DNA design

5' -CAGAGAAGATTAGCATGGCCCTGCGCAAGGATGACACGCAAAATTCGTGAAGCGTTCC (SEQ ID NO:25)
|||||
NH2 - GCGTTCCCTACT (SEQ ID NO:15)
|||||
5' -GAAGATTAGCATGGCCCT (SEQ ID NO:17)
|||||
TGTGCGTTTAAAGCACTTCG-5' (SEQ ID NO:26)
|||||
GCGTTTAAAGCACTTCGGA-5'

B. Factor V DNA design

5' -CGAGGCCG GAGGAATACAGG-HEX-3' (SEQ ID NO:28)
|||||
AGGACTACTTCTAATCTGTAAAGAGC-> (SEQ ID NO:30)
|||||
5' -TAGGACTACTTCTAATCTGTAAAGAGCAGATCCCTGGACAGGC GAGGAATACAGGTAATTTGTCCCTTGAAGTAACCTTTCAGAA-3' (SEQ ID NO:29)
|||||
GATCCCTGGACAGGCC (SEQ ID NO:31)
|||||
<-CAGGAAC TTCATTGGAAAAGTCTT (SEQ ID NO:32)

C. Factor II DNA design

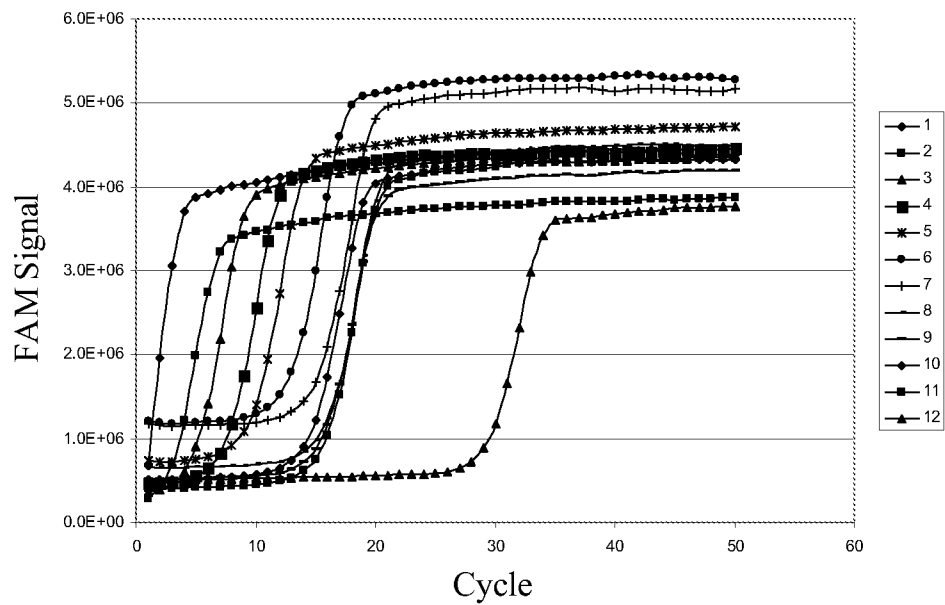
5' -GCCCCATGAATAGCACTGG CGTAACTCCGAG (SEQ ID NO:35)
|||||
5' -GCTGCCCCATGAATAGCACTGGGAGCATTGAGGCTCGCTGAGAGTCACCTTTATTGGGAACCATAGTTTGTAGAAAACACAAAAAT-3' (SEQ ID NO:36)
|||||
3' -TCGACTCTCAGTAAAAATAAC CTTGGTATCAAAATCTTTGTGTTTAA-5' (SEQ ID NO:37)
|||||
GAGGCGAG-5' (SEQ ID NO:34)
|||||
3' -HEX- (SEQ ID NO:38)

U6 design FRET = 5' -RED-TCT-Z28-TCGGCCTTTTGGCCGAGAGAGGACGCGCGGA-HEX-3' (SEQ ID NO:19)
Factor V design FRET = 5' -RED-TCT-Z28-AGCCGGTTTTCCGGCTGAGACGGCCTCGCG-HEX-3' (SEQ ID NO:33)
Factor II design FRET = 5' -FAM-TCT-Z28-AGCCGGTTTTCCGGCTGAGACTCCGGCTCCGT-HEX-3' (SEQ ID NO:6)

FIGURE 14 A-B

+ Invasive Oligo / + Afu FEN-1

A.



B.

