**NFκB inhibition mitigates serum amyloid A-induced pro-atherogenic responses in endothelial cells and leukocyte adhesion and adverse changes to endothelium function in isolated aorta**

Abigail VALLEJO,\* Belal CHAMI,\* Joanne M. DENNIS,\* Martin SIMONE,\* Gulfam AHMAD,\* Adrian I. ABDO,† Arpeeta SHARMA,‡ Waled A SHIHATA, Nathan MARTIN\*,*§* #£ Jaye P.F. CHIN-DUSTING, *§*‡#£ Judy B. de HAAN,‡*ɸ* and Paul K. WITTING.\*

*\*Discipline of Pathology, Sydney Medical School, The University of Sydney, NSW, 2006 Australia*

*†Heart Research Institute, Newton, NSW, 2053, Australia*

*‡Baker IDI Heart and Diabetes Institute, Victoria, Australia*

*§Department of Medicine, Monash University, Victoria, Australia*

*#Cardiovascular Disease Program, Biomedicine Discovery Institute, Monash University £Department of Pharmacology, Monash University, Victoria, Australia*

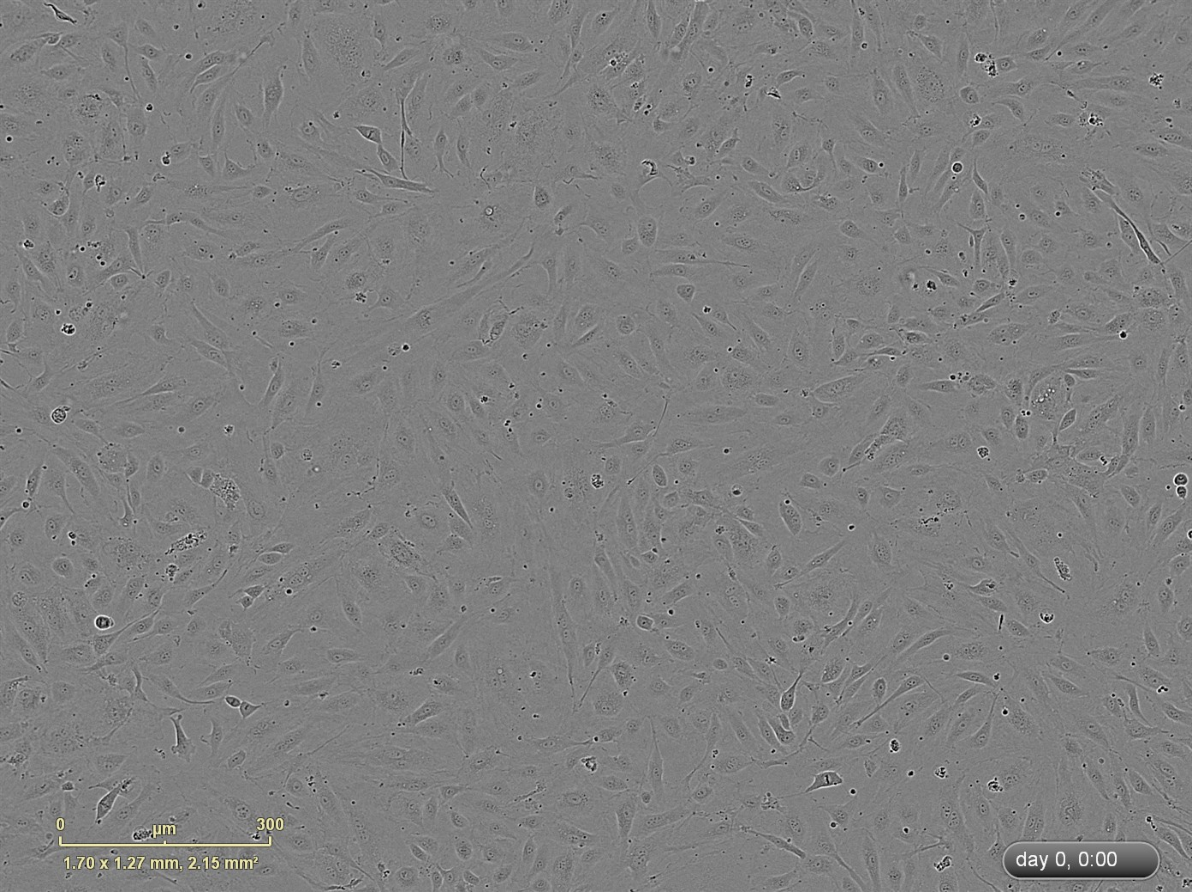
*ɸDepartment of Immunology, Monash Univeristy, Victoria, Australia.*

