

Metabolomics protocol

hPSC-Scs were treated with 5mM and 30mM glucose for 72 h and harvested for metabolomics analysis. Frozen total cell pellets from at least three biological repeats were submitted to the West Coast Metabolomics Center at the University of California, Davis. Agilent 7890C with Leco Pegasus HT was used for the study. Samples extracted using 1mL of 3:3:2 ACN:IPA:H₂O (v/v/v). Half of the sample was dried to completeness and then derivatized using 10 uL of 40 mg/mL of Methoxyamine in pyridine. They were shaken at 30C for 1.5 hours. Then 91 uL of MSTFA + FAMES to each sample and they were shaken at 37C for 0.5 hours to finish derivatization. Samples were then vialled, capped, and injected onto the instrument. A 7890C GC was used coupled with a LECO TOF. 0.5 uL of derivatized sample was injected using a splitless method onto a RESTEK RTX-5SIL MS column with an Intergra-Guard at 275C with a helium flow of 1 mL/min. The GC oven was set to hold at 50C for 1 min then ramped to 20C/min to 330C and then held for 5 min. The transfer line was set to 280C while the EI ion source was set to 250C. The Mass spec parameters collect data from 85m/z to 500m/z at an acquisition rate of 17 spectra/sec.