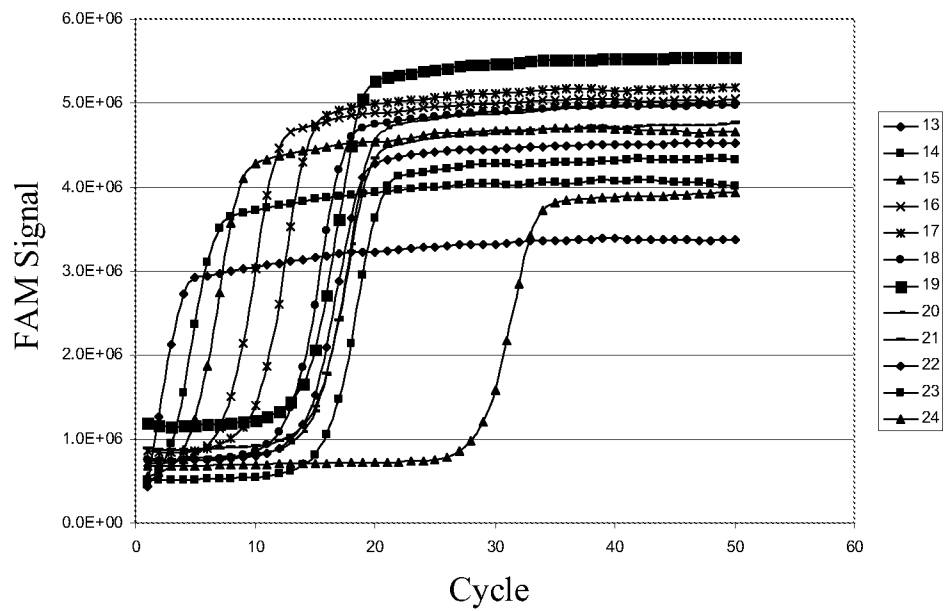
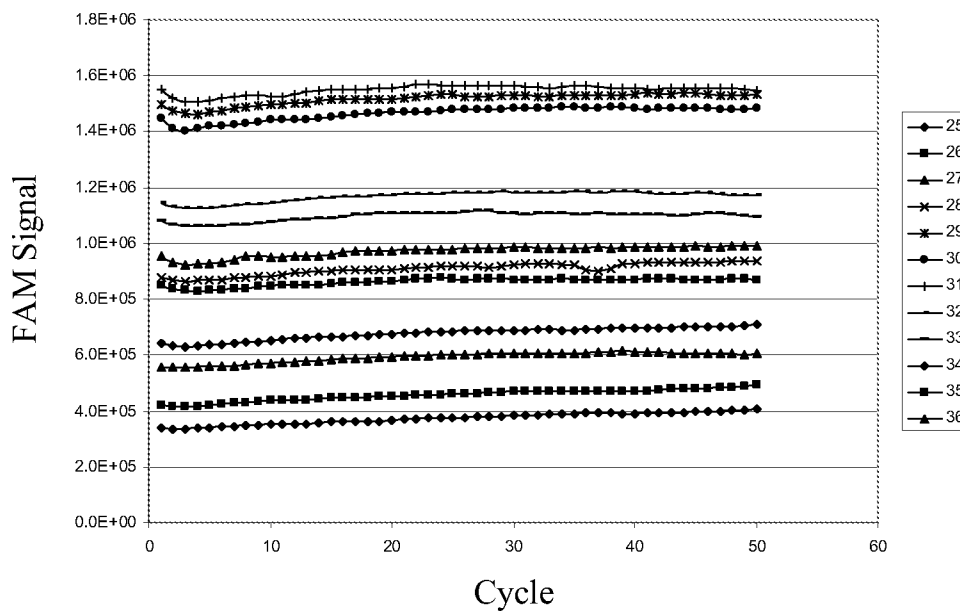


FIGURE 14 C-D

- Invasive Oligo / - Afu FEN-1

C.



D.

