Purification of HIV RNA from serum using a polymer capture matrix in a microfluidic device

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7.1 PCR

The PCR mix for amplification of the ssDNA oligonucleotide contained 26 µl of H_2O, 1 µl of recovered sample, 4.1 µl of 10x PCR buffer, 4.1 µl of 25 mM $MgCl_2$, 3.25 µl of dNTP mix (contains 10 mM of each nucleotide), 1.2 µl of each primer (10 µM stock solutions) and 0.6 µl of AmpliTaq Gold (PCR reagents from Applied Biosystems, Foster City, CA, primers from Operon Biotechnologies). Thermal cycling conditions were: 10 minutes at 95 °C, followed by 36 cycles of 95 °C for 30 seconds, 52 °C for 30 seconds and 62 °C for 1 minute. The RT-PCR mixture for amplification of recovered RNA contained 12.5 µl of 2X SuperScript™ III reaction mix, 1.0 µl of SuperScript™ III RT/Platinum *Taq* mix, 0.5 µl of each primer (10 µM stock solution), and 5 µl of recovered sample. Thermal cycling conditions for RT-PCR were: 30 minutes at 55 °C, 5 minutes at 94 °C, 42 cycles of 94 °C for 15 seconds, 55 °C for 30 seconds, 68 °C for 30 seconds, and a final extension at 68 °C for 5 minutes. Primers for PCR and RT-PCR are:

PCR

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5′ – TGC TTG ATG TCC CCC CAC T
5′ – TTG TAA AAC GAC GGC CAG T
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RT-PCR

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5′ – ACT GGG GGG ACA TCA AGC AGC CAT GCA AAT
5′ – TGC TAT GTC ACT TCC CCT TGG TTC TCT
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The ssDNA oligonucleotide target used in this study was:

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5′–  TTGTAAAACG ACGGCCAGTC ACACACACAC ACACACACAC ACACACACAC
     ACAGTGGGGG GACATCAAGCA
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7.2 Pfold Analysis of the HIV-B gag region

Pfold (http://www.daimi.au.dk/~compbio/pfold/) was used to analyze the HIV-B gag region for potential secondary structure. The red, italicized sequences are regions of secondary structure predicted by Pfold. The blue, underlined sequences are the SK462 and SK431 primer regions. The bold brackets indicate the capture sequences used in this study. The capture sequences were shorter than the primers to lower the dehybridization temperature necessary for the recovery step. Pfold predicts a six base region of the 22 bases targeted by the SK462 capture sequence to be hybridized to another region of the gag sequence.

CAAATGGTAC ATCAGGCCAT ATCACCTAGA ACTTTAAATG *CATGGGT*AA
AAGTAGTAGA AGAGAA*GGCT* TTC*AGCC*CAG AAGTAA*TACC* *CATGT*TTTCA
GCATTATCAG AAGGAGCCA*C* *CCCAC*AAGAT TTAAACACCA TGCTAAACAC
[AGTGGGGGGA CATCAAGCAG CC**]**ATGCAAAT GTTAAAAGAG ACCATCAATG
SK462
AGGAAGCTGC AGAATGGGAT AGAGTACATC CAGTGCATGC AG*GGCCT*ATT
GCACC*AGGCC* AGATG**[**AGAGA ACCAAGGGGA AGTGACAT**]**AG CAGGAACTAC
SK431
TAGTACCCTT CAGGAACAAA TAGGA*TGGAT* GACAAATA*AT* *CCA*CCT*ATCC*
*CAG*TAGGAGA AATTTATAAA AGATGGATAA TC*CTGGGAT*T AAATAAAATA
GTAAGAATGT ATAGCCCTAC CAGCATTCTG GACATAAGAC AAGGACCAAA
GGAACCC