Feb 4, 05

Sequencing of pET30a (empty plasmid) and pET30a/il3-SmGPCR by Parasitology-McGill sequencer

DH5α strain of *E. coli* was transformed with either **pET30a alone** or **pET30a/il3** ligate (il3 is a portion of the 3rd intracellular loop of SmGPCR). Glycerol stocks were made and kept at -121 °C. From 5-10ml O/N culture of transformed DH5α in LB+ Kanamycin (pET30a bears Kan resistant gene), a miniprep was performed to isolate the cloned plasmids. The miniprep products were sequenced with our new sequencer and I got the following:

(1) pET30a (alone) 645bp

GGGGACGTAATGACGATACATAACCCTCTAGAAATAATATTGTTAACTTTAAGAAGGAGATATACAT**ATG**CACCATC
ATCATCATCATTCTTCTGGTCTGGTGCCACGCGGTTCTGGTATGAAAGAAACCGCTGCTGCTAAATTCGAACGCCAGC
ACATGGACAGCCCAGATCTGGGTACCGACGACGACGACAAGGCCATGGCTGATATCGGATCCGAATTCGAGCTCCG
TCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCACTGAGATCCGGCTGCTAACAAAGCCCGAAAGGA
AGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTT
TTTTGCTGAAAGGAGGAACTATATCCGGATTGGCGAATGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGT
GTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTC
TCGCCACGTTCGCCGGCTTCCCCGTCAAGCTCTAAATCGGGGCTCCCTTTAGGGTCCGATTAGTGCTTACGCACTCAC
CCAAAACTGATAGGTATGTCCTATGGGC

(2) pET30a/il3 581bp

ATACCGATAACAAGTTCCCCTCTAGAAAATAATTTTGTTTAACTTTAAGAAGGAGATATACAT**ATG**CACCATCATCA
TCATCATTCTTCTGGTCTGGTGCCACGCGGTTCTGGTATGAAAGAAACCGCTGCTGCTAAATTCGAACGCCAGCACAT
GGACAGCCCAGATCTGGGTACCGACGACGACGACAAGGCCATGGCTGATATCGGATCCGAATTCATGCCCGAACCA
ACAGATCAAAAACTAAAAGTAATACAAATATTTAATATTTCAACGTCAGAGAAAGAATTACAAAAGGCGAATTGGA
AACCAACACCAAATGTAACATTGAAAAGTGAAACAAAACAACATCAACACGATTACTTACATAGAAGTAATGAAGA
ACAAAAGAATAAACTAGGAGACAAACAATTAATTAATTCTTCGAAATTACCATCATTAACATCATTTAGTATAGATC
ATATAAAGAAGCTTCGACGATTGAAATCTTTACCAAATTTTAAAACCAGAATACAATAAAACAGAACAATCACAAA
AGAATCTAATTTATTATCGACATGATCATTTCATATTTCATAGGG