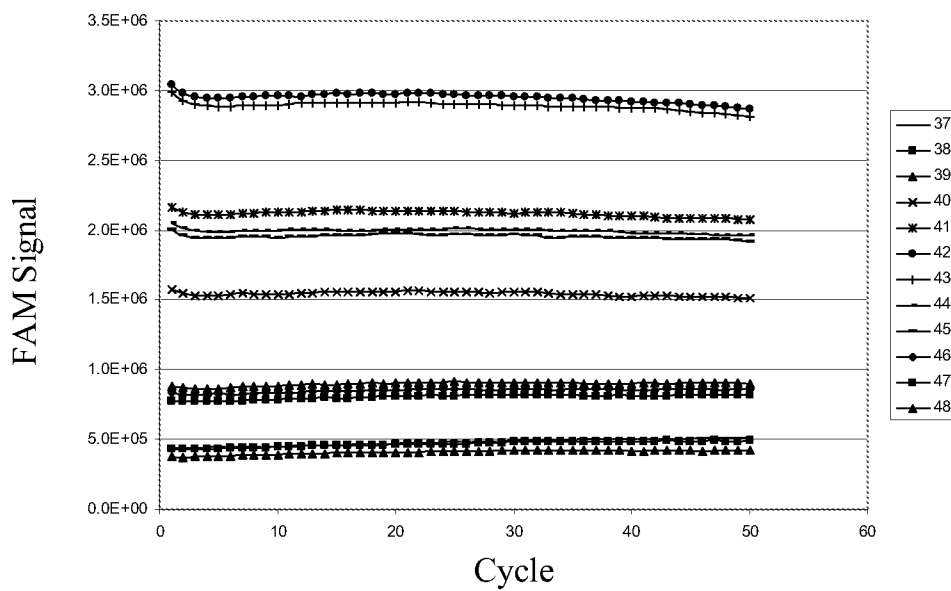


FIGURE 15A
Amplification Plots

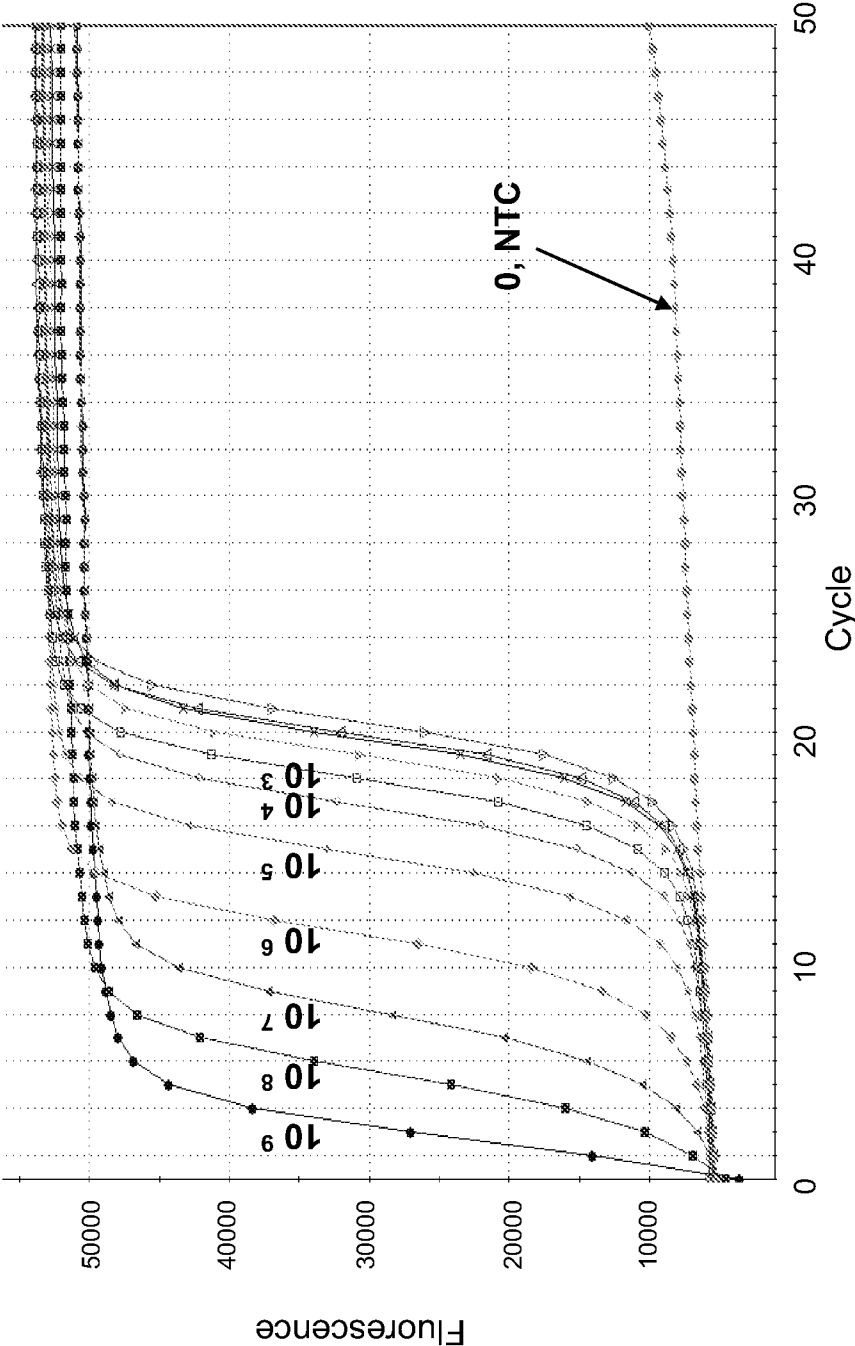


FIGURE 15B

Amplification Plots

