

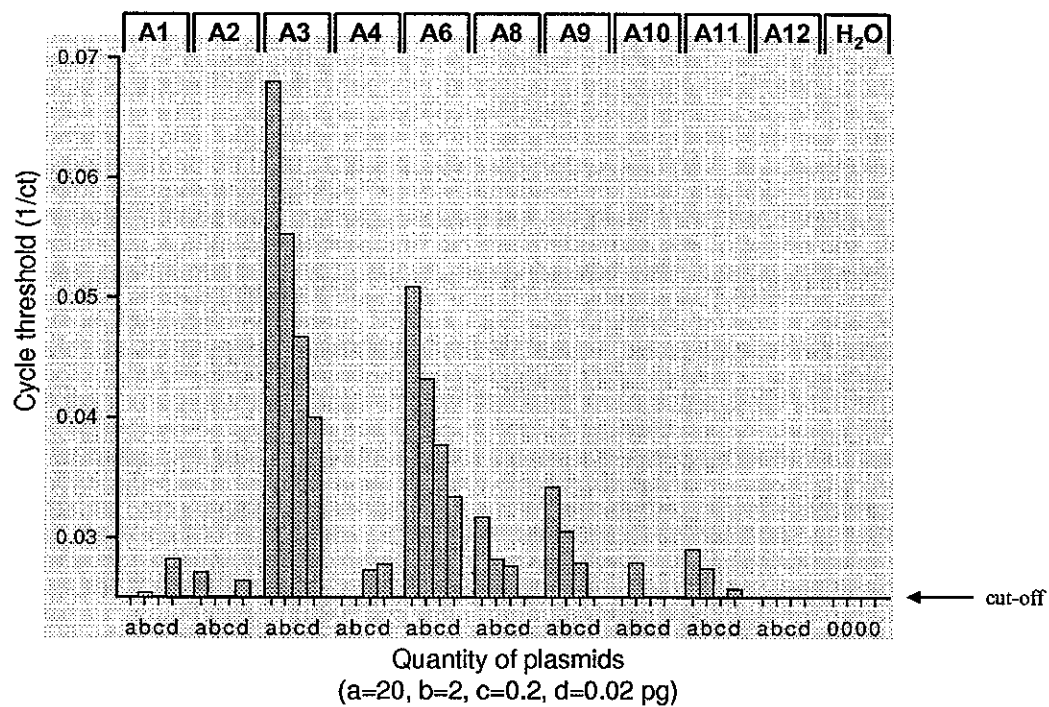
Figure 1

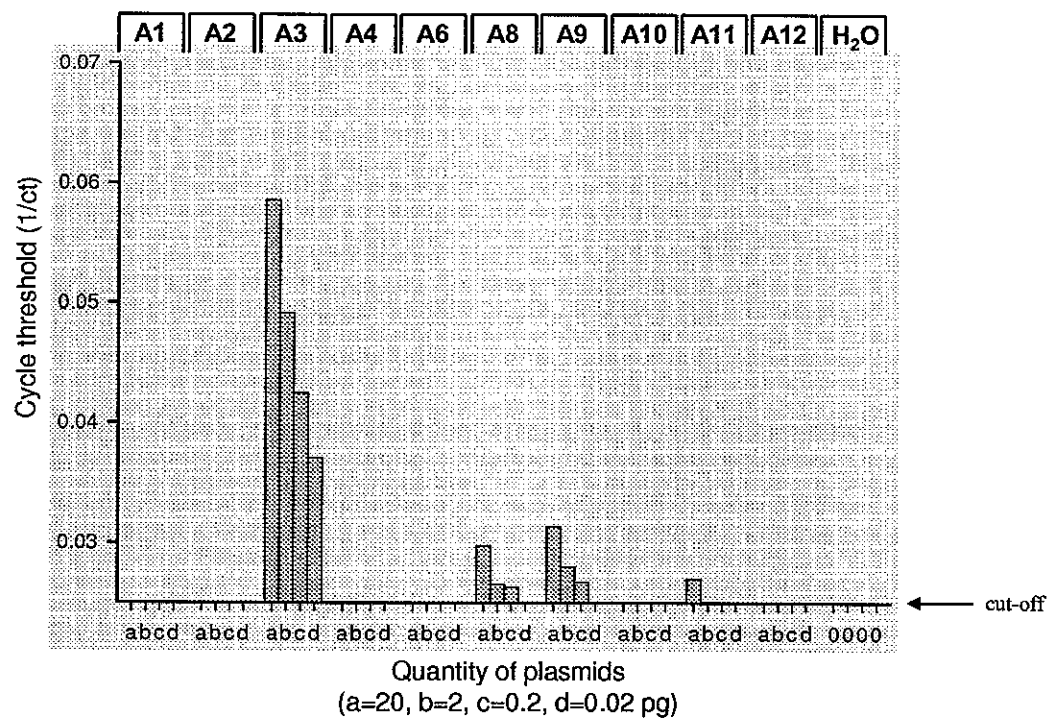
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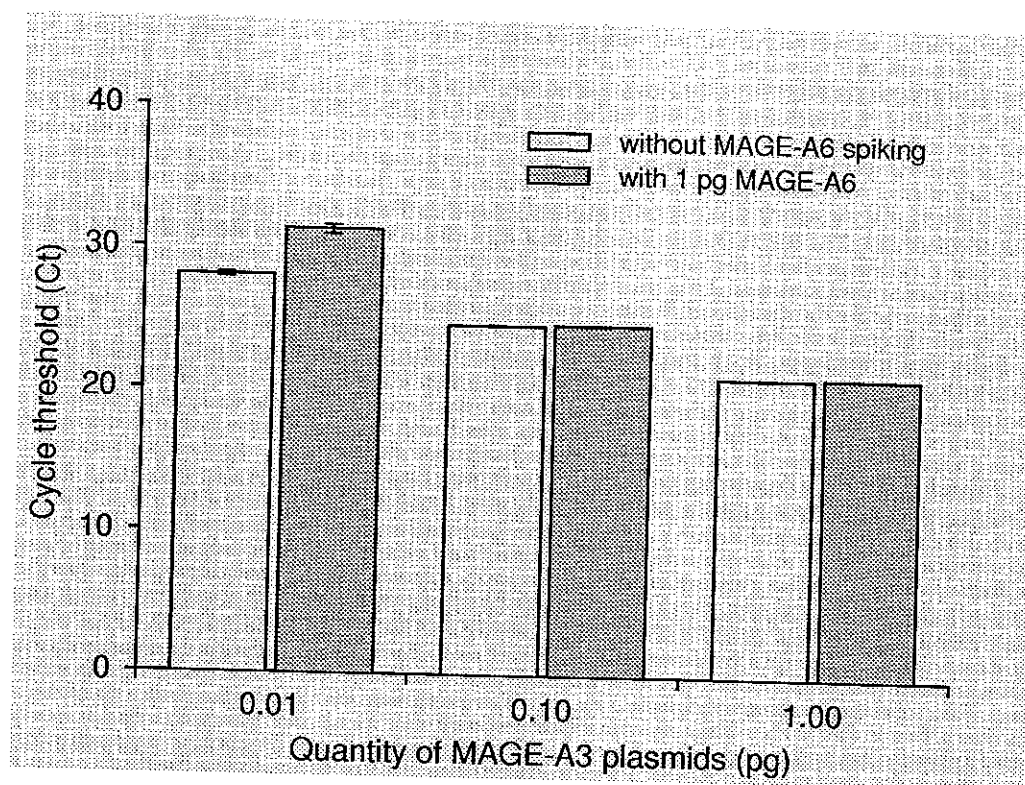
Figure 3