**Supplementary data**

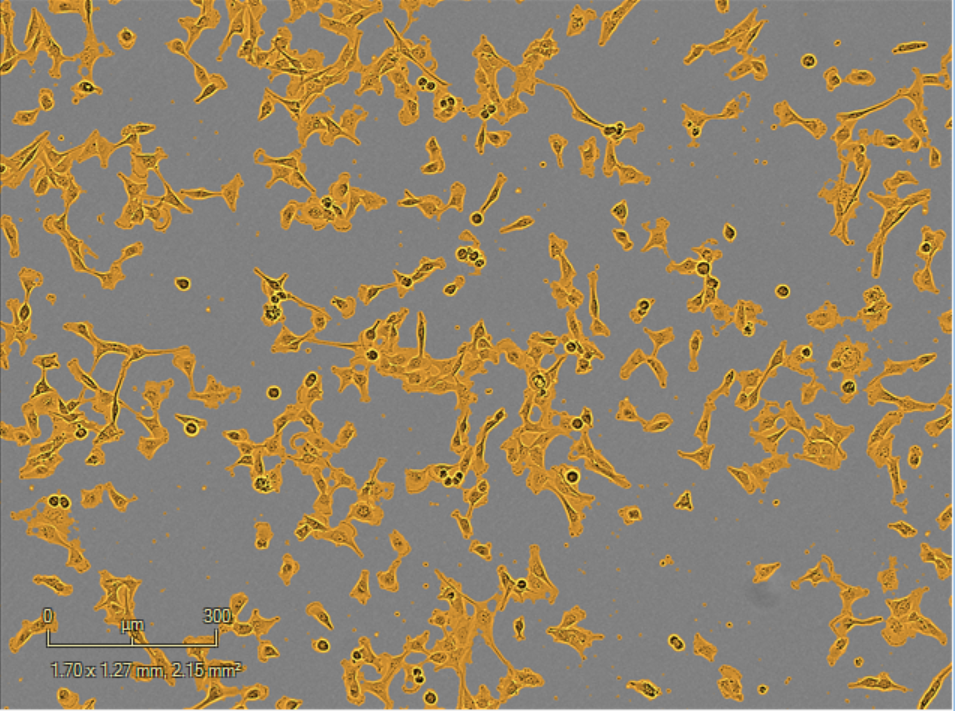
Table S1. HCtAE cell confluency as determined by imaging with an IncuCyte system.

|  |  |  |
| --- | --- | --- |
| Well | Treatment group | Confluency (%) |
| A1 | 1.1 Control | 56.85298 |
| B1 | 1.2 Control | 56.13226 |
| A2 | 1.1 SAA | 59.34018 |
| B2 | 1.2 SAA | 64.7347 |
| A3 | 1.1 BAY11 | 33.76793 |
| B3 | 1.2 BAY11 | 35.37162 |

**a** High-definition D phase-contrast images were acquired for each well in a 6-well plate using theThe IncuCyte Zoom® live cell imaging system (Essen BioScience, Australia). The system software calculated the average confluency of each individual field imaged to obtain the overall mean (n=4) level of confluency for the well expressed as a percentage of the total area imaged (%). Levels of confluency were then used to normalise total secretary IL-6 as determined by ELISA.