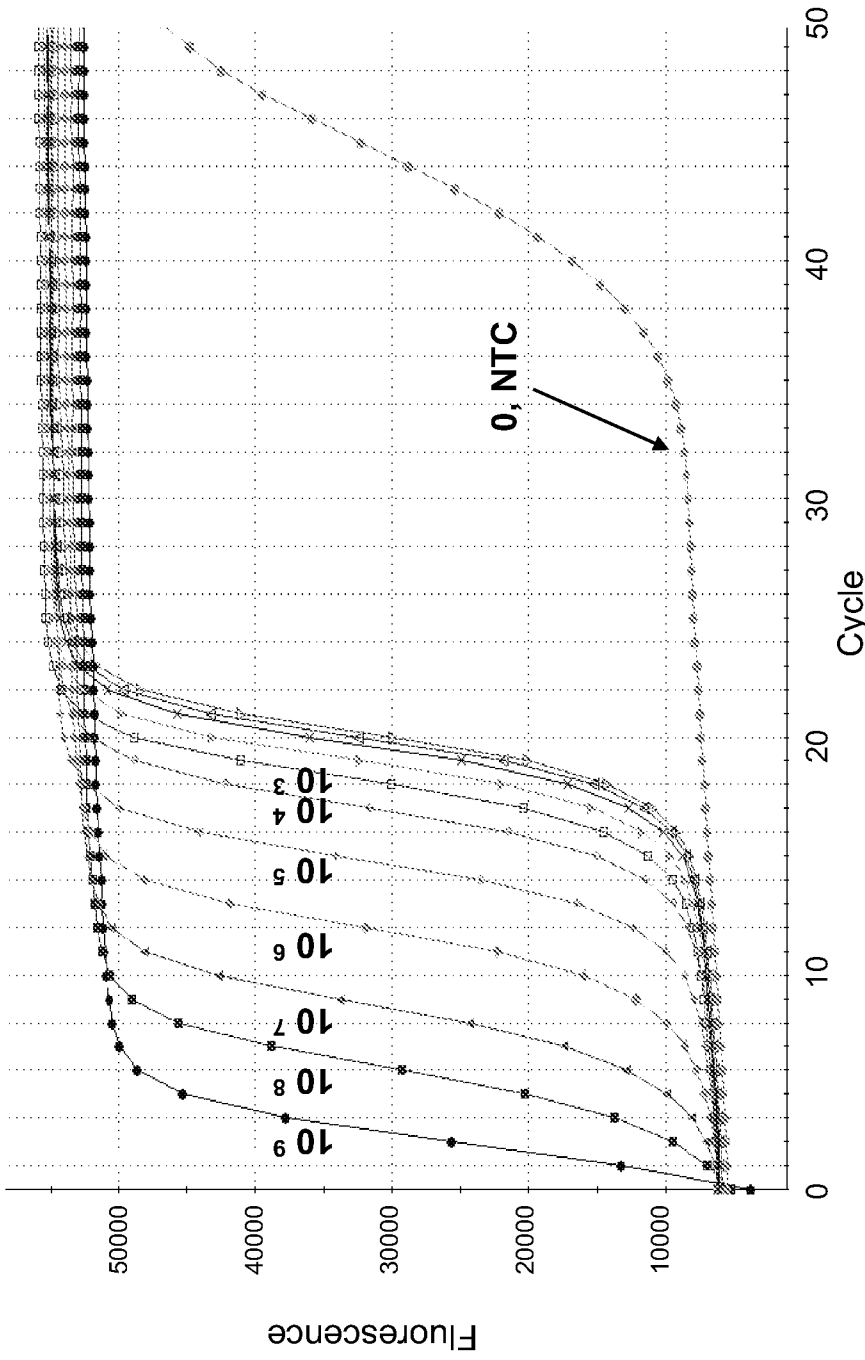


FIGURE 15C

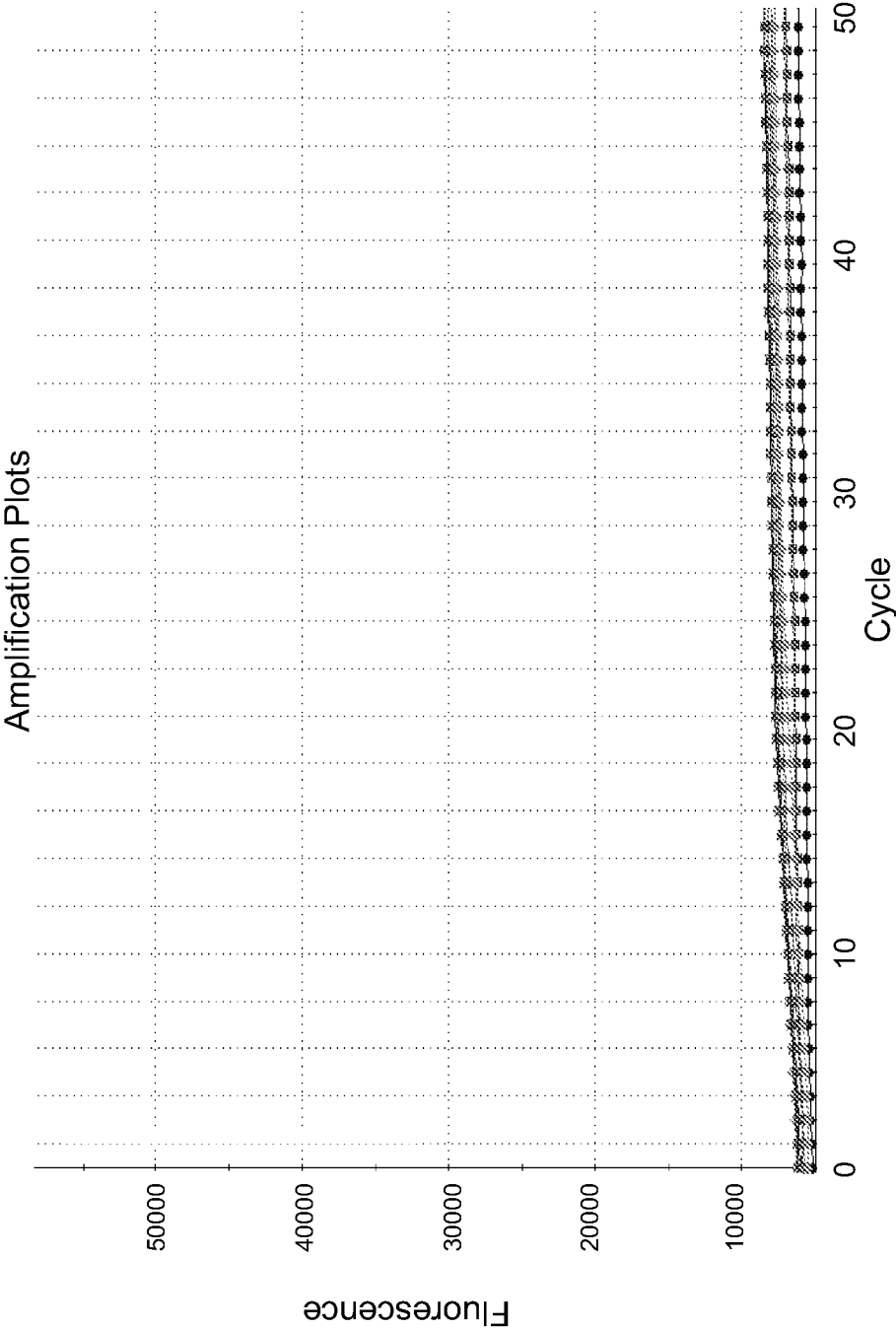


FIGURE 15D

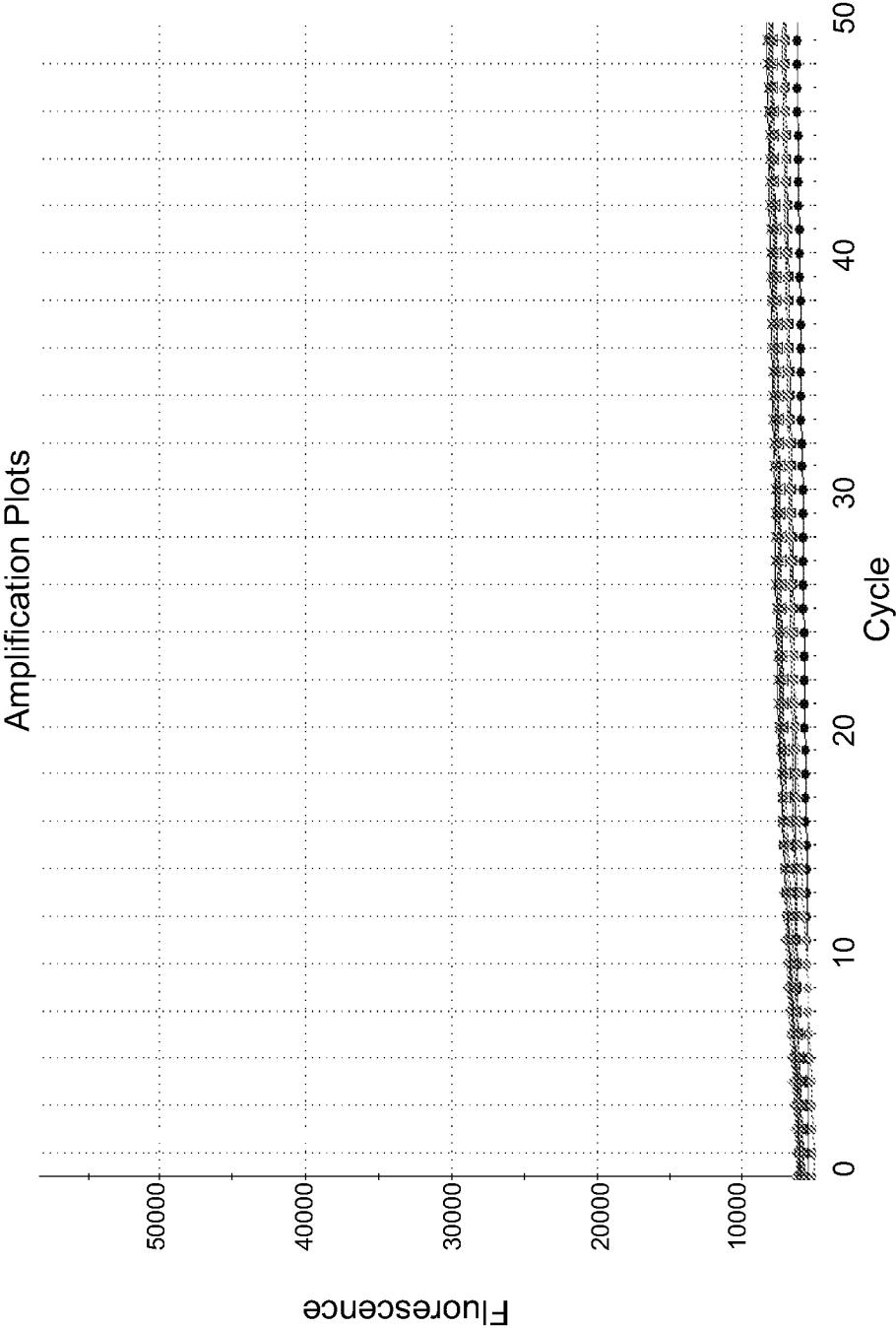


FIGURE 15E

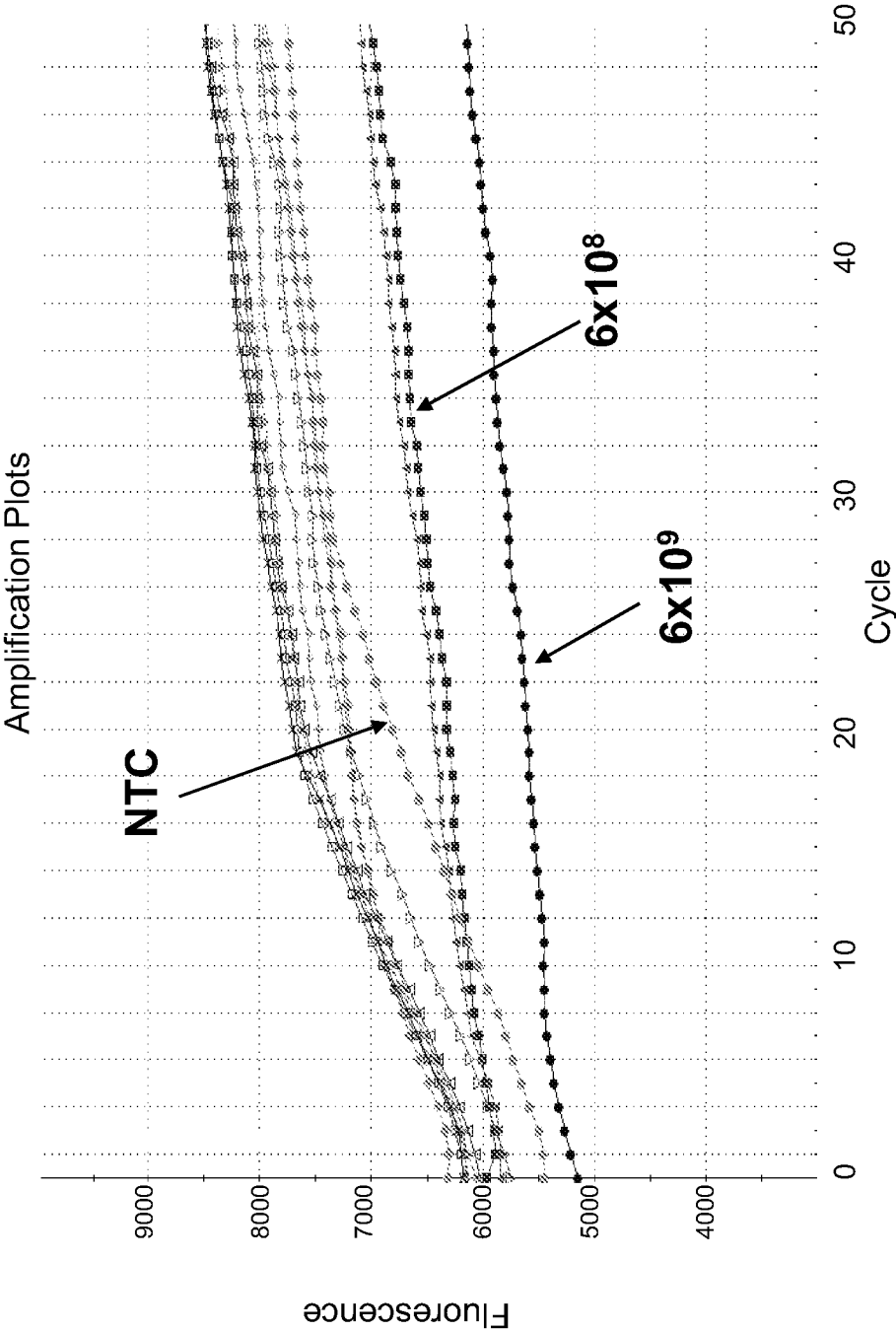


