

Construct Maps

pcDNA3.1

Courtesy of Drs. Linda Dong and Janine Steiger (CombinatoRx, Inc.)



Htt-Q23, [codon scheme], 1-3144, human

Cloning vector: pcDNA3.1(+), 5428 bp

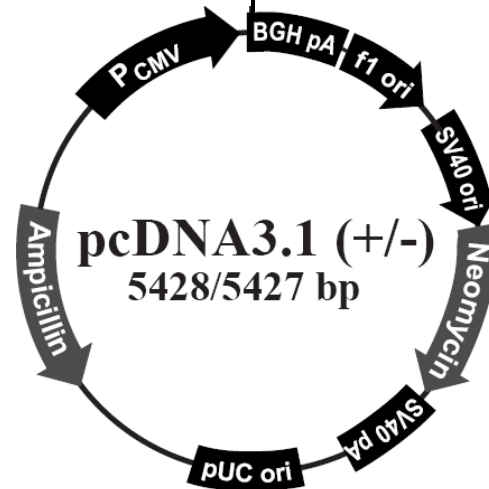
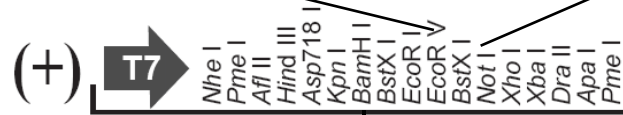
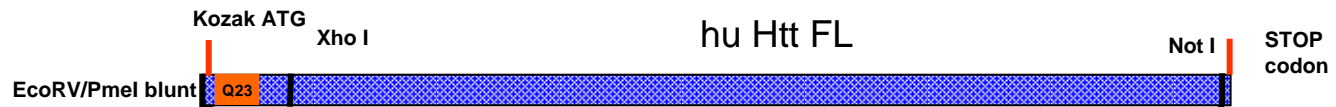
Insert: Full human Htt Q23 with XXXX codon, ~9.5 kb

Codon scheme: mix [CAA-CAG-CAG-CAA-CAG-CAA]_n; rdm: x/CAG/CAA/x; CAG: pure CAG.

Cloning site: fused EcoRV/PmeI at 5' and Not I at 3', RV and PmeI were deleted.

Diagnostic check: XhoI digestion should release two bands: 9 kb, 6 kb; Not I digestion should linearize the 15 kb band

Selection: Ampicillin



```

3' end of hCMV      putative transcriptional start
809 AAGCAGAGCT CTCTGGCTAA CTAGAGAACC CACTGCTTAC TGGCTTATCG AAATTAATAC
      T7 promoter/primer binding site      Nhe I      Pme I Afl II Hind III Asp718 I Kpn I
869 GACTCACTAT AGGGAGACCC AAGCTGGCTA GCGTTTAAAC TTAAGCTTGG TACCGAGCTC
      BamH I      BstX I* EcoR I      EcoR V      BstX I* Not I Xho I
929 GGATCCACTA GTCCAGTGTG GTGGAATTCT GCAGATATCC AGCACAGTGG CGGCCGCTCG
      Xba I      Apa I Pme I      pcDNA3.1/BGH reverse priming site
989 AGCTTAGAGG GCCCGTTTAA ACCCGCTGAT CAGCCTCGAC TGTGCCTTCT AGTTGCCAGC

1049 CATCTGTTGT TTGCCCCCTC CCCGTGCCTT CCTTGACCCT GGAAGGTGCC ACTCCCACTG
    
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Htt-Q73, [codon scheme], 1-3144, human

Cloning vector: pcDNA3.1(+), 5428 bp

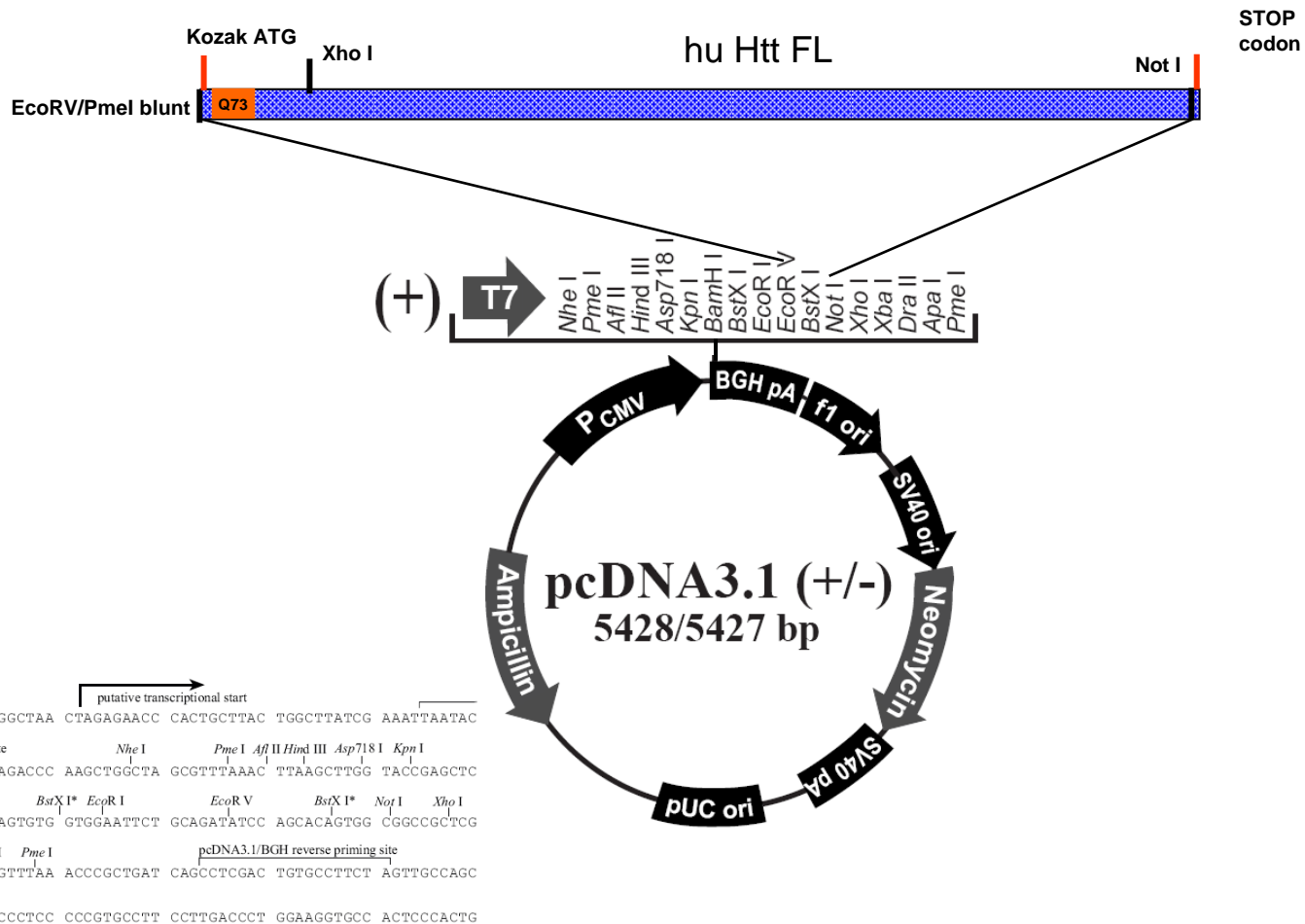
Insert: Full human Htt Q73 with XXX codon, ~9.6 kb

Codon scheme: mix [CAA-CAG-CAG-CAA-CAG-CAA]_n; rdm: x/CAG/CAA/x; CAG: pure CAG.

Cloning site: fused EcoRV/PmeI at 5' and Not I at 3', RV and PmeI were deleted.

Diagnostic check: XhoI digestion should release two bands: 9 kb, 6 kb; Not I digestion should linearize the 15 kb band.

Selection: Ampicillin



Htt-Q145, [codon scheme], 1-3144, human

Cloning vector: pcDNA3.1(+), 5428 bp

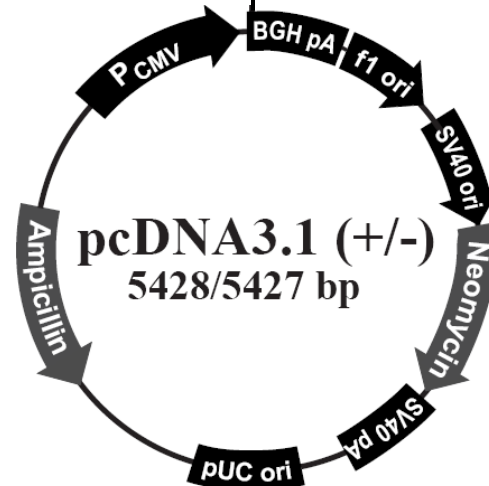
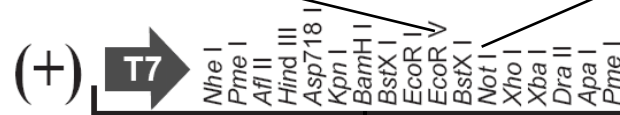
Insert: Full human Htt Q145 with XXX codon, ~9.8 kb

Codon scheme: mix [CAA-CAG-CAG-CAA-CAG-CAA]_n; rdm: x/CAG/CAA/x;

Cloning site: fused EcoRV/PmeI at 5' and Not I at 3', RV and PmeI were deleted.

Diagnostic check: XhoI digestion should release two bands: 9.2kb, 6kb; Not I digestion should linearize the 15.2kb band.

Selection: Ampicillin



```

      3' end of hCMV      putative transcriptional start
      |                  |
809  AAGCAGAGCT CTCTGGCTAA CTAGAGAACC CACTGCTTAC TGGCTTATCG AAATTAATAC
      T7 promoter/primer binding site      Nhe I      Pme I Afl II Hind III Asp718 I Kpn I
869  GACTCACTAT AGGGAGACCC AAGCTGGCTA GCGTTTAAAC TTAAGCTTGG TACCGAGCTC
      BamH I      BstX I* EcoR I      EcoR V      BstX I* Not I      Xho I
929  GGATCCACTA GTCCAGTGTG GTGGAATTCT GCAGATATCC AGCACAGTGG CGGCCGCTCG
      Xba I      Apa I      Pme I      pcDNA3.1/BGH reverse priming site
989  AGTCTAGAGG GCCCGTTTAA ACCCGCTGAT CAGCCTCGAC TGTGCCTTCT AGTTGCCAGC

