

SEQUENCE LISTING

<110> GlucoMetrix AG
 5 <120> Chimeric Polypeptide comprising a Membrane Protein and an Insulin Precursor
 <130> GLU 2002 PCT
 10 <150> 11000500.6
 <151> 2011-01-21
 <160> 12
 15 <170> PatentIn version 3.5
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 25 <400> 1
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 1 5 10 15
 30 Met Gly Leu Ala Ala Ile Gly Ala Ala Ile Gly Ile Gly Ile Leu Gly
 20 25 30
 35 Gly Lys Phe Leu Glu Gly Ala Ala Arg Gln Pro Asp Leu Ile Pro Leu
 35 40 45
 40 Leu Arg Thr Gln Phe Phe Ile Val Met Gly Leu Val Asp Ala Ile Pro
 50 55 60
 45 Met Ile Ala Val Gly Leu Gly Leu Tyr Val Met
 65 70 75
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 55 <400> 2
 Gly Leu Ala Ala Ile Gly Ala Ala Ile Gly Ile Gly Ile Leu Gly Gly
 1 5 10 15
 60 Lys Phe Leu Glu Gly Ala Ala Arg Gln Pro Asp Leu Ile Pro
 20 25 30
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5  <210> 3
   <211> 85
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   <220>
   <223> amino acid sequence comprising human insulin chains A and B being
10  separated by a fragment of the ATP synthase subunit Foc of E.
      coli

   <400> 3

15  Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu
      1             5             10             15

      Glu Asn Tyr Cys Asn Arg Lys Gly Leu Ala Ala Ile Gly Ala Ala Ile
20              20             25             30

      Gly Ile Gly Ile Leu Gly Gly Lys Phe Leu Glu Gly Ala Ala Arg Gln
25              35             40             45

      Pro Asp Leu Ile Pro Asn Met Phe Val Asn Gln His Leu Cys Gly Ser
30              50             55             60

      His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe
35              65             70             75             80

      Tyr Thr Pro Lys Thr
35              85

40  <210> 4
   <211> 89
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   <213> Artificial Sequence

   <220>
   <223> amino acid sequence comprising human insulin chains A and B being
45  separated by a fragment of the ATP synthase subunit Foc of E.
      coli and containing a His-tag of four histidine residues

   <400> 4

50  Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu
      1             5             10             15

      Glu Asn Tyr Cys Asn Arg Lys Gly Leu Ala Ala Ile Gly Ala Ala Ile
55              20             25             30

      Gly Ile Gly Ile Leu Gly Gly Lys Phe Leu Glu Gly Ala Ala Arg Gln
60              35             40             45

      Pro Asp Leu Ile Pro His His His His Asn Met Phe Val Asn Gln His
65              50             55             60

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Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu
 65 70 75 80

5 Arg Gly Phe Phe Tyr Thr Pro Lys Thr
 85

10 <210> 5
 <211> 164
 <212> PRT
 <213> Artificial Sequence

15 <220>
 <223> amino acid sequence of a chimeric polypeptide having the
 structure: P' - Met - insulin A-chain - Lys - Arg - C' - His-tag
 - Met - insulin B-chain

20 <400> 5
 Met Glu Asn Leu Asn Met Asp Leu Leu Tyr Met Ala Ala Ala Val Met
 1 5 10 15