FIGURE 15F

Amplification Plots

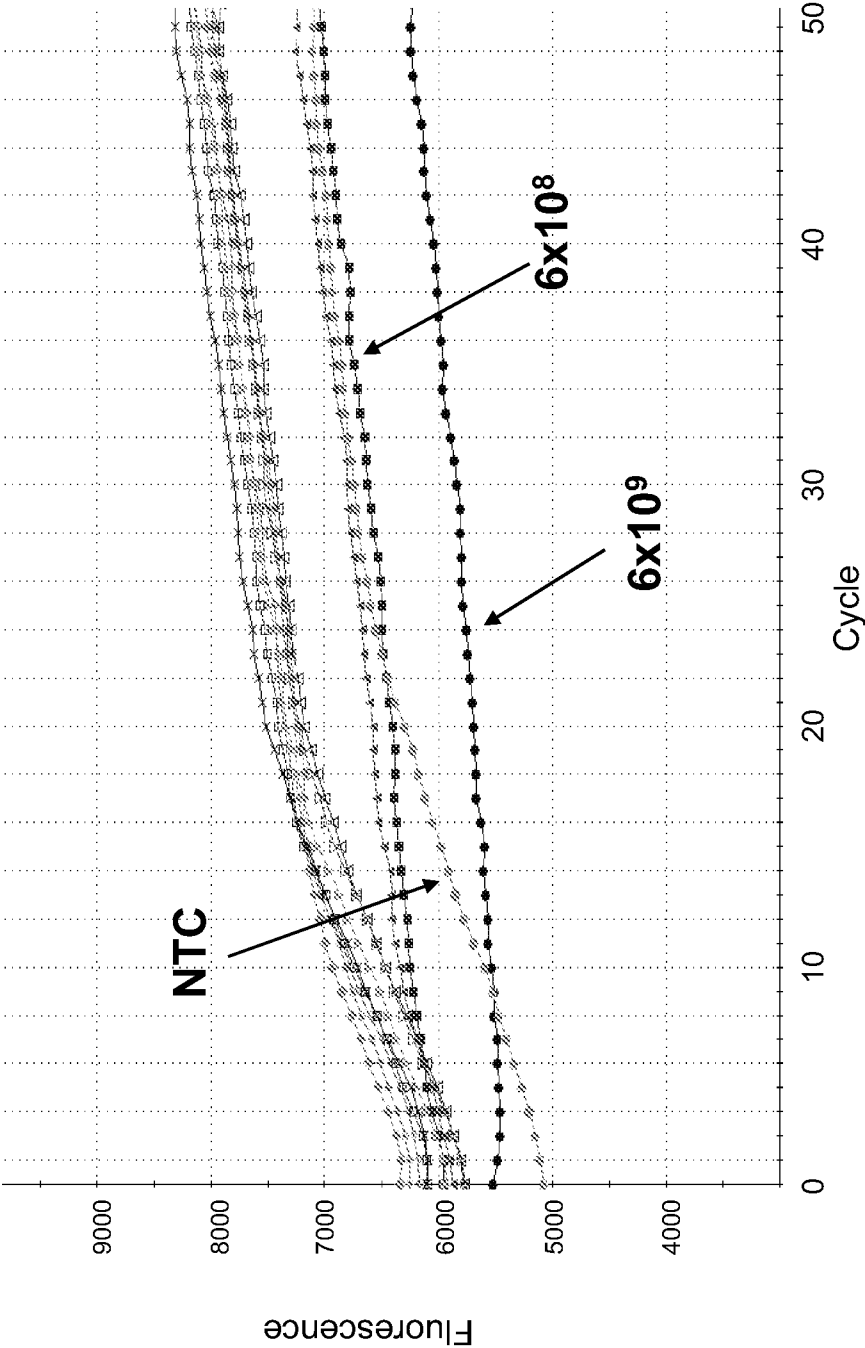


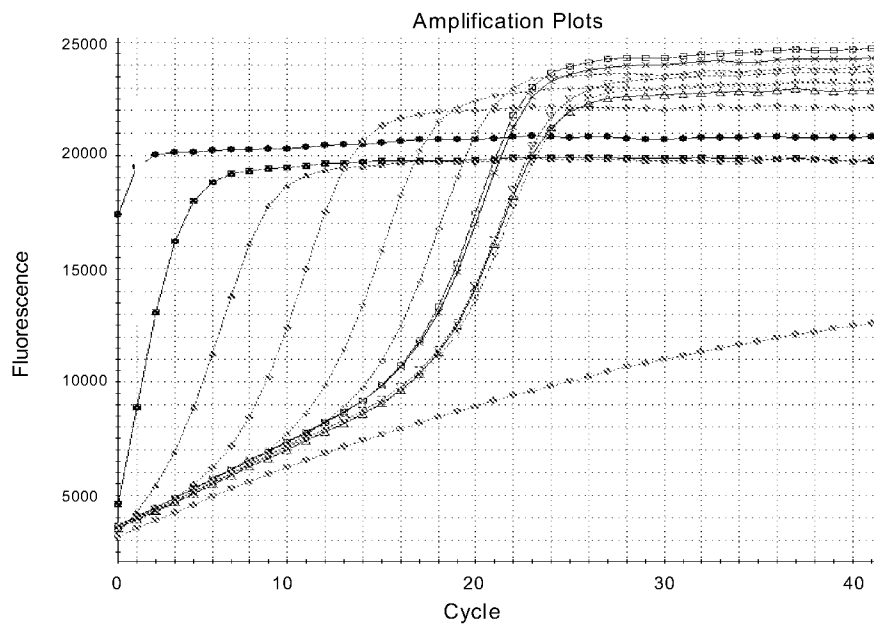
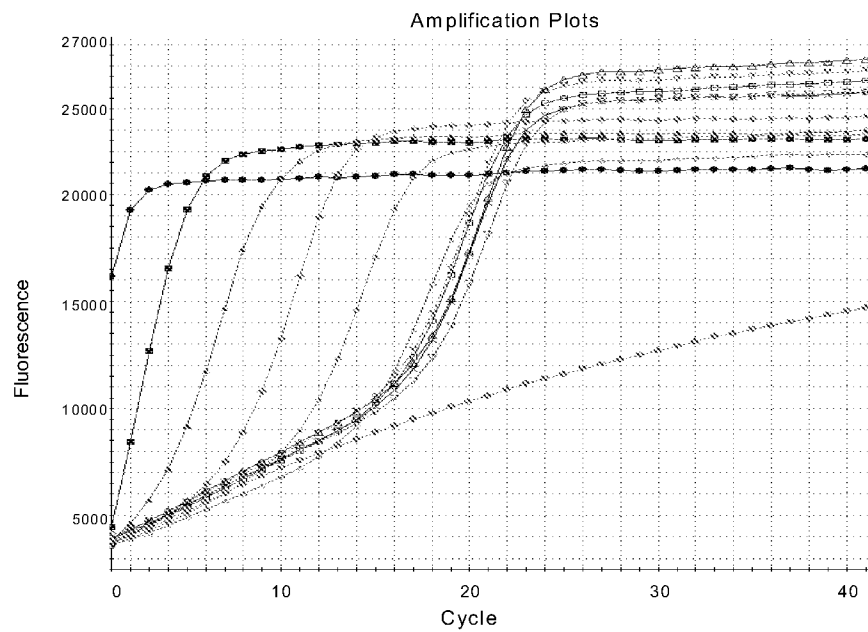