1049 CATCTGTTGT TTGCCCCCTC CCCGTGCCTT CCTTGACCCT GGAAGGTGCC ACTCCCACTG
    
```

Htt-Q23, [codon scheme], 1-90, human

Cloning vector: pcDNA3.1(+), 5428 bp

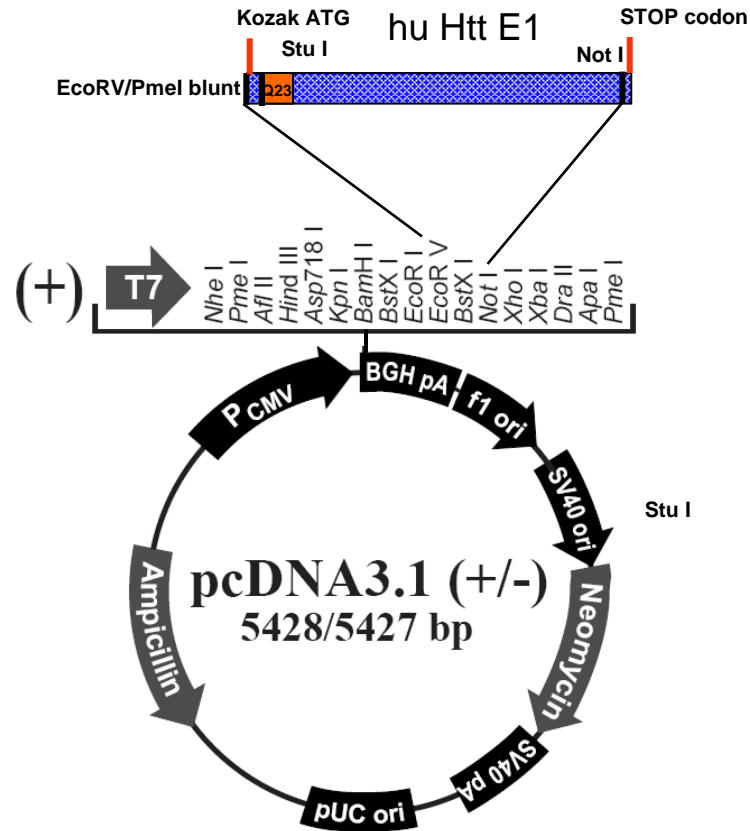
Insert: Human Htt Exon 1 with Q23 XXX codon, ~300 bp

Codon scheme: mix [CAA-CAG-CAG-CAA-CAG-CAA]_n; rdm: x/CAG/CAA/x; CAG: pure CAG

Cloning site: fused EcoRV/PmeI at 5' and Not I at 3', EcoRV and PmeI were deleted

Diagnostic check: BamHI / Not I digestion should release two bands (5.4kb,0.3kb); StuI digestion should release two bands: 4.4 kb, 1.3 kb.

Selection: Ampicillin



3' end of hCMV
 809 AAGCAGAGCT CTCTGGCTAA CTAGAGAACC CACTGCTTAC TGGCTTATCG AAATTAATAC
 putative transcriptional start
 T7 promoter/primer binding site
 869 GACTCACTAT AGGGAGACCC AAGCTGGCTA GCGTTTAAAC TTAAGCTTGG TACCGAGCTC
 Nhe I Pme I AfII Hind III Asp718 I Kpn I
 BamHI BstX I* EcoR I EcoR V BstX I* Not I Xho I
 929 GGATCCACTA GTCCAGTGTG GTGGAATTCT GCAGATATCC AGCACAGTGG CGGCCGCTCG
 Xba I Apa I Pme I
 989 AGTCTAGAGG GCCCGTTTAA ACCCGCTGAT CAGCCTCGAC TGTGCCTTCT AGTTGCCAGC
 pcDNA3.1/BGH reverse priming site
 1049 CATCTGTTGT TTGCCCCCTC CCCGTGCCTT CCTTGACCCT GGAAGGTGCC ACTCCCACTG

Htt-Q73, [codon scheme], 1-90, human

Cloning vector: pcDNA3.1(+), 5428 bp

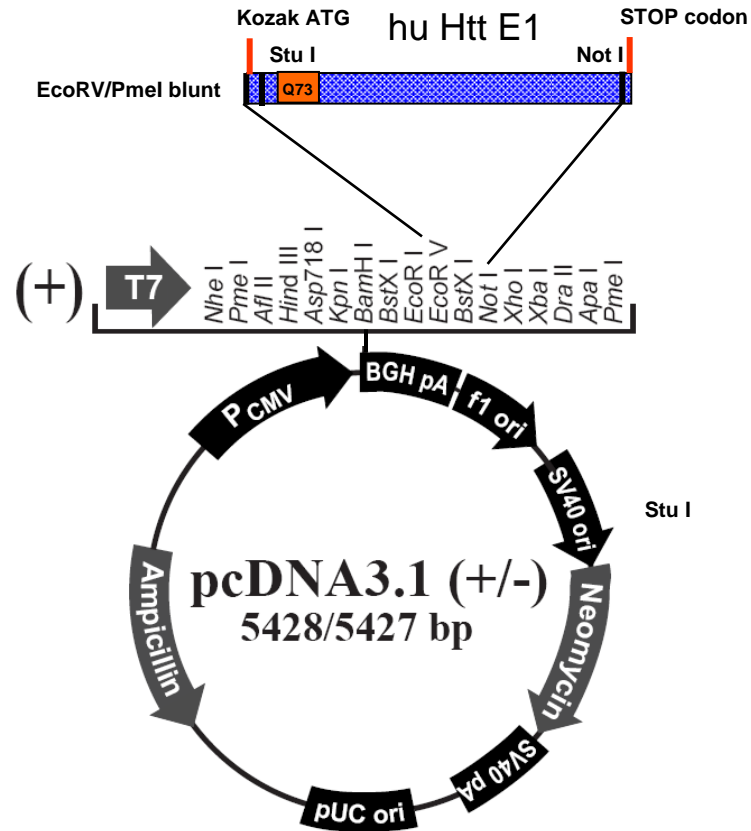
Insert: Human Htt Exon 1 with Q73 XXX codon, ~450 bp

Codon scheme: mix [CAA-CAG-CAG-CAA-CAG-CAA]_n; rdm: x/CAG/CAA/x; CAG: pure CAG

Cloning site: fused EcoRV/PmeI at 5' and Not I at 3', EcoRV and PmeI were deleted

Diagnostic check: BamHI / Not I digestion should release two bands (5.4kb,0.45kb); StuI digestion should release two bands: 4.4 kb, 1.5 kb;

Selection: Ampicillin



3' end of hCMV
 809 AAGCAGAGCT CTCTGGCTAA CTAGAGAACC CACTGCTTAC TGGCTTATCG AAATTAATAC
 putative transcriptional start
 T7 promoter/primer binding site
 869 GACTCACTAT AGGGAGACCC AAGCTGGCTA GCGTTTAAAC TTAAGCTTGG TACCGAGCTC
 Nhe I Pme I AfII Hind III Asp718 I Kpn I
 BamHI BstX I* EcoRV EcoRV BstX I* Not I Xho I
 929 GGATCCACTA GTCCAGTGTG GTGGAATTCT GCAGATATCC AGCACAGTGG CGGCCGCTCG
 Xba I Apa I Pme I
 989 AGCTTAGAGG GCCCGTTTAA ACCCGCTGAT CAGCCTCGAC TGTGCCTTCT AGTTGCCAGC
 pcDNA3.1/BGH reverse priming site
 1049 CATCTGTTGT TTGCCCCCTC CCCGTGCCTT CCTTGACCCT GGAAGGTGCC ACTCCCACTG

Htt-Q103, rdm, 1-90, human

Cloning vector: pcDNA3.1(+), 5428 bp

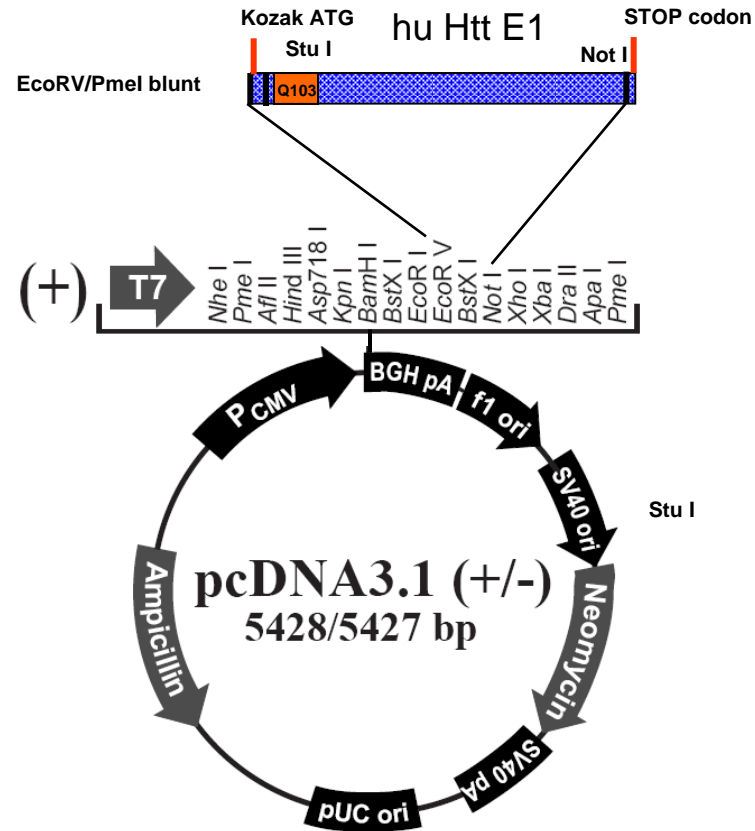
Insert: Human Htt Exon 1 with Q103 random (rdm) codon, ~540 bp

Codon scheme:; rdm: x/CAG/CAA/x;

Cloning site: fused EcoRV/PmeI at 5' and Not I at 3', EcoRV and PmeI were deleted

Diagnostic check: BamHI / Not I digestion should release two bands (5.4kb,0.5kb); StuI digestion should release two bands: 4.4 kb, 1.6 kb

Selection: Ampicillin