25 Met Gly Leu Ala Ala Ile Gly Ala Ala Ile Gly Ile Gly Ile Leu Gly
 20 25 30

30 Gly Lys Phe Leu Glu Gly Ala Ala Arg Gln Pro Asp Leu Ile Pro Leu
 35 40 45

35 Leu Arg Thr Gln Phe Phe Ile Val Met Gly Leu Val Asp Ala Ile Pro
 50 55 60

40 Met Ile Ala Val Gly Leu Gly Leu Tyr Val Met Gly Ile Val Glu Gln
 65 70 75 80

40 Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
 85 90 95

45 Arg Lys Gly Leu Ala Ala Ile Gly Ala Ala Ile Gly Ile Gly Ile Leu
 100 105 110

50 Gly Gly Lys Phe Leu Glu Gly Ala Ala Arg Gln Pro Asp Leu Ile Pro
 115 120 125

55 His His His His Asn Met Phe Val Asn Gln His Leu Cys Gly Ser His
 130 135 140

60 Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr
 145 150 155 160

60 Thr Pro Lys Thr

65 <210> 6

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<211> 495
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5  <220>
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        structure: P' - Met - insulin A-chain - Lys - Arg - C' - His-tag
        - Met - insulin B-chain

10  <220>
    <221> CDS
    <222> (1)..(492)

15  <400> 6
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    Met Glu Asn Leu Asn Met Asp Leu Leu Tyr Met Ala Ala Ala Val Met
    1          5          10          15

20  atg ggt ctg gcg gca atc ggt gct gcg atc ggt atc ggc atc ctc ggg      96
    Met Gly Leu Ala Ala Ile Gly Ala Ala Ile Gly Ile Gly Ile Leu Gly
          20          25          30

25  ggt aaa ttc ctg gaa ggc gca gcg cgt caa cct gat ctg att cct ctg      144
    Gly Lys Phe Leu Glu Gly Ala Ala Arg Gln Pro Asp Leu Ile Pro Leu
          35          40          45

30  ctg cgt act cag ttc ttt atc gtt atg ggt ctg gtg gat gct atc ccg      192
    Leu Arg Thr Gln Phe Phe Ile Val Met Gly Leu Val Asp Ala Ile Pro
          50          55          60

35  atg atc gct gta ggt ctg ggt ctg tac gtg atg ggc att gtt gaa cag      240
    Met Ile Ala Val Gly Leu Gly Leu Tyr Val Met Gly Ile Val Glu Gln
    65          70          75          80

40  tgc tgc acc tcg ata tgc agc ttg tat cag ttg gaa aat tat tgc aat      288
    Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
          85          90          95

45  agg aag ggt ctg gcg gca atc ggt gct gcg atc ggt atc ggc atc ctc      336
    Arg Lys Gly Leu Ala Ala Ile Gly Ala Ala Ile Gly Ile Gly Ile Leu
          100          105          110

50  ggg ggt aaa ttc ctg gaa ggc gca gcg cgt caa cct gat ctg att cct      384
    Gly Gly Lys Phe Leu Glu Gly Ala Ala Arg Gln Pro Asp Leu Ile Pro
          115          120          125

55  cat cac cac cat aat atg ttc gtt aat caa cac tta tgc ggt agt cac      432
    His His His His Asn Met Phe Val Asn Gln His Leu Cys Gly Ser His
          130          135          140

60  tta gtt gag gct tta tat tta gtt tgc ggt gaa cgt ggt ttc ttc tat      480
    Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr
    145          150          155          160