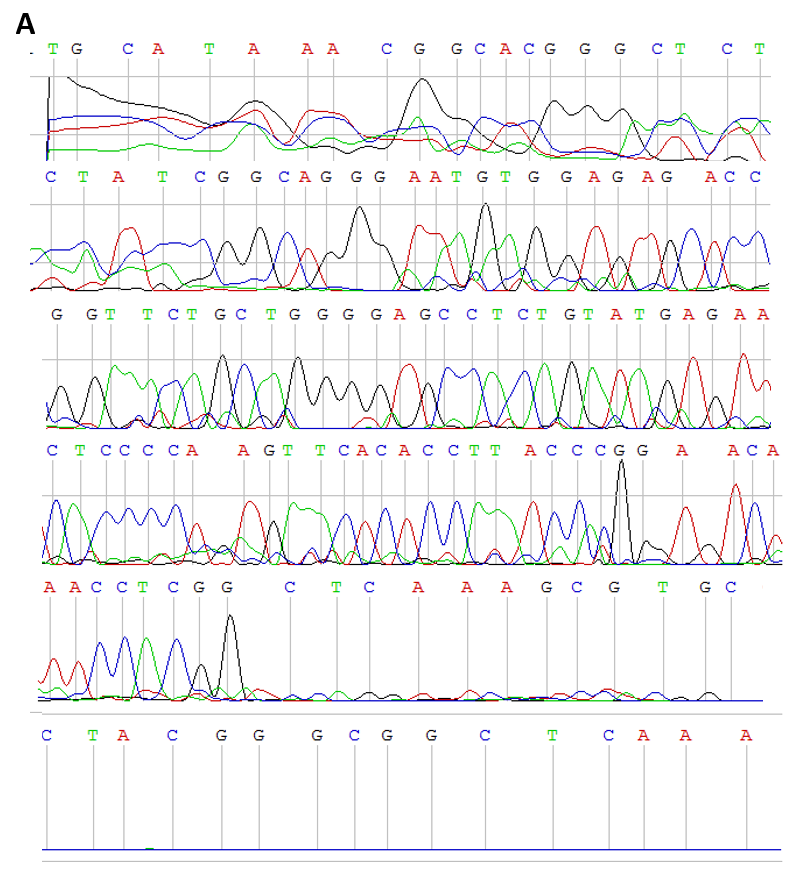
**(a)**

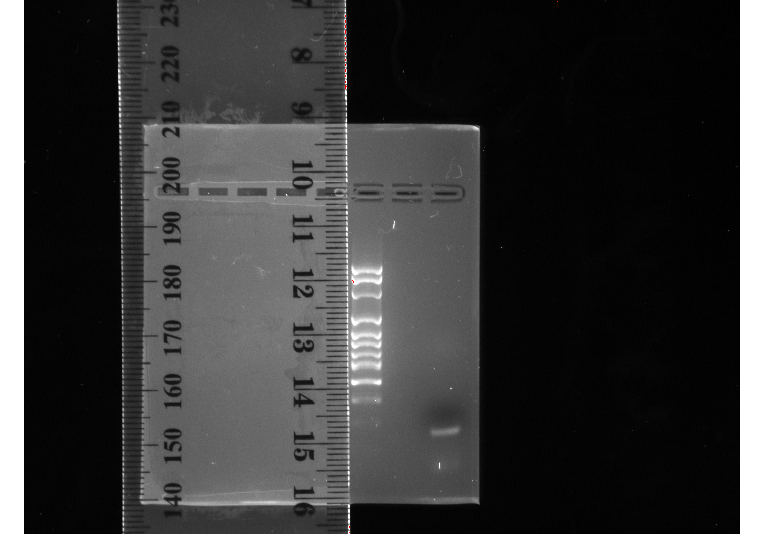


**(b)**

**Figure S1. Cultured HCtAE cell confluence as assessed with an IncuCyte imaging system**.

The IncuCyte Zoom® live cell imaging system (Essen BioScience, Australia) was used to measure the level of cell confluency (expressed as a percentage of the total area imaged) immediately prior to the time of harvest. (a) Representative field image of well A1 (*Table S1*); showing areal coverage at the 56% confluency level. (b) Representative field image of well B3 (*Table S1*) showing fewer cells in the same areal field (35%) – orange highlight shows confluence mask employed by the software to identify cells). Images were taken using a fixed 10*x* objective lens (Nikon, Australia).

**Figure S2. Gene sequencing**



**1**

**2**

**3**

**300**

**200**

**100**

**A**

**Figure S2(ii). Sequencing chromotagram for purified bands (Gentle Software)**

DNA from bands noted as *A* in Figure 1 were extracted from agarose gel, purified and sequenced. Figure shows good quality chromatography data for reliable sequence data.