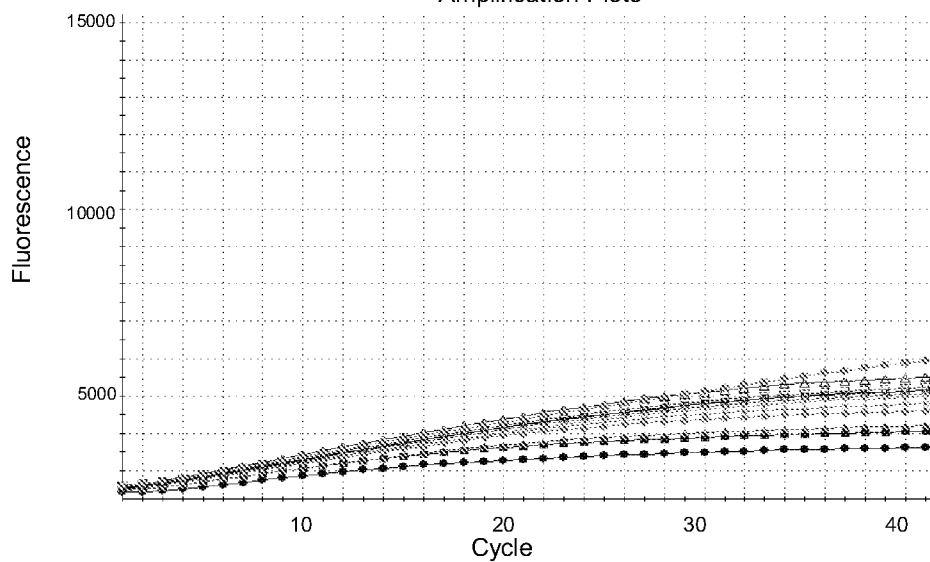
FIGURE 16 A-B**+ Invasive Oligo / + Afu FEN-1****A. 10-mer Probe; 56 degrees Celsius****B. 11-mer Probe; 56 degrees Celsius**

