

Transcriptome Analysis of Plasticity in the Anterior-Posterior Neural Axis in *Xenopus laevis* Embryos

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I. Introduction

The Anterior-Posterior axis directs the orientation of embryonic development during the formation of the neural tube. Early embryogenesis allows for a heightened ability of plasticity in the event of injury. The repatterning of the A-P Axis is crucial in early embryonic stages, specifically in healing and continuing healthy development of the neural tube. The results of this RNA-Seq analysis has suggested several candidate genes that contribute to the embryonic plasticity of the A-P axis in various crucial development stages. Three categories: healing, transplant, and repatterning, were used to group differentially expressed genes based on their type of perturbation. These inferential assumptions, investigated the effects of transplanting foreign tissue to the embryo, rotating the transplanted tissue, and injuring the original tissue, resulting in several candidate neurogenesis related genes that are conserved as well as some unique to each perturbation.

II. Methods

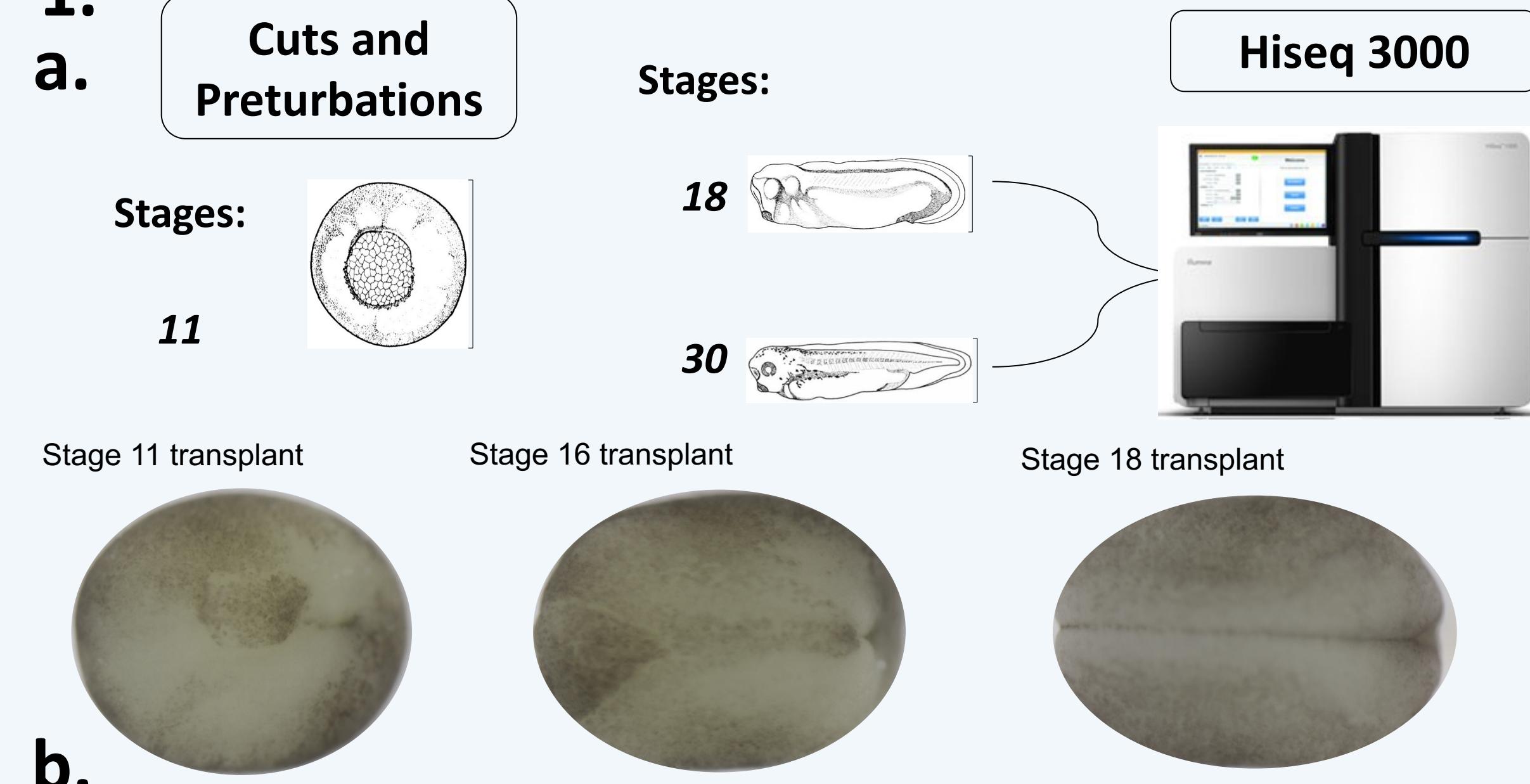
Presumptive neural tissue was removed from a labeled donor, rotated 180° and heterologously transplanted into an unlabeled host embryo. As a control for the rotation effects, tissue was removed from a labeled donor and heterologously transplanted into an unlabeled host embryo without rotation. As the sham may be slightly out of register when placed into a host embryo, an autologous ("selfie") transplant was performed where tissue was removed, then replaced back into the same embryo. RNA-Seq data was prepared with polyA selection, paired-end layout in the Illumina HiSeq 3000.