**Figure S2(i). RT-PCR gel for TF expression**

Lane 1. Hyperladder 100 Bp Plus

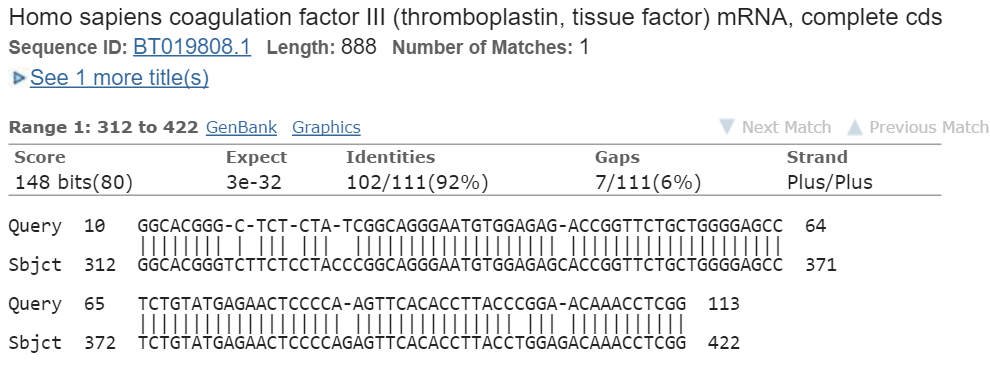
Lane 2. No sample

Lane 3. HCtAE cDNA

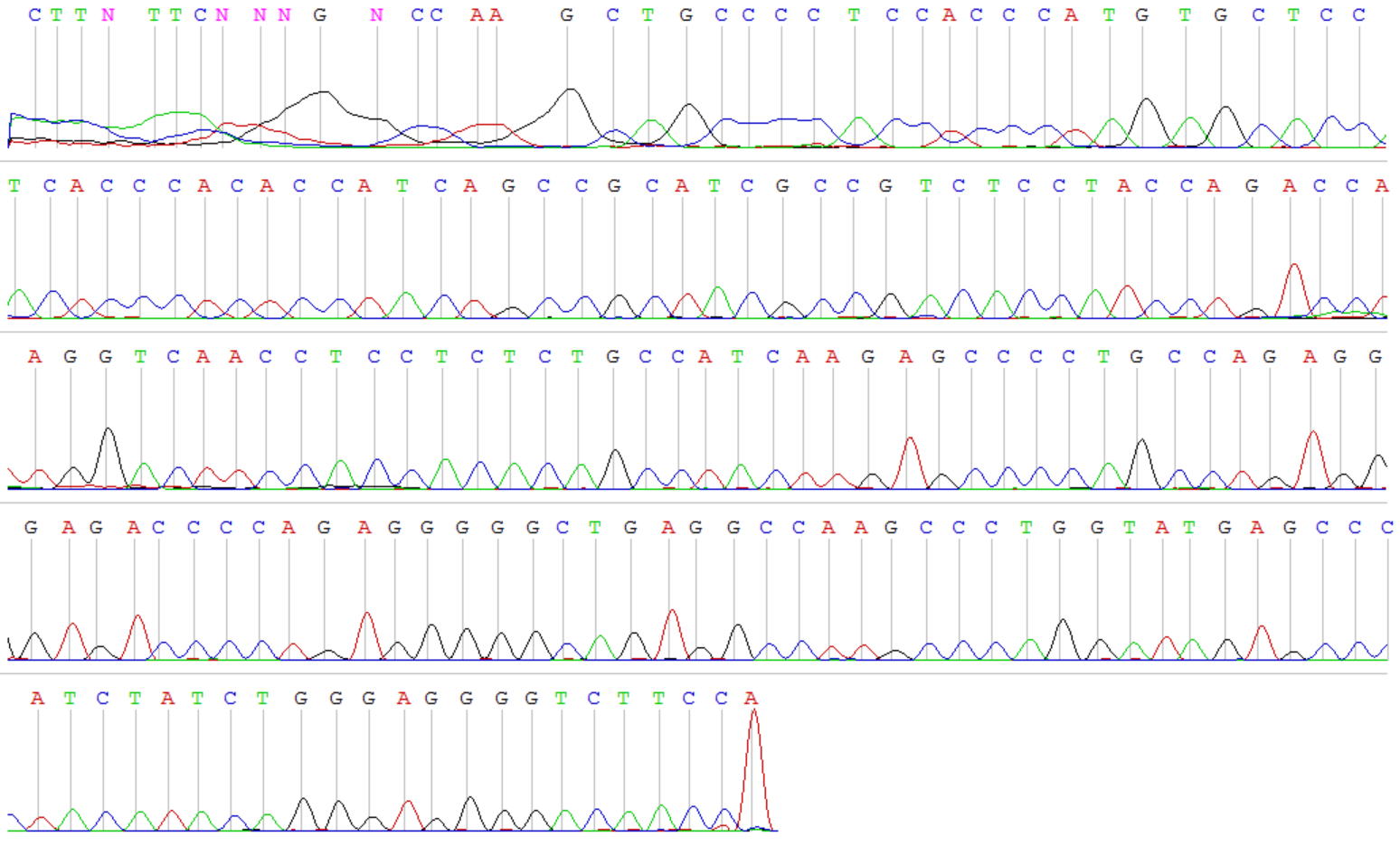
Note Band A corresponds to an amplification product of 157bp

