<110> Green Cross Lab Cell Corporation

<120> METHOD FOR CULTURING NATURAL KILLER CELLS DERIVED FROM CORD BLOODS WITH GENETICALLY ENGINEERED T CELLS

<130> FPD/201810-0021

<160> 25

<170> KoPatentIn 3.0

<210> 1
<211> 254
<212> PRT
<213> Artificial Sequence

<220>
<223> amino acid seqeunce for 4-1BBL

<400> 1

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Met Glu Tyr Ala Ser Asp Ala Ser Leu Asp Pro Glu Ala Pro Trp Pro
  1               5                  10                  15

Pro Ala Pro Arg Ala Arg Ala Cys Arg Val Leu Pro Trp Ala Leu Val
             20                  25                  30

Ala Gly Leu Leu Leu Leu Leu Leu Leu Ala Ala Ala Cys Ala Val Phe
         35                  40                  45

Leu Ala Cys Pro Trp Ala Val Ser Gly Ala Arg Ala Ser Pro Gly Ser
     50                  55                  60

Ala Ala Ser Pro Arg Leu Arg Glu Gly Pro Glu Leu Ser Pro Asp Asp
 65                  70                  75                  80

Pro Ala Gly Leu Leu Asp Leu Arg Gln Gly Met Phe Ala Gln Leu Val
                 85                  90                  95

Ala Gln Asn Val Leu Leu Ile Asp Gly Pro Leu Ser Trp Tyr Ser Asp
            100                 105                 110

Pro Gly Leu Ala Gly Val Ser Leu Thr Gly Gly Leu Ser Tyr Lys Glu
        115                 120                 125

Asp Thr Lys Glu Leu Val Val Ala Lys Ala Gly Val Tyr Tyr Val Phe
    130                 135                 140

Phe Gln Leu Glu Leu Arg Arg Val Val Ala Gly Glu Gly Ser Gly Ser
145                 150                 155                 160

Val Ser Leu Ala Leu His Leu Gln Pro Leu Arg Ser Ala Ala Gly Ala
                165                 170                 175
```

```
Ala Ala Leu Ala Leu Thr Val Asp Leu Pro Pro Ala Ser Ser Glu Ala
            180                 185                 190

Arg Asn Ser Ala Phe Gly Phe Gln Gly Arg Leu Leu His Leu Ser Ala
        195                 200                 205

Gly Gln Arg Leu Gly Val His Leu His Thr Glu Ala Arg Ala Arg His
    210                 215                 220

Ala Trp Gln Leu Thr Gln Gly Ala Thr Val Leu Gly Leu Phe Arg Val
225                 230                 235                 240

Thr Pro Glu Ile Pro Ala Gly Leu Pro Ser Pro Arg Ser Glu
                245                 250
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<210> 2
<211> 765
<212> DNA
<213> Artificial Sequence

<220>
<223> nucleotide sequence of 4-1BBL

<400> 2

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atggaatacg cctctgacgc ttcactggac cccgaagccc cgtggcctcc cgcgccccgc         60

gctcgcgcct gccgcgtact gccttgggcc ctggtcgcgg ggctgctgct gctgctgctg        120

ctcgctgccg cctgcgccgt cttcctcgcc tgcccctggg ccgtgtccgg ggctcgcgcc        180

tcgcccggct ccgcggccag cccgagactc cgcgagggtc ccgagctttc gcccgacgat        240

cccgccggcc tcttggacct gcggcagggc atgtttgcgc agctggtggc ccaaaatgtt        300

ctgctgatcg atgggcccct gagctggtac agtgacccag gcctggcagg cgtgtccctg        360

acgggggcc tgagctacaa agaggacacg aaggagctgg tggtggccaa ggctggagtc        420

tactatgtct tctttcaact agagctgcgg cgcgtggtgg ccggcgaggg ctcaggctcc        480

gtttcacttg cgctgcacct gcagccactg cgctctgctg ctggggccgc cgccctggct        540

ttgaccgtgg acctgccacc cgcctcctcc gaggctcgga actcggcctt cggtttccag        600

ggccgcttgc tgcacctgag tgccggccag cgcctgggcg tccatcttca cactgaggcc        660

agggcacgcc atgcctggca gcttacccag ggcgccacag tcttgggact cttccgggtg        720

acccccgaaa tcccagccgg actcccttca ccgaggtcgg aataa                        765
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<210> 3