```

      3' end of hCMV      putative transcriptional start
      |                  |
809  AAGCAGAGCT CTCTGGCTAA CTAGAGAACC CACTGCTTAC TGGCTTATCG AAATTAATAC
      T7 promoter/primer binding site      Nhe I      Pme I AfII Hind III Asp718 I Kpn I
869  GACTCACTAT AGGGAGACCC AAGCTGGCTA GCGTTTAAAC TTAAGCTTGG TACCGAGCTC
      BamHI I      BstX I* EcoR I      EcoR V      BstX I* Not I Xho I
929  GGATCCACTA GTCCAGTGTG GTGGAATTCT GCAGATATCC AGCACAGTGG CGGCCGCTCG
      Xba I      Apa I Pme I      pcDNA3.1/BGH reverse priming site
989  AGCTTAGAGG GCCCGTTTAA ACCCGTGAT CAGCCTCGAC TGTGCCTTCT AGTTGCCAGC

1049 CATCTGTGTG TTGCCCCCTC CCCGTGCCTT CCTTGACCCT GGAAGGTGCC ACTCCCACTG
  
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Htt-Q145, [codon scheme], 1-90, human

Cloning vector: pcDNA3.1(+), 5428 bp

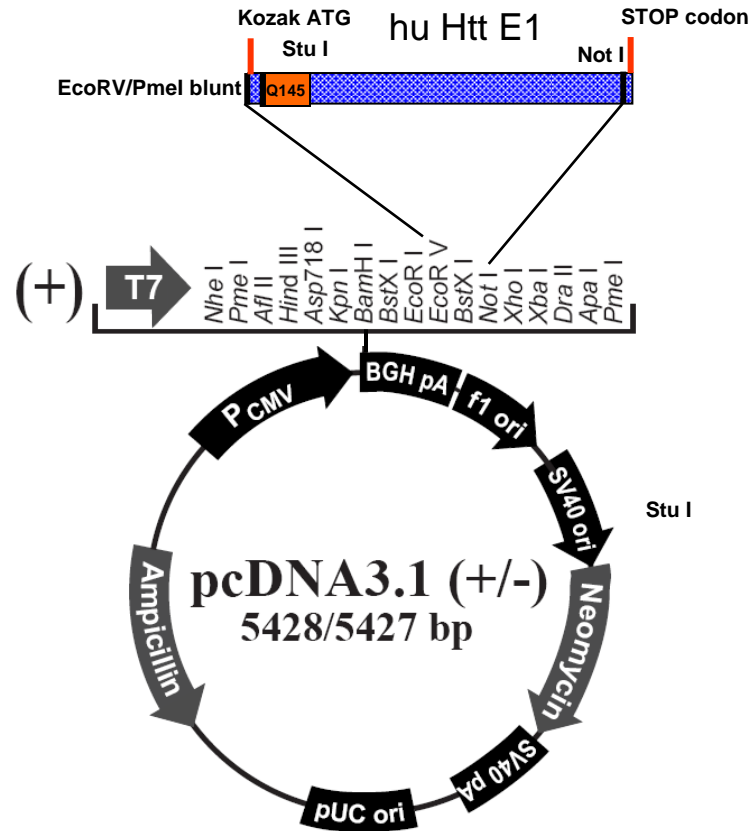
Insert: Human Htt Exon 1 with Q145 XXX codon, ~666 bp

Codon scheme: mix [CAA-CAG-CAG-CAA-CAG-CAA]_n; rdm: x/CAG/CAA/x;

Cloning site: fused EcoRV/PmeI at 5' and Not I at 3', EcoRV and PmeI were deleted

Diagnostic check: BamHI / Not I digestion should release two bands (5.4kb,0.66kb); StuI digestion should release two bands: 4.4 kb, 1.7 kb

Selection: Ampicillin



```

3' end of hCMV          putative transcriptional start
809 AAGCAGAGCT CTTGGCTAA CTAGAGAACC CACTGCTTAC TGGCTTATCG AAATTAATAC
      |
T7 promoter/primer binding site
869 GACTCACTAT AGGGAGACCC AAGCTGGCTA GCGTTTAAAC TTAAGCTTGG TACCGAGCTC
      |           |           |           |           |           |
BamHI1         BstX1* EcoR1         EcoRV         BstX1* NotI         XhoI
929 GGATCCACTA GTCCAGTGTG GTGGAATTCT GCAGATATCC AGCACAGTGG CGGCCGCTCG
      |           |           |           |           |           |
XbaI         ApaI PmeI         pcDNA3.1/BGH reverse priming site
989 AGCTTAGAGG GCCCGTTTAA ACCCGTGAT CAGCCTCGAC TGTGCCTTCT AGTTGCCAGC

1049 CATCTGTTGT TTGCCCCCTC CCCGTGCCTT CCTTGACCCT GGAAGGTGCC ACTCCCACTG
    
```


Construct Maps

pcDNA3.1-mycC

Courtesy of Drs. Linda Dong and Janine Steiger (CombinatoRx, Inc.)



Rapidly discover and develop drugs that delay or slow Huntington's disease

Htt-Q23-myc, [codon scheme], 1-3144, human

Cloning vector: pcDNA3.1(+) myc tagged frame C, ~5.5 kb

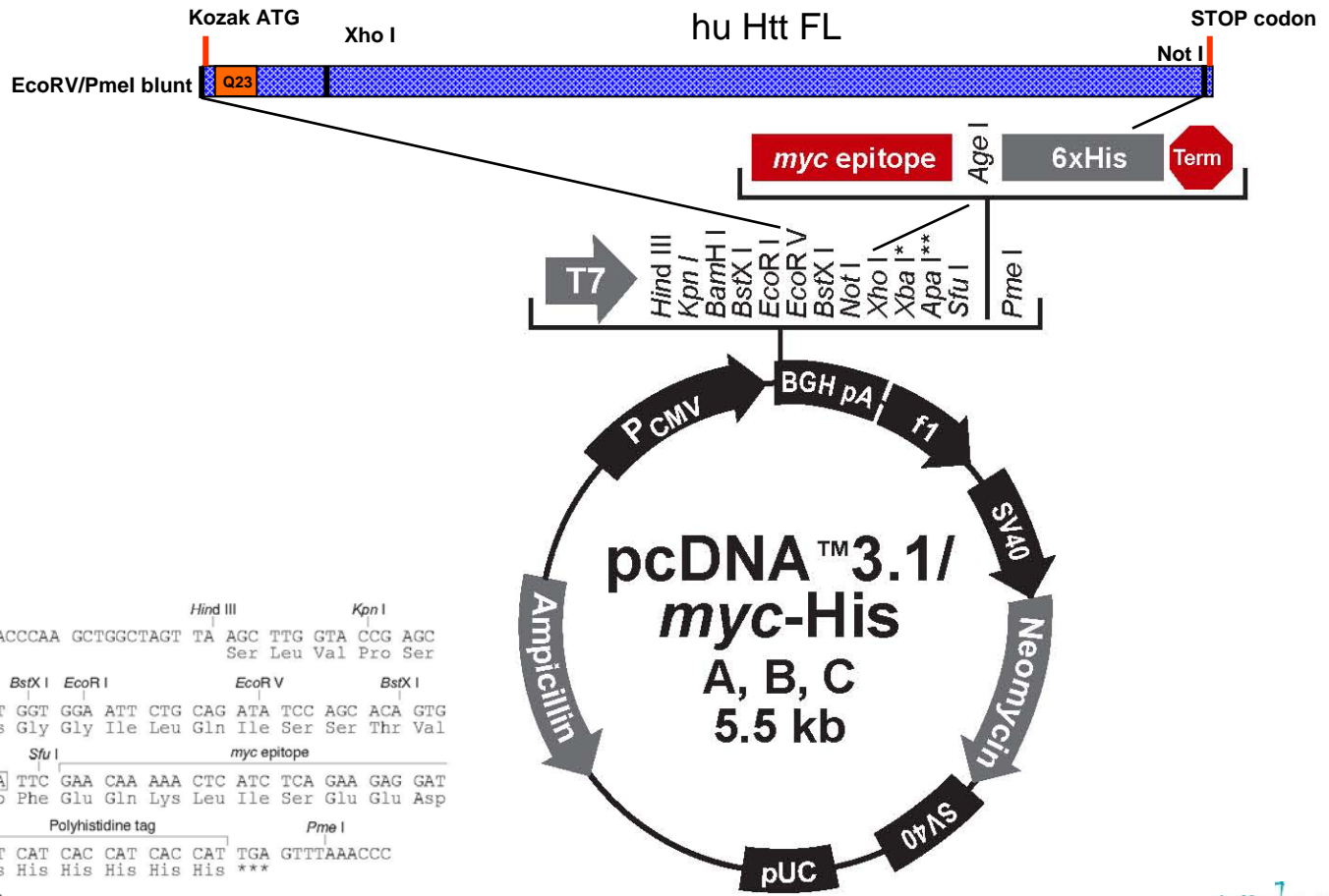
Insert: Human Htt full-length with Q23 XXXX codon, 9454 bp

Codon scheme: mix [CAA-CAG-CAG-CAA-CAG-CAA]_n; rdm: x/CAG/CAA/x; CAG: pure CAG.

Cloning site: fused EcoRV/PmeI at 5' and Not I at 3', EcoRV and PmeI were deleted

Diagnostic check: XhoI digestion should release two bands: 8.9 kb, 6 kb; Not I digestion should linearize the 15 kb band

Selection: Ampicillin



Htt-Q73-myc, [codon scheme], 1-3144, human

Cloning vector: pcDNA3.1(+) myc tagged frame C, ~5.5 kb

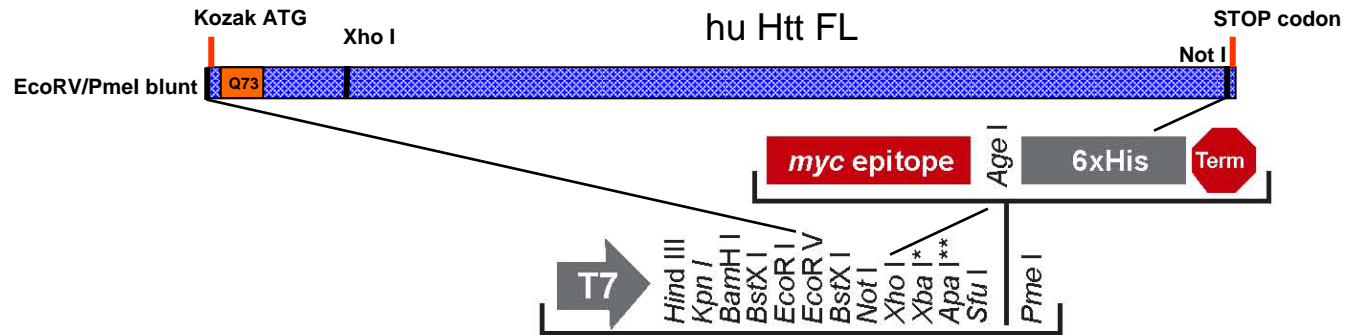
Insert: Human Htt full-length with Q73 XXXX codon, ~9.6 kb

Codon scheme: mix [CAA-CAG-CAG-CAA-CAG-CAA]_n; rdm: x/CAG/CAA/x; CAG: pure CAG.