***Experimentally determined Sequence***

TGCATAAACGGCACGGGCTCTCTATCGGCAGGGAATGTGGAGAGACCGGTTCTGCTGGGGAGCCTCTGTATGAGAACTCCCCAAGTTCACACCTTACCCGGAACAAACCTCGGCTCAAAGCGTGCCTACGGGCGGCTCAAATTATTGCATCTTTGCTGGGGGTCCCCCGGCGGGGGGGGGGGGAGGGCCAAAAGGAAATATTGCACG

**Figure S2(iii). Tissue factor sequencing result** 

**Figure S2(iv). Band A NCBI Nucleotide Blast result**



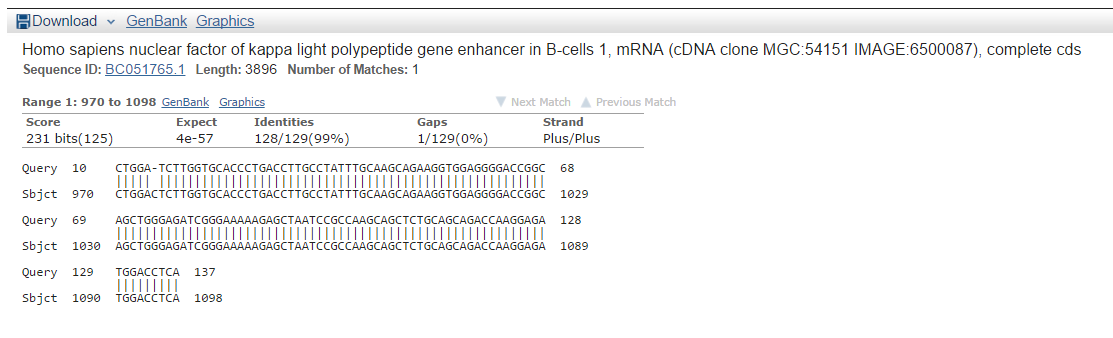
**Figure S2(i-iv). Sequencing Chromatogram for purified bands (Gentle Software)**

DNA from bands noted *A* Figure 1 were extracted from agarose gel, purified and sequenced. Figure shows good quality chromatography data for reliable sequence data. Percentage match obtained was 92% identity.

