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<110> Regeneron Pharmaceuticals, Inc.

<120> Non-Human Animals Having a Humanized Lymphocyte-Activation Gene 3

<130> 34031 (10212US01)

<150> 62/370,430

<151> 2016-08-03

<150> 62/258,181

<151> 2015-11-20

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<170> PatentIn version 3.5

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Trp	Ala	Gln	Glu	Gly	Ala	Pro	Val	His	Leu	Pro	Cys	Ser	Leu	Glu	Phe
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Pro	His	Leu	Asp	Pro	Asn	Phe	Leu	Arg	Arg	Gly	Trp	Val	Thr	Trp	Gln
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His Arg Pro Asp Ser Asp Gln Pro Ala Ser Ile Pro Ala Leu Asp Leu
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Leu Gln Gly Met Pro Ser Thr Arg Arg His Pro Pro His Arg Tyr Thr
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Val Leu Ser Val Ala Pro Gly Gly Leu Arg Ser Gly Arg Gln Pro Leu
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Ala Phe Val Arg Leu Pro Asp Arg Asp Phe Ser Cys Ser Leu Arg Leu
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Arg Val Gly Gln Ala Ser Met Ile Ala Ser Pro Pro Gly Thr Leu Lys
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Pro Ser Asp Trp Val Ile Leu Asn Cys Ser Phe Ser Arg Pro Asp Arg
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Pro Val Ser Val His Trp Phe Gln Gly Gln Ser Arg Val Pro Val His
195 200 205

Asn Ser Pro Arg His Tyr Leu Ala Glu Ser Phe Leu Leu Leu Pro Gln
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Val Ser Pro Leu Asp Ser Gly Thr Trp Gly Cys Val Leu Thr Tyr Arg
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Asp Gly Phe Asn Val Ser Ile Thr Tyr Asn Leu Lys Val Gln Gly Leu
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Glu Pro Val Ala Pro Leu Thr Val Tyr Ala Ala Glu Gly Ser Arg Val
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Glu Leu Pro Cys His Leu Pro Pro Val Val Gly Thr Pro Ser Leu Leu
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Gly Lys Ser Gly Asn Phe Thr Leu Gln Leu Glu Asn Val Gly Arg Ala
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Gln Ala Gly Thr Tyr Thr Cys Ser Ile His Leu Gln Gly Arg Gln Leu
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Ser Ala Ala Val Thr Leu Ala Val Ile Thr Val Thr Pro Lys Ser Phe
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Gly Leu Pro Gly Ser Pro Gln Lys Leu Leu Cys Glu Val Val Pro Ala
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Ser Gly Glu Gly Arg Phe Val Trp Arg Pro Leu Ser Asp Leu Ser Arg
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Ser Ser Leu Gly Pro Val Leu Glu Leu Gln Glu Ala Lys Leu Leu Ala
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Glu Gln Trp Gln Cys Gln Leu Tyr Glu Gly Gln Lys Leu Leu Gly Ala
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Ile Ser Gly Asp Leu Lys Gly Gly His Leu Phe Leu Ser Leu Ile Leu
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Gly Ile Arg Pro Pro Pro Val Gln Ser Lys Ile Glu Glu Leu Glu Arg
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Trp	Ala	Gln	Glu	Gly	Ala	Pro	Val	His	Leu	Pro	Cys	Ser	Leu	Lys	Ser
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Pro	Asn	Leu	Asp	Pro	Asn	Phe	Leu	Arg	Arg	Gly	Gly	Val	Ile	Trp	Gln
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His Gln Gly Met Pro Ser Pro Arg Gln Pro Ala Pro Gly Arg Tyr Thr
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Val Leu Ser Val Ala Pro Gly Gly Leu Arg Ser Gly Arg Gln Pro Leu
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Ser Leu Trp Leu Arg Pro Ala Leu Arg Thr Asp Ala Gly Glu Tyr His
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Ala Thr Val Arg Leu Pro Asn Arg Ala Leu Ser Cys Ser Leu Arg Leu
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Arg Val Gly Gln Ala Ser Met Ile Ala Ser Pro Ser Gly Val Leu Lys
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Leu Ser Asp Trp Val Leu Leu Asn Cys Ser Phe Ser Arg Pro Asp Arg
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Pro Val Ser Val His Trp Phe Gln Gly Gln Asn Arg Val Pro Val Tyr
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Asn Ser Pro Arg His Phe Leu Ala Glu Thr Phe Leu Leu Leu Pro Gln
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Val Ser Pro Leu Asp Ser Gly Thr Trp Gly Cys Val Leu Thr Tyr Arg
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Asp Gly Phe Asn Val Ser Ile Thr Tyr Asn Leu Lys Val Leu Gly Leu
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Glu Pro Val Ala Pro Leu Thr Val Tyr Ala Ala Glu Gly Ser Arg Val
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Glu Leu Pro Cys His Leu Pro Pro Gly Val Gly Thr Pro Ser Leu Leu
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Ile Ala Lys Trp Thr Pro Pro Gly Gly Gly Pro Glu Leu Pro Val Ala
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Gln Ala Gly Thr Tyr Thr Cys Ser Ile His Leu Gln Gly Gln Gln Leu
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Ser Gly Lys Glu Arg Phe Val Trp Arg Pro Leu Asn Asn Leu Ser Arg
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Ser Cys Pro Gly Pro Val Leu Glu Ile Gln Glu Ala Arg Leu Leu Ala
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Glu Arg Trp Gln Cys Gln Leu Tyr Glu Gly Gln Arg Leu Leu Gly Ala
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Thr Val Tyr Ala Ala Glu Ser Ser Ser Gly Ala His Ser Ala Arg Arg
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Ile Ser Gly Asp Leu Lys Gly Gly His Leu Val Leu Val Leu Ile Leu
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Gly Ala Leu Ser Leu Phe Leu Leu Val Ala Gly Ala Phe Gly Phe His
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Trp Trp Arg Lys Gln Leu Leu Leu Arg Arg Phe Ser Ala Leu Glu His
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<400> 6