<211> 223
<212> PRT
<213> Artificial Sequence

<220>
<223> amino acid seqeunce for membrane bound IL-21 (mbIL-21)

<400> 3

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Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
  1               5                  10                  15

His Ala Ala Arg Pro Gln Asp Arg His Met Ile Arg Met Arg Gln Leu
             20                  25                  30

Ile Asp Ile Val Asp Gln Leu Lys Asn Tyr Val Asn Asp Leu Val Pro
         35                  40                  45

Glu Phe Leu Pro Ala Pro Glu Asp Val Glu Thr Asn Cys Glu Trp Ser
     50                  55                  60

Ala Phe Ser Cys Phe Gln Lys Ala Gln Leu Lys Ser Ala Asn Thr Gly
 65                  70                  75                  80

Asn Asn Glu Arg Ile Ile Asn Val Ser Ile Lys Lys Leu Lys Arg Lys
                 85                  90                  95

Pro Pro Ser Thr Asn Ala Gly Arg Arg Gln Lys His Arg Leu Thr Cys
            100                 105                 110

Pro Ser Cys Asp Ser Tyr Glu Lys Lys Pro Pro Lys Glu Phe Leu Glu
        115                 120                 125

Arg Phe Lys Ser Leu Leu Gln Lys Met Ile His Gln His Leu Ser Ser
    130                 135                 140

Arg Thr His Gly Ser Glu Asp Ser Ala Lys Pro Thr Thr Thr Pro Ala
145                 150                 155                 160

Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser
                165                 170                 175

Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr
            180                 185                 190

Arg Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala
        195                 200                 205

Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr
    210                 215                 220
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<210> 4
<211> 672
<212> DNA
<213> Artificial Sequence

<220>
<223> nucleotide sequence of membrane bound IL-21 (mbIL-21)

<400> 4
atggctctgc ccgtcaccgc tctgctgctg cctctggccc tgctgctgca cgccgcaaga 60

ccacaggacc gccacatgat tcggatgagg cagctgatcg acattgtgga tcagctgaag 120

aactacgtga atgacctggt ccccgagttc ctgcccgctc ctgaggatgt cgaaacaaac 180

tgcgaatggt ctgcattcag ttgttttcag aaggctcagc tgaaatctgc aaacactgga 240

aacaatgagc gaatcattaa tgtgagcatc aagaaactga agcggaaacc cccttccact 300

aatgcaggcc ggagacagaa gcaccgactg acctgcccct catgtgacag ctatgaaaag 360

aaaccaccca aagagttcct ggagcggttc aagagcctgc tccagaaaat gatccaccag 420

cacctgagca gccggaccca cggcagcgag gattctgcca agcctaccac aactccagct 480

ccccgccctc caacccctgc accaacaatt gccagtcagc ccctgtcact gaggcctgaa 540

gcatgccgcc cagccgctgg cggagcagtc cacacacgag gcctggactt tgcttgtgat 600

atctacattt gggcacctct ggctggaact tgcggcgtcc tgctgctgtc cctggtcatc 660

accctgtatt ga 672

<210> 5
<211> 183
<212> PRT
<213> Artificial Sequence

<220>
<223> amino acid seqeunce for OX40L

<400> 5
Met Glu Arg Val Gln Pro Leu Glu Glu Asn Val Gly Asn Ala Ala Arg
1 5 10 15

Pro Arg Phe Glu Arg Asn Lys Leu Leu Leu Val Ala Ser Val Ile Gln
20 25 30

Gly Leu Gly Leu Leu Leu Cys Phe Thr Tyr Ile Cys Leu His Phe Ser
35 40 45

```
Ala Leu Gln Val Ser His Arg Tyr Pro Arg Ile Gln Ser Ile Lys Val
     50                  55                  60

Gln Phe Thr Glu Tyr Lys Lys Glu Lys Gly Phe Ile Leu Thr Ser Gln
 65                  70                  75                  80

Lys Glu Asp Glu Ile Met Lys Val Gln Asn Asn Ser Val Ile Ile Asn
                 85                  90                  95

Cys Asp Gly Phe Tyr Leu Ile Ser Leu Lys Gly Tyr Phe Ser Gln Glu
            100                 105                 110

Val Asn Ile Ser Leu His Tyr Gln Lys Asp Glu Glu Pro Leu Phe Gln
        115                 120                 125

Leu Lys Lys Val Arg Ser Val Asn Ser Leu Met Val Ala Ser Leu Thr
    130                 135                 140

Tyr Lys Asp Lys Val Tyr Leu Asn Val Thr Thr Asp Asn Thr Ser Leu
145                 150                 155                 160

Asp Asp Phe His Val Asn Gly Gly Glu Leu Ile Leu Ile His Gln Asn
                165                 170                 175

Pro Gly Glu Phe Cys Val Leu
            180
```