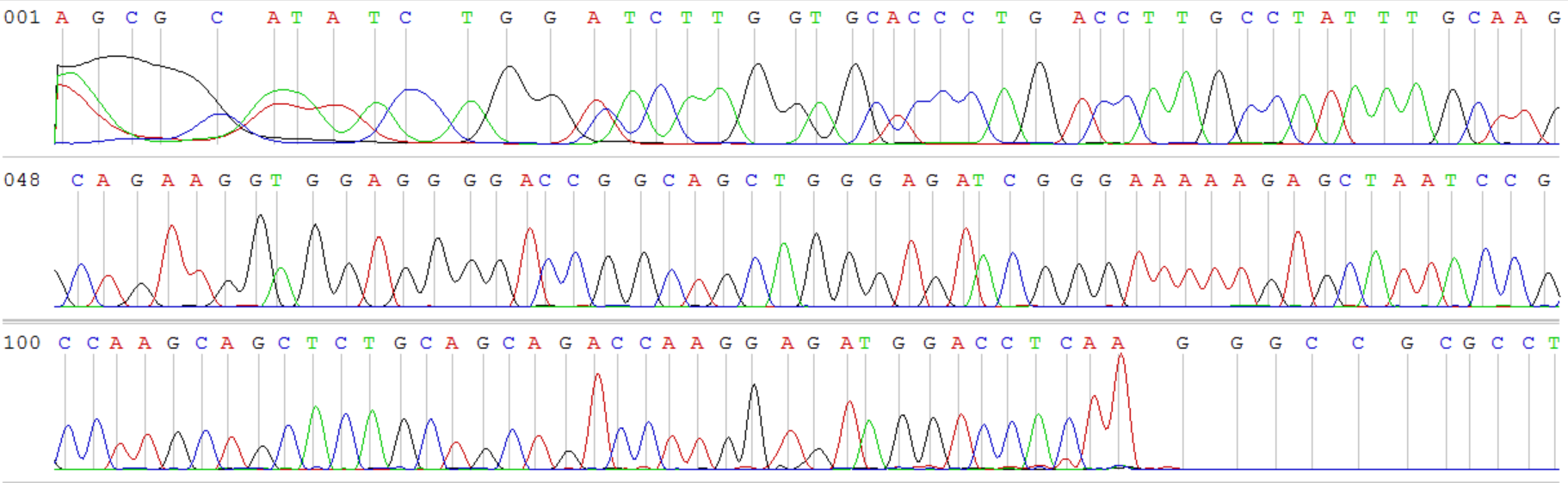
**Figure S3(i) NFKB sequencing result**

***Experimentally determined Sequence***

AGCGCATATCTGGATCTTGGTGCACCCTGACCTTGCCTATTTGCAAGCAGAAGGTGGAGGGGACCGGCAGCTGGGAGATCGGGAAAAAGAGCTAATCCGCCAAGCAGCTCTGCAGCAGACCAAGGAGATGGACCTCAAGGGCCGCGCCTAGGGGATTCGGAGGCGCCCGTGGGCGGCGCGGGGCCGGGGAGCAGAGCCATACTAGCGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGAGAC



**Figure S3(ii). NF*k*B Nucleotide Blast result**



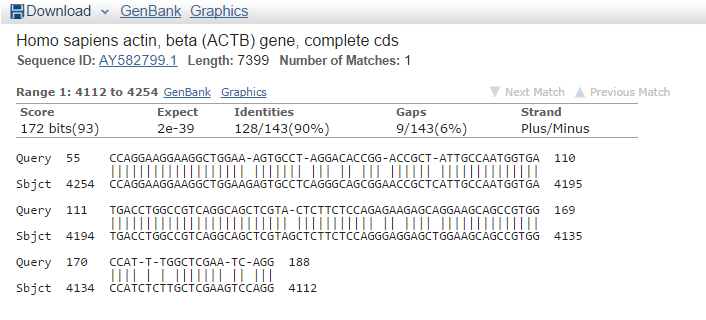
**Figure S3(iii). NF*k*B Sequencing Chromatogram**

DNA from a single band was extracted from agarose gel (as per figure S2(i) above), purified and sequenced. Figure shows good quality chromatography data for reliable sequence data. Percentage match obtained was 99% identity.