Ablations and transplants were done at stage 11, and the embryos were imaged and sequenced at stages 18 and 30. Stage 10/11 is the beginning of the gastrula stage where the embryo has begun differentiation and begins the development of the anterior-posterior axis [3]. Embryos were raised to Stage 18, the middle of the neurula stage when neurulation occurs, the folding of the neural plate into the neural tube. Stage 30 is the late Tailbud stage where neurulation is complete and tail formation begins.

All raw reads are processed through the pipeline in 1b. Read data was aligned to a reference genome and quantified into read counts with RNA-Seq programs. All read counts were clustered to determine unwanted variation, later removed through batch effect removal and filtered to have a mean of 5 counts for each gene. Differential expression was found through DESeq2 and a novel Bayesian method, BADER [5]. Batch correction with svr to remove effects between Rotations and Selfies data as they are from two separate conducted experiments.

1.

a. Cuts and Perturbations



Schematic of data (a) All transplants and ablations were done at stage 11 (12 not included in analysis), embryos were sequenced at stages 18 and 30 (Saha Lab, Chen Dong) (b) Analysis pipeline where reads are aligned to reference genome, count reads per gene, removing unwanted batch effects with empirical gene controls (svr), retrieve batch removed counts given log-expression values, retrieve normalized counts, plotting with R, inferential groups made afterwards

