

SEQUENCE LISTING

<110> QITAN TECHNOLOGY LTD., BEIJING

<120> MNEP MONOMER VARIANT AND APPLICATION THEREOF

<130> 1

<160> 18

<170> PatentIn version 3.5

<210> 1

<211> 191

<212> PRT

<213> Artificial Sequence

<220>

<223> wild-type Mnep monomer

<400> 1

Met Gly Leu Asp Asn Glu Leu Ser Leu Val Asp Gly Lys Asp Arg Thr

1 5 10 15

Leu Thr Ile Gln Gln Trp Asp Thr Phe Leu Asn Gly Val Phe Pro Leu

20 25 30

Asp Arg Asn Arg Leu Thr Arg Glu Trp Phe His Ser Gly Lys Ala Lys

35 40 45

Tyr Ile Val Ser Gly Pro Gly Ala Glu Asp Phe Glu Gly Val Leu Glu

50 55 60

Leu Gly Tyr Gln Val Gly Phe Pro Trp Ser Leu Gly Val Gly Ile Asn

65 70 75 80

Phe Ser Tyr Thr Thr Pro Asn Val Ala Leu Ser Gly Asp Pro Gly Phe

85 90 95

Gly Ile Thr Gly Ile Gly Gly Ala Leu Pro Val Ile Gly Glu Ile Ala

100 105 110

Thr Pro Pro Leu Phe Pro Gly Ala Ser Ile Ser Ala Asp Leu Gly Asn

115 120 125

Gly Pro Gly Ile Gln Glu Val Ala Thr Phe Ser Thr Glu Val Ala Gly

130 135 140

Pro Glu Gly Ala Val Ala Val Ser Asn Ala His Gly Thr Val Thr Gly

145 150 155 160

Ala Ala Gly Gly Val Leu Leu Arg Pro Phe Ala Arg Leu Val Ser Ser

165

170

175

Leu Gly Asp Ser Val Thr Thr Tyr Gly Glu Pro Trp Asn Met Asn

180

185

190

<210> 2

<211> 576

<212> DNA

<213> Artificial Sequence

<220>

<223> the nucleotide sequence encoding the Mnep monomer variant

<400> 2

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tggtttcaca gcggcaaagc caaatatatt gtgagcggcc cgggtgcaga agactttgaa 180

ggcgtgctgg agctgggcta tcaagttggt tttccgtgga gtctgggcgt ggcatcaac 240

tttagctaca ccacccgaa tgtggcactg agtaaatttc cgctgtttgg cattaccggc 300

attggcggca aactgccggt gattggcgaa attgcaacc cgccgctgtt tccgggtgca 360

agcattagcg ccgatttagg taacggtccg ggcatcaag aagttgccac cttagcacc 420

gaagtggctg gtccggaagg tgccgttgcc gtgagcaatg ctcattggcac tgtgactggg 480

gccgctgggtg gcgttttact gcgccggttt gcccgtttag tgagcagtct gggtgatagc 540

gtgaccacct atggcgaacc gtggaatatg aactaa 576

<210> 3

<211> 863

<212> PRT

<213> Artificial Sequence

<220>

<223> helicase EF8813-1

<400> 3

Met Gly Ser Ser His His His His His Ser Ser Gly Leu Val Pro

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Arg Gly Ser His Met Lys Ile Glu Ser Leu Asp Leu Pro Asp Glu Val