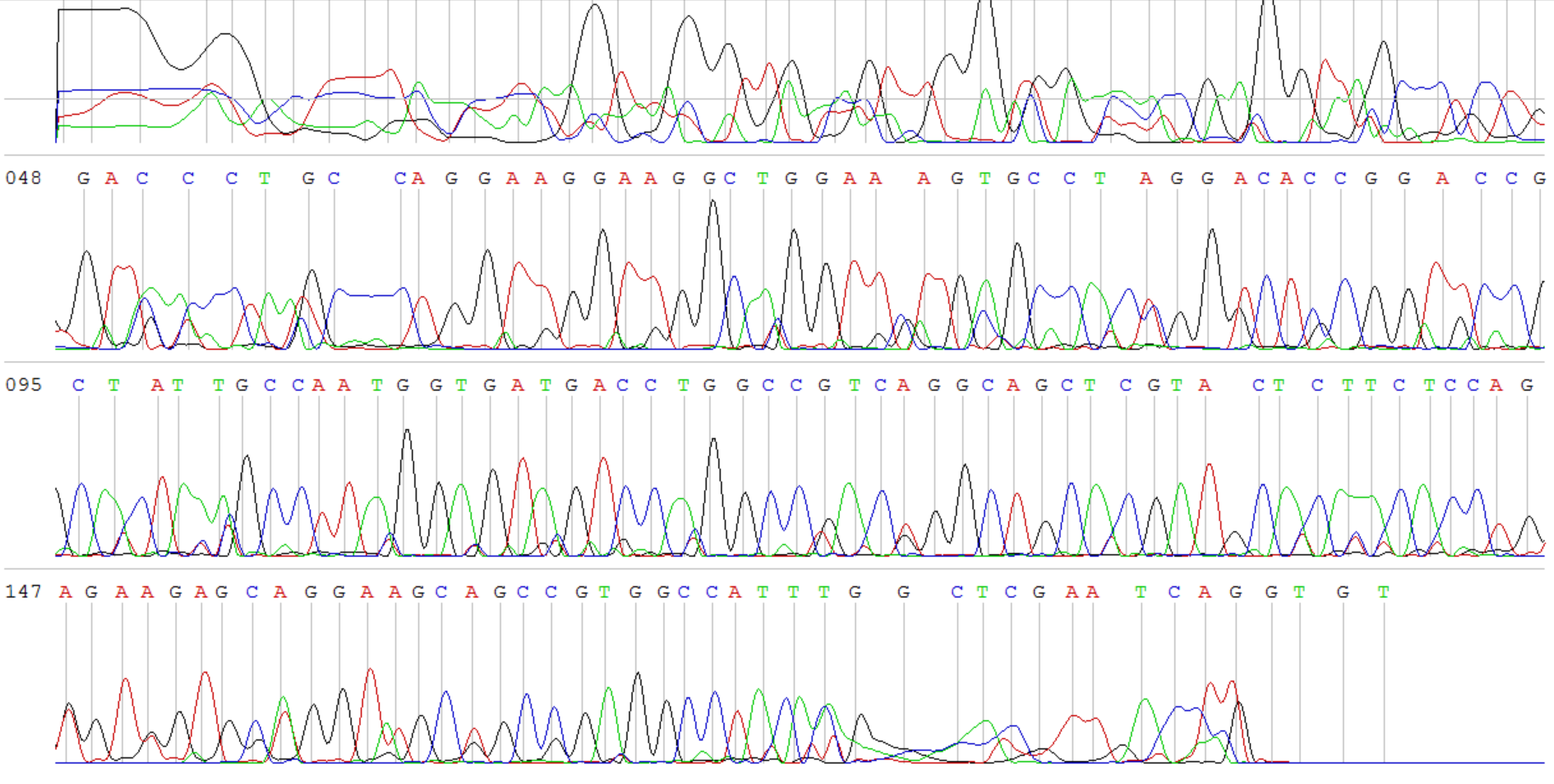
**Figure S3(i). Beta actin house keeping gene**

***Experimentally determined Sequence***

TGGTCTCATATTCATTGAAGGAGCGAAGGTGTCTCGTGGATGCCCAGGACCCTGCCAGGAAGGAAGGCTGGAAAGTGCCTAGGACACCGGACCGCTATTGCCAATGGTGATGACCTGGCCGTCAGGCAGCTCGTACTCTTCTCCAGAGAAGAGCAGGAAGCAGCCGTGGCCATTTGGCTCGAATCAGGTGT



**Figure S3(ii). Beta actin Nucleotide Blast**



**Figure S3(iii). Beta actin Sequencing Chromatogram**

DNA from a single band was extracted from agarose gel (as per figure S2(i) above), purified and sequenced. Figure shows good quality chromatography data for reliable sequence data. Percentage match obtained was 90% identity.