If aligned together

```
pET30a alone sequencing  1    GGGGACGTAATGACGATACATAACCCTCTAGAAA-TAATATTGTT-AAC 47
pET30ail3 sequencing     1          ATACCGATAACAAGTTCCCCTCTAGAAAATAATTTTGTTTAAC 43

pET30a alone sequencing  48   TTTAAGAAGGAGATATACATATGCACCATCATCATCATCATTCTTCTGG 96
pET30ail3 sequencing     44   TTTAAGAAGGAGATATACATATGCACCATCATCATCATCATTCTTCTGG 92

pET30a alone sequencing  97   TCTGGTGCCACGCGGTTCTGGTATGAAAGAAACCGCTGCTGCTAAATTC 145
pET30ail3 sequencing     93   TCTGGTGCCACGCGGTTCTGGTATGAAAGAAACCGCTGCTGCTAAATTC 141

pET30a alone sequencing  146  GAACGCCAGCACATGGACAGCCCAGATCTGGGTACCGACGACGACGACA 194
pET30ail3 sequencing     142  GAACGCCAGCACATGGACAGCCCAGATCTGGGTACCGACGACGACGACA 190

pET30a alone sequencing  195  AGGCCATGGCTGATATCGGATCCGAATTCGAGCTCCGTCGACAAGCTTG 243
pET30ail3 sequencing     191  AGGCCATGGCTGATATCGGATCCGAATTCATGCCCGAACCAACAGAT-- 237

pET30a alone sequencing  244  CGGCCGCACTCGAGCACCACCACCACCACCACTGAGATC--CGGCTGCT 290
pET30ail3 sequencing     238  ----CAAAAACTAAAAGTAATACAAATATTTAATATTTCAACGTCAGAG 282

pET30a alone sequencing  291  AACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGC-TG-AGCA 337
pET30ail3 sequencing     283  AAAGAATTACAAAAGGC----GAATTGGAAACCAACACCAAATGTAACA 327

pET30a alone sequencing  338  ATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTT 386
pET30ail3 sequencing     328  TTGAAAAGTGAAACAAAACA--ACATCAACACGATTACT------TACA 368

pET30a alone sequencing  387  TTGCTGAAAGGAGGAACTATATCCGGATTGGCGAATGGGACGCGCCCTG 435
pET30ail3 sequencing     369  TAGAAGTAATGAAGAACAAAA----GAATAAACTAGGAGACAAACAAT- 412

pET30a alone sequencing  436  TAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACC 484
```

```
pET30ail3 sequencing      413 TA-------ATTAATTCT--------TCGAAATTACCATCATTAACATC 446

pET30a alone sequencing   485 GCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTT 533
pET30ail3 sequencing      447 ATTTAGTATAGATCATATAAAGAAGCTTCGACGATTGAAATCTTTACCA 495

pET30a alone sequencing   534 CCTTTCTCGCCACGTTCGCCGGCTTCCCCGTCAAGCTCTAAATCGGGGC 582
pET30ail3 sequencing      496 AATTTTAAAACCAGAATACAATAAAACAGAACAATCACAAAA----GAA 540

pET30a alone sequencing   583 TCCCTTTAGGGTCCGATTAGTGCTTACGCACTCACCCAAAACTGATAGG 631
pET30ail3 sequencing      541 TCTAATTTATTATCGA------CATGATCATTT---CATATTTCATAGG 580

pET30a alone sequencing   632 TATGTCCTATGGGC 645
pET30ail3 sequencing      581 G              581
```

Thus, the inserted il3 sequence is

ATGCCCGAACCAACAGATCAAAAACTAAAAGTAATACAAATATTTAATATTTCAACGTCAGAGAAAGAATTAC
AAAAGGCGAATTGGAAACCAACACCAAATGTAACATTGAAAAGTGAAACAAAACAACATCAACACGATTACTT
ACATAGAAGTAATGAAGAACAAAAGAATAAACTAGGAGACAAACAATTAATTAATTCTTCGAAATTACCATCA
TTAACATCATTTAGTATAGATCATATAAAGAAGCTTCGACGATTGAAATCTTTACCAAATTTTAAAACCAGAAT
ACAATAAAACAGAACAATCACAAAAGAATCTAATTTATTATCGACATGATCATTTCATATTTCATAGGG

and the protein sequence is:

MPEPTDQKLKVIQIFNISTSEKELQKANWKPTPNVTLKSETKQHQHDYLHRSNEEQKNKLGDKQLINSSKLPSLTSFSI
DHIKKLRRLKSLPNFKTRIQ

Alignment between pET30a-il3 (theoretical) and sequenced one (i.e. experimental) at protein level

```
pET30ail3 protein sequencing (experiment)  1    MHHHHHHSSGLVPRGSGMKETAAAKFERQHM 31
pET30ail3 protein sequence (theoretical)   1    MHHHHHHSSGLVPRGSGMKETAAAKFERQHM 31

pET30ail3 protein sequencing (experiment)  32   DSPDLGTDDDDKAMADIGSEFMPEPTDQKLK 62
pET30ail3 protein sequence (theoretical)   32   DSPDLGTDDDDKAMADIGSEFMPEPTDQKLK 62

pET30ail3 protein sequencing (experiment)  63   VIQIFNISTSEKELQKANWKPTPNVTLKSET 93
pET30ail3 protein sequence (theoretical)   63   VIQIFNISTSEKELQKANWEPTPNVTLKSET 93

pET30ail3 protein sequencing (experiment)  94   KQHQHDYLHRSNEEQKNKLGDKQLINSSKLP 124
pET30ail3 protein sequence (theoretical)   94   KQHQHDYLHRSNEEQKNKLGDKQLINSSKLP 124

pET30ail3 protein sequencing (experiment)  125 SLTSFSIDHIKKLRRLKSLPNFKTRIQ     151
pET30ail3 protein sequence (theoretical)   125 SLTSFSIDHIKKLRRLKSLPNFKTEYNKTEQ 155

pET30ail3 protein sequencing (experiment)  152                                 151
pET30ail3 protein sequence (theoretical)   156 SQKNLIYYRHDHFIFHREQKTAAALEHHHH 186

pET30ail3 protein sequencing (experiment)  152    151
pET30ail3 protein sequence (theoretical)   187 HH 188
```