<210> 6
<211> 552
<212> DNA
<213> Artificial Sequence

<220>
<223> nucleotide sequence of OX40L

<400> 6

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atggaacggg tgcaacctct ggaggagaat gtgggcaatg cagcccggcc aagatttgag         60

aggaacaagc tactgctggt ggcctctgtg atacagggac tggggctcct cctgtgcttc        120

acctacatct gcctgcactt cagcgctctt caggtgtcac accggtatcc ccgcatacaa        180

agtatcaagg tccagtttac cgagtataag aaagagaagg gtttcatcct cactagtcag        240

aaggaggatg aaatcatgaa ggtgcagaac aactcagtta ttattaactg tgacggcttt        300

tatctgatca gcctgaaggg ctacttctcc caggaagtaa acatcagcct gcattaccag        360

aaggatgagg agcccctgtt tcagctgaag aaggtccggt ccgtgaactc ccttatggtc        420

gcctctctga cgtacaagga caaggtgtac ctcaatgtga ccacagacaa tacatccctg        480
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gatgacttcc atgtgaatgg cggagaactg attctgattc accagaaccc tggggagttc 540

tgtgtcttgt ga 552

<210> 7
<211> 705
<212> DNA
<213> Homo sapiens

<400> 7
atgagcactg aaagcatgat ccgggacgtg gagctggccg aggaggcgct ccccaagaag 60

acaggggggc cccagggctc caggcggtgc ttgttcctca gcctcttctc cttcctgatc 120

gtggcaggcg ccaccacgct cttctgcctg ctgcactttg gagtgatcgg cccccagagg 180

gaagagttcc ccagggacct ctctctaatc agccctctgg cccaggcagt cagatcatct 240

tctcgaaccc cgagtgacaa gcctgtagcc catgttgtag caaaccctca agctgagggg 300

cagctccagt ggctgaaccg ccgggccaat gccctcctgg ccaatggcgt ggagctgaga 360

gataaccagc tggtggtgcc atcagagggc ctgtacctca tctactccca ggtcctcttc 420

aagggccaag gctgcccctc cacccatgtg ctcctcaccc acaccatcag ccgcatcgcc 480

gtctcctacc agaccaaggt caacctcctc tctgccatca agagcccctg ccagagggag 540

accccagagg gggctgaggc caagccctgg tatgagccca tctatctggg aggggtcttc 600

cagctggaga agggtgaccg actcagcgct gagatcaatc ggcccgacta tctcgacttt 660

gccgagtctg ggcaggtcta ctttgggatc attgccctgt gagga 705

<210> 8
<211> 233
<212> PRT
<213> Artificial Sequence

<220>
<223> amino acid seqeunce for mutated TNF alpha (mTNF-a)

<400> 8
Met Ser Thr Glu Ser Met Ile Arg Asp Val Glu Leu Ala Glu Glu Ala
1 5 10 15