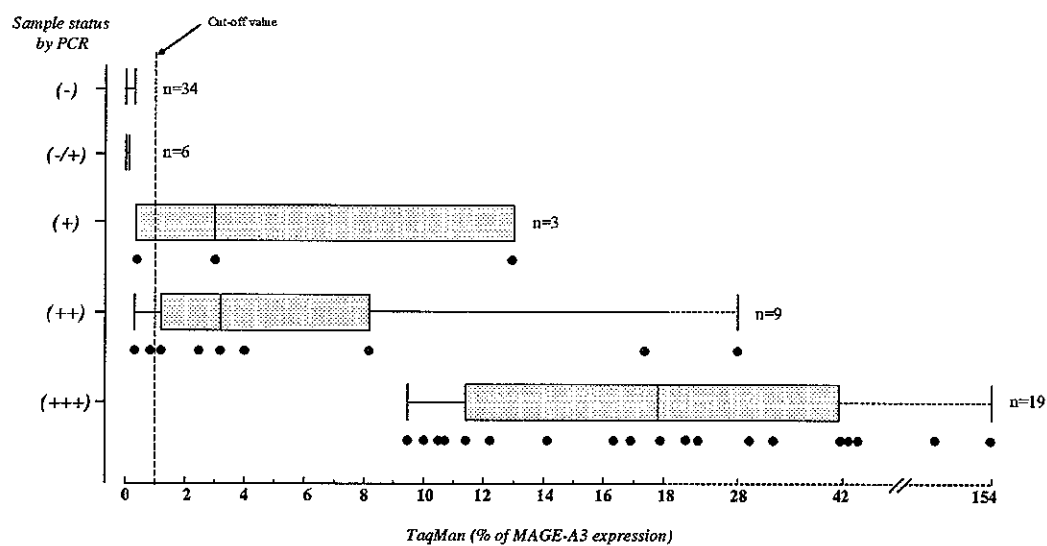
Figure 4

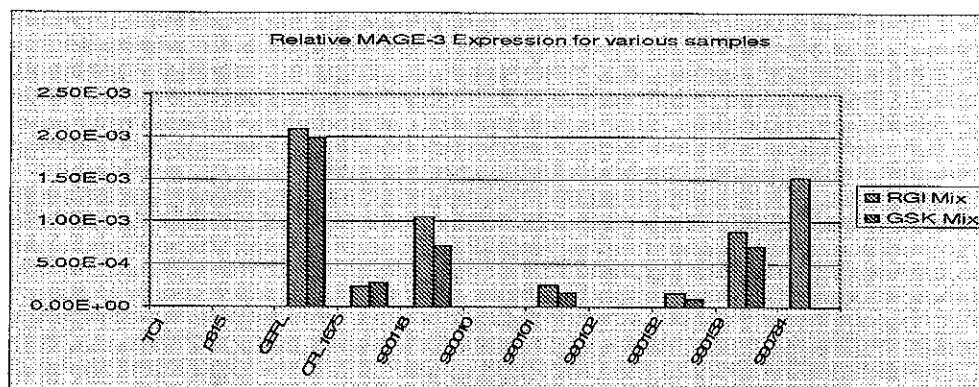
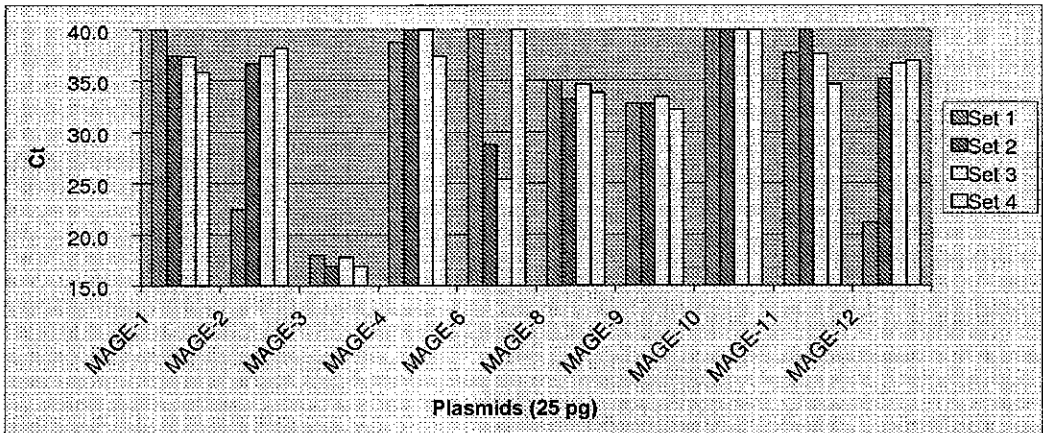
Figure 5

Figure 6a



5

Figure 6b

10

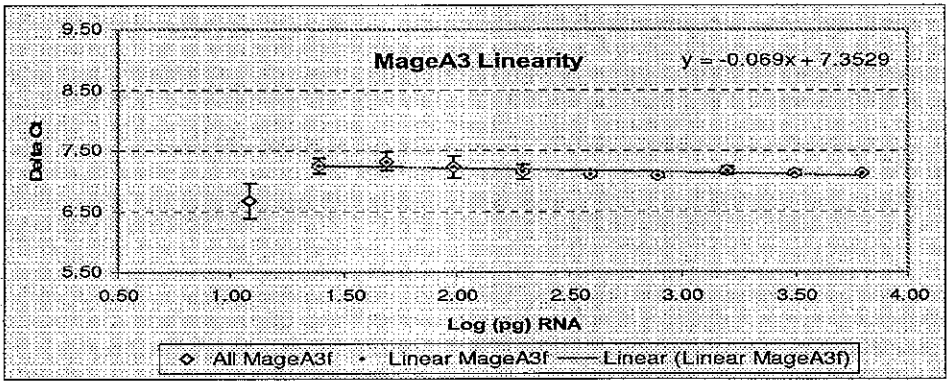


Figure 7

5

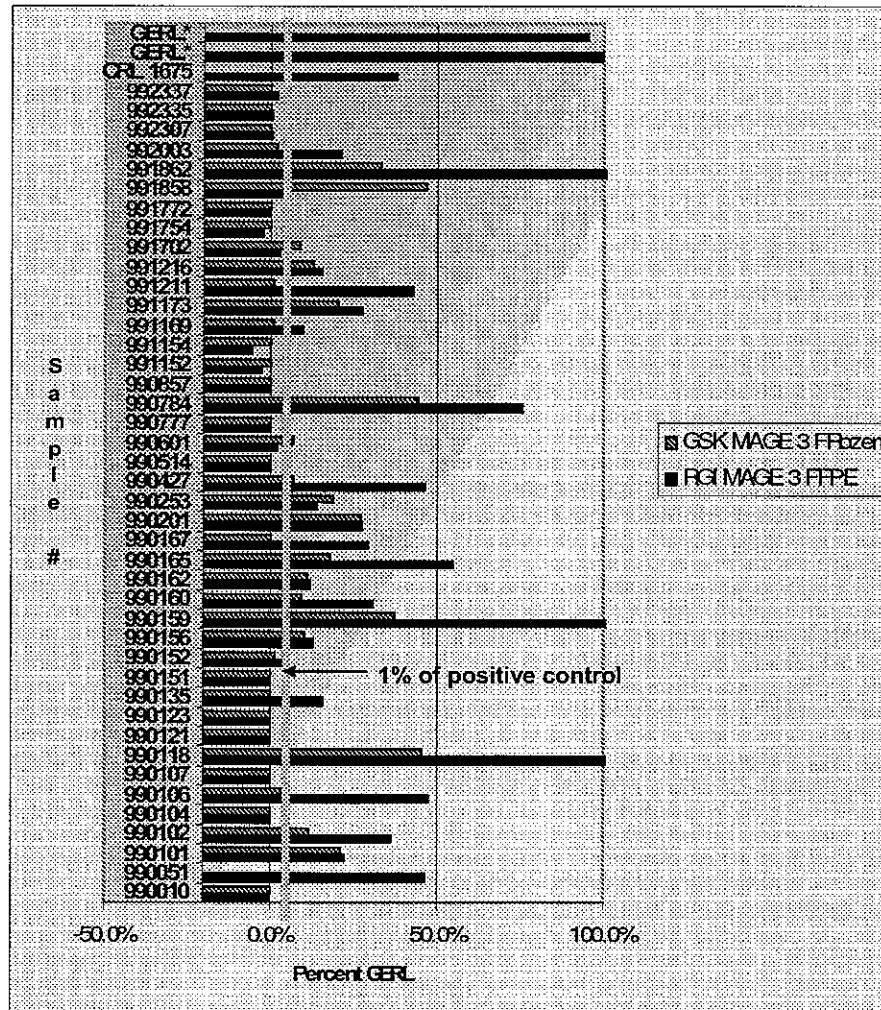


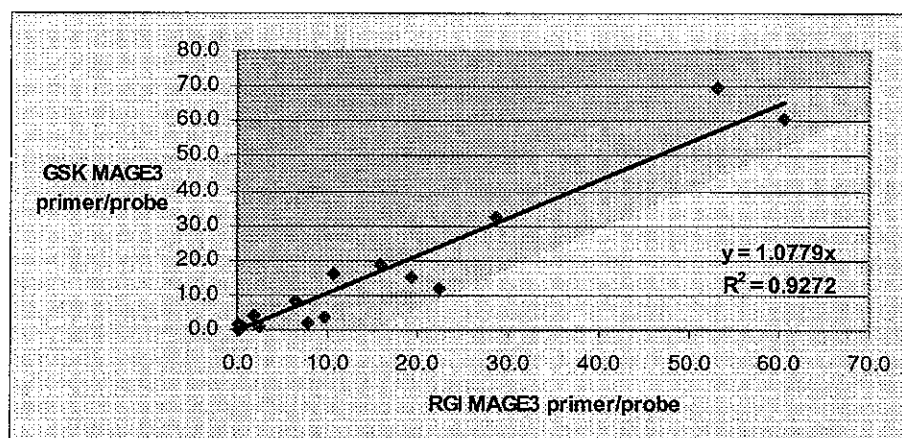
Figure 8

Figure 9

Nucleotide sequence encoding fusion protein of Protein D fragment, Mage3 fragment, and histidine tail, and its respective amino acid sequence (SEQ ID NO: 35 and 36).

5		atg gat cca aaa act tta gcc ctt tct tta tta gca gct ggc gta cta	48
		Met Asp Pro Lys Thr Leu Ala Leu Ser Leu Leu Ala Ala Gly Val Leu	
	1	5 10 15	
10		gca ggt tgt agc agc cat tca tca aat atg gcg aat acc caa atg aaa	96
		Ala Gly Cys Ser Ser His Ser Ser Asn Met Ala Asn Thr Gln Met Lys	
		20 25 30	
15		tca gac aaa atc att att gct cac cgt ggt gct agc ggt tat tta cca	144
		Ser Asp Lys Ile Ile Ile Ala His Arg Gly Ala Ser Gly Tyr Leu Pro	
		35 40 45	
20		gag cat acg tta gaa tct aaa gca ctt gcg ttt gca caa cag gct gat	192
		Glu His Thr Leu Glu Ser Lys Ala Leu Ala Phe Ala Gln Gln Ala Asp	
		50 55 60	
25		tat tta gag caa gat tta gca atg act aag gat ggt cgt tta gtg gtt	240
		Tyr Leu Glu Gln Asp Leu Ala Met Thr Lys Asp Gly Arg Leu Val Val	
		65 70 75 80	
30		att cac gat cac ttt tta gat ggc ttg act gat gtt gcg aaa aaa ttc	288
		Ile His Asp His Phe Leu Asp Gly Leu Thr Asp Val Ala Lys Lys Phe	
		85 90 95	
		cca cat cgt cat cgt aaa gat ggc cgt tac tat gtc atc gac ttt acc	336
		Pro His Arg His Arg Lys Asp Gly Arg Tyr Tyr Val Ile Asp Phe Thr	
		100 105 110	
35		tta aaa gaa att caa agt tta gaa atg aca gaa aac ttt gaa acc atg	384
		Leu Lys Glu Ile Gln Ser Leu Glu Met Thr Glu Asn Phe Glu Thr Met	
		115 120 125	
40		gat ctg gaa cag cgt agt cag cac tgc aag cct gaa gaa ggc ctt gag	432
		Asp Leu Glu Gln Arg Ser Gln His Cys Lys Pro Glu Glu Gly Leu Glu	
		130 135 140	
		gcc cga gga gag gcc ctg gcc ctg gtg ggt gcg cag gct cct gct act	480
		Ala Arg Gly Glu Ala Leu Gly Leu Val Gly Ala Gln Ala Pro Ala Thr	

