



cDNA Cloning

Objective

To clone the human Folate Receptor 1 (hFOLR1) cDNA with a His N-terminal tag into an expression vector.

Starting material

Cells pellet

Methods

1. RNA extraction from HeLa cells with RNeasy (Qiagen)
2. Primers design and synthesis. His-tag introduced into the 5' primer .
3. RT-PCR with SuperScript III One-Step RT-PCR System (Invitrogen)
4. NheI/ NotI cloning into pcDNA3.1. Transformation into OneShot TOP10F'.
5. Mini-prep DNA digestion to identify a clone with correct size insert.
6. Double-strand sequence of the positive clone and comparison to known sequence.

Primers

N-term

5' (GCT AGC ATG CAC CAC CAC CAC CAC CAC TTG CAG TGT CGA CCC TGG AG) 3'

C-term

5' (TGC GGC CGC TCA GCT GAG CAG CCA CAG CAG) 3'

Results

DNA consensus sequence

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GCTAGCATGCACCACCACCACCACCCTTGCATGAGCAGTGTCGACCCTGGAGGAAGA
ATGCCTGCTGTTCTACCAACACCAGCCAGGAAGCCCATAAGGATGTTTCCTACCTATATA
GATTCAACTGGAACCACTGTGGAGAGATGGCACCTGCCTGCAAACGGCATTTCATCCAG
GACACCTGCCTCTACGAGTGCTCCCCCACTTGGGGCCCTGGATCCAGCAGGTGGATC
AGAGCTGGCGCAAAGAGCGGGTACTGAACGTGCCCTGTGCAAAGAGGACTGTGAGCA
ATGGTGGGAAGATTGTCGCACCTCCTACACCTGCAAGAGCAACTGGCACAAGGGCTGG
AACTGGACTTCAGGGTTTAACAAGTGCAGTGGGAGCTGCCTGCCAACCTTCCATTT
CTACTTCCCCACACCCACTGTTCTGTGCAATGAAATCTGGACTCACTCCTACAAGGTCA
GCAACTACAGCCGAGGGAGTGGCCGCTGCATCCAGATGTGGTTTCGACCCAGCCCAGG
GCAACCCCAATGAGGAGGTGGCGAGGTTCTATGCTGCAGCCATGAGTGGGGCTGGGC
CCTGGGCAGCCTGGCCTTTCCTGCTTAGCCTGGCCCTAATGCTGCTGTGGCTGCTCAG
CTGAGCGGCCGC
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Protein consensus sequence

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MHHHHHHEQCRPWKNACCSTNTSQAHKDVSYLRFNWNHCGEMAPACKRHFIQDTCL
YECSPNLGPWIQQVDQSWRKERVNLNPLCKEDCEQWWEDCRTSYTCKSNWHKGNWNTS
GFNKCAVGAACQPFHFYFPTPTVLCNEIWTHSYKVSNSYRSGRGIQMWFDPAQGNPNEE
VARFYAAAMSGAGPWAAWPFLLSLALMLLWLLS
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Conclusions

Folate Receptor cDNA as been cloned into pCDNA3.1 with an N-terminal His-tag. Sequence confirmed that no mutations have been artificially introduced in the coding sequence.