2. Differentially Expressed Genes in 3 Experiments Conservation

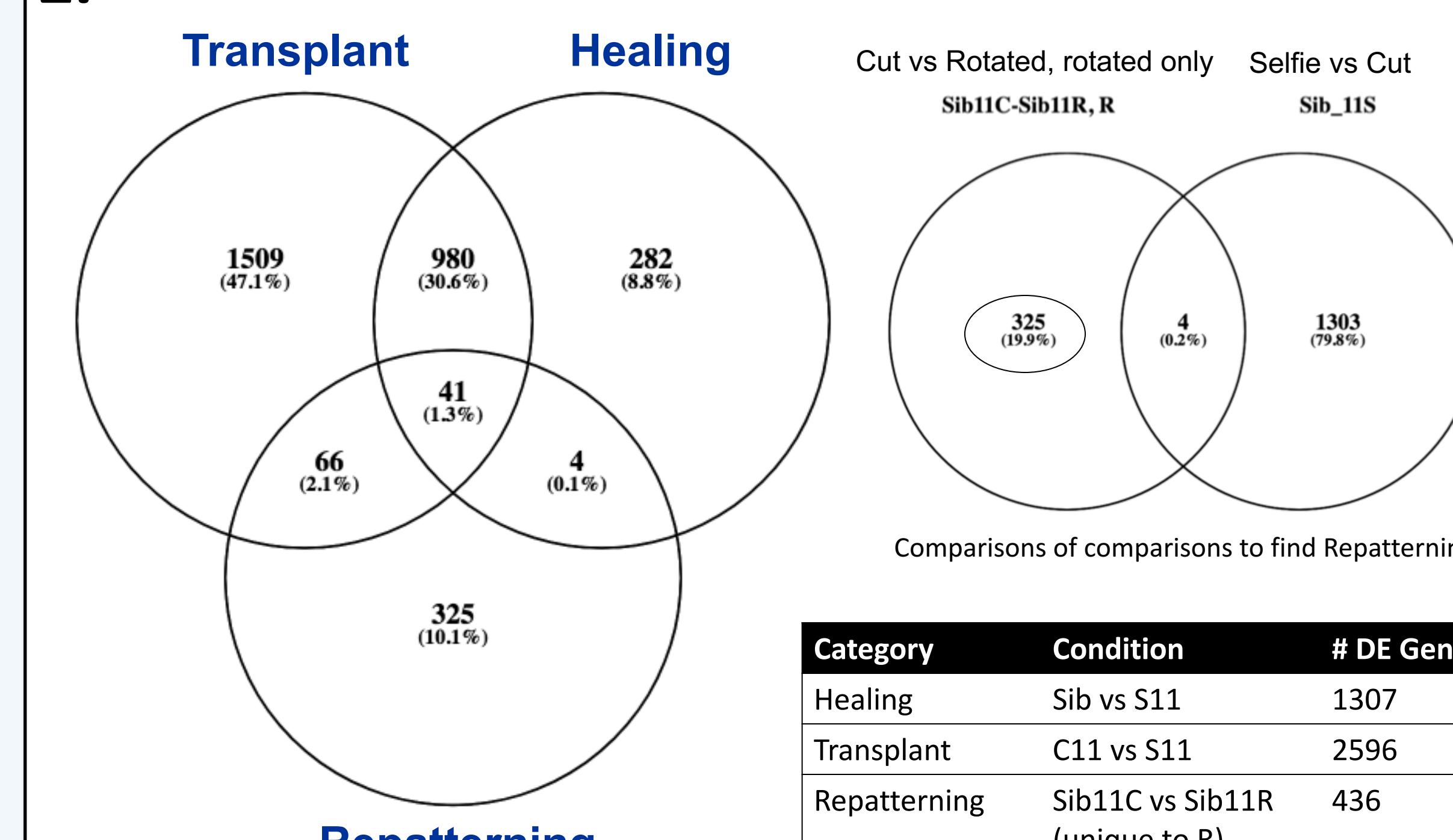
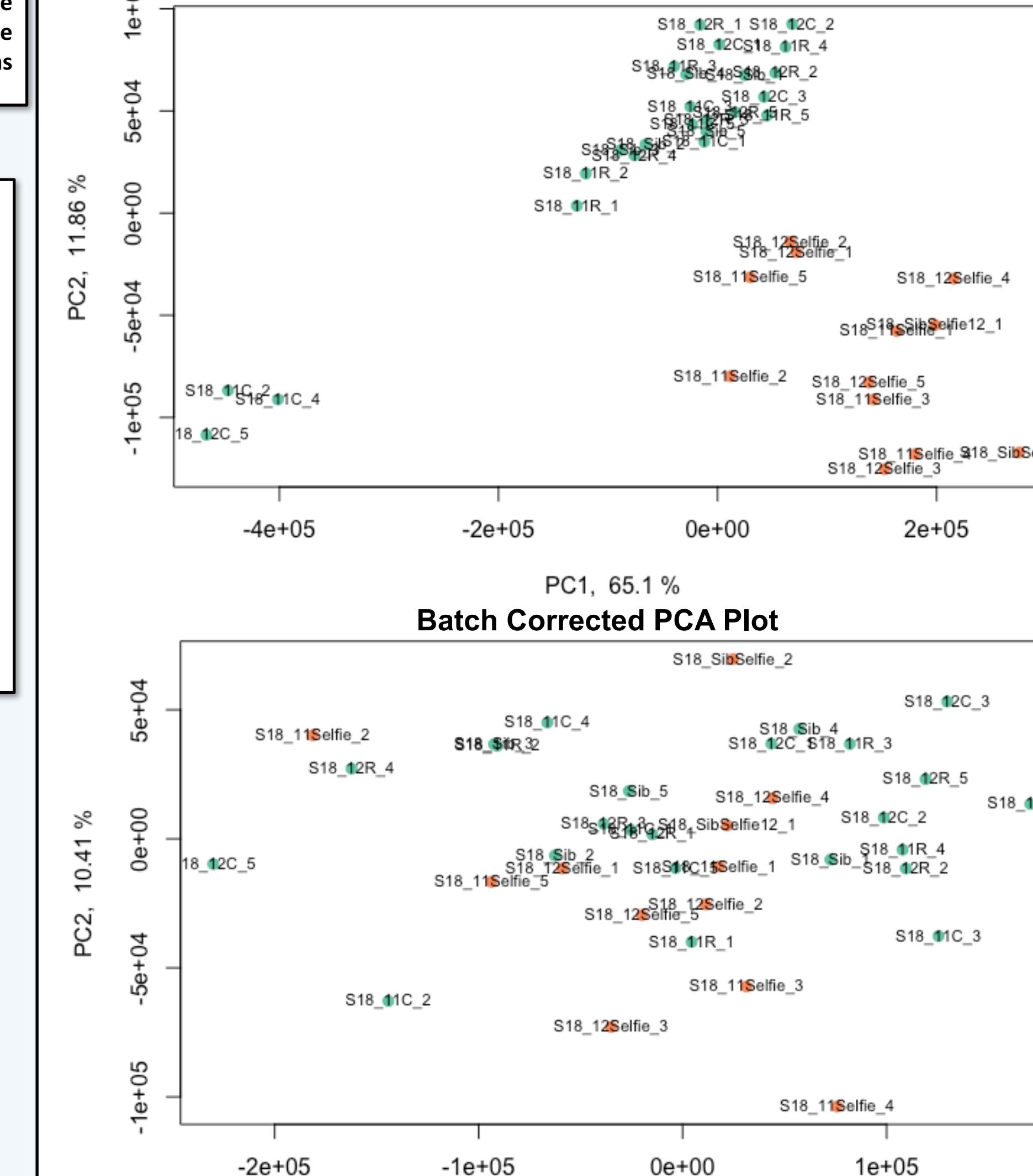