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	145	150	155	160	
	gag gag cag gag gct gcc tcc tcc tct tct act cta gtt gaa gtc acc				528
	Glu Glu Gln Glu Ala Ala Ser Ser Ser Ser Thr Leu Val Glu Val Thr				
5		165	170	175	
	ctg ggg gag gtg cct gct gcc gag tca cca gat cct ccc cag agt cct				576
	Leu Gly Glu Val Pro Ala Ala Glu Ser Pro Asp Pro Pro Gln Ser Pro				
		180	185	190	
10	cag gga gcc tcc agc ctc ccc act acc atg aac tac cct ctc tgg agc				624
	Gln Gly Ala Ser Ser Leu Pro Thr Thr Met Asn Tyr Pro Leu Trp Ser				
		195	200	205	
15	caa tcc tat gag gac tcc agc aac caa gaa gag gag ggg cca agc acc				672
	Gln Ser Tyr Glu Asp Ser Ser Asn Gln Glu Glu Glu Gly Pro Ser Thr				
		210	215	220	
	ttc cct gac ctg gag tcc gag ttc caa gca gca ctc agt agg aag gtg				720
20	Phe Pro Asp Leu Glu Ser Glu Phe Gln Ala Ala Leu Ser Arg Lys Val				
		225	230	235	240
	gcc gaa ttg gtt cat ttt ctg ctc ctc aag tat cga gcc agg gag ccg				768
	Ala Glu Leu Val His Phe Leu Leu Leu Lys Tyr Arg Ala Arg Glu Pro				
25		245	250	255	
	gtc aca aag gca gaa atg ctg ggg agt gtc gtc gga aat tgg cag tat				816
	Val Thr Lys Ala Glu Met Leu Gly Ser Val Val Gly Asn Trp Gln Tyr				
		260	265	270	
30	ttc ttt cct gtg atc ttc agc aaa gct tcc agt tcc ttg cag ctg gtc				864
	Phe Phe Pro Val Ile Phe Ser Lys Ala Ser Ser Ser Leu Gln Leu Val				
		275	280	285	
35	ttt ggc atc gag ctg atg gaa gtg gac ccc atc ggc cac ttg tac atc				912
	Phe Gly Ile Glu Leu Met Glu Val Asp Pro Ile Gly His Leu Tyr Ile				
		290	295	300	
	ttt gcc acc tgc ctg ggc ctc tcc tac gat ggc ctg ctg ggt gac aat				960
40	Phe Ala Thr Cys Leu Gly Leu Ser Tyr Asp Gly Leu Leu Gly Asp Asn				
		305	310	315	320
	cag atc atg ccc aag gca ggc ctc ctg ata atc gtc ctg gcc ata atc				1008
	Gln Ile Met Pro Lys Ala Gly Leu Leu Ile Ile Val Leu Ala Ile Ile				
45		325	330	335	

	gca aga gag ggc gac tgt gcc cct gag gag aaa atc tgg gag gag ctg	1056
	Ala Arg Glu Gly Asp Cys Ala Pro Glu Glu Lys Ile Trp Glu Glu Leu	
	340 345 350	
5	agt gtg tta gag gtg ttt gag ggg agg gaa gac agt atc ttg ggg gat	1104
	Ser Val Leu Glu Val Phe Glu Gly Arg Glu Asp Ser Ile Leu Gly Asp	
	355 360 365	
10	ccc aag aag ctg ctc acc caa cat ttc gtg cag gaa aac tac ctg gag	1152
	Pro Lys Lys Leu Leu Thr Gln His Phe Val Gln Glu Asn Tyr Leu Glu	
	370 375 380	
	tac cgg cag gtc ccc ggc agt gat cct gca tgt tat gaa ttc ctg tgg	1200
15	Tyr Arg Gln Val Pro Gly Ser Asp Pro Ala Cys Tyr Glu Phe Leu Trp	
	385 390 395 400	
	ggt cca agg gcc ctc gtt gaa acc agc tat gtg aaa gtc ctg cac cat	1248
	Gly Pro Arg Ala Leu Val Glu Thr Ser Tyr Val Lys Val Leu His His	
20	405 410 415	
	atg gta aag atc agt gga gga cct cac att tcc tac cca ccc ctg cat	1296
	Met Val Lys Ile Ser Gly Gly Pro His Ile Ser Tyr Pro Pro Leu His	
	420 425 430	
25	gag tgg gtt ttg aga gag ggg gaa gag ggc ggt cat cac cat cac cat	1344
	Glu Trp Val Leu Arg Glu Gly Glu Glu Gly Gly His His His His	
	435 440 445	
30	cac cat taa	1353
	His His	
	450	

Figure 10

Fusion protein of NS1-MAGE3, and Histidine tail (SEQ ID NO:37)

5

Met Asp Pro Asn Thr Val Ser Ser Phe Gln Val Asp Cys Phe Leu Trp
 1 5 10 15

10 His Val Arg Lys Arg Val Ala Asp Gln Glu Leu Gly Asp Ala Pro Phe
 20 25 30

Leu Asp Arg Leu Arg Arg Asp Gln Lys Ser Leu Arg Gly Arg Gly Ser
 35 40 45

15 Thr Leu Gly Leu Asp Ile Glu Thr Ala Thr Arg Ala Gly Lys Gln Ile
 50 55 60

Val Glu Arg Ile Leu Lys Glu Glu Ser Asp Glu Ala Leu Lys Met Thr
 65 70 75 80

Met Asp Leu Glu Gln Arg Ser Gln His Cys Lys Pro Glu Glu Gly Leu
 85 90 95

20 Glu Ala Arg Gly Glu Ala Leu Gly Leu Val Gly Ala Gln Ala Pro Ala
 100 105 110

Thr Glu Glu Gln Glu Ala Ala Ser Ser Ser Ser Thr Leu Val Glu Val
 115 120 125

25 Thr Leu Gly Glu Val Pro Ala Ala Glu Ser Pro Asp Pro Pro Gln Ser
 130 135 140

Pro Gln Gly Ala Ser Ser Leu Pro Thr Thr Met Asn Tyr Pro Leu Trp
 145 150 155 160

Ser Gln Ser Tyr Glu Asp Ser Ser Asn Gln Glu Glu Gly Pro Ser
 165 170 175

30 Thr Phe Pro Asp Leu Glu Ser Glu Phe Gln Ala Ala Leu Ser Arg Lys
 180 185 190

Val Ala Glu Leu Val His Phe Leu Leu Leu Lys Tyr Arg Ala Arg Glu
 195 200 205

35 Pro Val Thr Lys Ala Glu Met Leu Gly Ser Val Val Gly Asn Trp Gln
 210 215 220

Tyr Phe Phe Pro Val Ile Phe Ser Lys Ala Ser Ser Ser Leu Gln Leu
 225 230 235 240

Val Phe Gly Ile Glu Leu Met Glu Val Asp Pro Ile Gly His Leu Tyr
 245 250 255

40 Ile Phe Ala Thr Cys Leu Gly Leu Ser Tyr Asp Gly Leu Leu Gly Asp
 260 265 270

Asn Gln Ile Met Pro Lys Ala Gly Leu Leu Ile Ile Val Leu Ala Ile
 275 280 285

45 Ile Ala Arg Glu Gly Asp Cys Ala Pro Glu Glu Lys Ile Trp Glu Glu
 290 295 300

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Leu Ser Val Leu Glu Val Phe Glu Gly Arg Glu Asp Ser Ile Leu Gly
305 310 315 320
Asp Pro Lys Lys Leu Leu Thr Gln His Phe Val Gln Glu Asn Tyr Leu
325 330 335
5 Glu Tyr Arg Gln Val Pro Gly Ser Asp Pro Ala Cys Tyr Glu Phe Leu
340 345 350
Trp Gly Pro Arg Ala Leu Val Glu Thr Ser Tyr Val Lys Val Leu His
355 360 365
His Met Val Lys Ile Ser Gly Gly Pro His Ile Ser Tyr Pro Pro Leu
10 370 375 380
His Glu Trp Val Leu Arg Glu Gly Glu Glu Gly Gly His His His His
385 390 395 400
His His His

15

Figure 11

DNA coding fusion protein NS1-MAGE3-His (SEQ ID NO:38)

	atggatccaa acactgtgtc aagctttcag gtagattgct ttcttttgca tgtccgcaaa	60
5	cgagttgcag accaagaact aggtgatgcc ccattccttg atcggcttcg ccgagatcag	120
	aaatccctaa gaggaagggg cagcactctt ggtctggaca tcgagacagc cacacgtgct	180
	ggaaagcaga tagtggagcg gattctgaaa gaagaatccg atgaggcact taaaatgacc	240
	atggatctcg aacagcgtag tcagcactgc aagcctgaag aaggccttga ggcccagga	300
	gaggccctgg gcctggtggg tgcgcaggct cctgctactg aggagcagga ggctgcctcc	360
10	tcctcttcta ctctagttga agtcaccctg ggggaggtgc ctgctgccga gtcaccagat	420
	cctccccaga gtccctcaggg agcctccage ctcccacta ccatgaacta ccctctctgg	480
	agccaatcct atgaggactc cagcaaccaa gaagaggagg ggccaagcac cttccctgac	540
	ctggagtcgg agttccaagc agcactcagt aggaaggtgg ccgaattggt tcattttctg	600
	ctcctcaagt atcgagccag ggagccggtc acaaaggcag aaatgctggg gagtgtcgtc	660
15	ggaaattggc agtatttctt tcctgtgatc ttcagcaaag ctccagttc cttgcagctg	720
	gtcttttgca tcgagctgat ggaagtggac cccatcggcc acttgtacat ctttgccacc	780
	tgcctgggccc tctcctacga tggcctgctg ggtgacaatc agatcatgcc caaggcaggc	840
	ctcctgataa tcgtcctggc cataatcgca agagagggcg actgtgcccc tgaggagaaa	900
	atctgggagg agctgagtggt gttagaggtg tttagagggga gggaagacag tatcttgggg	960
20	gatcccaaga agctgctcac ccaacatttc gtgcaggaaa actacctgga gtaccggcag	1020
	gtccccggca gtgatcctgc atgttatgaa ttctgtggg gtccaagggc cctcgttgaa	1080
	accagctatg tgaaagtect gcaccatatg gtaaagatca gtggaggacc tcacatttcc	1140
	taccaccccc tgcattgagtg ggttttgaga gagggggaag agggcggtca tcaccatcac	1200
25	catcaccatt aa	1212