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Trp	Ala	Gln	Glu	Gly	Ala	Pro	Ala	Gln	Leu	Pro	Cys	Ser	Pro	Thr	Ile
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Pro	Leu	Gln	Asp	Leu	Ser	Leu	Leu	Arg	Arg	Ala	Gly	Val	Thr	Trp	Gln
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Gly Ser Leu Arg Ala Ser Asp Trp Val Ile Leu Asn Cys Ser Phe Ser
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Arg Pro Asp Arg Pro Ala Ser Val His Trp Phe Arg Asn Arg Gly Gln
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Gly Arg Val Pro Val Arg Glu Ser Pro His His His Leu Ala Glu Ser
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Phe Leu Phe Leu Pro Gln Val Ser Pro Met Asp Ser Gly Pro Trp Gly
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Cys Ile Leu Thr Tyr Arg Asp Gly Phe Asn Val Ser Ile Met Tyr Asn
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Leu Thr Val Leu Gly Leu Glu Pro Pro Thr Pro Leu Thr Val Tyr Ala
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Gly Ala Gly Ser Arg Val Gly Leu Pro Cys Arg Leu Pro Ala Gly Val
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Gly Thr Arg Ser Phe Leu Thr Ala Lys Trp Thr Pro Pro Gly Gly Gly
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Pro Asp Leu Leu Val Thr Gly Asp Asn Gly Asp Phe Thr Leu Arg Leu
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Glu Asp Val Ser Gln Ala Gln Ala Gly Thr Tyr Thr Cys His Ile His
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Leu Gln Glu Gln Gln Leu Asn Ala Thr Val Thr Leu Ala Ile Ile Thr
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Val Thr Pro Lys Ser Phe Gly Ser Pro Gly Ser Leu Gly Lys Leu Leu
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Cys Glu Val Thr Pro Val Ser Gly Gln Glu Arg Phe Val Trp Ser Ser
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Gln Glu Ala Gln Leu Leu Ser Gln Pro Trp Gln Cys Gln Leu Tyr Gln
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Gly Glu Arg Leu Leu Gly Ala Ala Val Tyr Phe Thr Glu Leu Ser Ser
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Pro Gly Ala Gln Arg Ser Gly Arg Ala Pro Gly Ala Leu Pro Ala Gly
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His Leu Leu Leu Phe Leu Ile Leu Gly Val Leu Ser Leu Leu Leu Leu
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Val Thr Gly Ala Phe Gly Phe His Leu Trp Arg Arg Gln Trp Arg Pro
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Arg Arg Phe Ser Ala Leu Glu Gln Gly Ile His Pro Pro Gln Ala Gln
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 <213> Artificial Sequence

