Figure 3.3.1 - Bacterial growth of EGFP plasmids. The pcDNA3-EGFP vector was spread on an LB plate containing 100 µg/mL ampicillin and incubated for 24 hours at 37°C. This image was taken on an Alpha Innotech FluorChem Q gel doc using a CY2 filter (Excitation 489/Emission 506 nm).
Figure 3.3.2 - Electrophoresis of EGFP vectors. pcDNA3-EGFP (A-B) and pHygEGFP (C-D) vectors were purified by mini-prep from 3 mL of overnight culture in 2YT media containing 100 µg/mL of ampicillin. 1 µg from each of the purified DNA samples, together with a negative control containing the DNA/RNA free water used to prepare samples (E), were then separated on a 0.8% agarose gel containing 0.5 µg/mL of ethidium bromide at 150 V for 45 minutes. The bands were visualised under UV trans-illumination on a gel doc.
Figure 3.3.3 - Transfection of IMR-32 cells with EGFP. Purified pcDNA3-EGFP DNA was transfected into confluent IMR-32 cells using jetPRIME® transfection reagent and incubated at 37°C for 24 hours. The control (A) and transfected (B) cells were trypsinised, washed three times with PBS, and re-suspended in 250 µL of PBS. This image was taken on an Alpha Innotech FluorChem Q gel doc using a CY2 filter.
Figure 3.3.4 - Flow cytometry analysis of IMR-32 cells transfected with pcDNA3-EGFP. Purified pcDNA3-EGFP DNA was transfected into confluent IMR-32 cells using jetPRIME® transfection reagent alongside non-transfected controls and incubated at 37°C for 24 (A+B), 30 (C+D) and 46 (E+F) hours. The cells were trypsinised, washed three times with PBS, and re-suspended in 250 µL of PBS. Using an Accuri C6 flow cytometer, 10,000 cells were counted and gated accordingly: R1 is the viable population of cells, M1 is the population of non-transfected cells, and M2 is the population of transfected cells. (N=1).
Figure 3.3.5 - Restriction digest of hBCATm entry vector pENTR221. The hBCATm entry vector was purified by mini-prep from 3 mL of overnight culture in 2YT media containing 50 µg/mL of kanamycin sulphate. A total of 1 µg of hBCATm entry vector pENTR221 was incubated with the PacI and Ascl restriction enzyme/s for 5 minutes at 37°C in Fast digest green buffer before being loaded onto a 0.8% agarose gel containing 0.5 µg/mL of ethidium bromide and separated at 150 V for 45 minutes. The bands were visualised under UV trans-illumination on a gel doc. Lane A; entry vector, Lane B; digest with PacI, Lane C; digest with Ascl, Lane D; digest with PacI and Ascl, Lane E- G; entry vector, Lane H; digest with PacI and Ascl, Lane I; negative control.
The hBCATm expression vector was purified by mini-prep from 3 mL of overnight culture in 2YT media containing 100 µg/mL of ampicillin. A total of 1 µg of hBCATm expression vector was incubated with the Pacl and Ascl restriction enzymes for 5 minutes at 37°C in Fast digest green buffer before being loaded onto a 0.8% agarose gel containing 0.5 µg/mL of ethidium bromide. The DNA was separated at 150 V for 45 minutes and the bands were visualised under UV trans-illumination on a gel doc. **Lane A-D;** expression vector, **Lane E;** digest with Pacl and Ascl, **Lane F;** expression vector, **Lane G;** digest with Pacl and Ascl, **Lane H;** entry vector, **Lane I;** entry vector digest with Pacl and Ascl, **Lane J;** Gus LR positive control vector, **Lane K;** negative control.
Figure 3.3.7 - Western blot analysis of IMR-32 cell lysate transfected with hBCATm-His and bound to His Mag Sepharose Ni. IMR-32 cells were transfected with hBCATm expression vector and incubated at 37°C for 9, 24, 32 and 48 hours. As a negative control IMR-32 cells were also transfected with Gus expression vector for 48 hours. From the 48 hour hBCATm and Gus expression vector transfections, 150 µL of the IMR-32 cell lysate was incubated with 75 µL of His Mag Sepharose Ni for 30 minutes and the bound proteins eluted. With the exception of the eluted precipitation proteins from which 20 µL was taken, 20 µg of total protein from each of the samples was separated by SDS-PAGE along with 20 ng of BCATm-His pure protein. After transfer to a PVDF membrane, primary anti-BCATm (Abcam) (1:5000) was added overnight, followed by secondary anti-rabbit HRP (1:1000) for 1 hour (A). Densitometry analysis was carried out to demonstrate the increase in expression of hBCATm-His over the various time points (B) (N=1).
Figure 3.3.8 - Restriction digest of hBCATc expression vector. The hBCATc expression vector was purified by mini-prep from 3 mL of overnight culture in 2YT media containing 100 µg/mL of ampicillin. A total of 1 µg of hBCATc expression vector was incubated with the PacI and Ascl restriction enzymes for 5 minutes at 37°C in Fast digest green buffer before being loaded onto a 0.8% agarose gel containing 0.5 µg/mL of ethidium bromide. The DNA was separated at 150 V for 45 minutes and the bands were visualised under UV transillumination on a gel doc. **Lane A+D:** expression vector, **Lane B+E:** digest with PacI, **Lane C+F:** digest with PacI and Ascl, **Lane G:** GFP control vector, **Lane H:** negative control.
<table>
<thead>
<tr>
<th>Media</th>
<th>Transfection Control</th>
<th>0.5 µg hBCATc</th>
<th>1 µg hBCATc</th>
<th>1.5 µg hBCATc</th>
<th>2.5 µg hBCATc</th>
<th>Media</th>
<th>Transfection Control</th>
<th>0.5 µg hBCATm</th>
<th>1 µg hBCATm</th>
<th>1.5 µg hBCATm</th>
<th>2.5 µg hBCATm</th>
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**Figure 3.3.9 - Western blot analysis of SH-SY5Y cells transfected with hBCAT plasmids.** SH-SY5Y cells were transfected with varying concentrations of hBCAT expression vector or a control GFP plasmid for 48 hours. Cell lysates were collected using RIPA extraction and 10 µg of sample was separated on a 12% SDS-PAGE gel and transferred to PVDF membrane for western blot analysis. The membrane was probed with either anti-hBCATc or anti-hBCATm (1:3000) overnight followed by a 1 hour incubation with anti-rabbit HRP (1:2000). The bands were visualised using BM chemiluminescent HRP substrate and exposed to Hyperfilm. (N=3)