Figure 12

Fusion protein of CLYTA-MAGE3-Histidine (SEQ ID NO:39)

```

5  Met Lys Gly Gly Ile Val His Ser Asp Gly Ser Tyr Pro Lys Asp Lys
   1             5             10             15
   Phe Glu Lys Ile Asn Gly Thr Trp Tyr Tyr Phe Asp Ser Ser Gly Tyr
      20             25             30
   Met Leu Ala Asp Arg Trp Arg Lys His Thr Asp Gly Asn Trp Tyr Trp
10      35             40             45
   Phe Asp Asn Ser Gly Glu Met Ala Thr Gly Trp Lys Lys Ile Ala Asp
      50             55             60
   Lys Trp Tyr Tyr Phe Asn Glu Glu Gly Ala Met Lys Thr Gly Trp Val
   65             70             75             80
15  Lys Tyr Lys Asp Thr Trp Tyr Tyr Leu Asp Ala Lys Glu Gly Ala Met
      85             90             95
   Val Ser Asn Ala Phe Ile Gln Ser Ala Asp Gly Thr Gly Trp Tyr Tyr
      100            105            110
   Leu Lys Pro Asp Gly Thr Leu Ala Asp Arg Pro Glu Leu Ala Ser Met
20      115            120            125
   Leu Asp Met Asp Leu Glu Gln Arg Ser Gln His Cys Lys Pro Glu Glu
      130            135            140
   Gly Leu Glu Ala Arg Gly Glu Ala Leu Gly Leu Val Gly Ala Gln Ala
   145            150            155            160
25  Pro Ala Thr Glu Glu Gln Glu Ala Ala Ser Ser Ser Ser Thr Leu Val
      165            170            175
   Glu Val Thr Leu Gly Glu Val Pro Ala Ala Glu Ser Pro Asp Pro Pro
      180            185            190
   Gln Ser Pro Gln Gly Ala Ser Ser Leu Pro Thr Thr Met Asn Tyr Pro
30      195            200            205
   Leu Trp Ser Gln Ser Tyr Glu Asp Ser Ser Asn Gln Glu Glu Glu Gly
      210            215            220
   Pro Ser Thr Phe Pro Asp Leu Glu Ser Glu Phe Gln Ala Ala Leu Ser
   225            230            235            240
35  Arg Lys Val Ala Glu Leu Val His Phe Leu Leu Leu Lys Tyr Arg Ala
      245            250            255
   Arg Glu Pro Val Thr Lys Ala Glu Met Leu Gly Ser Val Val Gly Asn
      260            265            270
   Trp Gln Tyr Phe Phe Pro Val Ile Phe Ser Lys Ala Ser Ser Ser Leu
40      275            280            285
   Gln Leu Val Phe Gly Ile Glu Leu Met Glu Val Asp Pro Ile Gly His
      290            295            300
   Leu Tyr Ile Phe Ala Thr Cys Leu Gly Leu Ser Tyr Asp Gly Leu Leu
   305            310            315            320
45  Gly Asp Asn Gln Ile Met Pro Lys Ala Gly Leu Leu Ile Ile Val Leu

```

[illegible]

Figure 13

DNA for fusion protein CLYTA-MAGE3-His (SEQ ID NO:40)

	atgaaagggg gaattgtaca ttcagacggc ttttatccaa aagacaagtt tgagaaaatc	60
5	aatggcactt ggtactactt tgacagttca ggctatatgc ttgcagaccg ctggagggaag	120
	cacacagacg gcaactggta ctggttcgac aactcaggcg aaatggctac aggctggaag	180
	aaaatcgctg ataagtggta ctatttcaac gaagaagggtg ccatgaagac aggctgggtc	240
	aagtacaagg acacttggta ctacttagac gctaaagaag gcgccatggt atcaaatgcc	300
	tttatccagt cagcggacgg aacaggctgg tactacctca aaccagacgg aacactggca	360
10	gacaggccag aattggccag catgctggac atggatctgg aacagcgtag tcagcactgc	420
	aagcctgaag aagccttga ggcccagga gaggccctgg gcctggtggg tgcgcaggct	480
	cctgctactg aggagcagga ggctgcctcc tctcttcta ctctagtga agtcaccctg	540
	ggggagggtg ctgctgccga gtcaccagat cctccccaga gtcctcaggg agcctccagc	600
	ctccccacta ccatgaacta cctctctctg agccaatcct atgaggactc cagcaaccaa	660
15	gaagaggagg ggccaagcac cttccctgac ctggagtctg agttccaagc agcactcagt	720
	aggaagggtg ccaagtttgt tcatcttctg ctctcaagt atcgagccag ggagccggtc	780
	acaaaggcag aaatgctggg gagtgtctgc ggaaattggc agtacttctt tctgtgatc	840
	ttcagcaaa cttccgattc cttgcagctg gtctttggca tcgagctgat ggaagtggac	900
	cccacgggc acgtgtacat ctttgccacc tgctggggc tctctacga tggcctgctg	960
20	ggtgacaatc agatcatgcc caagacaggc ttcttgataa tcctcctggc cataatcgca	1020
	aaagagggcg actgtgcccc tgaggagaaa atctgggagg agctgagtgt gttagagggtg	1080
	tttgagggga gggaagacag tatcttcggg gatcccaaga agctgctcac ccaatatttc	1140
	gtgcaggaaa actacctgga gtaccggcag gtccccggca gtgatcctgc atgctatgag	1200
	ttcctgtggg gtccaagggc cctcattgaa accagctatg tgaaagtcct gcaccatattg	1260
25	gtaaagatca gtggaggacc tcgcatttcc taccactcc tgcatgagtg ggctttgaga	1320
	gagggggaag agggcgggtc tcaccatcac catcaccatt aa	1362

Figure 15

Comparison of MAGE-A3 (SEQ ID NO:51) and MAGE-A6 (SEQ ID NO:52)

MAGE-A3 ccaagcagcactcagt aggaaggtggccgagttggttcattttctgctcctcaagtatcg
 MAGE-A6 ccaagcagcactcagt aggaaggtggccagttggttcattttctgctcctcaagtatcg
 MAGEA3I-623F E3 MAGE3 TMF
 MAGE-A3 agccaggagccgggtcacaaaggcagaaatgctggggaggtcgtcgcggaattggcagta
 MAGE-A6 agccaggagccgggtcacaaaggcagaaatgctggggaggtcgtcgcggaattggcagta
 MAGEA3I-651Tm E3 MAGE3 TMP MAGEA3I-697R
 MAGE-A3 tctctttcctgtgatcttcagcaaaacttcagttcctgcagctggtctttgcatcga
 MAGE-A6 tctctttcctgtgatcttcagcaaaacttcagttcctgcagctggtctttgcatcga
 E3 MAGE3 TMR
 MAGE-A3 gctgatggaagtggaccccatcgccacttgtacatctttgccacctgcctgggacctc
 MAGE-A6 gctgatggaagtggaccccatcgccacttgtacatctttgccacctgcctgggacctc
 MAGEA3-775F MAGEA3-801TMCF
 MAGE-A3 ctacgatggcctgctgggtgacaatcagatcatgccaaaggcagcctcctgataatcgt
 MAGE-A6 ctacgatggcctgctgggtgacaatcagatcatgccaaaggcagcctcctgataatcgt
 MAGEA3-849R
 MAGE-A3 cctgggcataatcgcaagagagggccactgtgccctgaggagaaaatctgggaggagct
 MAGE-A6 cctgggcatatcgcaaaagagggcgactgtgccctgaggagaaaatctgggaggagct
 MAGE-A3 gagtgtgttagaggtgtttgaggggaggggaagacagtatcttggggatcccaagaagct
 MAGE-A6 gagtgtgttagaggtgtttgaggggaggggaagacagtatcttggggatcccaagaagct
 MAGEA3e-950F
 MAGE-A3 gctcagccaacatttcgtgcaggaaactacctggagtaccggcaggtccccggcagtga
 MAGE-A6 gctcacccaatatttcgtgcaggaaactacctggagtaccggcaggtccccggcagtga
 MAGEA3e-1000Tmc MAGEA3e-1037R
 MAGE-A3 tcttgcattgttatgaacttctgtgggtccaaggccctcgttgaaccagctatgtgaa
 MAGE-A6 tcttgcattgttatgaacttctgtgggtccaaggccctcgttgaaccagctatgtgaa
 MAGE-A3 agtcctgcaccatattggtaaagatcagtgaggacctcacatttctacccacctgca
 MAGE-A6 agtcctgcaccatattggtaaagatcagtgaggacctcacatttctacccacctgca

Figure 16

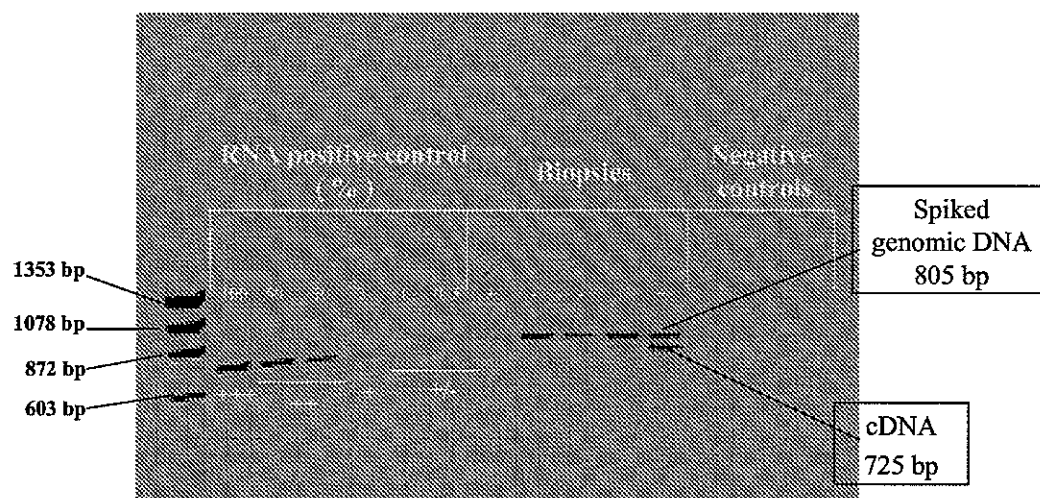


Figure 17: Graphical Comparison of Ct values from MAGE-A Plasmids using
20, 2, 0.2 0.02 pg of DNA

