**I. Complete Sequence of Construct (both strands and amino acid sequence): Using 4 pc. Assembly**

**5’-GTCTCGATCC CGCGAAATAA TACGACTCAC TATAGGGAGC ACACAACGAG TTCCCTCTAG AAATAATTTT GTTTAACTTT**

**3’-cagagctagg gcgctttatt atgctgagtg atatccctcg tgtgttgctc aagggagatc tttattaaaa caaattgaaa**

**AA**GA**AGGAGG TATACCATG-3’**

**ttCTTCCTCC ATATGGTAC-5’**

001 010 020

**Q V Q L Q E S G G G L V Q A G G S L R L**

**CAG GTT CAG CTG CAA GAA AGC GGT GGT GGT CTG GTT CAG GCA GGC GGT AGC CTG CGT CTG**

**gtc caa gtc gac gtt ctt tcg cca cca cca gac caa gtc cgt ccg cca tcg gac gca gac**

021 **027 028 029** 030 **032 033 034** **036** 040

**S C A A S G R A A S G H G H Y G M G W F**

**AGC TGT GCA AGC AGC GGT DKT DMA DMA TCA GGT MWK DRT MWK TAT DKT ATG GGT TGG TTT**

**tcg aca cgt tcg tcg cca gca cgt cgt agt cca gta cca gta ata cca TAC CCA ACC AAA**

041 050  **055** **056**  060

**R Q V P G K E R E F V A A I R W S G K E**

**CGT CAG GTT CCG ggt aaa gaa cgt gaa ttt gtt gca gca att dkt dkk agc ggt aaa gaa**

**GCA GTC CAA GGC CCA TTT CTT GCA CTT AAA CAA CGT CGT TAA ZMA ZMM TCG CCA TTT CTT**

061 070 080

**T W Y K D S V K G R F T I S R D N A K T**

**acc tgg tat aaa gat agc gtg aaa GGT CGT TTT ACC ATC AGC CGT GAT AAT GCA AAA** **ACC**

**TGG ACC ATA TTT CTA TCG CAC TTT CCA GCA AAA TGG TAG TCG GCA CTA att acg ttt TTG**

081 090 100

**T V Y L Q M N S L K G E D T A V Y Y C A**

**ACC GTT TAC CTG CAG ATG AAT AGT CTG AAA GGT GAA GAT ACG GCA GTG TAT TAT TGT GCA**

**gtg gca aat gga cgt cta ctt atc aga ctt tcc act tct** **tgc** **CGT CAC ATA ATA ACA CGT**

101 **103 104 106 107** 110 120

**A R P V R V A D I S L P V G F D Y W G Q**

**GCA CGT mmk dwt cgt dwt dma gat att agc ctg ccg gtt ggt ttt gat tat tgg ggc cag**

**CGT GCA KKM ZWA GCA ZWA ZKT** **CTA TAA TCG GAC GGC CAA CCA AAA CTA ATA ACC CCG GTC**

121 128

**G T Q V T V S S**

**ggg acc cag gtt acc gtt agc agc**

**CCC TGG GTC CAA TGG CAA TCG TCG**

**NbPRBS1: (108 bases) \*Current Oligo**

**5’-GTCTCGATCC CGCGAAATAA TACGACTCAC TATAGGGAGC ACACAACGAG TTCCCTCTAG AAATAATTTT GTTTAACTTT**

**AA**GA**AGGAGG TATACCATG GGT CAG CTG-3’**

**NbPRBS1: (108 bases + 8 bases NE + - 9 bases (old Nb) + 24 bases (new Nb overlap) = 131 bases \*Desired Oligo**

**(Post PCR)**

**5’- NCTTCTCG GTCTCGATCC CGCGAAATAA TACGACTCAC TATAGGGAGC ACACAACGAG TTCCCTCTAG AAATAATTTT GTTTAACTTT**

**AA**GA**AGGAGG TATACCATG CAG GTT CAG CTG CAA GAA AGC GGT -3’**

**Synthesize:**

**NbCDS1: (132 bases)**

**5’ -CAG GTT CAG CTG CAA GAA AGC GGT GGT GGT CTG GTT CAG GCA GGC GGT AGC CTG CGT CTG**

**AGC TGT GCA AGC AGC GGT DKT DMA DMA TCA GGT MWK DRT MWK TAT DKT ATG GGT TGG TTT**

**CGT CAG GTT CCG -3’**

**NbCDS2: (120 bases)**

**3’ TAC CCA ACC AAA**

**GCA GTC CAA GGC CCA TTT CTT GCA CTT AAA CAA CGT CGT TAA ZMA ZMM TCG CCA TTT CTT**

**TGG ACC ATA TTT CTA TCG CAC TTT CCA GCA AAA TGG TAG TCG GCA CTA-5’**

**Synthesize:**

**5’ ATC ACG GCT GAT**

**GGT AAA ACG ACC TTT CAC GCT ATC TTT ATA CCA GGT TTC TTT ACC GCT MMZ AMZ AAT TGC**

**TGC AAC AAA TTC ACG TTC TTT ACC CGG AAC CTG ACG AAA CCA ACC CAT -3’**

**NbCDS3: (102 bases)**

**5’ -GGT CGT TTT ACC ATC AGC CGT GAT AAT GCA AAA** **ACC**

**ACC GTT TAC CTG CAG ATG AAT AGT CTG AAA GGT GAA GAT ACG GCA GTG TAT TAT TGT GCA**

**GCA CGT -3’**

**NbCDS4: (102 bases) + NE Site (8 bases) = 110 bases**

**3’- CGT CAC ATA ATA ACA CGT**

**CGT GCA KKM ZWA GCA ZWA ZKT** **CTA TAA TCG GAC GGC CAA CCA AAA CTA ATA ACC CCG GTC**

**CCC TGG GTC CAA TGG CAA TCG TCG NCTTCTCG -5’**

**Synthesize:**

**5’- GCTCTTCN GCT GCT AAC GGT AAC CTG GGT CCC CTG GCC CCA ATA ATC AAA ACC AAC CGG**

**CAG GCT AAT ATC TKZ** **AWZ ACG AWZ MKK ACG TGC TGC ACA ATA ATA CAC TGC -3’**

**II. Complete Sequence of Construct (both strands and amino acid sequence): Using OP Assembly**

Nt.BspQI  

