METADATA

Bulk RNA sequencing (RNAseq) method and representative results

THP-1-derived macrophages were exposed to microbial stimuli in agonist mode, or in antagonist mode upon stimulation with a known nociceptive compound, the *Escherichia coli* lipopolysaccharide (LPS, Sigma). Cells cultured in RPMI media with or without LPS spike were used as controls. After 24 h exposure, THP-1 cells were collected in RNAlater and processed by Azenta for RNA extraction, library preparation, RNA PolyA selection, and Illumina HiSeq 2x150bp bulk mRNA sequencing (20-30M reads/sample). The read counts were quality controlled and analyzed with the online RNAseq pipeline iDEP version 0.96 (PMCID: PMC6299935). Using the Human GRCh38.p12 reference genome, differentially expressed genes (DEGs) were tabulated using the DESeq2 software (PMCID: PMC4302049). In agonist mode, the DEG transcriptional profiles were calculated using cells in RPMI as reference control. In antagonist mode, cells exposed to LPS were used as reference. The DEGs were subjected to a 0.03 failure detection rate (FDR) with a $\log_2$ fold change cutoff of 1 for significance. Average results of the DEG analysis for representative members in the diversity panel belonging to microbial orders showing the broadest range of activity in pain are reported. These include DEG profiles (total of 20) of cells exposed in agonist and antagonist mode to well tolerated dilutions of: two species of Coriobacteriales, two species of Eggerthellales, two species of Bacteroidales, two species of Lactobacillales, six species of Clostridiales, one species of Erysipelotrichales, one species of Tissierellales, one species of Fusobacteriales, one species of Enterobacterales, and one species of Verrucomicrobiales, as well as the DEG profile of cells challenged with LPS as compared to THP-1 cells in RPMI (baseline).