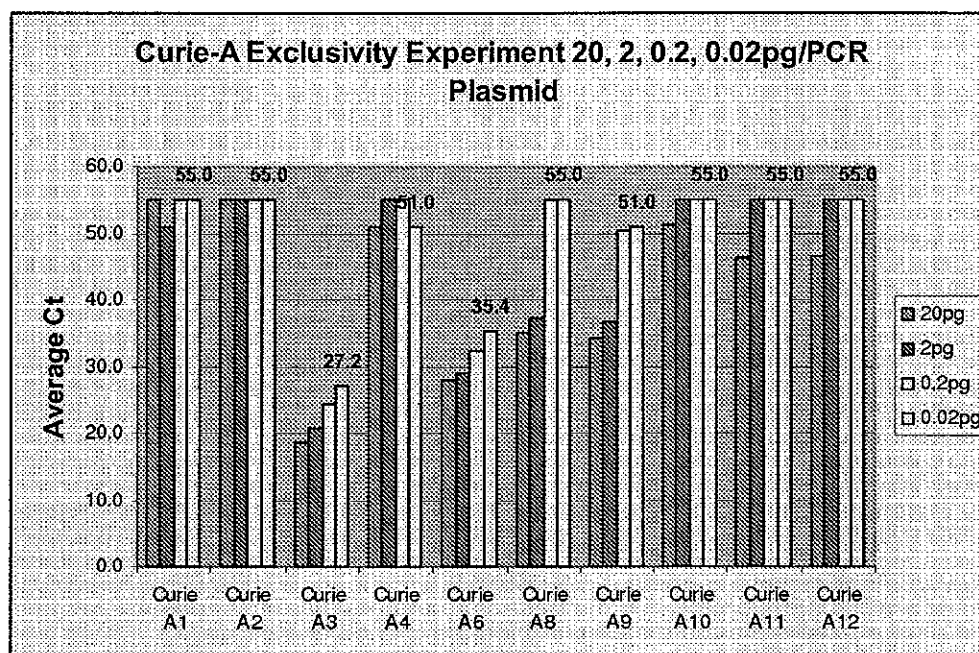


Figure 18: Graphical Comparison of Delta Ct values Relative to MAGE-A3 for other MAGE-A Plasmids using 20, 2, 0.2 0.02 pg of DNA

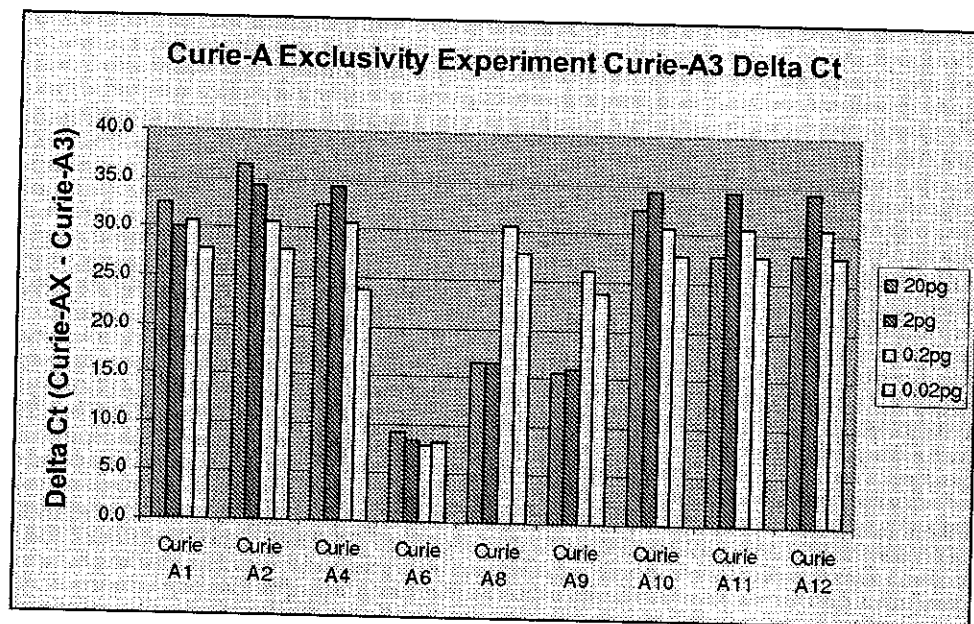


Figure 19: Linearity of FFPE Xenograft GERL RNA - 2- fold serial dilutions from 100 to 0.1 ng of RNA Input

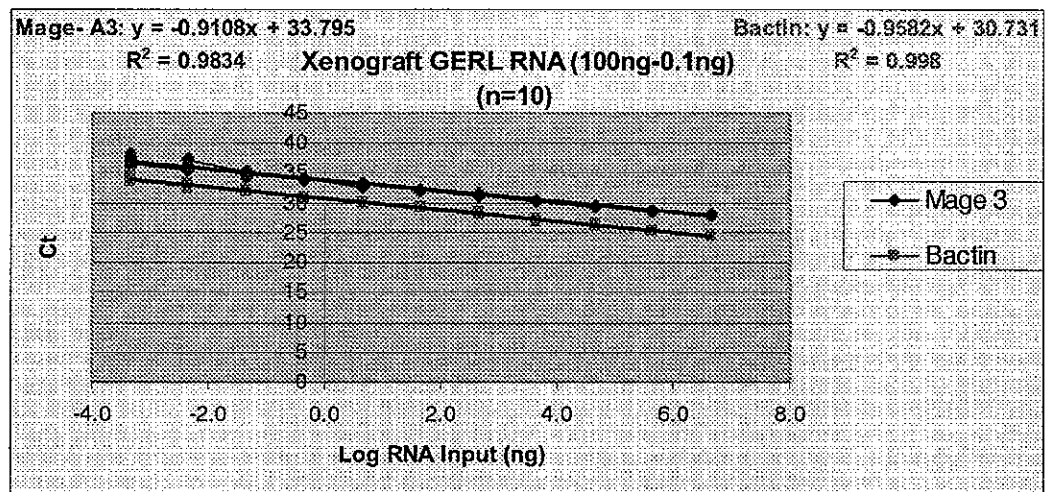


Figure 20: Delta Ct vs. Log (Base 2) RNA input for FFPE Xenograft GERL RNA

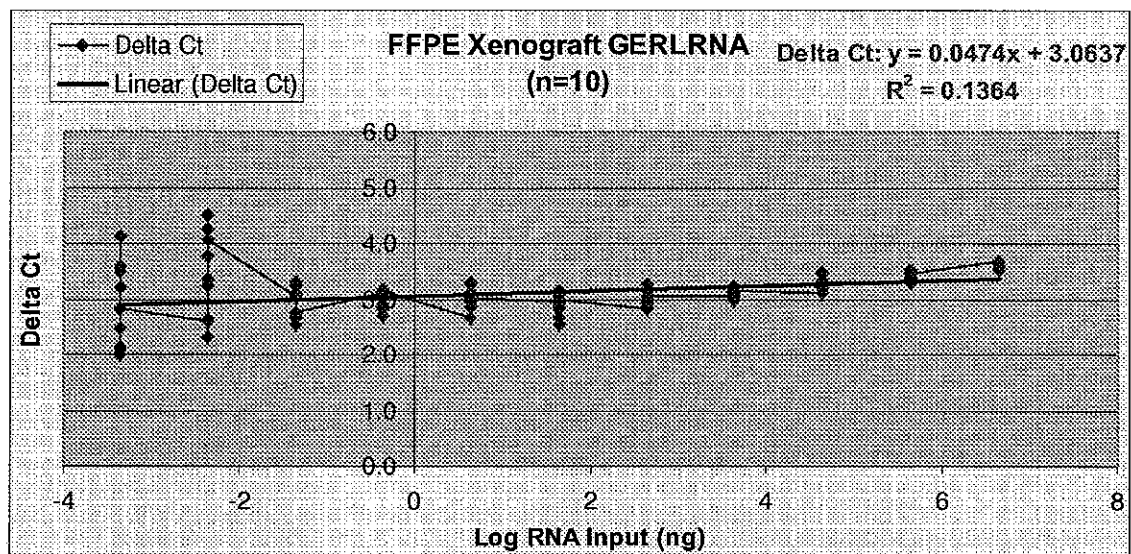


Table 1. Oligonucleotide primers used for semi-quantitative MAGE RT-PCR

SEQ ID NOs	Name	Sequence	Specificity	Amplicon	
				gDNA	mRNA
SEQ ID NO: 1	MAGE3-E2F	TGGAGGACCAGAGGCCCCC	MAGE-A3	805 bp	725 bp
SEQ ID NO: 2	MAGE3-E3R	GGACGATTATCAGGAGGCCTGC			
SEQ ID NO: 18	B-actin-E4F	GGCATCGTGATGGACTCCG	β -actin	615 bp + 615 bp	830 bp
SEQ ID NO: 19	B-actin-E6R	GCTGGAAGGTGGACAGCGA			

F= forward, R=Reverse, bp=base pairs

Table 2. Oligonucleotide primers and probes used for Taqman quantitative RT-PCR

	Name	Sequence	Primer	Dye
Exon 3 MAGE-A3 specific primers				
SEQ ID NO: 3	E3 MAGE3 TMF	GTCGTCGGAAATTGGCAGTAT	Forward	FAM; NFQ-MGB
SEQ ID NO: 4	E3 MAGE3 TMR	TGGGGTCCACTTCCATCAG	Reverse	
SEQ ID NO: 13	E3 MAGE3 TMP	AAAGCTTCCAGTTCCTT	Probe	
Intron 3 MAGE-A3 specific primers				
SEQ ID NO: 5	I3 MAGE3 TMF	TAAGCCTTTGTTAGAGCCTCCAA	Forward	NED; NFQ-MGB
SEQ ID NO: 6	I3 MAGE3 TMR	GGAGAGAGGGAGCATGTGAGA	Reverse	
SEQ ID NO: 14	I3 MAGE3 TMP	TTCCATTCACTACTCAG	Probe	
Beta-actin specific primers				
SEQ ID NO: 20	B-actin- TMF	CTGGAACGGTGAAGGTGACA	Forward	VIC; NFQ-MGB
SEQ ID NO: 21	B-actin- TMR	CGGCCACATTGTGAACTTTG	Reverse	
SEQ ID NO: 22	B-actin- TMP	TGCTCGCTCCAACC	Probe	

Table 3

RT-PCR	TaqMan PCR		<i>Total</i>
	Negative	Positive	
Negative	40	0	40
Positive	3	28	31
Total	43	28	71

Table 4

Ct Values						
	MAGE-3 exon		MAGE-3 intron		Beta-Actin	
	RGI	GSK	RGI	GSK	RGI	GSK
<i>TC1</i>	40.00	40.00	40.00	40.00	39.25	37.40
<i>p815</i>	34.07	33.94	40.00	40.00	33.22	32.76
<i>GERL</i>	31.77	31.38	34.45	33.83	23.11	22.69
<i>CRL 1675</i>	33.19	32.90	34.63	34.53	21.68	21.43
<i>990118</i>	33.11	32.99	35.13	34.23	23.63	23.17
<i>990010</i>	37.42	40.00	40.00	40.00	23.03	22.67
<i>990101</i>	35.81	36.10	37.94	38.57	23.86	23.48
<i>990102</i>	38.19	38.74	40.00	39.42	26.94	26.56
<i>990133</i>	Quantity Not Sufficient					
<i>990152</i>	35.97	37.61	39.22	38.44	23.36	23.00
<i>990159</i>	36.80	36.73	37.77	37.52	26.66	26.27
<i>990784</i>	35.90	38.13	36.77	37.90	26.54	26.09