Cloning site: fused EcoRV/PmeI at 5' and Not I at 3', EcoRV and PmeI were deleted

Diagnostic check: XhoI digestion should release two bands: 8.9 kb, 6 kb; Not I digestion should linearize the 15 kb band

Selection: Ampicillin



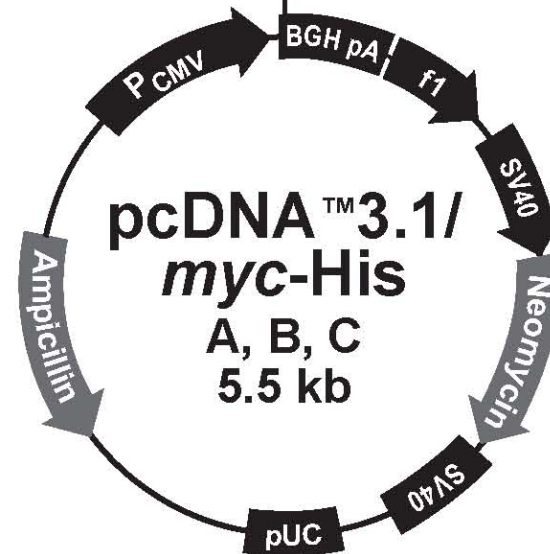
861 ATTAATACGA CTCACTATAG GGAGACCCAA GCTGGCTAGT TA AGC TTG GTA CCG AGC
 Ser Leu Val Pro Ser

918 TCG GAT CCA CTA GTC CAG TGT GGT GGA ATT CTG CAG ATA TCC AGC ACA GTG
 Ser Asp Pro Leu Val Gln Cys Gly Gly Ile Leu Gln Ile Ser Ser Thr Val

969 GCG GCC GCT CGA GGT CAC CCA TTC GAA CAA AAA CTC ATC TCA GAA GAG GAT
 Ala Ala Ala Arg Gly His Pro Phe Glu Gln Lys Leu Ile Ser Glu Glu Asp

1020 CTG AAT ATG CAT ACC GGT CAT CAT CAC CAT CAC CAT TGA GTTTAAACCC
 Leu Asn Met His Thr Gly His His His His His His ***

1069 GCTGATCAGC CTCGACTGTG CCTTCTAGTT GC



Htt-Q145-myc, [codon scheme], 1-3144, human

Cloning vector: pcDNA3.1(+) myc tagged frame C, ~5.5 kb

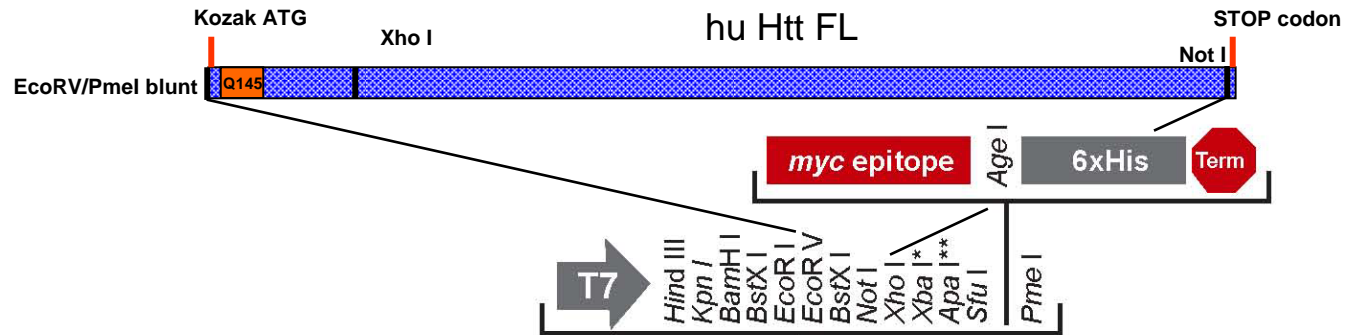
Insert: Human Htt full-length with Q145 XXXX codon, ~9.8 kb

Codon scheme: mix [CAA-CAG-CAG-CAA-CAG-CAA]_n; rdm: x/CAG/CAA/x; CAG: pure CAG.

Cloning site: fused EcoRV/PmeI at 5' and Not I at 3', EcoRV and PmeI were deleted

Diagnostic check: XhoI digestion should release two bands: 9.2 kb, 6 kb; Not I digestion should linearize the 15.2 kb band

Selection: Ampicillin



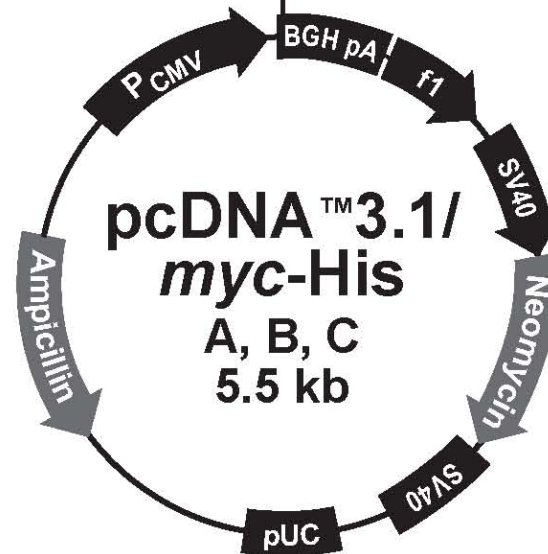
861 ATTAATACGA CTCACTATAG GGAGACCCAA GCTGGCTAGT TA AGC TTG GTA CCG AGC
 Ser Leu Val Pro Ser
 Hind III Kpn I

918 TCG GAT CCA CTA GTC CAG TGT GGT GGA ATT CTG CAG ATA TCC AGC ACA GTG
 Ser Asp Pro Leu Val Gln Cys Gly Gly Ile Leu Gln Ile Ser Ser Thr Val
 BamHI BstXI EcoRI EcoRV BstXI

969 GCG GCC GCT CGA GGT CAC CCA TTC GAA CAA AAA CTC ATC TCA GAA GAG GAT
 Ala Ala Ala Arg Gly His Pro Phe Glu Gln Lys Leu Ile Ser Glu Glu Asp
 NotI XhoI BstE II SfuI myc epitope

1020 CTG AAT ATG CAT ACC GGT CAT CAT CAC CAT CAC CAT TGA GTTTAAACCC
 Leu Asn Met His Thr Gly His His His His His His ***
 Age I Polyhistidine tag Pme I

1069 GCTGATCAGC CTCGACTGTG CCTTCTAGTT GC
 BGH Reverse priming site



Htt-Q23-myc, [codon scheme], 1-90, human

Cloning vector: pcDNA3.1(+) myc tagged frame C, ~5.5 kb

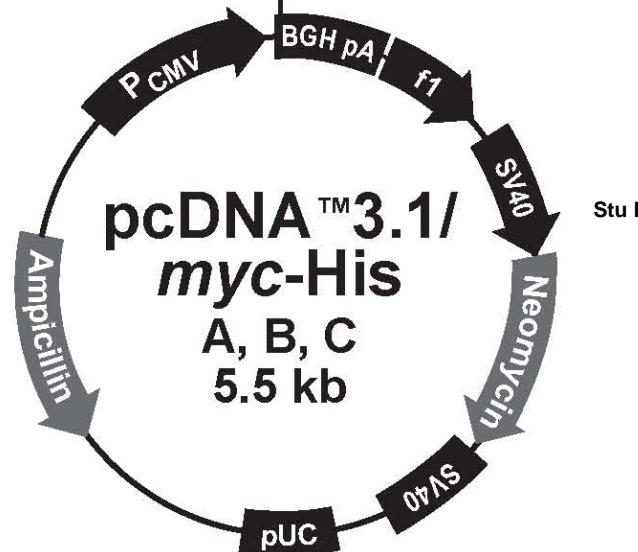
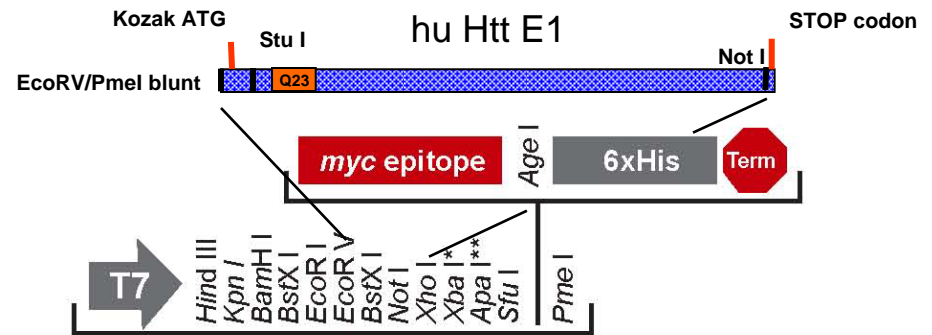
Insert: Human Htt Exon 1 with Q23 XXXX codon, ~ 300 bp

Codon scheme: mix [CAA-CAG-CAG-CAA-CAG-CAA]_n; rdm: x/CAG/CAA/x; CAG: pure CAG.

Cloning site: fused EcoRV/PmeI at 5' and Not I at 3', EcoRV and PmeI were deleted

Diagnostic check: BamHI / Not I digestion should release two bands (5.4kb,0.3kb); StuI digestion should release two bands: 4.4 kb, 1.3 kb.