Leu Pro Lys Lys Thr Gly Gly Pro Gln Gly Ser Arg Arg Cys Leu Phe
20 25 30

Leu Ser Leu Phe Ser Phe Leu Ile Val Ala Gly Ala Thr Thr Leu Phe
35 40 45

```
Cys Leu Leu His Phe Gly Val Ile Gly Pro Gln Arg Glu Glu Phe Pro
     50                  55                  60

Arg Asp Leu Ser Leu Ile Ser Pro Leu Ala Gln Pro Val Arg Ser Ser
 65                  70                  75                  80

Ser Arg Thr Pro Ser Asp Lys Pro Val Ala His Val Val Ala Asn Pro
                 85                  90                  95

Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn Ala Leu
            100                 105                 110

Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val Val Pro Ser
        115                 120                 125

Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly Gln Gly
    130                 135                 140

Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser Arg Ile Ala
145                 150                 155                 160

Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile Lys Ser Pro
                165                 170                 175

Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu
            180                 185                 190

Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Arg Leu
        195                 200                 205

Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe Ala Glu Ser Gly
    210                 215                 220

Gln Val Tyr Phe Gly Ile Ile Ala Leu
225                 230
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<210> 9
<211> 702
<212> DNA
<213> Artificial Sequence

<220>
<223> nucleotide sequence of mutated TNF alpha (mTNF-a)

<400> 9

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atgagcactg aaagcatgat ccgggacgtg gagctggccg aggaggcgct ccccaagaag         60

acaggggggc cccagggctc caggcggtgc ttgttcctca gcctcttctc cttcctgatc        120

gtggcaggcg ccaccacgct cttctgcctg ctgcactttg gagtgatcgg cccccagagg        180
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gaagagttcc ccagggacct ctctctaatc agccctctgg cccagccggt cagatcatct 240

tctcgaaccc cgagtgacaa gcctgtagcc catgttgtag caaaccctca agctgagggg 300

cagctccagt ggctgaaccg ccgggccaat gccctcctgg ccaatggcgt ggagctgaga 360

gataaccagc tggtggtgcc atcagagggc ctgtacctca tctactccca ggtcctcttc 420

aagggccaag gctgcccctc cacccatgtg ctcctcaccc acaccatcag ccgcatcgcc 480

gtctcctacc agaccaaggt caacctcctc tctgccatca agagcccctg ccagagggag 540

accccagagg gggctgaggc caagccctgg tatgagccca tctatctggg aggggtcttc 600

cagctggaga agggtgaccg actcagcgct gagatcaatc ggcccgacta tctcgacttt 660

gccgagtctg ggcaggtcta ctttgggatc attgccctgt ga 702

<210> 10
<211> 46
<212> DNA
<213> Artificial Sequence

<220>
<223> Forward primer for 4-1BBL (PCR)

<400> 10
tctagagcta gcgaattcgc caccatggaa tacgcctctg acgctt 46

<210> 11
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Reverse primer for 4-1BBL (PCR)

<400> 11
ttcgcggccg cggatcctta ttccgacctc ggtgaagg 38

<210> 12
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Forward primer for mbIL-21 (PCR)

<400> 12
tagagctagc gaattcgcca ccgccaccat ggctctgccc 40

<210> 13
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Reverse primer for mbIL-21 (PCR)

<400> 13
tcgcggccgc ggatcctcaa tacagggtga tgacc 35

<210> 14
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Forward primer for OX40L (PCR)

<400> 14
tagagctagc gaattcgcca ccatggaacg ggtgcaac 38

<210> 15
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Reverse primer for OX40L (PCR)

<400> 15
tcgcggccgc ggatcctcac aagacacaga actcccc 37

<210> 16
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Forward primer for mTNF-a (PCR)

<400> 16
tagagctagc gaattcgcca ccgccaccat ggctctgccc 40

<210> 17
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Reverse primer for mTNF-a (PCR)

<400> 17
tcgcggccgc ggatcctcac agggcaatga tccc 34

<210> 18
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Forward primer for 4-1BBL (RT-qPCR)

<400> 18
tctgagacag ggcatgtttg 20

<210> 19
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Reverse primer for 4-1BBL (RT-qPCR)

<400> 19
ccaccagttc tttggtgtcc 20

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

<220>
<223> Forward primer for mTNF-a (RT-qPCR)

<400> 20
aacctcctct ctgccatcaa 20

<210> 21
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Reverse primer for mTNF-a (RT-qPCR)

<400> 21
atagtcgggc cgattgatct 20

<210> 22
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Forward primer for mbIL-21 (RT-qPCR)

<400> 22
tggaaacaat gagcgaatca 20

<210> 23
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Reverse primer for mbIL-21 (RT-qPCR)

<400> 23
aaccgctcca ggaactcttt 20

<210> 24
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Forward primer for hTOP1 (RT-qPCR)

<400> 24
ccagacggaa gctcggaaac 20

<210> 25
<211> 22

<212> DNA
<213> Artificial Sequence

<220>
<223> Reverse primer for hTOP1 (RT-qPCR)

<400> 25
gtccaggagg ctctatcttg aa 22