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BE504

Final Project Proposal

**Introduction**

GABA-A receptors are ligand-gated ion channels selective for chloride. Intracellular neuronal chloride concentrations during embryonic development have been found to be elevated in many organisms. In a mature neuron, GABA binding causes an increase in chloride ion conductance and hyperpolarizes the cell membrane because chloride has a reversal potential lower than the membrane potential. However, during embryonic development, intracellular neuronal chloride concentrations are elevated in many organisms, causing GABA to be an excitatory neurotransmitter as opposed to an inhibitory one as found in adulthood. This sequence is observed in a wide range of brain structures and animal species suggesting that it has been conserved throughout evolution. It is mediated primarily by a developmentally regulated expression of the NKCC1 and KCC2 chloride importer and exporter respectively.

**Objective**

Since previous research has already confirmed the developmental changes associated with the GABA neurotransmitter, the primary objective of this project is to use the ES and NPC data used in class and observe the statistical power of various normalization and testing methods. In addition, it would provide insight into the magnitude of differential expression for the genes involved.

**Hypotheses**  
 Mean counts for gene expression will not be different for the genes associated with the chloride ion importer/exporter  
 Mean counts for gene expression will be different for the genes associated with the chloride ion importer/exporter

**Methods**

Data: ES and NPC counts file provided for assignment

- Various correlation values in addition to basic descriptive statistics  
- Various transformation methods (log, sqareroot, etc)  
- Median-of-ratio normalization  
- RPKM/TPM normalization  
- Fisher’s exact test  
- Poisson log-ratio test  
- Negative binomial log-ratio test (possibly?)  
- Various multiple testing correction methods (Bonferroni, Benjamini-Hochberg, etc)

Questions:

- How to find gene in the final.ESNPC.txt file?   
- Will the number of genes be too small to perform significant analysis?

Proposal is well written and interesting. There is a reference file linking ucsc gene id's to the gene names. You will need an annotated list of all possible ligand-gated ion channels. I encourage you to cast your net widely and look at all possible ion channel genes in the data provided. Also, refine your hypothesis - do you think these genes will be high or low in ES cells compared to NPCs?

NKCC1: (slc12a2) Na-K-Cl cotransporter, is a protein that aids in active transport. Membrane transport proteins that transport Na, K and Cl ions. They move each solute in the same direction, so considered symporters.

KCC2: (slc12a5) Cl-K symporter 5 is a neuron-specific chloride potassium symporter responsible for establishing the chloride ion gradient in neurons through the maintenance of low intracellular chloride concentrations.

Slc12a1: uc008mcf.1, uc008mci.1, uc008mcg.1, uc008mch.1, uc008mcj.1 – All counts are 0, excluded  
Slc12a2: uc008ezm.2 – Important one  
Slc12a3: uc009mwc.2, uc009mwd.2, uc009mwe.2, uc009mwf.2 – All counts are 0, excluded  
Slc12a4: uc009ner.1, uc009nes.1 – All counts are 0, excluded  
Slc12a5: uc008nwu.2, uc012cjf.1 – Important one  
Slc12a6: uc008los.2, uc008lot.2, uc008lou.2, uc008low.2, uc008lox.2, uc008lov.2 – has counts  
Slc12a7: uc007rea.1, uc007reb.1, uc007rec.1, uc007red.1 – has counts  
Slc12a8: uc007zad.1, uc007zae.1, uc007zaf.1, uc007zag.1, uc009acf.2 – has counts  
Slc12a9: uc009ace.2 – has large counts

Number of significant genes for t-test after correction: 0 out of 14  
Number of significant genes for Fisher's exact test after correction: 5 out of 14  
Number of significant genes for K-S test after correction: 0 out of 14  
Number of significant genes for Poisson LRT after correction: 7 out of 14

Names of genes that have significant p-values from Fisher  
'uc008lov.2': upregulation in ES, slc12a6  
'uc008low.2': upregulation in ES, slc12a6  
'uc008lox.2': upregulation in ES, slc12a6  
'uc007reb.1': upregulation in ES, slc12a7  
'uc007zad.1': upregulation in ES, slc12a8  
Names of genes that have significant p-values from Poisson LRT:   
'uc008ezm.2': upregulation, slc12a2 (NKCC1, correct test result)  
'uc008nwu.2': ??, slc12a5   
'uc008los.2': ??, slc12a6  
'uc008lov.2': upregulation, slc12a6  
'uc007red.1': upregulation, slc12a7  
'uc009acf.2': ??, slc12a8  
'uc009ace.2': ??, slc12a9