

9869SG3315-seq1-000001
SEQUENCE LISTING

<110> Agency For Science Technology and Research

<120> A Method to Up-Regulate Cancer Stem Cell Markers For The
Generation of Antigen-Specific Cytotoxic Effector T Cells

<130> 9869SG3314

<160> 205

<170> PatentIn version 3.5

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 OKSM-eGFP cassette in genome (forward primer)

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<223> Amplify mRNA BMP2 (forward primer)

<400> 119
ggtggaatga ctggattg 18

<210> 120
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Amplify mRNA BMP2 (reverse primer)

<400> 120
gcatcgagat agcactg 17

<210> 121
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Amplify mRNA BMP4 (forward primer)

<400> 121
ctggtccacc acaatgtgac 20

<210> 122
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Amplify mRNA BMP4 (reverse primer)

<400> 122
cgatcggcta atcctgacat 20

<210> 123
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Amplify mRNA RHPN2 (forward primer)

<400> 123
gagaacgacg gctacttttcg ga 22

<210> 124
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Amplify mRNA RHPN2 (reverse primer)

<400> 124
agctcttcct tgagcatctg ca 22

<210> 125
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Amplify mRNA SCG5 (forward primer)

<400> 125
atatccagct caccaggcca tgaa 24

<210> 126
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Amplify mRNA SCG5 (reverse primer)

<400> 126
tgttgtctcc agtcaactct gccca 24

<210> 127
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Amplify mRNA GREM1 (forward primer)

<400> 127
gtcacactca actgccctga 20

<210> 128
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Amplify mRNA GREM1 (reverse primer)

<400> 128
ggtgaggtgg gtttctggta 20

<210> 129
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Amplify mRNA FMN1 (forward primer)

<400> 129
cagcatctct gttcctccat ca 22

<210> 130
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Amplify mRNA FMN1 (reverse primer)

<400> 130
agtccttcc attatgccta cc 22

<210> 131
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Construct PGK-Luc-Intron to examine intron 2 (2.4kb)

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transcriptional regulation activity of CD24 (forward primer)

<400> 131
gcgctctaga ctgcagggat tagcgcctgg ag 32

<210> 132
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Construct PGK-Luc-Intron to examine intron 2 (2.4kb)
transcriptional regulation activity of CD24 (reverse primer)

<400> 132
atatggccgg cccacatca cagctacctc catgtac 37

<210> 133
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Construct CD24-derived luciferase reporter to examine core
promoter activity of CD24 (forward primer)

<400> 133
gatgatatcg ataccagccg ggtaccagca g 31

<210> 134
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Construct CD24-derived luciferase reporter to examine core
promoter activity of CD24 (reverse primer)

<400> 134
gataagcttt caggatgctg ggtgcttgga g 31

<210> 135
<211> 33
<212> DNA

<213> Artificial Sequence

<220>

<223> Construct CD24-derived luciferase reporter to examine core promoter activity of CD24 (forward primer)

<400> 135

gatgatatcg cagtctgagt ggcaatgcac ttg

33

<210> 136

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> Construct CD24-derived luciferase reporter to examine core promoter activity of CD24 (reverse primer)

<400> 136

gataagcttt caggatgctg ggtgcttgga g

31

<210> 137

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> Construct CD24-derived luciferase reporter to examine core promoter activity of CD24 (forward primer)

<400> 137

gatgatatcc ctgtagtttg cagcgtcagg ca

32

<210> 138

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> Construct CD24-derived luciferase reporter to examine core promoter activity of CD24 (reverse primer)

<400> 138

gataagcttt caggatgctg ggtgcttgga g

31

<210> 139
 <211> 31
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Construct CD24-derived luciferase reporter to examine core promoter activity of CD24 (forward primer)

 <400> 139
 gatgatatcc cacgcccggc caaagtatct c 31

 <210> 140
 <211> 31
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Construct CD24-derived luciferase reporter to examine core promoter activity of CD24 (reverse primer)