Selection: Ampicillin



861 ATTAATACGA CTCACTATAG GGAGACCCAA GCTGGCTAGT TA AGC TTG GTA CCG AGC
 Ser Leu Val Pro Ser

918 TCG GAT CCA CTA GTC CAG TGT GGT GGA ATT CTG CAG ATA TCC AGC ACA GTG
 Ser Asp Pro Leu Val Gln Cys Gly Gly Ile Leu Gln Ile Ser Ser Thr Val

969 GCG GCC GCT CGA GGT CAC CCA TTC GAA CAA AAA CTC ATC TCA GAA GAG GAT
 Ala Ala Ala Arg Gly His Pro Phe Glu Gln Lys Leu Ile Ser Ser Glu Glu Asp

1020 CTG AAT ATG CAT ACC GGT CAT CAT CAC CAT CAC CAT TGA GTTTAAACCC
 Leu Asn Met His Thr Gly His His His His His His ***

1069 GCTGATCAGC CTCGACTGTG CCTTCTAGTT GC

Htt-Q73-myc, [codon scheme], 1-90, human

Cloning vector: pcDNA3.1(+) myc tagged frame C, ~5.5 kb

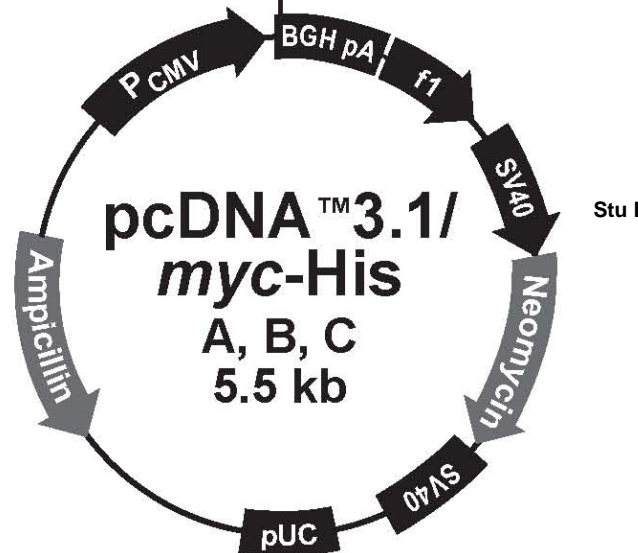
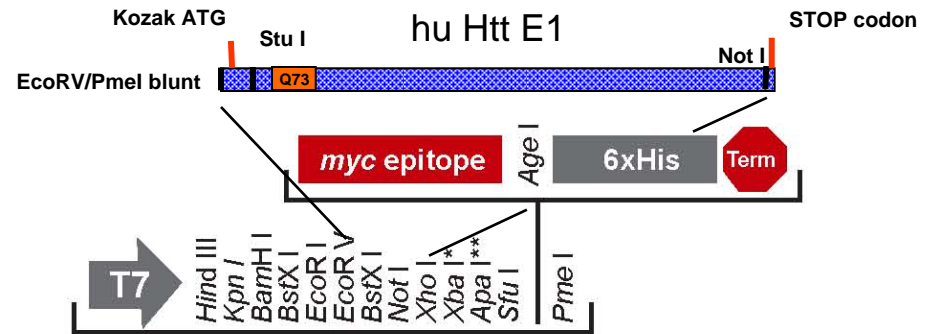
Insert: Human Htt Exon 1 with Q73 XXXX codon, ~ 450 bp

Codon scheme: mix [CAA-CAG-CAG-CAA-CAG-CAA]_n; rdm: x/CAG/CAA/x; CAG: pure CAG.

Cloning site: fused EcoRV/PmeI at 5' and Not I at 3', EcoRV and PmeI were deleted

Diagnostic check: BamHI / Not I digestion should release two bands (5.4kb,0.45kb); StuI digestion should release two bands: 4.4 kb, 1.5 kb

Selection: Ampicillin



861 ATTAATACGA CTCACTATAG GGAGACCCAA GCTGGCTAGT TA AGC TTG GTA CCG AGC
 Ser Leu Val Pro Ser

918 TCG GAT CCA CTA GTC CAG TGT GGT GGA ATT CTG CAG ATA TCC AGC ACA GTG
 Ser Asp Pro Leu Val Gln Cys Gly Gly Ile Leu Gln Ile Ser Ser Thr Val

969 GCG GCC GCT CGA GGT CAC CCA TTC GAA CAA AAA CTC ATC TCA GAA GAG GAT
 Ala Ala Ala Arg Gly His Pro Phe Glu Gln Lys Leu Ile Ser Glu Glu Asp

1020 CTG AAT ATG CAT ACC GGT CAT CAT CAC CAT CAC CAT TGA GTTTAAACCC
 Leu Asn Met His Thr Gly His His His His His His ***

1069 GCTGATCAGC CTCGACTGTG CCTTCTAGTT GC

Htt-Q103-myc, rdm 1-90, human

Cloning vector: pcDNA3.1(+) myc tagged frame C, ~5.5 kb

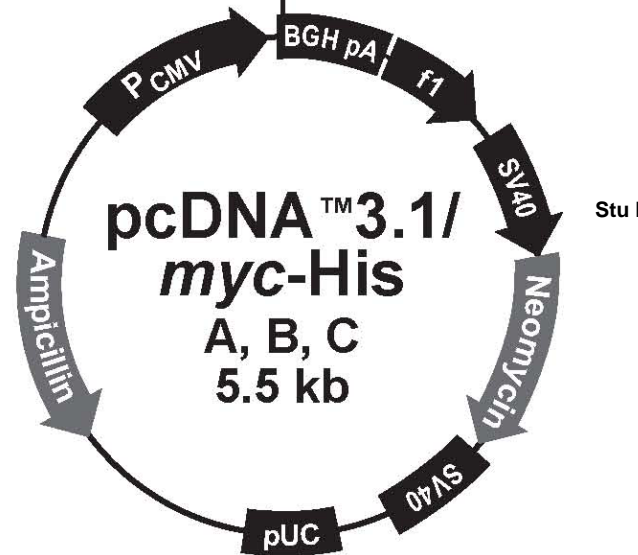
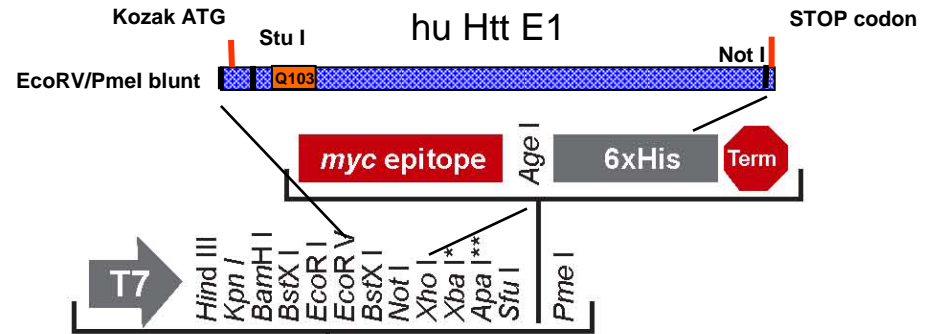
Insert: Human Htt Exon 1 with Q103 rdm codon, ~ 540 bp

Codon scheme: rdm: x/CAG/CAA/x;

Cloning site: fused EcoRV/PmeI at 5' and Not I at 3', EcoRV and PmeI were deleted

Diagnostic check: BamHI / Not I digestion should release two bands (5.4kb,0.5kb); StuI digestion should release two bands: 4.4 kb, 1.6 kb

Selection: Ampicillin



861 ATTAATACGA CTCACTATAG GGAGACCCAA GCTGGCTAGT TA AGC TTG GTA CCG AGC
 Ser Leu Val Pro Ser

918 TCG GAT CCA CTA GTC CAG TGT GGT GGA ATT CTG CAG ATA TCC AGC ACA GTG
 Ser Asp Pro Leu Val Gln Cys Gly Gly Ile Leu Gln Ile Ser Ser Thr Val

969 GCG GCC GCT CGA GGT CAC CCA TTC GAA CAA AAA CTC ATC TCA GAA GAG GAT
 Ala Ala Ala Arg Gly His Pro Phe Glu Gln Lys Leu Ile Ser Glu Glu Asp

1020 CTG AAT ATG CAT ACC GGT CAT CAT CAC CAT CAC CAT TGA GTTTAAACCC
 Leu Asn Met His Thr Gly His His His His His His ***

1069 GCTGATCAGC CTCGACTGTG CCTTCTAGTT GC

Htt-Q145-myc, [codon scheme], 1-90, human

Cloning vector: pcDNA3.1(+) myc tagged frame C, ~5.5 kb

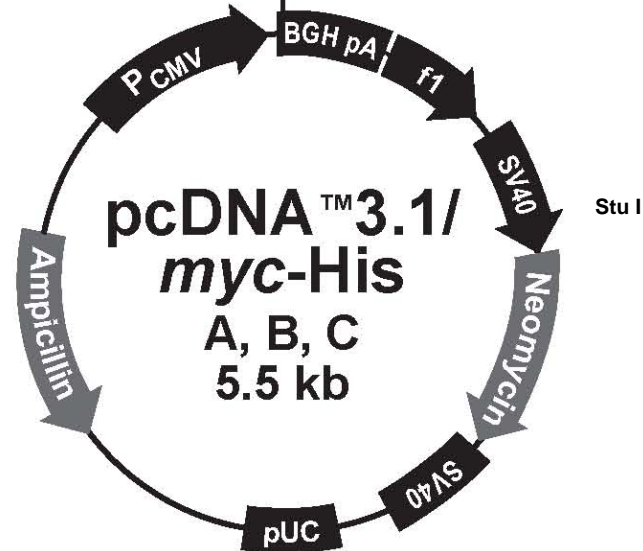
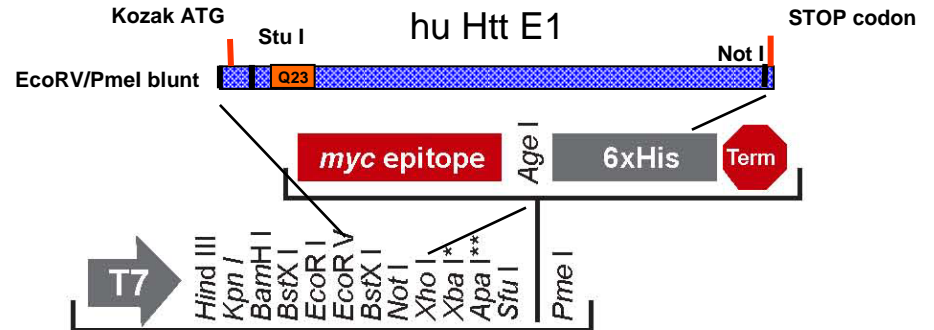
Insert: Human Htt Exon 1 with Q73 XXXX codon, ~ 666 bp

Codon scheme: mix [CAA-CAG-CAG-CAA-CAG-CAA]_n; rdm: x/CAG/CAA/x; CAG: pure CAG.