Table 5

<u>SEQ ID NO</u>	<u>SEQUENCE SET/NAME</u>	<u>SEQUENCE</u>	<u>5' LABEL</u>	<u>3' LABEL</u>
	<u>SET 1 (ds)</u>			
15	MAGEA3-801Tmc	CAG GAG GCC TGC CTT	6-FAM	MGBNFQ
7	MAGEA3-775F	TGCTGGGTGACAATCAGATCAT	Amplicon size: 75bp	
8	MAGEA3-849R	CGCCCTCTCTTGCGATTATG		
	<u>SET 2 (ds)</u>			
16	MAGEA3e-1000Tmc	TTCATAACATGCAGGATCAC	6-FAM	MGBNFQ
9	MAGEA3e-950F	CCAACATTTTCGTGCAGGAA	Amplicon: 88bp; Tm 82 C	
10	MAGEA3e-1037R	CCTTGGACCCACAGGAA		
	<u>SET 3 (ds)</u>			
17	MAGEA3f-651Tm	CCT GTG ATC TTC AGC AAA	6-FAM	MGBNFQ
11	MAGEA3f-623F	TGTCGTCGGAATTGGCAGTAT	Amplicon size: 75 bp; 79 C	
12	MAGEA3f-697R	CAAAGACCAGCTGCAAGGAACT		

Table 7
COBAS™ TaqMan MAGE-A3 Primer and Probe Sequences

COBAS™ TaqMan MAGE-A3 Primer Sequences	
MAGEA3f-623F: (HW_MAGEA3_F; SEQ ID NO: 11)	5 'TGTCGTCGGAAATTGGCAGTAT3 '
MAGEA3f- 697R:(HW_MAGEA3_R; SEQ ID NO: 12)	5 'CAAAGACCAGCTGCAAGGAACT3 '
COBAS™ TaqMan MAGE-A3 Probe Sequence	
MAGEA3F-646MOD SEQ ID NO: 53	5 ' - ELFLLLFFLQGLGALFLLFAGFAAAGFLLFP - 3 '

E= FAM Reporter Dye, F= 5-Methyl dC, Q = BHQ2 Quencher, L = 5-Propynyl dU,
P= Phosphate, I = HEX Reporter

MAGEA3F-646MOD probe sequence comprises the following modified nucleotides
(SEQ ID NO:54): 5' - TCTTTCCTGTGATCTTCAGCAAAGCTTC - 3'

Table 8*COBAS TaqMan beta-actin Primer and Probe Sequences*

TaqMan beta-actin Primer Sequences	
RGI_BACT_F2: (SEQ ID NO: 55)	5'-GAGCGCGGCTACAGCTT-3'
RGI_BACT_R2: (SEQ ID NO: 56)	5'-TCCTTAATGTCACGCACGATTT-3'
TaqMan beta-actin Probe Sequence	
HW_RGIBACT_H: (SEQ ID NO: 57)	5'-IACCACCAQCGGCCGAGCGGP-3'

E= FAM Reporter Dye, F= 5-Methyl dC, Q = BHQ2 Quencher, L = 5-Propynyl dU,
P= Phosphate, I = HEX Reporter

Table 9*Sample & Control Valid CT range*

Controls	Valid Ct Range
FFPET Sample Mage Gene 50ng FAM	<35.2
FFPET Sample Bactin Gene 50n HEX	<32.1
100% Gerl Mage Gene 50ng FAM	26.0-29.5
100% Gerl Bactin Gene 50ng HEX	24.0-27.0
1% Gerl Mage Gene 0.5ng FAM	33.0-35.5
1% Gerl Bactin Gene 0.5ng HEX	29.0-32.5
100% UHR Mage Gene 50ng FAM	25.0-28.0
100% UHR Bactin Gene 50ng HEX	22.0-25.0
1% UHR Mage Gene 0.5ng FAM	31.0-33.0
1% UHR Bactin Gene 0.5ng HEX	27.0-29.0
P53 Positive Control DNA 20ng FAM	26.5-29.0
Negative Control (NC)	>38.0

Table 10*Thermal cycling profile*

PCR Thermal Profile for MAGE-A3 Exclusivity Experiment

Steps	Description	Temperature	Time	Cycle number
1	UNG Decontamination	50°C	5 min.	1X
2	Denaturation	95°C	15 sec.	2X
	Annealing	63°C	25 sec.	
3	Denaturation	92°C	15 sec.	53X
	Annealing	63°C	50 sec.	
4	Post Cycle	40°C	2 min.	1X

Table 11

RT-PCR Thermal Profile for Linearity and RT-PCR Efficiency, Analytical Sensitivity (Limit of Detection), Method Correlation, and Reproducibility Experiments

Steps	Description	Temperature	Time	Cycle number
1	UNG Decontamination	50°C	5 min.	1X
2	Denaturation	95°C	1 min.	1X
3	Reverse Transcription	60°C	20 min.	1X
4	Denaturation	95°C	15 sec.	2X
	Annealing	63°C	25 sec.	
6	Denaturation	92°C	15 sec.	53X
	Annealing	63°C	50 sec.	
7	Post Cycle	40°C	2 min.	1X

Relatively high anneal temperature of 63°C was implemented to improve MAGE-A3 specificity relative to the other MAGE-A family members.

Table 12: Ct values from MAGE-A Plasmids using 20, 2, 0.2 0.02 pg of DNA

Input	Curie A1	Curie A2	Curie A3	Curie A4	Curie A6	Curie A8	Curie A9	Curie A10	Curie A11	Curie A12
20pg	55.0	55.0	18.5	51.0	27.8	35.1	34.1	51.1	46.4	46.5
2pg	50.9	55.0	20.7	55.0	29.1	37.2	36.7	55.0	55.0	55.0
0.2pg	55.0	55.0	24.4	55.0	32.2	55.0	50.5	55.0	55.0	55.0
0.02pg	55.0	55.0	27.2	51.0	35.4	55.0	51.0	55.0	55.0	55.0

Table 13: Delta Ct Values Relative to MAGE-A3 for other MAGE-A Plasmids using 20, 2, 0.2 0.02 pg of DNA

Input	Curie A1	Curie A2	Curie A3	Curie A4	Curie A6	Curie A8	Curie A9	Curie A10	Curie A11	Curie A12
20pg	32.6	36.5	0.0	32.5	9.3	16.6	15.6	32.6	27.8	28.0
2pg	30.1	34.3	0.0	34.3	8.4	16.5	16.0	34.3	34.3	34.3
0.2pg	30.6	30.6	0.0	30.6	7.8	30.6	26.1	30.6	30.6	30.6
0.02pg	27.8	27.8	0.0	23.8	8.2	27.8	23.8	27.8	27.8	27.8

Table 14: MAGE-A3 Exclusivity Experiment Validation

Control	Run1	Run2	Run3	Run4	Run5	Run6	Run7	Run8
P53 (20ng)	28.3	28.3	28.6	28.2	28.4	28.5	28.6	28.5
NC	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0
NC	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0
NC	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0

Control Cts were within validated range. Experiment passed validation.

Tables 15 and 16: MAGE-A3 Exclusivity Experiment Details

Item/Reagent	ID/Lot
MMX (Universal)	CW120406
Primer/Probe	JL011507
MgOAc MnOAc Blend	CW012607
p53 0.4ng/ul	120506HL
H ₂ O	C08E41
P-1000	3250
P-200	3248
P-20	3244
Biosafety Hood	6

k-tray	Lot	CTM48
72910	6004153	1093
72512	6004153	1093
72867	6004153	1012
72561	6004153	1012
78933	6004153	1074
78804	6004153	1074
78950	6004153	108
78852	6004153	108

Digital Data Storage Location: J:\Discovery Research\GeneExpProfile\GSK\IDE Experiments\Mage Exclusivity
Notebook References: HL38027, JL38664, KM34771

Table 17: Linearity MAGE-A3 and β -Actin Ct and Delta Ct from 10 replicates at 11 Levels

Xenograft GERL-b in Water (n=10)										
		Curie-A3			Bactin			Delta Ct		
Dilution	RNA input (ng)	Av Ct	STD	%CV	Av Ct	STD	%CV	Delta Ct	Stdev	%CV
1	100	28.0	0.097	0.35%	24.4	0.086	0.35%	3.6	0.05	1.47%
2	50	28.7	0.087	0.30%	25.3	0.044	0.17%	3.4	0.07	1.97%
3	25	29.6	0.100	0.34%	26.3	0.069	0.26%	3.3	0.11	3.30%
4	12.5	30.3	0.073	0.24%	27.2	0.070	0.26%	3.1	0.07	2.31%
5	6.25	31.4	0.126	0.40%	28.3	0.075	0.27%	3.1	0.14	4.61%
6	3.13	32.1	0.158	0.49%	29.2	0.034	0.12%	2.9	0.18	6.27%
7	1.56	33.1	0.219	0.66%	30.0	0.073	0.24%	3.0	0.19	6.41%
8	0.78	33.9	0.162	0.48%	31.0	0.057	0.18%	2.9	0.17	5.91%
9	0.39	34.9	0.347	0.99%	32.0	0.169	0.53%	3.0	0.27	9.02%
10	0.20	36.3	0.682	1.88%	33.0	0.223	0.68%	3.3	0.78	23.49%
11	0.10	36.9	0.745	2.02%	34.0	0.244	0.72%	2.9	0.73	25.04%

Table 18: RT-PCR Amplification Efficiency of MAGE-A3 and β -actin

100 - 0.1 ng RNA Input	Slope	AE	% Efficiency
MAGE-A3	-0.9108	2.14	107.0
β -actin	-0.9582	2.06	103.1

Table 19: Linearity / RT-PCR Efficiency Experiment Validation

NC	Run1	Run2	Run3	Run4	Run5	Run6	Run7	Run7	Run7	Run7
MAGE-FAM	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0
MAGE-FAM	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0
MAGE-FAM	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	NA
MAGE-FAM	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	NA
Bactin-HEX	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0
Bactin-HEX	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0
Bactin-HEX	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	NA
Bactin-HEX	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	NA

Control Cts were within validated range. Experiment passed validation.