<220>
 <223> exemplary humanized Lag-3 amino acid sequence

<400> 8

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			20					25					30		

Trp	Ala	Gln	Glu	Gly	Ala	Pro	Ala	Gln	Leu	Pro	Cys	Ser	Pro	Thr	Ile
		35					40					45			

Pro	Leu	Gln	Asp	Leu	Ser	Leu	Leu	Arg	Arg	Ala	Gly	Val	Thr	Trp	Gln
	50					55					60				

His Gln Pro Asp Ser Gly Pro Pro Ala Ala Ala Pro Gly His Pro Leu
65 70 75 80

Ala Pro Gly Pro His Pro Ala Ala Pro Ser Ser Trp Gly Pro Arg Pro
85 90 95

Arg Arg Tyr Thr Val Leu Ser Val Gly Pro Gly Gly Leu Arg Ser Gly
100 105 110

Arg Leu Pro Leu Gln Pro Arg Val Gln Leu Asp Glu Arg Gly Arg Gln
115 120 125

Arg Gly Asp Phe Ser Leu Trp Leu Arg Pro Ala Arg Arg Ala Asp Ala
130 135 140

Gly Glu Tyr Arg Ala Ala Val His Leu Arg Asp Arg Ala Leu Ser Cys
145 150 155 160

Arg Leu Arg Leu Arg Leu Gly Gln Ala Ser Met Thr Ala Ser Pro Pro
165 170 175

Gly Ser Leu Arg Ala Ser Asp Trp Val Ile Leu Asn Cys Ser Phe Ser
180 185 190

Arg Pro Asp Arg Pro Ala Ser Val His Trp Phe Arg Asn Arg Gly Gln
195 200 205

Gly Arg Val Pro Val Arg Glu Ser Pro His His His Leu Ala Glu Ser
210 215 220

Phe Leu Phe Leu Pro Gln Val Ser Pro Met Asp Ser Gly Pro Trp Gly
225 230 235 240

Cys Ile Leu Thr Tyr Arg Asp Gly Phe Asn Val Ser Ile Met Tyr Asn
245 250 255

Leu Thr Val Leu Gly Leu Glu Pro Val Ala Pro Leu Thr Val Tyr Ala
260 265 270

Ala Glu Gly Ser Arg Val Glu Leu Pro Cys His Leu Pro Pro Gly Val
275 280 285

Gly Thr Pro Ser Leu Leu Ile Ala Lys Trp Thr Pro Pro Gly Gly Gly
290 295 300

Pro Glu Leu Pro Val Ala Gly Lys Ser Gly Asn Phe Thr Leu His Leu
305 310 315 320

Glu Ala Val Gly Leu Ala Gln Ala Gly Thr Tyr Thr Cys Ser Ile His
325 330 335

Leu Gln Gly Gln Gln Leu Asn Ala Thr Val Thr Leu Ala Val Ile Thr
340 345 350

Val Thr Pro Lys Ser Phe Gly Leu Pro Gly Ser Arg Gly Lys Leu Leu
355 360 365

Cys Glu Val Thr Pro Ala Ser Gly Lys Glu Arg Phe Val Trp Arg Pro
370 375 380

Leu Asn Asn Leu Ser Arg Ser Cys Pro Gly Pro Val Leu Glu Ile Gln
385 390 395 400

Glu Ala Arg Leu Leu Ala Glu Arg Trp Gln Cys Gln Leu Tyr Glu Gly
405 410 415

Gln Arg Leu Leu Gly Ala Thr Val Tyr Ala Ala Glu Ser Ser Ser Gly
420 425 430

Ala His Ser Ala Arg Arg Ile Ser Gly Asp Leu Lys Gly Gly His Leu
435 440 445

Val Leu Val Leu Ile Leu Gly Ala Leu Ser Leu Phe Leu Leu Val Ala
450 455 460

Gly Ala Phe Gly Phe His Trp Trp Arg Lys Gln Leu Leu Leu Arg Arg
465 470 475 480

Phe Ser Ala Leu Glu His Gly Ile Gln Pro Phe Pro Ala Gln Arg Lys
485 490 495

Ile Glu Glu Leu Glu Arg Glu Leu Glu Thr Glu Met Gly Gln Glu Pro
500 505 510

Glu Pro Glu Pro Glu Pro Gln Leu Glu Pro Glu Pro Arg Gln Leu
515 520 525

<210> 9
<211> 1741
<212> DNA
<213> Artificial Sequence

<220>
<223> exemplary synthetic DNA fragment for humanization (1,741 bp
including exons 2-4 and part of intron 4 of a human LAG3 gene