20 25 30

Lys Gln Phe Tyr Leu Asp Ser Gly Ile Leu Glu Leu Tyr Pro Pro Gln

35 40 45

Ala Glu Ala Val Glu Lys Gly Leu Leu Glu Gly Arg Asn Leu Leu Ala

50 55 60

Ala Ile Pro Thr Ala Ser Gly Lys Thr Leu Leu Ala Glu Leu Ala Met

65 70 75 80

Leu Lys Ser Ile Leu Asn Gly Gly Lys Ala Leu Tyr Ile Val Pro Leu

85 90 95

Arg Ala Leu Ala Ser Glu Lys Phe Lys Arg Phe Arg Glu Phe Ser Lys

100 105 110

Leu Gly Ile Arg Val Gly Ile Ser Thr Gly Asp Tyr Asp Leu Arg Asp

115 120 125

Glu Gly Leu Gly Val Asn Asp Ile Ile Val Ala Thr Ser Glu Lys Thr

130 135 140

Asp Ser Leu Leu Arg Asn Glu Thr Val Trp Met Gln Glu Ile Ser Val

145 150 155 160

Val Val Ala Asp Glu Val His Leu Ile Asp Ser Pro Asp Arg Gly Pro

165 170 175

Thr Leu Glu Ile Thr Leu Ala Lys Leu Arg Lys Met Asn Pro Ser Cys

180 185 190

Gln Ile Leu Ala Leu Ser Ala Thr Ile Gly Asn Ala Asp Glu Leu Ala

195 200 205

Ala Trp Leu Glu Ala Gly Leu Val Leu Ser Glu Trp Arg Pro Thr Glu

210 215 220

Leu Arg Glu Gly Val Phe Phe Asn Gly Thr Phe Tyr Cys Lys Asp Arg

225 230 235 240

Glu Lys Ser Ile Glu Gln Ser Thr Lys Asp Glu Ala Val Asn Leu Val

245 250 255

Leu Asp Thr Leu Arg Glu Asp Gly Gln Cys Leu Val Phe Glu Asn Ser

260 265 270

Arg Lys Asn Cys Met Ala Phe Ala Lys Lys Ala Ser Ser Ala Val Lys

275 280 285

Lys Ile Leu Ser Ala Glu Asp Lys Glu Ala Leu Ala Glu Ile Ala Asp

290 295 300

Glu Val Leu Glu Asn Ser Glu Thr Asp Thr Ser Ala Ala Leu Ala Ala

305 310 315 320

Cys Ile Arg Ser Gly Thr Ala Phe His His Ala Gly Leu Thr Thr Pro

325 330 335

Leu Arg Glu Leu Val Glu Asp Gly Phe Arg Ala Gly Lys Ile Lys Leu

340 345 350

Ile Ser Ser Thr Pro Thr Leu Ala Ala Gly Leu Asn Leu Pro Ala Arg

355 360 365

Arg Val Val Ile Arg Ser Tyr Arg Arg Tyr Ser Ser Glu Asp Gly Met

370 375 380

Gln Pro Ile Pro Val Ile Glu Tyr Lys Gln Met Ala Gly Arg Ala Gly

385 390 395 400

Arg Pro Arg Leu Asp Pro Tyr Gly Glu Ala Val Leu Val Ala Lys Ser

405 410 415

Tyr Glu Glu Phe Val Phe Leu Phe Arg Asn Tyr Ile Glu Ala Asp Ala

420 425 430

Glu Asp Ile Trp Ser Lys Leu Gly Thr Glu Asn Ala Leu Arg Thr His
435 440 445

Val Leu Ser Thr Ile Ser Asn Gly Phe Ala Arg Thr Lys Glu Glu Leu
450 455 460

Met Glu Phe Leu Glu Ala Thr Phe Phe Ala Phe Gln Tyr Ser Asn Phe
465 470 475 480

Gly Leu Ser Thr Val Val Asp Glu Cys Leu Asn Phe Leu Arg Gln Glu
485 490 495

Glu Met Leu Glu Lys Thr Asp Thr Leu Ile Ser Thr Ser Phe Gly Lys
500 505 510

Leu Val Ser Lys Leu Tyr Ile Asp Pro Leu Ser Ala Ala Arg Ile Val
515 520 525

Lys Gly Leu Lys Glu Ala Lys Ile Leu Thr Glu Leu Thr Leu Leu His
530 535 540

Leu Val Cys Ser Thr Pro Asp Met Arg Leu Leu Tyr Met Arg Asn Gln
545 550 555 560

Asp Tyr Gln Asp Ile Asn Asp Tyr Val Ile Ala His Ala Asp Glu Phe

565 570 575