Tables 20, 21 and 22: Linearity / RT-PCR Efficiency Experiment Details

Isolation Reagents	Lot Number	Expiration Date
Buffer PKD	121100445	01/2007
Proteinase K	12199252	01/2007
Buffer RBC	12199582	01/2007
gDNA Eliminator Column	12198612	01/2007
Ethanol	64209 (Sigma)	01/2007
RNeasy MinElute Column	121100265	01/2007
DNase I	12411639	01/2007
RDD	11877821	01/2007
Buffer RWT	Research Lot	01/2007
Buffer RPE	12199510	01/2007
RNase-free Water	12199577	01/2007

Item/Reagent	ID/Lot
MMX (Universal)	CW120406
Primer/Probe	JL011507
MgOAc MnOAc Blend	HW121906
Gerl-b	11507
Stac-b	11507
UHR	760380
H ₂ O	C08E41
P-1000	3250
P-200	3248
P-20	3244
Biosafety Hood	6

k-tray	Lot	CTM48
78848/78790	6004153	1012
78817/78864	6004153	1069
72893/72957	6004153	1071
78944/78871	6004153	105
78793/78813	6004153	1067
78847/78919	6004153	1072
79010	6004153	1074

Digital Data Storage Location: J:\Discovery Research\GeneExpProfile\GSK\IDE Experiments\Linearity\RMS
 Notebook References: JL38664, KM34771

Table 23: MAGE-A3 Ct Hit Rate, N = 24 for Each Condition

Total Input	0.5%Gerl 99.5%Stac	1%Gerl 99%Stac	5%Gerl 95%Stac
12.5ng	0/24	4/24	24/24
25ng	6/24	20/24	24/24
50ng	23/24	24/24	24/24
100ng	18/24	24/24	24/24

Table 24: MAGE-A3 Ct Hit Rate Percentage, N = 24 for Each Condition

Total Input	0.5%Gerl 99.5%Stac	1%Gerl 99%Stac	5%Gerl 95%Stac
12.5ng	0%	16.7%	100%
25ng	25%	83.3%	100%
50ng	95.8%	100%	100%
100ng	75%	100%	100%

Table 25: β -actin Ct Hit Rate, N = 24 for Each Condition

Total Input	0.5%Gerl 99.5%Stac	1%Gerl 99%Stac	5%Gerl 95%Stac
12.5ng	24/24	24/24	24/24
25ng	24/24	24/24	24/24
50ng	24/24	24/24	24/24
100ng	24/24	24/24	24/24

Table 26: β -actin Ct Hit Rate Percentage, N = 24 for Each Condition

Total Input	0.5%Gerl 99.5%Stac	1%Gerl 99%Stac	5%Gerl 95%Stac
12.5ng	100%	100%	100%
25ng	100%	100%	100%
50ng	100%	100%	100%
100ng	100%	100%	100%

Table 27: MAGE Gene FAM Control Cts

Control	Run4C	Run4D	Run5A	Run6C	Run6D	Run7A	Run8C	Run8D	Run9A	Run7B	Run10C	Run10D	Run11A	Run11B	Run12B	Run12BB
1% Gerl	33.20	33.73	33.29	33.01	33.60	34.03	33.34	33.08	33.34	33.50	33.11	33.55	34.96	34.21	34.21	35.11
1%UHR	32.00	31.79	32.28	31.87	31.79	32.03	31.92	31.69	31.60	31.64	31.57	31.89	31.87	31.70	31.96	31.91
100% Gerl	27.35	27.31	27.36	27.34	27.38	27.57	27.76	27.62	28.04	27.51	27.75	28.02	27.83	27.60	27.82	28.05
100% UHR	25.33	25.34	25.39	25.31	25.34	25.48	25.65	25.44	25.92	25.63	25.65	25.76	26.06	25.92	25.97	26.20
NC	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0

Table 28: β -actin HEX Control Cts

Control	Run4C	Run4D	Run5A	Run6C	Run6D	Run7A	Run8C	Run8D	Run9A	Run7B	Run10C	Run10D	Run11A	Run11B	Run12B	Run12BB
1% Gerl	30.30	30.81	30.76	29.65	30.56	30.83	30.55	30.49	30.44	30.68	30.41	30.93	31.47	31.58	31.39	31.23
1%UHR	27.58	27.38	27.75	27.74	27.90	27.71	27.62	27.52	27.43	27.62	27.28	27.31	27.82	27.65	27.64	27.71
100% Gerl	24.82	24.57	24.74	24.71	24.70	24.93	25.07	24.78	25.41	24.82	25.00	25.23	25.04	24.89	25.04	25.15
100% UHR	22.21	22.16	22.22	22.20	22.16	22.36	22.63	22.28	22.83	22.59	22.41	22.45	22.92	22.67	22.71	22.99
NC	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0	>38.0

Control Cts are within validated range. Experiment passed validation.

Table 29; Table 30; and Table 31: Limit of Detection Experiment Details

Gerl & Stac Isolation Reagents	Lot Number	Expiration Date
Buffer PKD	121100445	12/14/2007
Proteinase K	12199252	12/11/2007
Buffer RBC	124101094	12/18/2007
gDNA Eliminator Column	12198612	12/15/2007
Ethanol	06H07GA	2/15/2008
RNeasy MinElute Column	121100265	12/6/2007
DNase I	124119201	12/6/2007
RDD	124115915	12/6/2007
Buffer RWT	124114997	12/18/2007
Buffer RPE	12199510	12/15/2007
RNase-free Water	12199577	12/15/2007

Item/Reagent	ID/Lot
MMX (Universal)	CW120406
Primer/Probe	JL011507
MgOAc MnOAc Blend	CW012607
H ₂ O	C08E41
UHR	760380
GERL	RGI 12307
STAC	RGI 12307
P-1000	3302
P-1000	3303
P-1000	3304
P-1000	3250
P-1000	3369
P-1000	1499
P-200	3308
P-200	3248
P-200	3305
P-200	1506
P-20	3245
P-20	3301
P-20	3244
ELPH-200	3313
ELPH-1000	3369
Biosafety Hood	4,5,6,1574

k-tray	Lot	CTM48
78839/78841	6004153	1012
78856/78870	6004153	105
71641/71692	6004153	1012
78920/71697	6004153	1092
71755/71690	6004153	105
71659/71630	6004153	1093
54608/54631	6004153	1012
54671/54677	6004153	101
54580/54649	6004153	105
54587/54282	6004153	1092
54592/54619	6004153	1012
54481/54585	6004153	105

Digital Data Storage Location: J:\Discovery Research\GeneExpProfile\GSK\IDE Experiments\LOD Experiments
 Notebook References: JL38664, LC38028, HL38027, KM38662, HL38661

Table 32: Cross Validation Summary of Samples

Cross Validation Results	COBAS	Prototype
Total Samples Available for Testing	131	131
Sample without gDNA	123	123
Samples OOR	4	10
Insufficient RNA Samples	2	5
Remaining Samples	117	108

OOR = MAGE-A3 Expression of the sample above the threshold, but MAGE-A3 Ct outside of the acceptable range.

Insufficient RNA Samples = β -actin Ct outside the acceptable range.

Table 33: COBAS and prototype Tests Positive and Negative by Comparator Percent Agreement

prototype vs. COBAS	COBAS Positive	COBAS Negative
prototype Positive	59	5
prototype Negative	4	39

98/107 91.6% Concordance

Positive by Comparator Percent Agreement 59/ 64 or 92.2 %

Negative by Comparator Percent Agreement 39/43 or 90.7 %

Table 34: COBAS and prototype Tests Positive and Negative by Comparator Percent Agreement with frozen Test to Resolve Discordant Results

RGI vs. RMS with GSK Results to Resolve Discordance	RMS Positive	RMS Negative
RGI Positive	63	1
RGI Negative	1	42

105/107 98.1 % Concordance

Positive by Comparator Percent Agreement 63/64 or 98.4 %

Negative by Comparator Percent Agreement 42/43 or 97.7 %

Table 35: Experiment Control MAGE Gene Cts

Control	Run1	Run2	Run3	Run4	Run5	Run6	Run7	Run8	RunA	RunB	Run7RX
1%Gerl	34.25	34.12	34.25	33.56	33.95	34.10	34.39	34.12	33.89	34.34	33.67
1%Gerl	33.58	34.27	34.18	33.57	34.42	34.37	34.22	34.20	33.80	33.54	NA
100%Gerl	27.49	27.63	27.64	27.61	27.58	27.80	29.10	27.67	27.45	27.71	27.84
100%Gerl	27.57	27.65	27.98	27.82	27.85	27.77	28.34	27.80	27.41	27.81	NA
1%UHR	31.52	31.51	31.78	32.24	32.21	32.47	32.76	31.99	31.85	32.13	32.19
1%UHR	31.24	31.96	32.12	32.28	32.31	32.50	32.27	32.03	32.12	31.99	NA
100&UHR	25.46	25.50	25.44	25.49	25.60	25.43	27.18	25.96	25.35	25.70	26.06
100%UHR	25.50	25.50	25.68	25.57	25.54	25.54	26.54	25.75	25.34	25.74	NA
NC	>38	>38	>38	>38	>38	>38	>38	>38	>38	>38	>38
NC	>38	>38	>38	>38	>38	>38	>38	>38	>38	>38	NA

Table 36: Experiment Control β -actin Gene Cts

Control	Run1	Run2	Run3	Run4	Run5	Run6	Run7	Run8	RunA	RunB	Run7RX
1%Gerl	31.08	31.09	31.06	31.08	31.42	31.33	31.94	31.36	31.15	31.53	31.12
1%Gerl	30.91	31.84	31.03	31.02	32.02	31.47	31.39	30.74	31.10	31.04	NA
100%Gerl	24.88	24.95	24.86	25.15	25.03	25.15	26.87	24.93	24.84	25.07	25.06
100%Gerl	24.80	24.63	25.07	25.13	25.17	25.07	25.98	24.57	24.68	25.16	NA
1%UHR	27.50	27.65	27.82	28.03	28.33	28.61	28.49	27.80	28.10	28.14	28.06
1%UHR	27.31	27.95	27.95	28.03	28.41	28.37	28.47	27.39	28.08	28.07	NA
100&UHR	22.30	22.33	22.37	22.39	22.51	22.44	24.35	22.71	22.19	22.50	22.97
100%UHR	22.36	22.32	22.58	22.44	22.36	22.40	23.59	22.40	22.22	22.57	NA
NC	>38	>38	>38	>38	>38	>38	>38	>38	>38	>38	>38
NC	>38	>38	>38	>38	>38	>38	>38	>38	>38	>38	NA

Control Cts are within validated range. Experiment passed validation.