Figure 2a) The Venn diagram showing the shared and unique genes found between the three categories: healing, transplant, repatterning. When observing each category, all of the genes were DE, filtered by a p-value of 0.05. Done with strict regulations for 'transplant' category, only those unique to R in Sibs vs 11R and Sibs vs 11C overlap comparison. DE genes found using DESeq2.

III. Results

3. Clustering of Batch Corrected Data with Siblings and Conditions



4. Unique Genes to Gene Groups

	GO Enrichment	Log2fold change
Healing Specific	Cellular/developmental pro.	0.307400912
akna	Cell projection organization	0.85762617
odf3	Forebrain/head developm.	0.647935759
Transplant Specific	GO Enrichment	Log2fold change
yap1	cellular resp. to DNA damage stim.	0.208805378
osigin1.L	oxidative stress response protein	-0.668296194
id3	somitogenesis	0.149684125
Repatterning Specific	GO Enrichment	Log2 fold change
foxn4	Cell fate commitment	0.67755328
sox8	epithelium development	-0.388583069
spdef	embryonic morphogenesis	0.643610531

Figure 4: Several unique genes for each of the three gene groups, with their specific GO enrichment related term. Log2 fold is the regulation of the gene within the cell, this is an average of all of the replicates, a positive value signifies upregulation while a negative value means downregulation.