 <400> 140
 gataagcttt caggatgctg ggtgcttgga g 31

 <210> 141
 <211> 38
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> CD24 promoter region bearing a Sox2 binding site (forward primer)

 <400> 141
 tggcaggtcc cgggaaacaa aggaaacttg ggcccggc 38

 <210> 142
 <211> 38
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> CD24 promoter region bearing a Sox2 binding site (reverse primer)

<400> 142
gccgggcccc agtttccttt gtttcccggg acctgcca 38

<210> 143
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> CD24 promoter region bearing two Sox2 binding sites (forward primer)

<400> 143
ccgggaaaca aaggaaactc cgggaaacaa aggaaact 38

<210> 144
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> CD24 promoter region bearing two Sox2 binding sites (reverse primer)

<400> 144
agtttccttt gtttcccgga gtttcctttg tttcccgg 38

<210> 145
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> CD24 promoter region bearing no Sox2 binding site (none relevant sequence 1) (forward primer)

<400> 145
ccagccgggt accagcagcc ggcggcgccc gcccacct 38

<210> 146
<211> 38
<212> DNA
<213> Artificial Sequence

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<220>

<223> CD24 promoter region bearing no Sox2 binding site (none relevant sequence 1) (reverse primer)

<400> 146

agggtgggcgg gcgccgccgg ctgctggtac ccggctgg 38

<210> 147

<211> 38

<212> DNA

<213> Artificial Sequence

<220>

<223> CD24 promoter region bearing no Sox2 binding site (none relevant sequence 2) (forward primer)

<400> 147

gctttcctgg tatataaggt ctgccggct cgccgcgc 38

<210> 148

<211> 38

<212> DNA

<213> Artificial Sequence

<220>

<223> CD24 promoter region bearing no Sox2 binding site (none relevant sequence 2) (reverse primer)

<400> 148

gcgcggcgag ccggcgagac cttatatacc aggaaaagc 38

<210> 149

<211> 38

<212> DNA

<213> Artificial Sequence

<220>

<223> CD24 promoter region bearing a mutated Sox2 binding site (forward primer)

<400> 149

tggcaggtcc cgggaaaagg aggaaacttg ggcccggc 38

<210> 150

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<211> 38
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> CD24 promoter region bearing a mutated Sox2 binding site (reverse primer)

<400> 150
 gccgggcca agtttcctcc ttttcccggg acctgcca 38

<210> 151
 <211> 38
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> CD24 promoter region bearing a consensus Sox2 binding site (positive control) (forward primer)

<400> 151
 tggcaggtcc atttgcataa caaagagttg ggccccggc 38

<210> 152
 <211> 38
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> CD24 promoter region bearing a consensus Sox2 binding site (positive control) (reverse primer)

<400> 152
 gccgggcca actctttggtt atgcaaattg acctgcca 38

<210> 153
 <211> 26
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Construct CRISPR to knockout miR205 binding site on CD24 3'-UTR (forward primer)

<400> 153

aaacgagttt catgtacaag atgagt 26

<210> 154
 <211> 26
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Construct CRISPR to knockout miR205 binding site on CD24 3'-UTR
 (reverse primer)

<400> 154
 taaaactcat cttgtacatg aaactc 26

<210> 155
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> T7E1 assay to quantify CRISPR-mediated cleavage efficiency on
 targeted CD24 site (forward primer)

<400> 155
 cagtcaacag ccagtctctt cgtg 24

<210> 156
 <211> 23
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> T7E1 assay to quantify CRISPR-mediated cleavage efficiency on
 targeted CD24 site (reverse primer)

<400> 156
 catcattgcc ctggcacatg tca 23

<210> 157
 <211> 34
 <212> DNA
 <213> Artificial Sequence

<220>

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<223> Construct left homologous arm bearing CD24 sequences (forward primer)

<400> 157

gcgctacgta ccaggataga cagtgaccca atga

34

<210> 158

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> Construct left homologous arm bearing CD24 sequences (reverse primer)

