WNV i Flavivirus prajmeri

Quantitative RT-PCR for WNV and house-keeping gene (β-actin)

Primers : **WNproC-F 10 (5'-CCTGTGTGAGCTGACAAACTTAGT-3')**
WNproC-R 153 (5'-GCGTTTTAGCATATTGACAGCC-3')
ACTBFrw966 : (5'-CAGCACAATGAAGATCAAGATCATC-3')
ACTBrev1096 : (5'-CGGACTCATCGTACTCCTGCTT-3')

Resuspend primers with milliQ water to obtain a 100µM stock solution and store at -20°C (FMDV L3 laboratory).

Probes :
Classical TaqMan probes :
WNproC-probe (5'-FAM-CCTGGTTTCTTAGACATCGAGATCT -TAMRA-3')
ACTB1042-67 probe (5'-VIC-TCGCTGTCCACCTTCCAGCAGATGT-TAMRA-3')

Protokol iz IFI Riems, Germany, regarding modified primers from Linke et al., 2007. Nemam prajmere i probu za house keepeng gene

WNV (Flavivirus specific prajmers)
Tamás Bakonyi, Éva Ivanics, Károly Erdélyi, Krisztina Ursu, Emöke Ferenczi, Herbert Weissenböck, and Norbert Nowotny (2006): Lineage 1 and 2 Strains of Encephalitic West Nile Virus, Central Europe. Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 12, No. 4, April 2006
JEV (Japanise encephalitis virus) -group specific oligonucleotide primer pair designed on the nonstructural protein **5 (NS5) and 3′-**untranslated regions (UTR) of WNV **(ISTI KAO I WEISSENBOCK 2002 broj 2 ispod)**
forward primer: **WNV F 5'- GARTGGATGACVACRGAAGACATGCT- 3'**
reverse primer: **WNV R 5′-GGGGTCTCCTCTAACCTCTAGTCCTT- 3′;**

WNV (Flavivirus specific prajmers)
Weissenböck, H., Kolodziejek, J., Url, A., Lussy, H., Rebel-Bauder, B. & Nowotny, N. (2002). Emergence of Usutu virus, an African mosquito-borne flavivirus of the Japanese encephalitis virus group, central Europe. Emerging Infectious Diseases. 8 , 652 _/656.
After aligning available nucleotide (nt) sequences of various mosquito-borne flaviviruses and determining highly conserved genomic regions, we designed three pairs of oligonucleotide primers (to amplify a wide range of mosquito-borne flaviviruses) and used them in the reverse transcription-polymerase chain reaction (RT-PCR) assays:
5'-TACAACATGATGGGVAARAGAGAGA-3' (nt position 9031–9055 of WNV GenBank accession no. NC 001563) and
5'-AGCATGTCTTCYGTBGTCATCCAYT- 3' (nt position 10115–10091) **(OVE NE UZIMATI ZA SADA)**
resulting in a 1,084-bp amplification product;

Weissenböck et al., 2002, Bakonyi et al 2006 Imam ove
WNV W2F **5'-GARTGGATGACVACRGAAGACATGCT-3'** (nt position 10090–10115)
WNV W2R **5'-GGGGTCTCCTCTAACCTCTAGTCCTT-3'** (nt position 10832–10807),
amplifying a **743-bp** PCR product; and
Program: 95°C for 15 min, the cDNA was amplified in 40 cycles of heat denaturation at 94°C for 40 s, primer annealing at 57°C for 50 s, and DNA extension at 72°C for 1 min

Weissenböck et al., 2002 Imam i ove
WNV W3F **5'- GCCACCGGAAGTTGAGTAGA-3'** (nt position 10460–10479 of WNV no. NC 001563) and
WNV W3R **5'-GCTGGTTGTGCAGAGCAGAA-3'** (nt position 10908–10889),
resulting in a **449-bp** amplicon.
Reverse transcription was performed for 30 min at 50°C. Following an initial denaturation for 15 min at 95°C, the reaction mixture was subjected to 45 cycles of heat denaturation at 94°C for 30 s, primer annealing at 60°C for 30 s, and DNA extension at 72°C for 1 min, completed by a final extension of 10 min at 72°C.