Tables 37, 38 and 39- Method Correlation / Cross Validation Experiment Details

Isolation Reagents	Lot Number	Expiration Date
Buffer PKD	121100445	12/14/2007
Proteinase K	12199252	12/11/2007
Buffer RBC	124101094	12/18/2007
gDNA Eliminator Column	12198612	12/15/2007
Ethanol	06A49GA	8/14/2007
RNeasy MinElute Column	121100265	12/6/2007
DNase I	124119201	12/6/2007
RDD	124115915	12/6/2007
Buffer RWT	124114997	12/18/2007
Buffer RPE	12199510	12/15/2007
RNase-free Water	12199577	12/15/2007

Item/Reagent	ID/Lot
MMX (Universal)	CW120406
Primer/Probe	JL011507
MgOAc MnOAc Blend	CW012607
H ₂ O	C08E41
UHR	760380
GERL	RGI 12307
STAC	RGI 12307
P-200	3248
P-200	3305
P-200	3308
P-20	3244
P-20	3245
P-20	3301
P-2	3307
P-1000	3303
P-1000	3304
P1000	3250
P-10	3306
ELPH P-1000	3314
Biosafety Hood	4,5,6

k-tray	Lot	CTM48
72568/72637	6004153	1092
72993/72552	6004153	1095
72498/72495	6004153	105
72583/72540	6004153	1092
72490/72596	6004153	1093
72590/72641	6004153	1012
73016/72592	6004153	1069
72930/78783	6004153	1071
72620/72541	6004153	1012
72530/75252	6004153	1067
54581	6004153	1012

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 Notebook References: JL38664, LC38028, HL38027, KM38662, KM34771

Table 40: Run 1 — MAGE-A3 Expression Threshold from GERL RNA Controls

GERL RNA Control	100% β -actin	1% Curie	Delta Ct	Curie-A3 Threshold
Replicate1	24.56	33.48	-8.92	2.06E-03
Replicate2	24.47	33.29	-8.82	2.21E-03
Average	24.51	33.39	-8.87	2.13E-03

Table 41: Run 1 — MAGE-A3 Expression Call for Reproducibility Specimens

RGI Accession #	Sample ID	Mage A3 CT	Bactin CT	Delta	Expression	% Gerl	Mage Call
AGG-07-0000580	990105	46.0	24.4	-21.6	3.25383E-07	0.00	Negative
AGG-07-0000580	990105	36.3	24.5	-11.9	0.000268087	0.13	Negative
AGG-07-0000581	990112	46.0	24.3	-21.7	2.96142E-07	0.00	Negative
AGG-07-0000581	990112	36.5	24.4	-12.1	0.000226088	0.11	Negative
AGG-07-0000582	990116	36.6	26.2	-10.3	0.000770965	0.36	Negative
AGG-07-0000582	990116	36.0	26.0	-10.1	0.000936414	0.44	Negative
AGG-07-0000583	990117	46.0	23.6	-22.4	1.8283E-07	0.00	Negative
AGG-07-0000583	990117	37.7	23.6	-14.1	5.75628E-05	0.03	Negative
AGG-07-0000584	990131	36.1	25.8	-10.3	0.000804481	0.38	Negative
AGG-07-0000584	990131	36.2	25.7	-10.5	0.000692747	0.32	Negative
AGG-07-0000585	990619	32.9	25.4	-7.5	0.005560602	2.61	Positive
AGG-07-0000585	990619	33.1	25.5	-7.7	0.004921188	2.31	Positive
AGG-07-0000586	991157	29.2	22.2	-7.0	0.007818891	3.66	Positive
AGG-07-0000586	991157	29.3	22.3	-7.0	0.007812905	3.66	Positive
AGG-07-0000587	991158	26.4	24.4	-2.0	0.250968781	117.61	Positive
AGG-07-0000587	991158	26.4	24.3	-2.1	0.238331954	111.69	Positive
AGG-07-0000588	990130	29.0	25.4	-3.6	0.081955519	38.41	Positive
AGG-07-0000588	990130	29.2	25.6	-3.6	0.083954419	39.34	Positive
AGG-07-0000589	990136	28.3	24.8	-3.5	0.087129355	40.83	Positive
AGG-07-0000589	990136	28.2	24.7	-3.6	0.084384326	39.54	Positive
AGG-07-0000590	395	31.3	24.1	-7.3	0.006411876	3.00	Positive
AGG-07-0000590	395	31.5	24.1	-7.5	0.005674828	2.66	Positive
AGG-07-0000591	512	28.6	24.5	-4.1	0.057999545	27.18	Positive
AGG-07-0000591	512	28.6	24.4	-4.2	0.053251449	24.95	Positive

Table 42: Run 2 — MAGE-A3 Expression Threshold from GERL RNA Controls

GERL RNA Control	100% β -actin	1% Curie	Delta Ct	Curie-A3 Threshold
Replicate1	25.11	34.05	-8.94	2.04E-03
Replicate2	25.32	34.18	-8.86	2.15E-03
Average	25.21	34.11	-8.90	2.09E-03

Table 43: Run 2 — MAGE-A3 Expression Call for Reproducibility Specimens

RGI Accession #	Sample ID	Mage A3 CT	Bactin CT	Delta	Expression	% Gerl	Mage Call
AGG-07-0000568	990105	36.1	26.1	-10.0	0.001006688	0.48	Negative
AGG-07-0000568	990105	46.0	26.1	-19.9	1.0005E-06	0.00	Negative
AGG-07-0000569	990112	46.0	26.1	-19.9	9.96715E-07	0.00	Negative
AGG-07-0000569	990112	36.8	26.2	-10.5	0.000674353	0.32	Negative
AGG-07-0000570	990116	36.9	27.2	-9.7	0.001187625	0.57	Negative
AGG-07-0000570	990116	36.6	27.0	-9.6	0.001318477	0.63	Negative
AGG-07-0000571	990117	35.7	24.6	-11.0	0.000474819	0.23	Negative
AGG-07-0000571	990117	34.8	24.7	-10.1	0.000887085	0.42	Negative
AGG-07-0000572	990131	46.0	26.5	-19.5	1.3292E-06	0.00	Negative
AGG-07-0000572	990131	46.0	26.3	-19.7	1.17885E-06	0.00	Negative
AGG-07-0000573	990619	34.6	28.5	-6.1	0.014100789	6.74	Positive
AGG-07-0000573	990619	34.1	28.7	-5.5	0.022281952	10.65	Positive
AGG-07-0000574	991157	31.1	23.3	-7.8	0.004582971	2.19	Positive
AGG-07-0000574	991157	31.1	23.5	-7.6	0.005138918	2.46	Positive
AGG-07-0000575	991158	28.8	25.6	-3.2	0.108292659	51.75	Positive
AGG-07-0000575	991158	28.9	25.6	-3.3	0.102863278	49.15	Positive
AGG-07-0000576	990130	31.1	26.4	-4.7	0.039418402	18.84	Positive
AGG-07-0000576	990130	31.3	26.7	-4.6	0.040843351	19.52	Positive
AGG-07-0000577	990136	31.8	27.0	-4.8	0.034810768	16.63	Positive
AGG-07-0000577	990136	31.6	26.8	-4.8	0.035601088	17.01	Positive
AGG-07-0000578	395	33.6	25.6	-8.0	0.004032587	1.93	Positive
AGG-07-0000578	395	33.0	25.3	-7.6	0.005000295	2.39	Positive
AGG-07-0000579	512	31.2	25.6	-5.6	0.020284412	9.69	Positive
AGG-07-0000579	512	31.2	25.4	-5.8	0.018051401	8.63	Positive

Table 44: Reproducibility Validation

Control	Run 1 Control Cts		Run 2 Control Cts	
	MAGE Ct	β -actin Ct	MAGE Ct	β -actin Ct
100% Gerl	26.5	24.6	28.1	25.1
100% Gerl	26.5	24.5	28.3	25.3
1% Gerl	33.5	32.2	34.0	30.5
1% Gerl	33.3	31.9	34.2	30.6
100% UHR	24.8	21.3	26.1	22.8
100% UHR	24.6	21.2	26.3	23.0
1% UHR	31.6	28.6	32.1	27.3
1% UHR	31.6	28.5	32.0	27.3
NC	>38.0	>38.0	>38.0	>38.0
NC	>38.0	>38.0	>38.0	>38.0

Control Cts are within validated range. Experiment passed validation.

Reproducibility Experiment Details - Tables 45, 46 and 47

Isolation Reagents Run1	Lot Number	Expiration Date
Buffer PKD	121100445	12/14/2007
Proteinase K	12199252	12/11/2007
Buffer RBC	124101094	12/18/2007
gDNA Eliminator Column	12198612	12/15/2007
Ethanol	06H07GA	2/15/2008
RNeasy MinElute Column	121100265	12/6/2007
DNase I	124119201	12/6/2007
RDD	124115915	12/6/2007
Buffer RWT	124114997	12/18/2007
Buffer RPE	12199510	12/15/2007
RNase-free Water	12199577	12/15/2007

Isolation Reagents Run2	Lot Number	Expiration Date
Buffer PKD	124113180	2/15/2008
Proteinase K	124125018	2/15/2008
Buffer RBC	124114320	2/15/2008
gDNA Eliminator Column	124125864	2/15/2008
Ethanol	06H07GA	2/15/2008
RNeasy MinElute Column	124123928	2/15/2008
DNase I	124124041	2/15/2008
RDD	124122839	2/15/2008
Buffer RWT	124123852	2/15/2008
Buffer RPE	124119688	2/15/2008
RNase-free Water	124123699	2/15/2008

Item/Reagent	ID/Lot
MMX (Universal)	CW120406
Primer/Probe	JL011507
MgOAc MnOAc Blend	CW012607
H ₂ O	C08E41
H ₂ O	EMD Lot# 46201
UHR	260400
UHR	760380
GERL	RGI 12307
STAC	RGI 12307
Rainin PipetLite P-1000	3302
Rainin PipetLite P-200	3307
Rainin PipetLite P-20	3244
Rainin PipetLite P-10	3306
Rainin PipetLite P-1000	3303
Rainin PipetLite P-200	3305
Rainin PipetLite P-2	3307
Biosafety Hood	4,5

k-tray	Lot	CTM48
54562/54586	6004153	102
54669/54591	6004153	105

Digital Data Storage Location: J:\Discovery Research\GeneExpProfile\GSK\IDE Experiments\Reproducibility
 Notebook References: JL38664, LC38028, KM38662