<400> 158

atatgtcgac cttgtacatg aaactccagc agat

34

<210> 159

<211> 37

<212> DNA

<213> Artificial Sequence

<220>

<223> Construct right homologous arm bearing CD24 sequences (forward primer)

<400> 159

atatgcggcc gcgaaggaga ggcaacatcc aaaatag

37

<210> 160

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Construct right homologous arm bearing CD24 sequences (reverse primer)

<400> 160

gcgcttcgaa tcaagcctgt aatcccagca ctttg

35

<210> 161

<211> 25

<212> DNA
 <213> Artificial Sequence

 <220>
 <223> Check for site-specific integration of mCherry transgene on CD24
 (forward primer)

 <400> 161
 gacatcacct cccacaacga ggact 25

 <210> 162
 <211> 25
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Check for site-specific integration of mCherry transgene on CD24
 (reverse primer)

 <400> 162
 agcatcagtg tgtgaccatg cgaac 25

 <210> 163
 <211> 20
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Amplify mRNA CD24 on chromosome 6 (forward primer)

 <400> 163
 gagcaatggt ggccaggctc 20

 <210> 164
 <211> 25
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Amplify mRNA CD24 on chromosome 6 (reverse primer)

 <400> 164
 ggattggggtt tagaagatgg ggaaa 25

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<210> 165
 <211> 20
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Amplify mRNA CD24 on chromosome Y (forward primer)

 <400> 165
 gagcaatggg gccaggctt 20

 <210> 166
 <211> 25
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Amplify mRNA CD24 on chromosome Y (reverse primer)

 <400> 166
 ggattgggtt tagaagatgg ggaag 25

 <210> 167
 <211> 20
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Amplify mRNA CD24 on chromosome 15 (forward primer)

 <400> 167
 atccagccag acacgctgca 20

 <210> 168
 <211> 23
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Amplify mRNA CD24 on chromosome 15 (reverse primer)

 <400> 168
 cagtagctgg atttggggca gtc 23

<210> 169
 <211> 22
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Amplify truncated mRNA CD24 expressed by chromosome 6 (forward primer)

 <400> 169
 tctggaagtc caatgtggca ag 22

 <210> 170
 <211> 25
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Amplify truncated mRNA CD24 expressed by chromosome 6 (reverse primer)

 <400> 170
 cactggaagt tcccttctca tgtac 25

 <210> 171
 <211> 24
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Quantify precursor stem-loop hsa-mir-205 expression (forward primer)

 <400> 171
 agatcctcag acaatccatg tgct 24

 <210> 172
 <211> 20
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Quantify precursor stem-loop hsa-mir-205 expression (reverse primer)

<400> 172
agctccatgc ctcctgaact 20

<210> 173
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> cDNA synthesis of mature miR205-5p (adapter)

<400> 173
tccgtcgttc taatgcgaac agactccg 28

<210> 174
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Quantify mature miR205-5p expression (forward primer)

<400> 174
cctccatcct tcattccacc g 21

<210> 175
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Quantify mature miR205-5p expression (reverse primer)

<400> 175
gtttccgtcg ttctaatacg aa 22

<210> 176
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Quantify miR205HG expression (forward primer)

<400> 176
ctcaagtacc catcttggag gg 22

<210> 177
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Quantify miR205HG expression (reverse primer)

<400> 177
cacgcacact ccagatgtct c 21

<210> 178
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Construct CMV-miR205 (forward primer)

<400> 178
gcgcgaattc aaagatcctc agacaatcca tgtgct 36

<210> 179
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Construct CMV-miR205 (reverse primer)

<400> 179
atatactagt tgtcagctcc atgcctcctg aac 33

<210> 180
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Construct CMV-miR205HG (co-expressing miR205) (forward primer)

<400> 180
gcgcgaattc gaaatgggct gaggccctct tg 32

<210> 181
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Construct CMV-miR205HG (co-expressing miR205) (reverse primer)

<400> 181
atatactagt tttttcagta gacaagcaac ttttag 36

<210> 182
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Construct PGK-Luc-UTR to examine 3'-UTR (211bp) transcriptional regulation activity of CD24 (forward primer)