Cloning site: fused EcoRV/PmeI at 5' and Not I at 3', EcoRV and PmeI were deleted

Diagnostic check: BamHI / Not I digestion should release two bands (5.4kb,0.66kb); StuI digestion should release two bands: 4.4 kb, 1.7 kb

Selection: Ampicillin



861 ATTAATACGA CTCACTATAG GGAGACCCAA GCTGGCTAGT TA AGC TTG GTA CCG AGC
 Ser Leu Val Pro Ser

918 TCG GAT CCA CTA GTC CAG TGT GGT GGA ATT CTG CAG ATA TCC AGC ACA GTG
 Ser Asp Pro Leu Val Gln Cys Gly Gly Ile Leu Gln Ile Ser Ser Thr Val

969 GCG GCC GCT CGA GGT CAC CCA TTC GAA CAA AAA CTC ATC TCA GAA GAG GAT
 Ala Ala Ala Arg Gly His Pro Phe Glu Gln Lys Leu Ile Ser Glu Glu Asp

1020 CTG AAT ATG CAT ACC GGT CAT CAT CAC CAT CAC CAT TGA GTTTAAACCC
 Leu Asn Met His Thr Gly His His His His His His ***

1069 GCTGATCAGC CTCGACTGTG CCTTCTAGTT GC

Construct Maps

pcDNA5/FRT

Courtesy of Drs. Linda Dong and Janine Steiger (CombinatoRx, Inc.)



Rapidly discover and develop drugs that delay or slow Huntington's disease

Htt-Q23-FRT, [codon scheme], 1-3144, human

Cloning vector: pcDNA5/FRT, 5070 bp

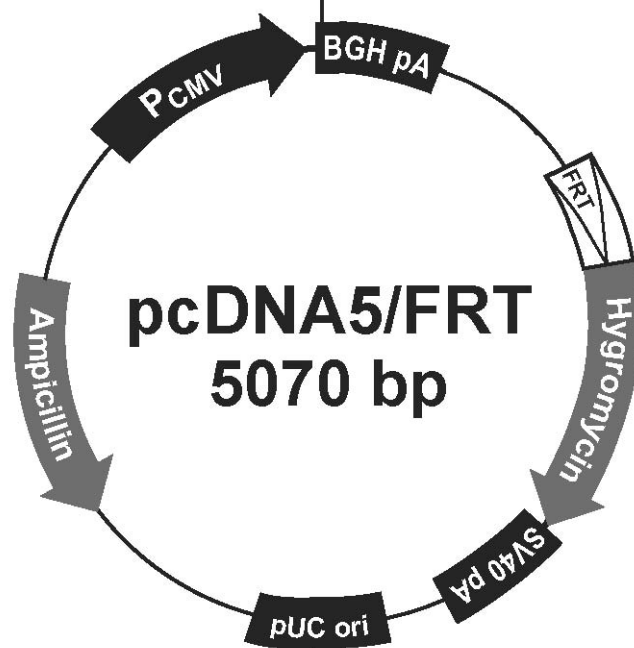
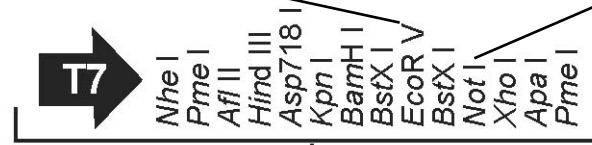
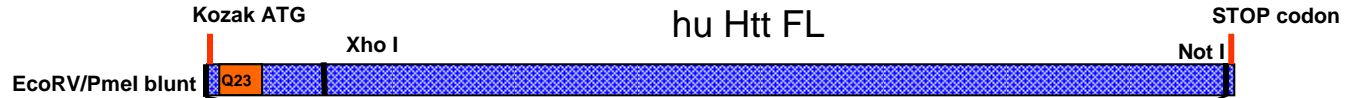
Insert: Full human Htt Q23 with XXXX codon, ~9.5 kb

Codon scheme: mix [CAA-CAG-CAG-CAA-CAG-CAA]_n; rdm: x/CAG/CAA/x; CAG: pure CAG.

Cloning site: fused EcoRV/PmeI at 5' and Not I at 3', RV and PmeI were deleted.

Diagnostic check: XhoI digestion should release two bands: 9 kb, 6 kb; Not I digestion should linearize the 15 kb band

Selection: Ampicillin



809 AAGCAGAGCT C TCTGGCTAA CTAGAGAACC CACTGCTTAC TGGCTTATCG AAATTAATAC
 3' end of hCMV putative transcriptional start
 T7 promoter/primer binding site Nhe I Pme I Afl II Hind III Asp718 I Kpn I
 869 GACTCACTAT AGGGAGACCC AAGCTGGCTA GCGTTTAAAC TTAAGCTTGG TACCGAGCTC
 BamH I BstX I* EcoR I EcoR V BstX I* Not I Xho I
 929 GGATCCACTA GTCCAGTGTG GTGGAATTCT GCAGATATCC AGCACAGTGG CGGCCGCTCG
 Xba I Apa I Pme I pcDNA3.1/BGH reverse priming site
 989 AGCTTAGAGG GCCCGTTTAA ACCCGCTGAT CAGCCTCGAC TGTGCCTTCT AGTTGCCAGC
 1049 CATCTGTTGT TTGCCCCCTC CCCGTGCCTT CCTTGACCCT GGAAGGTGCC ACTCCCACTG



Htt-Q73-FRT, [codon scheme], 1-3144, human

Cloning vector: pcDNA5/FRT, 5070 bp

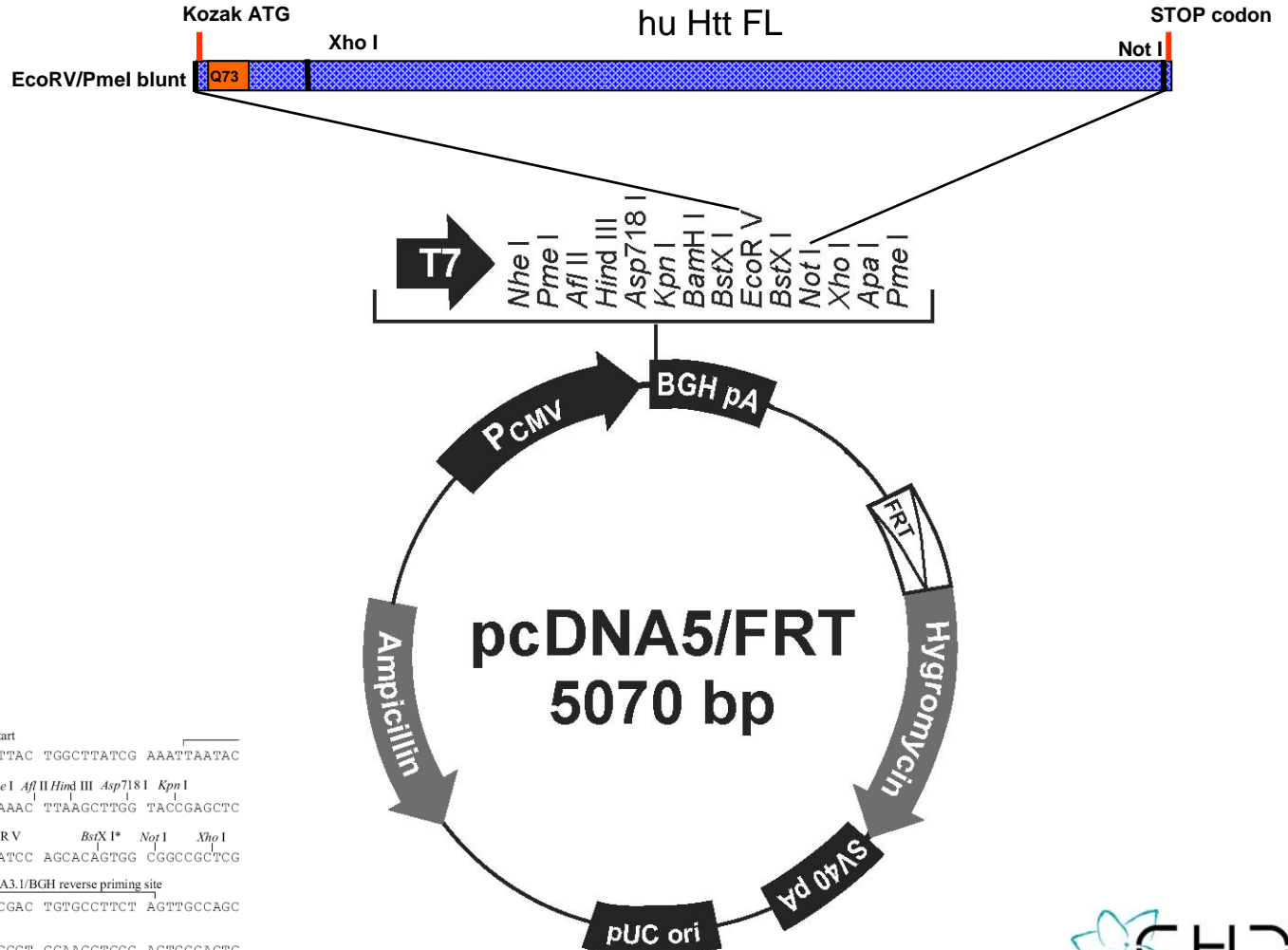
Insert: Full human Htt Q73 with XXX codon, ~9.6 kb

Codon scheme: mix [CAA-CAG-CAG-CAA-CAG-CAA]_n; rdm: x/CAG/CAA/x; CAG: pure CAG.

Cloning site: fused EcoRV/PmeI at 5' and Not I at 3', RV and PmeI were deleted.

Diagnostic check: XhoI digestion should release two bands: 9 kb, 6 kb; Not I digestion should linearize the 15 kb band.