Val Arg Val Pro Ser Pro Phe Asn Tyr Thr Glu Tyr Glu Trp Phe Leu

580 585 590

Gly Glu Val Lys Thr Ser Leu Leu Leu Val Asp Trp Ile His Glu Lys

595 600 605

Ser Glu Asn Glu Ile Cys Leu Lys Phe Gly Ile Gly Glu Gly Asp Ile

610 615 620

His Ala Ile Ala Asp Ile Ala Glu Trp Leu Met His Val Thr Ala Gln

625 630 635 640

Leu Ala Arg Leu Leu Glu Leu Lys Gly Ala Lys Glu Ala Ala Glu Leu

645 650 655

Glu Lys Arg Ile His Tyr Gly Ala Ser Pro Glu Leu Met Asp Leu Leu

660 665 670

Asp Ile Arg Gly Ile Gly Arg Met Arg Ala Arg Lys Leu Tyr Glu Ser

675 680 685

Gly Phe Arg Ser Ser Ala Glu Leu Ala Gly Ala Asp Pro Val Lys Val

690 695 700

Ala Ala Leu Leu Gly Pro Lys Ile Ala Asp Arg Ile Phe Lys Gln Ile

705 710 715 720

Gly Arg Arg Glu Val Leu Pro Glu Ile Ala Glu Pro Thr Leu Pro Glu

725 730 735

Lys Ser Pro Ser Ser Gly Gln Lys Thr Ile Asn Asp Tyr Gly Thr Gly

740 745 750

Gly Gly Gly Ser Trp Lys Glu Trp Leu Glu Arg Lys Val Gly Glu Gly

755 760 765

Arg Ala Arg Arg Leu Ile Glu Tyr Phe Gly Ser Ala Gly Glu Val Gly

770 775 780

Lys Leu Val Glu Asn Ala Glu Val Ser Lys Leu Leu Glu Val Pro Gly

785 790 795 800

Ile Gly Asp Glu Ala Val Ala Arg Leu Val Pro Gly Tyr Lys Thr Leu
805 810 815

Arg Asp Ala Gly Leu Thr Pro Ala Glu Ala Glu Arg Val Leu Lys Arg
820 825 830

Tyr Gly Ser Val Ser Lys Val Gln Glu Gly Ala Thr Pro Asp Glu Leu
835 840 845

Arg Glu Leu Gly Leu Gly Asp Ala Lys Ile Ala Arg Ile Leu Gly
850 855 860

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<211> 2592
<212> DNA
<213> Artificial Sequence

<220>
<223> helicase EF8813-1

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attttagaac tgtaccgcc gcaggcagaa gcagtagaaa aaggcctgtt agaaggacgt 180

aatctgctgg cagcaattcc gaccgcaagc ggtaagacac tgctggctga attagcaatg 240

ctgaaaagca tactgaatgg gggaaaagca ctgtatattg ttccgctgag agcactggca 300

tcagaaaaat ttaaactgtt tagagagttc agcaagttag gtataagagt gggatttagc 360

acaggagact atgacctgag agatgaaggt ctgggtgta atgatattat tgtggcaaca 420

agcgaaaaaa ccgatagcct gctgcgtaat gaaaccgtt ggatgcagga aattagcgtt 480

gttggtgcag atgaagtca tctgattgat agcccggatc gtggtccgac cctggaaatt 540

acctggcaa aactgcgtaa aatgaatccg agctgtcaga ttctggcact gagcgcaacc 600

attggaatg cagatgaact ggcagcatgg ctggaagcag gtctggttct gagcgaatgg 660

cgtccgaccg aactgcgtga aggtgtttt tttaatggta cattttattg taaagatcgt 720

gaaaaaagca ttgaacagag caccaaagat gaagcagtta atctggttct ggataccctg 780

cgtgaagatg gtcagtgtct ggtttttgaa aatagccgta aaaattgtat ggcatttgca 840

aaaaaagcaa gcagcgcagt taaaaaaatt ctgagcgag aagataaaga agcactggca 900

gaaattgcag atgaagttct ggaaaatagc gaaaccgata ccagcgagc actggcagca 960

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cgtccgctgc tggaccctta tggatgaagca gttctggtg caaaaagcta tgaagaattt 1260