<400> 182
gcgctctaga cttaagagac tcaggccaag aaacg 35

<210> 183
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Construct PGK-Luc-UTR to examine 3'-UTR (211bp) transcriptional regulation activity of CD24 (reverse primer)

<400> 183
atatggccgg ccggcatcca tcatctagtc aaacctc 37

<210> 184
<211> 35
<212> DNA
<213> Artificial Sequence

<220>

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<223> Construct PGK-Luc-UTR to examine 3'-UTR transcriptional regulation activity of CD24 (forward primer)

<400> 184

gcgctctaga cttaagagac tcaggccaag aaacg

35

<210> 185

<211> 39

<212> DNA

<213> Artificial Sequence

<220>

<223> Construct PGK-Luc-UTR to examine 3'-UTR transcriptional regulation activity of CD24 (reverse primer)

<400> 185

atatggccgg cccaagccac attcaaggaa atcatgtct

39

<210> 186

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Construct CRISPR to knockout CD24 ORF (forward primer)

<400> 186

aaacggacat gggcagagca atgggt

26

<210> 187

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Construct CRISPR to knockout CD24 ORF (reverse primer)

<400> 187

taaaacccat tgctctgccc atgtcc

26

<210> 188

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> T7E1 assay to quantify CRISPR-mediated cleavage efficiency on targeted CD24 site (forward primer)

<400> 188

cacgtcacgg ctattgtggc tttc

24

<210> 189

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> T7E1 assay to quantify CRISPR-mediated cleavage efficiency on targeted CD24 site (reverse primer)

<400> 189

gcctctgggt gaaagtggga agtag

25

<210> 190

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Construct left homologous arm bearing CD24 sequences (forward primer)

<400> 190

gcgctacgta gctcacagaa caaagcaagg gcttc

35

<210> 191

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Construct left homologous arm bearing CD24 sequences (reverse primer)

<400> 191

atatgtcgac ttgctctgcc catgtcccct

30

<210> 192
 <211> 31
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Construct right homologous arm bearing CD24 sequences (forward primer)

<400> 192
 atatgcggcc gctggtggcc aggctcgggc t 31

<210> 193
 <211> 36
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Construct right homologous arm bearing CD24 sequences (reverse primer)

<400> 193
 gcgcttcgaa aggatctagg gagaccgcgc tggtag 36

<210> 194
 <211> 25
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Check for site-specific integration of mCherry transgene on CD24 (forward primer)

<400> 194
 gacatcacct cccacaacga ggact 25

<210> 195
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Check for site-specific integration of mCherry transgene on CD24 (reverse primer)

<400> 195
atcctccaaa cccgaactga ccca 24

<210> 196
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Amplify mRNA CD24 (foward primer)

<400> 196
gagcaatggt ggccaggctc 20

<210> 197
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Amplify mRNA CD24 (reverse primer)

<400> 197
ggattgggtt tagaagatgg ggaaa 25

<210> 198
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Amplify mRNA DPP4 (forward primer)

<400> 198
cattcacttt tgaaaagttc tccctagaaa g 31

<210> 199
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Amplify mRNA DPP4 (reverse primer)

<400> 199
cgtttttgtg cagttttaaa atgtgtgc 28

<210> 200
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Amplify mRNA ITGB1 (forward primer)

<400> 200
ggttctcata tgaacctaac tgggtcaaa 28

<210> 201
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Amplify mRNA ITGB1(reverse primer)

<400> 201
atcatgttta taaaagcaat agaaggtacg gt 32

<210> 202
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Amplify mRNA KIT (forward primer)

<400> 202
aagtgatct atttttccct ttctcc 26

<210> 203
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Amplify mRNA KIT (reverse primer)

<400> 203
tttcatactg accaaaaactc agcct 25

<210> 204
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Amplify mRNA MET (forward primer)

<400> 204
gactcctaca acccgaatac tgc 23

<210> 205
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Amplify mRNA MET (reverse primer)

<400> 205
catagtgctc cccaatgaaa gtagag 26