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<210> 10

<211> 7522

<212> DNA

<213> Artificial Sequence

<220>

<223> exemplary humanized Lag3 allele including a selection cassette

<400> 10

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<210> 11
 <211> 2292
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> a humanized Lag3 allele after recombinase-mediated excision of a selection cassette

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ctctgggcat cttttagctg ctgtctctct tacttttatt tattttattg tgtgtttatt	2100
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attggattcc ctggaacttg acctacagac agtcatgaga taccatatgg gtgctgggaa	2220
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tcccttggct gt	2292

<210> 12
<211> 160
<212> DNA
<213> Artificial Sequence

<220>

<223> the nucleotide sequence across the upstream insertion point
between a mouse Lag-3 genomic sequence and a human LAG-3 genomic
sequence

<400> 12
catgatgttt ctttcttagg aaagccaggg catttctcta ttctccaatc tcttggtca 60
atgcccttgg cctctctttt gttccactag tgaagcctct ccagccaggg gctgaggtcc 120
cgggtggtgtg ggcccaggag ggggctcctg cccagctccc 160

<210> 13
<211> 190
<212> DNA
<213> Artificial Sequence

<220>

<223> the nucleotide sequence comprising a human LAG-3 genomic sequence
contiguous with the 5' end of a self-deleting neomycin cassette

<400> 13
ttcacatttg accacaactc cttcctgccc cccttgctcac ctcccctaac gtcgagataa 60
cttcgtataa tgtatgctat acgaagttat atgcatggcc tccgcgccgg gttttggcgc 120
ctcccgcggg cgccccctc ctcacggcga gcgctgccac gtcagacgaa gggcgagcgc 180
agcgtcctga 190

<210> 14
<211> 221
<212> DNA
<213> Artificial Sequence

<220>

<223> the nucleotide sequence across the downstream insertion point
comprising the 3' end of a self-deleting neomycin cassette
contiguous with a mouse Lag-3 genomic sequence

<400> 14
tttactgca ttctagtgtt ggtttgtcca aactcatcaa tgtatcttat catgtctgga 60
ataacttcgt ataatgtatg ctatacgaag ttatgctagt aactataacg gtcctaaggt 120
agcgagctag cgacccccaa aacttttctca gctgcgtgtg gtctcactcc acatcacttt 180

gtttcagtgt ccaaaccatt ttctctctgg gcatctttta g 221

<210> 15
<211> 278
<212> DNA
<213> Artificial Sequence

<220>
<223> the nucleotide sequence across the upstream insertion point after deletion of a neomycin cassette

<400> 15
acgtctccat catgtataac ctactgttc tgggtaactc cccactctg cttcacattt 60
gaccacaact ccttcctgcc ccccttgta cctcccctaa cgtcgagata acttcgtata 120
atgtatgcta tacgaagtta tgctagtaac tataacggtc ctaaggtagc gagctagcga 180
cccccaaac tttctcagct gcgtgtggtc tcactccaca tcactttggtt tcagtgtcca 240
aaccattttc tctctgggca tcttttagct gctgtctc 278

<210> 16
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<212> DNA
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<220>
<223> Synthetic Oligonucleotide

<400> 16
gaggctgctg acggtcaag 19

<210> 17
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 17
ttaggcaggt taactttatc ctcaaagca 29

<210> 18
<211> 23
<212> DNA
<213> Artificial Sequence

<220>

<223> Synthetic Oligonucleotide

<400> 18

gccacgaaga agatgcactc aag

23

<210> 19

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Oligonucleotide

<400> 19

gtcccgggtc tcttgagat

20

<210> 20

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Oligonucleotide

<400> 20

ccaccataa acatcccag gttca

26

<210> 21

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Oligonucleotide

<400> 21

ccgctcattc caagtcagtt c

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<210> 22

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Oligonucleotide

<400> 22

cggttggtg tcaagagaac

20

<210> 23
<211> 23
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<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 23
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<210> 24
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<400> 24
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<210> 25
<211> 20
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<220>
<223> Synthetic Oligonucleotide

<400> 25
agccctgctg tggtgggaaa 20

<210> 26
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 26
tgtttccagt gggctgatga agtc 24

<210> 27
<211> 18
<212> DNA
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<220>

<223> Synthetic Oligonucleotide

<400> 27

tggcagtcac tgtgcaag

18