FIGURE 16 C-D

- Invasive Oligo / - Afu FEN-1

C. 10-mer Probe; 56 degrees Celsius

Amplification Plots



D. 11-mer Probe; 56 degrees Celsius

Amplification Plots

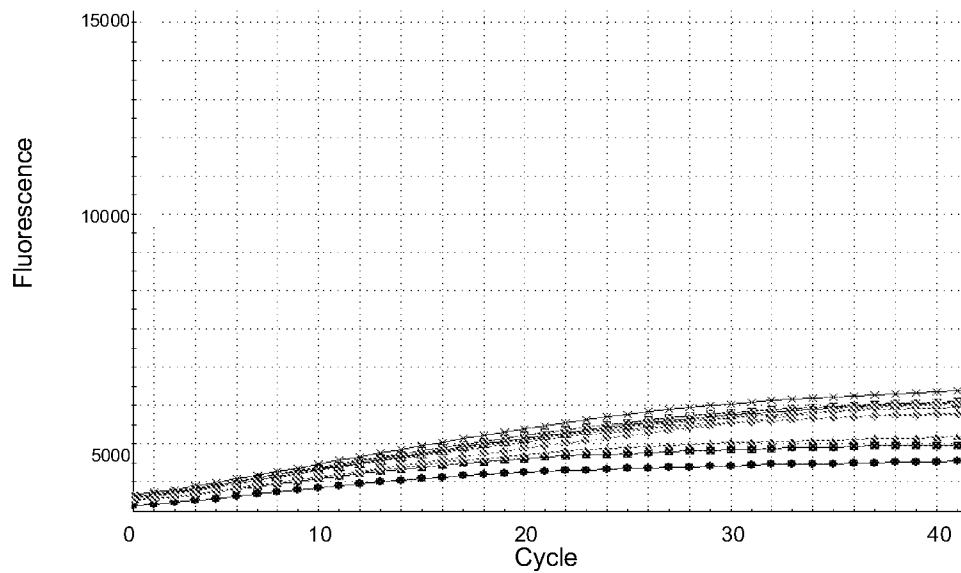
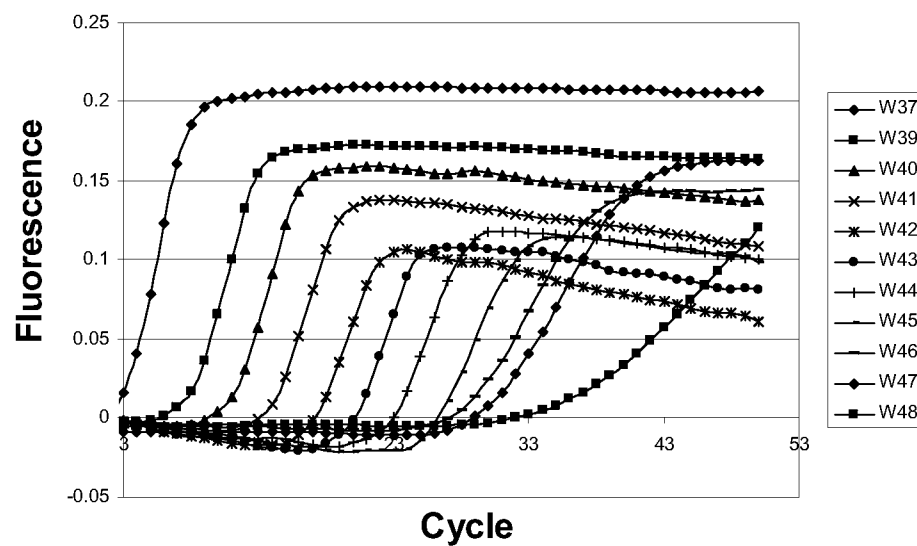


FIGURE 17 A-B

+ Invasive Oligo / + Afu FEN-1

A.