001 010 020

**Q V Q L Q E S G G G L V Q A G G S L R L**

**CAG GTT CAG CTG CAA GAA AGC GGT GGT GGT CTG GTT CAG GCA GGC GGT AGC CTG CGT CTG**

**gtc caa gtc gac gtt ctt tcg cca cca cca gac caa gtc cgt ccg cca tcg gac gca gac**

021 **027 028 029** 030 **032 033 034** **036** 040

**S C A A S G R A A S G H G H Y G M G W F**

**AGC TGT GCA AGC AGC GGT DKT DMA DMA TCA GGT MWK DRT MWK TAT DKT ATG GGT TGG TTT**

**tcg aca cgt tcg tcg cca gca cgt cgt agt cca gta cca gta ata cca tac cca acc aaa**

041 050  **055** **056**  060

**R Q V P G K E R E F V A A I R W S G K E**

**CGT CAG GTT CCG GGT AAA GAA CGT GAA TTT GTT GCA GCA ATT DKT DKK AGC GGT AAA GAA**

**gca gtc caa ggc cca ttt ctt gca ctt aaa caa cgt cgt taa cgt tgg TCG CCA TTT CTT**

061 070 080

**T W Y K D S V K G R F T I S R D N A K T**

**ACC TGG TAT AAA gat agc gtg aaa ggt cgt ttt acc atc agc cgt gat aat gca aaa** **acc**

**TGG ACC ATA TTT CTA TCG CAC TTT CCA GCA AAA TGG TAG TCG GCA CTA ATT ACG TTT TTG**

081 090 100

**T V Y L Q M N S L K G E D T A V Y Y C A**

**acc gtt tac ctg cag atg aat agt ctg aaa ggt gaa gat acg GCA GTG TAT TAT TGT GCA**

**GTG GCA AAT GGA CGT CTA CTT ATC AGA CTT TCC ACT TCT** **TGC** **CGT CAC ATA ATA ACA CGT**

101 **103 104 106 107** 110 120

**A R P V R V A D I S L P V G F D Y W G Q**

**GCA CGT CCG GTT CGT GTT GCA GAT ATT AGC CTG CCG GTT GGT TTT GAT TAT TGG GGC CAG**

**CGT GCA MMK DWA GCA DWA DMT** **CTA TAA TCG GAC GGC CAA CCA AAA CTA ATA ACC CCG GTC**

121 128

**G T Q V T V S S**

**GGG ACC CAG GTT ACC GTT AGC AGC**

**CCC TGG GTC CAA TGG CAA TCG TCG**

**NbCDS1: (132 bases + 8 bases NE site) = 140 bases**

**5’ - GCTCTTCN CAG GTT CAG CTG CAA GAA AGC GGT GGT GGT CTG GTT CAG GCA GGC GGT AGC CTG CGT CTG**

**AGC TGT GCA AGC AGC GGT DKT DMA DMA TCA GGT MWK DRT MWK TAT DKT ATG GGT TGG TTT**

**CGT CAG GTT CCG- 3’**

**NbCDS2: (84 bases)**

**5’ -ATG GGT TGG TTT**

**CGT CAG GTT CCG GGT AAA GAA CGT GAA TTT GTT GCA GCA ATT DKT DKK AGC GGT AAA GAA**

**ACC TGG TAT AAA- 3’**

**NbCDS3: (138 bases + 8 bases NE site) = 146 bases**

**3’ -TCG CCA TTT CTT**

**TGG ACC ATA TTT CTA TCG CAC TTT CCA GCA AAA TGG TAG TCG GCA CTA ATT ACG TTT TTG**

**GTG GCA AAT GGA CGT CTA CTT ATC AGA CTT TCC ACT TCT** **TGC** **CGT CAC ATA ATA ACA CGT**

**CGT GCA NCTTCTCG -5’**

**Synthesize:**

**5’ – GCTCTTCN ACG TGC TGC ACA ATA ATA CAC TGC CGT TCT TCA CCT TTC AGA CTA TTC ATC TGC AGG TAA ACG GTG GTT TTT GCA TTA ATC ACG GCT GAT GGT AAA ACG ACC TTT CAC GCT ATC TTT ATA CCA GGT TTC TTT ACC GCT -3’**

**NbCDS4: (102 bases + 8 bases NE site) = 110 bases**

**3’ -CGT CAC ATA ATA ACA CGT**

**CGT GCA MMK DWA GCA DWA DMT** **CTA TAA TCG GAC GGC CAA CCA AAA CTA ATA ACC CCG GTC**

**CCC TGG GTC CAA TGG CAA TCG TCG NCTTCTCG - 5’**

**Synthesize:**

**5’ -GCTCTTCN GCT GCT AAC GGT AAC CTG GGT CCC CTG GCC CCA ATA ATC AAA ACC AAC CGG CAG GCT AAT ATC TMD AWD ACG AWD KMM ACG TGC TGC ACA ATA ATA CAC TGC - 3’**