65  acc cct aaa act tag      495
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<210> 7
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<220>
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 10 Met Gly Leu Ala Ala Ile Gly Ala Ala Ile Gly Ile Gly Ile Leu Gly
 20 25 30
 15 Gly Lys Phe Leu Glu Gly Ala Ala Arg Gln Pro Asp Leu Ile Pro Leu
 35 40 45
 20 Leu Arg Thr Gln Phe Phe Ile Val Met Gly Leu Val Asp Ala Ile Pro
 50 55 60
 Met Ile Ala Val Gly Leu Gly Leu Tyr Val Met Gly Ile Val Glu Gln
 65 70 75 80
 25 Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
 85 90 95
 30 Arg Lys Gly Leu Ala Ala Ile Gly Ala Ala Ile Gly Ile Gly Ile Leu
 100 105 110
 35 Gly Gly Lys Phe Leu Glu Gly Ala Ala Arg Gln Pro Asp Leu Ile Pro
 115 120 125
 40 His His His His Asn Met Phe Val Asn Gln His Leu Cys Gly Ser His
 130 135 140
 Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr
 145 150 155 160
 45 Thr Pro Lys Thr
 50 <210> 8
 <211> 774
 <212> DNA
 <213> Artificial Sequence
 55 <220>
 <223> artificial sequence coding for a chimeric peptide having the
 structure: P' - Met - insulin A-chain - Lys - Arg - C' - His-tag
 - Met - insulin B-chain - Stop - rbs
 60 <220>
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10	atg ggt ctg gcg gca atc ggt gct gcg atc ggt atc ggc atc ctc ggg Met Gly Leu Ala Ala Ile Gly Ala Ala Ile Gly Ile Gly Ile Leu Gly 20 25 30	96
15	ggg aaa ttc ctg gaa ggc gca gcg cgt caa cct gat ctg att cct ctg Gly Lys Phe Leu Glu Gly Ala Ala Arg Gln Pro Asp Leu Ile Pro Leu 35 40 45	144
20	ctg cgt act cag ttc ttt atc gtt atg ggt ctg gtg gat gct atc ccg Leu Arg Thr Gln Phe Phe Ile Val Met Gly Leu Val Asp Ala Ile Pro 50 55 60	192
25	atg atc gct gta ggt ctg ggt ctg tac gtg atg ggc att gtt gaa cag Met Ile Ala Val Gly Leu Gly Leu Tyr Val Met Gly Ile Val Glu Gln 65 70 75 80	240
30	tgc tgc acc tcg ata tgc agc ttg tat cag ttg gaa aat tat tgc aat Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn 85 90 95	288
35	agg aag ggt ctg gcg gca atc ggt gct gcg atc ggt atc ggc atc ctc Arg Lys Gly Leu Ala Ala Ile Gly Ala Ala Ile Gly Ile Gly Ile Leu 100 105 110	336
40	ggg ggt aaa ttc ctg gaa ggc gca gcg cgt caa cct gat ctg att cct Gly Gly Lys Phe Leu Glu Gly Ala Ala Arg Gln Pro Asp Leu Ile Pro 115 120 125	384
45	cat cac cac cat aat atg ttc gtt aat caa cac tta tgc ggt agt cac His His His His Asn Met Phe Val Asn Gln His Leu Cys Gly Ser His 130 135 140	432
50	tta gtt gag gct tta tat tta gtt tgc ggt gaa cgt ggt ttc ttc tat Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr 145 150 155 160	480
55	acc cct aaa act tagtaagcgt tgcttttatt taaagagcaa tatcagaacg Thr Pro Lys Thr	532
60	ttaactaaat agaggcattg tgt atg aat ctt aac gca aca atc ctc ggc cag Met Asn Leu Asn Ala Thr Ile Leu Gly Gln 165 170	585
65	gcc atc gcg ttt gtc ctg ttc gtt ctg ttc tct atg aag tac gta tgg Ala Ile Ala Phe Val Leu Phe Val Leu Phe Ser Met Lys Tyr Val Trp 175 180 185 190	633
70	ccg cct tta atg gca gcc atc gaa aaa cgt caa aaa gaa att gct gac Pro Pro Leu Met Ala Ala Ile Glu Lys Arg Gln Lys Glu Ile Ala Asp 195 200 205	681
75	ggc ctt gct tcc gca gaa cga gca cat aag gac ctt gac ctt gca aag Gly Leu Ala Ser Ala Glu Arg Ala His Lys Asp Leu Asp Leu Ala Lys 210 215 220	729
80	gcc agc gcg acc gac cag ctg aaa aaa gcg aaa gcg gaa gct tag	774

Ala Ser Ala Thr Asp Gln Leu Lys Lys Ala Lys Ala Glu Ala
 225 230 235

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10 <220>
 <223> Synthetic Construct
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 1 5 10 15