Selection: Ampicillin



809 AAGCAGAGCT C TCTGGCTAA CTAGAGAACC CACTGCTTAC TGGCTTATCG AAATTAATAC
 putative transcriptional start
 T7 promoter/primer binding site Nhe I Pme I Afl II Hind III Asp718 I Kpn I
 869 GACTCACTAT AGGGAGACCC AAGCTGGCTA GCGTTTAAAC TTAAGCTTGG TACCGAGCTC
 BamH I BstX I* EcoR I EcoR V BstX I* Not I Xho I
 929 GGATCCACTA GTCCAGTGTG GTGGAATTCT GCAGATATCC AGCACAGTGG CGGCCGCTCG
 Xba I Apa I Pme I pcDNA3.1/BGH reverse priming site
 989 AGCTTAGAGG GCCCGTTTAA ACCCGCTGAT CAGCCTCGAC TGTGCCTTCT AGTTGCCAGC
 1049 CATCTGTGTG TTGCCCCCTC CCCGTGCTT CCTTGACCCT GGAAGGTGCC ACTCCCACTG

Htt-Q145-FRT, [codon scheme], 1-3144, human

Cloning vector: pcDNA5/FRT, 5070 bp

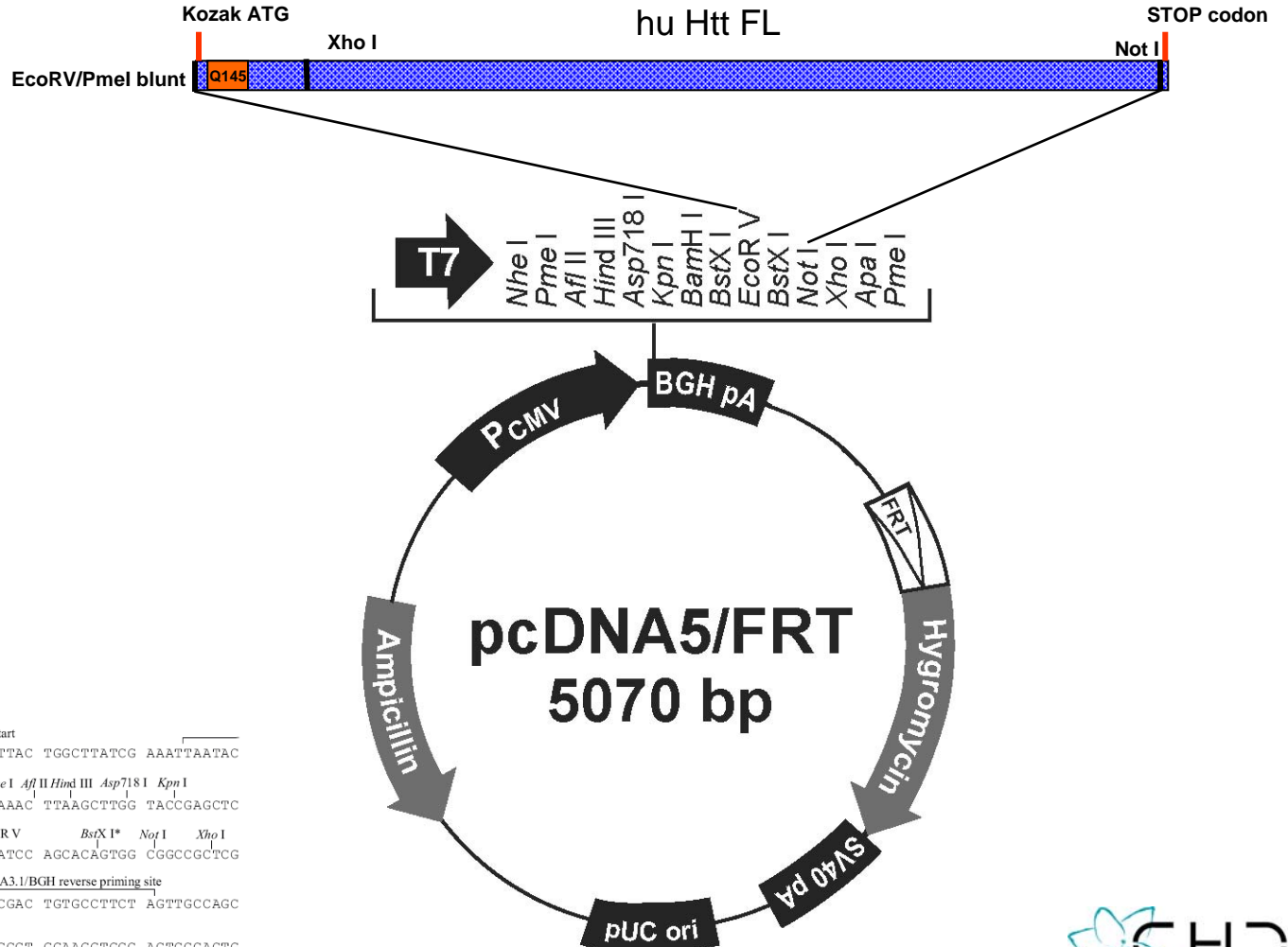
Insert: Full human Htt Q145 with XXX codon, ~9.8 kb

Codon scheme: mix [CAA-CAG-CAG-CAA-CAG-CAA]_n; rdm: x/CAG/CAA/x;

Cloning site: fused EcoRV/PmeI at 5' and Not I at 3', RV and PmeI were deleted.

Diagnostic check: XhoI digestion should release two bands: 9.2kb, 6kb; Not I digestion should linearize the 15.2kb band.

Selection: Ampicillin



3' end of hCMV | putative transcriptional start

809 AAGCAGAGCT CTCTGGCTAA CTAGAGAACC CACTGCTTAC TGGCTTATCG AAATTAATAC

T7 promoter/primer binding site | Nhe I | Pme I | Afl II | Hind III | Asp718 I | Kpn I

869 GACTCACTAT AGGGAGACCC AAGCTGGCTA GCGTTTAAAC TTAAGCTTGG TACCGAGCTC

BamH I | BstX I* | EcoR I | EcoR V | BstX I* | Not I | Xho I

929 GGATCCACTA GTCCAGTGTG GTGGAATTC TGCAGATATCC AGCACAGTGG CGGCCGCTCG

Xba I | Apa I | Pme I | pcDNA3.1/BGH reverse priming site

989 AGCTTAGAGG GCCCGTTTAA ACCCGCTGAT CAGCCTCGAC TGTGCCTTCT AGTTGCCAGC

1049 CATCTGTTGT TTGCCCCCTC CCCGTGCTT CCTTGACCCT GGAAGGTGCC ACTCCCACTG

Htt-Q23-FRT, [codon scheme], 1-90, human

Cloning vector: pcDNA5/FRT, 5070 bp

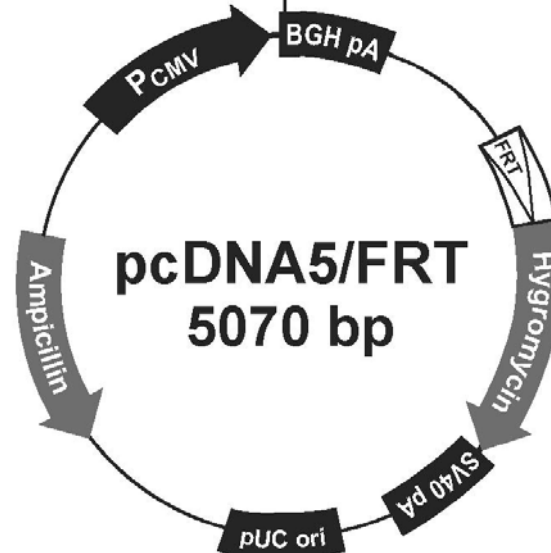
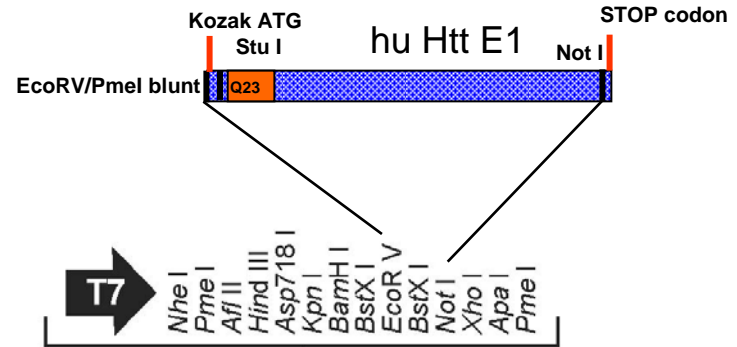
Insert: Human Htt Exon 1 with Q23 XXX codon, ~300 bp

Codon scheme: mix [CAA-CAG-CAG-CAA-CAG-CAA]_n; rdm: x/CAG/CAA/x; CAG: pure CAG

Cloning site: fused EcoRV/PmeI at 5' and Not I at 3', EcoRV and PmeI were deleted

Diagnostic check: BamHI/NotI digest should release two bands: 5.0kb + 0.3 kb.

Selection: Ampicillin



3' end of hCMV putative transcriptional start

809 AAGCAGAGCT CTCTGGCTAA CTAGAGAACC CACTGCTTAC TGGCTTATCG AAATTAATAC

T7 promoter/primer binding site Nhe I Pme I Afl II Hind III Asp718 I Kpn I

869 GACTCACTAT AGGGAGACCC AAGCTGGCTA GCGTTTAAAC TTAAGCTTGG TACCGAGCTC

BamH I BstX I* EcoR I EcoR V BstX I* Not I Xho I

929 GGATCCACTA GTCCAGTGTG GTGGAATTCT GCAGATATCC AGCACAGTGG CGGCCGCTCG

Xba I Apa I Pme I pcDNA3.1/BGH reverse priming site

989 AGCTTAGAGG GCCCGTTTAA ACCCGCTGAT CAGCCTCGAC TGTGCCTTCT AGTTGCCAGC

1049 CATCTGTTGT TTGCCCCCTC CCCGTGCCTT CCTTGACCCT GGAAGGTGCC ACTCCCACTG

Htt-Q73-FRT, [codon scheme], 1-90, human

Cloning vector: pcDNA5/FRT, 5070 bp

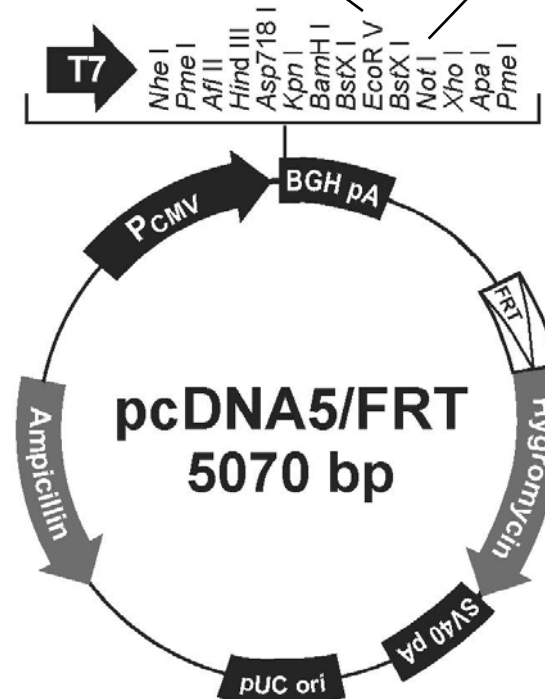
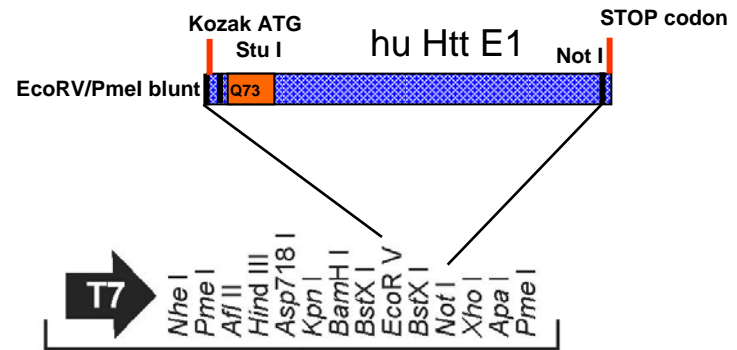
Insert: Human Htt Exon 1 with Q73 XXX codon, ~450 bp