B.

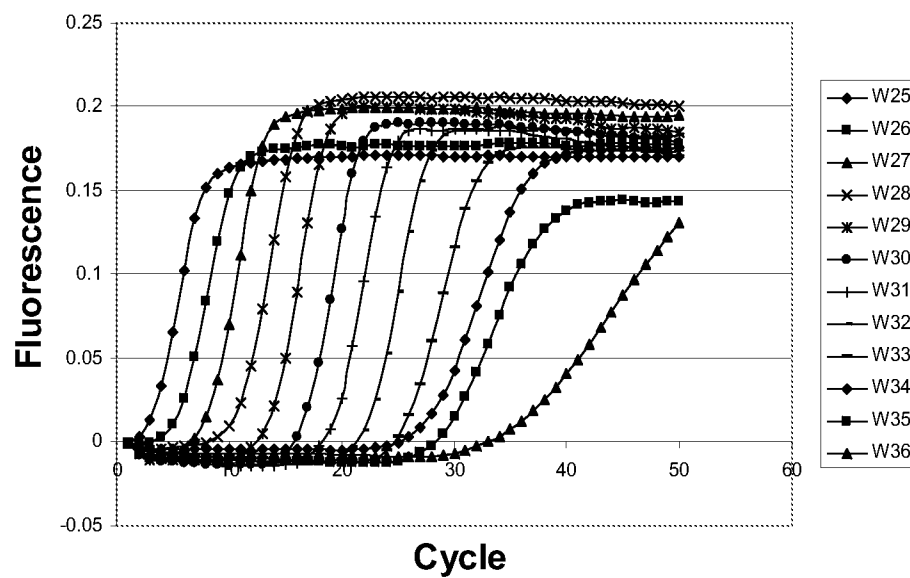
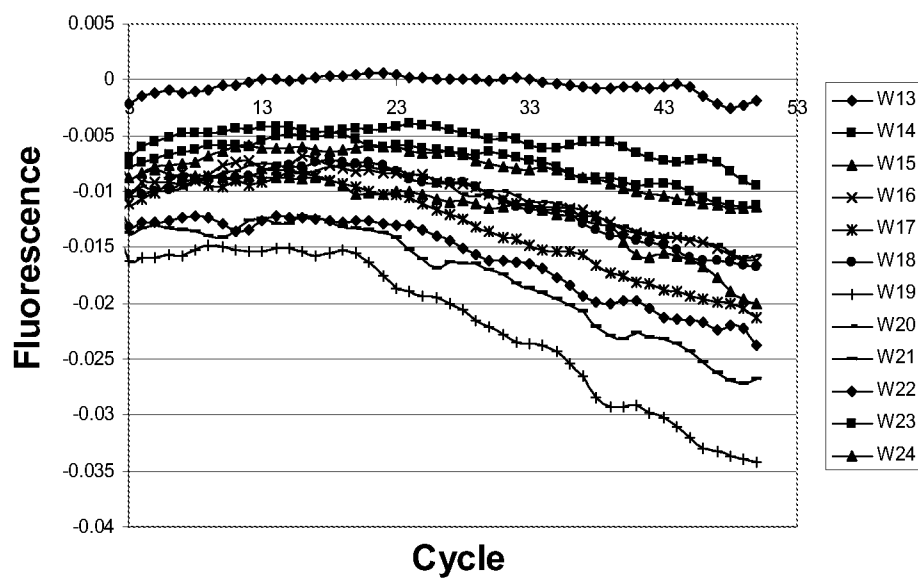


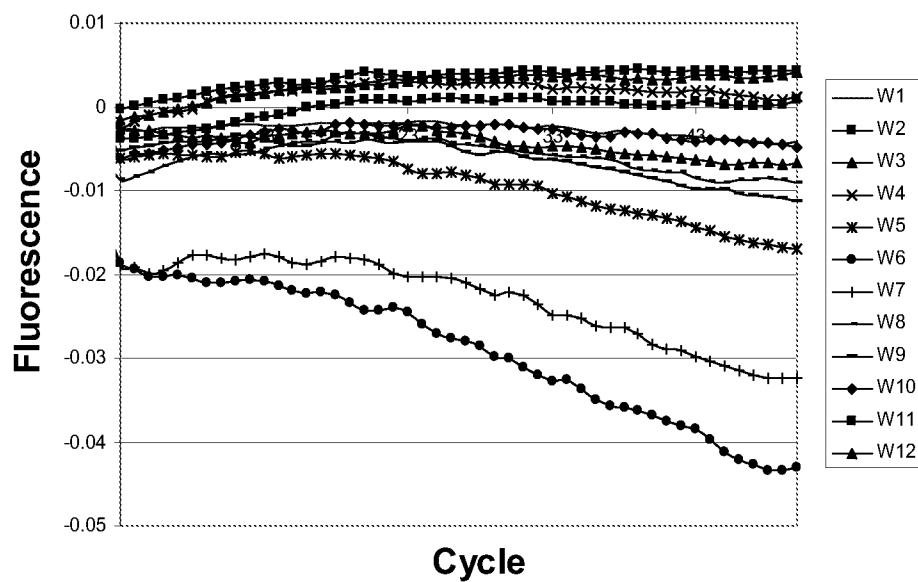
FIGURE 17 C-D

- Invasive Oligo / - Afu FEN-1

C.



D.



SEQUENCE LISTING

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 Allawi, Hatim T.
 Lyamichev, Victor I.

<120> Compositions and Methods for Analysis of Nucleic Acid Molecules
 During Amplification Reactions

<130> FORS-30277/WO-1/ORD

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 <151> 2008-03-15

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