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ctggcacgtc tgctggaact gaaaggtgca aaagaagcag cagaactgga aaaacgtatt 1980

cattatggtg caagcccgga actgatggat ctgctggata ttcgtggtat tggctgtatg 2040

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ctgaccccg gagaagcaga ggcgtgctg aagcgctacg gcagcgtcag caaagtgcag 2520

gaaggcgcaa cgccggacga attgcgcgag ttaggtctgg gcgacgcaa gattgccgcg 2580

attctgggtt aa 2592

<210> 5

<211> 54

<212> DNA

<213> Artificial Sequence

<220>

<223> X2

<400> 5

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<210> 6

<211> 45

<212> DNA

<213> Artificial Sequence

<220>

<223> X2

<400> 6

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<210> 7

<211> 15

<212> DNA

<213> Artificial Sequence

<220>

<223> X2

<400> 7

tttttgcagc agcat 15

<210> 8

<211> 45

<212> DNA

<213> Artificial Sequence

<220>

<223> cX2-80-15

<400> 8

aaccaaaaaa agatgggtgca ccaatgagaa cgggatagtg agaaa 45

<210> 9

<211> 40

<212> DNA

<213> Artificial Sequence

<220>

<223> cX2-80-15

<400> 9

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<210> 10

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> S1T

<400> 10

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<210> 11

<211> 576

<212> DNA

<213> Artificial Sequence

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<223> the nucleotide sequence encoding the Mnep monomer variant

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tggtttcaca gcggcaaagc caaatatatt gtgagcgcc cgggtgcaga agactttgaa 180

ggcgtgctgg agctgggcta tcaagttggt tttccgtgga gtctgggcgt ggcatcaac 240

tttagctaca ccacccgaa tgtggcactg agtaaatttc cgctgtttgg cattaccggc 300

attggcggca aactgccgtt gattggcgaa attgcaacc cgccgtgtt tccgggtgca 360

agcattagcg ccgatttagg taacgtccg ggcattcaag aagttgccac cttagcacc 420

gaagtggctg gtccggaagg tgccgttgc gtgagcaatg ctcattggcac tgtgactggt 480

gccgctggtg gcgttttact gcgccgttt gccggttag tgagcagtct gggatagc 540

gtgaccacct atggcgaacc gtggaatatg aactaa 576

<210> 12

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> X

<400> 12

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<210> 13

<211> 79

<212> DNA

<213> Artificial Sequence

<220>

<223> X

<400> 13

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caggtcggtg gtcgcagta 79

<210> 14

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> S1MC

<400> 14

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<210> 15

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> S1MC

<400> 15

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<210> 16

<211> 84

<212> DNA

<213> Artificial Sequence

<220>

<223> Point mutation primers

<400> 16

ccacccgaa tgtggcactg agtaaatttc cgctgtttgg cattaccggc attggcggca 60

aactgccggt gattggcgaa attg 84

<210> 17

<211> 84

<212> DNA

<213> Artificial Sequence

<220>

<223> Point mutation primers

<400> 17

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tactcagtgc cacattcggg gtgg 84

<210> 18

<211> 191

<212> PRT

<213> Artificial Sequence

<220>

<223> the Mnep monomer variant

<400> 18

Met Gly Leu Asp Asn Glu Leu Ser Leu Val Asp Gly Lys Asp Arg Thr

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20 25 30

Asp Arg Asn Arg Leu Thr Arg Glu Trp Phe His Ser Gly Lys Ala Lys

35 40 45

Tyr Ile Val Ser Gly Pro Gly Ala Glu Asp Phe Glu Gly Val Leu Glu

50 55 60

Leu Gly Tyr Gln Val Gly Phe Pro Trp Ser Leu Gly Val Gly Ile Asn

65 70 75 80

Phe Ser Tyr Thr Thr Pro Asn Val Ala Leu Ser Lys Phe Pro Leu Phe

85 90 95

Gly Ile Thr Gly Ile Gly Gly Lys Leu Pro Val Ile Gly Glu Ile Ala

100 105 110

Thr Pro Pro Leu Phe Pro Gly Ala Ser Ile Ser Ala Asp Leu Gly Asn

115 120 125

Gly Pro Gly Ile Gln Glu Val Ala Thr Phe Ser Thr Glu Val Ala Gly

130 135 140

Pro Glu Gly Ala Val Ala Val Ser Asn Ala His Gly Thr Val Thr Gly

145 150 155 160

Ala Ala Gly Gly Val Leu Leu Arg Pro Phe Ala Arg Leu Val Ser Ser

165 170 175

Leu Gly Asp Ser Val Thr Thr Tyr Gly Glu Pro Trp Asn Met Asn

180 185 190