5. Differentially Expressed Genes with Bayesian Statistics

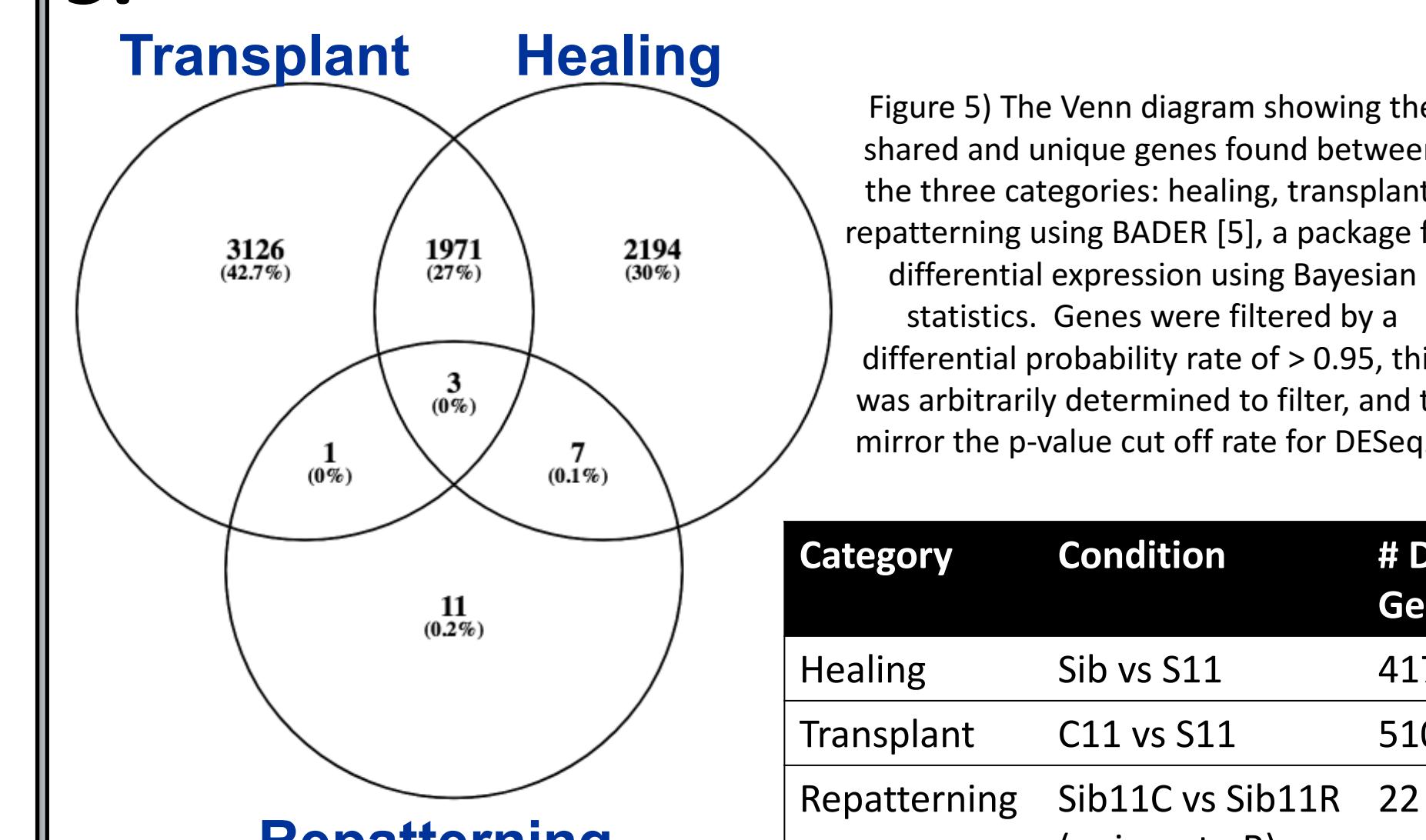
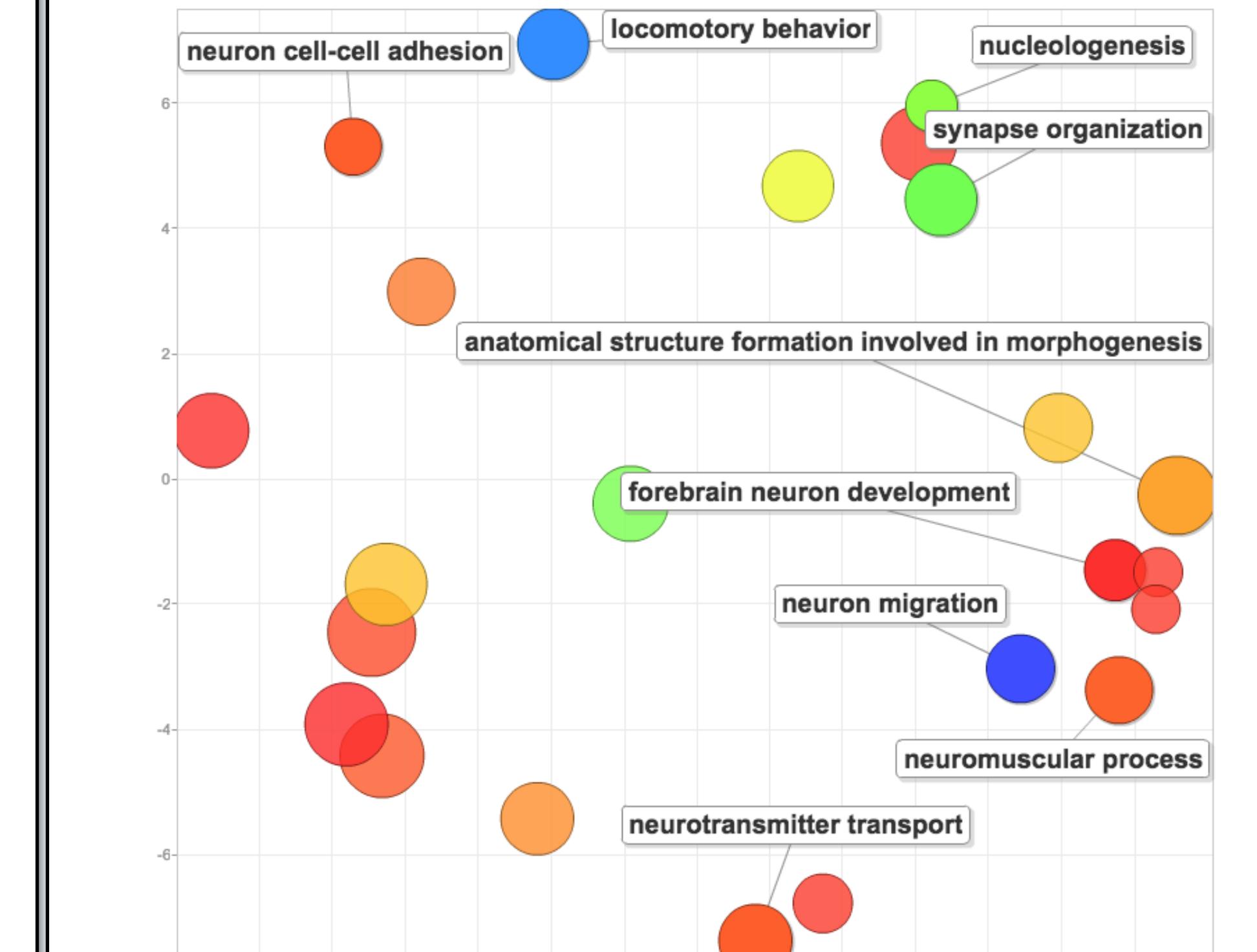


Figure 5) The Venn diagram showing the shared and unique genes found between the three categories: healing, transplant, repatterning using BADER [5], a package for differential expression using Bayesian statistics. Genes were filtered by a differential probability rate of > 0.95, this was arbitrarily determined to filter, and to mirror the p-value cut off rate for DESeq2.

6. GO Analysis of "Healing" genes from Sibling vs Selfie



PCA: The majority of the functions are related to basic embryonic development such as neuron cell-cell adhesion or migration.

Healing follows the typical pattern of growth and utilizes cell processes that undergo during healthy development without perturbation. Morphogenesis is interesting as it suggests the construction of the neural tube. At this early stage (18), an injury is likely simple to repair as other structures are building and the presence of continual checks and repairs.

7. Posterior Distribution of Log Fold Change

gene afth2.L gene ireb2.L

gene osgin1.L gene foxn1.L

probability

probability