Codon scheme: mix [CAA-CAG-CAG-CAA-CAG-CAA]_n; rdm: x/CAG/CAA/x; CAG: pure CAG

Cloning site: fused EcoRV/PmeI at 5' and Not I at 3', EcoRV and PmeI were deleted

Diagnostic check: BamHI/NotI digest should release two bands: 5.0kb + 0.45 kb

Selection: Ampicillin



```

      3' end of hCMV      putative transcriptional start
      |                  |
809  AAGCAGAGCT CTCTGGCTAA CTAGAGAACC CACTGCTTAC TGGCTTATCG AAATTAATAC
      T7 promoter/primer binding site      Nhe I      Pme I Afl II Hind III Asp718 I Kpn I
869  GACTCACTAT AGGGAGACCC AAGCTGGCTA GCGTTTAAAC TTAAGCTTGG TACCGAGCTC
      BamH I      BstX I* EcoR I      EcoR V      BstX I* Not I Xho I
929  GGATCCACTA GTCCAGTGTG GTGGAATTCT GCAGATATCC AGCACAGTGG CGGCCGCTCG
      Xba I      Apa I Pme I      pcDNA3.1/BGH reverse priming site
989  AGCTTAGAGG GCCCGTTTAA ACCCGCTGAT CAGCCTCGAC TGTGCCTTCT AGTTGCCAGC

1049 CATCTGTTGT TTGCCCCCTC CCCGTGCCTT CCTTGACCCT GGAAGGTGCC ACTCCCACTG
    
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Htt-Q103-FRT, rdm, 1-90, human

Cloning vector: pcDNA5/FRT, 5070 bp

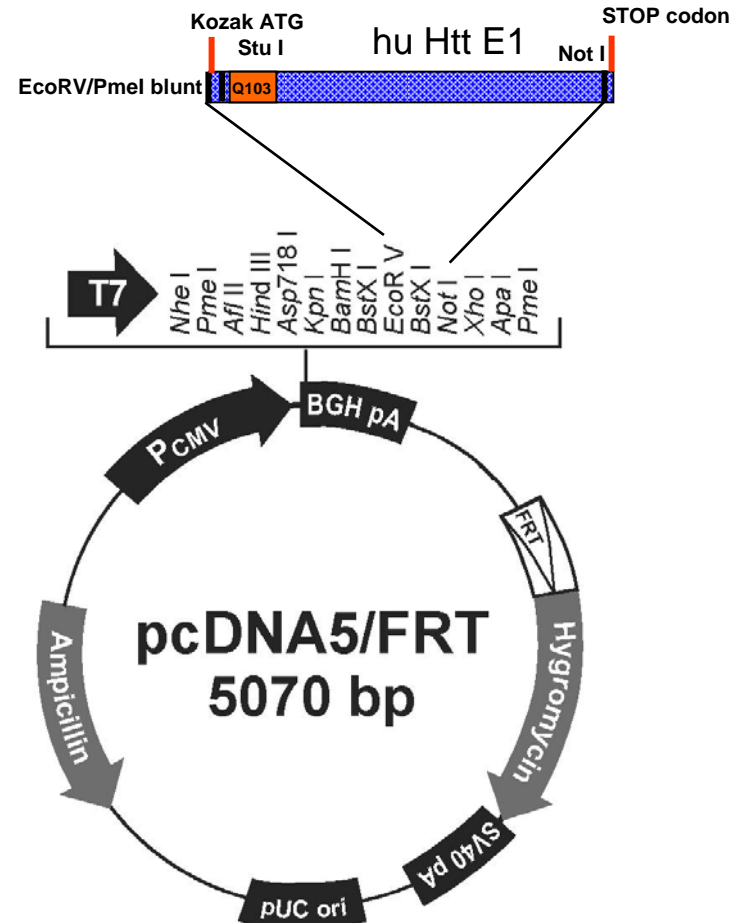
Insert: Human Htt Exon 1 with Q103 random (rdm) codon, ~540 bp

Codon scheme:; rdm: x/CAG/CAA/x;

Cloning site: fused EcoRV/PmeI at 5' and Not I at 3', EcoRV and PmeI were deleted

Diagnostic check: BamHI/NotI digest should release two bands: 5.0kb + 0.5 kb

Selection: Ampicillin



```

      3' end of hCMV      putative transcriptional start
      |                  |
809  AAGCAGAGCT CTCTGGCTAA CTAGAGAACC CACTGCTTAC TGGCTTATCG AAATTAATAC
      T7 promoter/primer binding site      Nhe I      Pme I Afl II Hind III Asp718 I Kpn I
869  GACTCACTAT AGGGAGACCC AAGCTGGCTA GCGTTTAAAC TTAAGCTTGG TACCGAGCTC
      BamH I      BstX I* EcoR I      EcoR V      BstX I* Not I Xho I
929  GGATCCACTA GTCCAGTGTG GTGGAATTC TGCAGATATCC AGCACAGTGG CGGCCGCTCG
      Xba I      Apa I Pme I      pcDNA3.1/BGH reverse priming site
989  AGCTTAGAGG GCCCGTTTAA ACCCGCTGAT CAGCCTCGAC TGTGCCTTCT AGTTGCCAGC

1049 CATCTGTTGT TTGCCCCCTC CCCGTGCCTT CCTTGACCCT GGAAGGTGCC ACTCCCACTG
    
```

Htt-Q145-FRT, [codon scheme], 1-90, human

Cloning vector: pcDNA5/FRT, 5070 bp

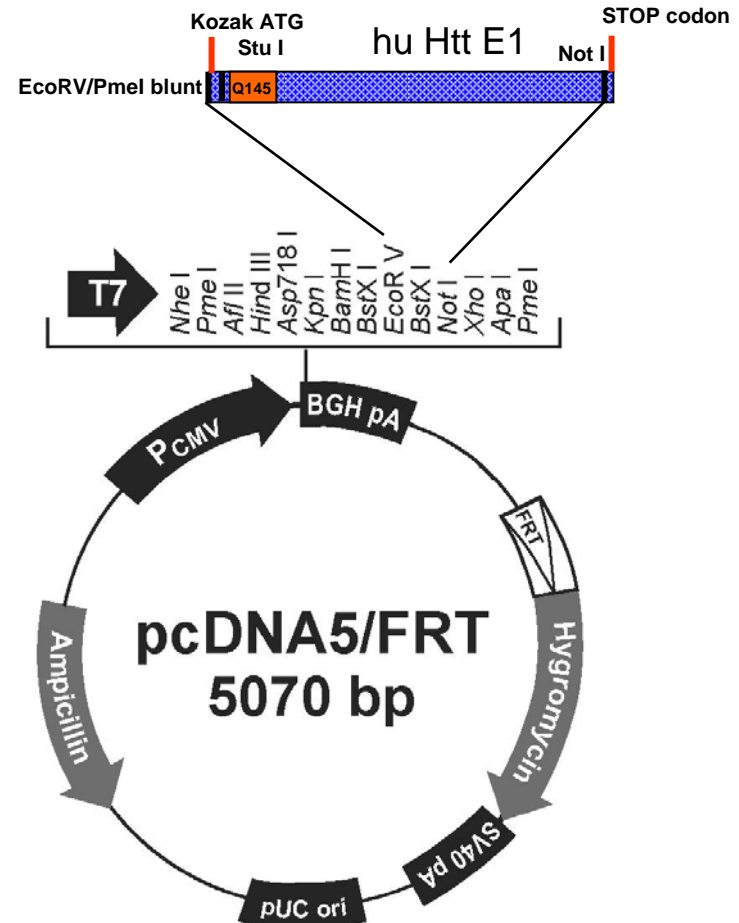
Insert: Human Htt Exon 1 with Q145 XXX codon, ~666 bp

Codon scheme: mix [CAA-CAG-CAG-CAA-CAG-CAA]_n; rdm: x/CAG/CAA/x;

Cloning site: fused EcoRV/PmeI at 5' and Not I at 3', EcoRV and PmeI were deleted

Diagnostic check: BamHI/NotI digest should release two bands: 5.0kb +0.66kb

Selection: Ampicillin



3' end of hCMV putative transcriptional start

809 AAGCAGAGCT CTCTGGCTAA CTAGAGAACC CACTGCTTAC TGGCTTATCG AAATTAATAC

T7 promoter/primer binding site Nhe I Pme I Afl II Hind III Asp718 I Kpn I

869 GACTCACTAT AGGGAGACCC AAGCTGGCTA GCGTTTAAAC TTAAGCTTGG TACCGAGCTC

BamH I BstX I* EcoR I EcoR V BstX I* Not I Xho I

929 GGATCCACTA GTCCAGTGTG GTGGAATTC TGCAGATATCC AGCACAGTGG CGGCCGCTCG

Xba I Apa I Pme I pcDNA3.1/BGH reverse priming site

989 AGTCTAGAGG GCCCGTTTAA ACCCGTGAT CAGCCTGAC TGTGCCTTCT AGTTGCCAGC

1049 CATCTGTTGT TTGCCCTCC CCCGTGCCTT CCTTGACCCT GGAAGGTGCC ACTCCCACTG