20 Met Gly Leu Ala Ala Ile Gly Ala Ala Ile Gly Ile Gly Ile Leu Gly
 20 25 30

25 Gly Lys Phe Leu Glu Gly Ala Ala Arg Gln Pro Asp Leu Ile Pro Leu
 35 40 45

30 Leu Arg Thr Gln Phe Phe Ile Val Met Gly Leu Val Asp Ala Ile Pro
 50 55 60

35 Met Ile Ala Val Gly Leu Gly Leu Tyr Val Met Gly Ile Val Glu Gln
 65 70 75 80

40 Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
 85 90 95

45 Arg Lys Gly Leu Ala Ala Ile Gly Ala Ala Ile Gly Ile Gly Ile Leu
 100 105 110

50 Gly Gly Lys Phe Leu Glu Gly Ala Ala Arg Gln Pro Asp Leu Ile Pro
 115 120 125

55 His His His His Asn Met Phe Val Asn Gln His Leu Cys Gly Ser His
 130 135 140

60 Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr
 145 150 155 160

65 Thr Pro Lys Thr

60 <210> 10
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 <212> PRT
 <213> Artificial Sequence

65 <220>
 <223> Synthetic Construct

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  1          5          10          15

Phe Val Leu Phe Ser Met Lys Tyr Val Trp Pro Pro Leu Met Ala Ala
10      20          25          30

Ile Glu Lys Arg Gln Lys Glu Ile Ala Asp Gly Leu Ala Ser Ala Glu
15      35          40          45

Arg Ala His Lys Asp Leu Asp Leu Ala Lys Ala Ser Ala Thr Asp Gln
20      50          55          60

Leu Lys Lys Ala Lys Ala Glu Ala
25      65          70

<210> 11
<211> 333
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<220>
30 <223> human proinsulin coding sequence

<220>
35 <221> CDS
    <222> (1)..(330)

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    Met Ala Leu Trp Met Arg Leu Leu Pro Leu Leu Ala Leu Leu Ala Leu
    1          5          10          15          48

tgg gga cct gac cca gcc gca gcc ttt gtg aac caa cac ctg tgc ggc
45 Trp Gly Pro Asp Pro Ala Ala Ala Phe Val Asn Gln His Leu Cys Gly
    20          25          30          96

tca cac ctg gtg gaa gct ctc tac cta gtg tgc ggg gaa cga ggc ttc
50 Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
    35          40          45          144

ttc tac aca ccc aag acc cgc cgg gag gca gag gac ctg cag gtg ggg
55 Phe Tyr Thr Pro Lys Thr Arg Arg Glu Ala Glu Asp Leu Gln Val Gly
    50          55          60          192

cag gtg gag ctg ggc ggg ggc cct ggt gca ggc agc ctg cag ccc ttg
60 Gln Val Glu Leu Gly Gly Gly Pro Gly Ala Gly Ser Leu Gln Pro Leu
    65          70          75          80          240

gcc ctg gag ggg tcc ctg cag aag cgt ggc att gtg gaa caa tgc tgt
65 Ala Leu Glu Gly Ser Leu Gln Lys Arg Gly Ile Val Glu Gln Cys Cys
    85          90          95          288

acc agc atc tgc tcc ctc tac cag ctg gag aac tac tgc aac tag
70 Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
    100          105          110          333
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<210> 12
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 <212> PRT
 5 <213> Artificial Sequence

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 <223> Synthetic Construct
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 15 Trp Gly Pro Asp Pro Ala Ala Ala Phe Val Asn Gln His Leu Cys Gly
 20 25 30
 20 Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
 35 40 45
 25 Phe Tyr Thr Pro Lys Thr Arg Arg Glu Ala Glu Asp Leu Gln Val Gly
 50 55 60
 30 Gln Val Glu Leu Gly Gly Gly Pro Gly Ala Gly Ser Leu Gln Pro Leu
 65 70 75 80
 35 Ala Leu Glu Gly Ser Leu Gln Lys Arg Gly Ile Val Glu Gln Cys Cys
 85 90 95
 40 Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
 100 105 110