

Comparing Gene Expression Of Islets From Diabetic, Heterozygous, and Wild-Type Mice To Identify Genes Impacted By Dedifferentiation

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Goal of Experiment

Type 2 diabetes is influenced by dedifferentiation resulting from the loss of function of pancreatic β -cells.

Establish db/db as an animal model for dedifferentiation by using RNA sequencing to compare the gene expression profile in islets isolated from wild-type, db/+, and db/db mice.



Region of Interest

Cell functions:

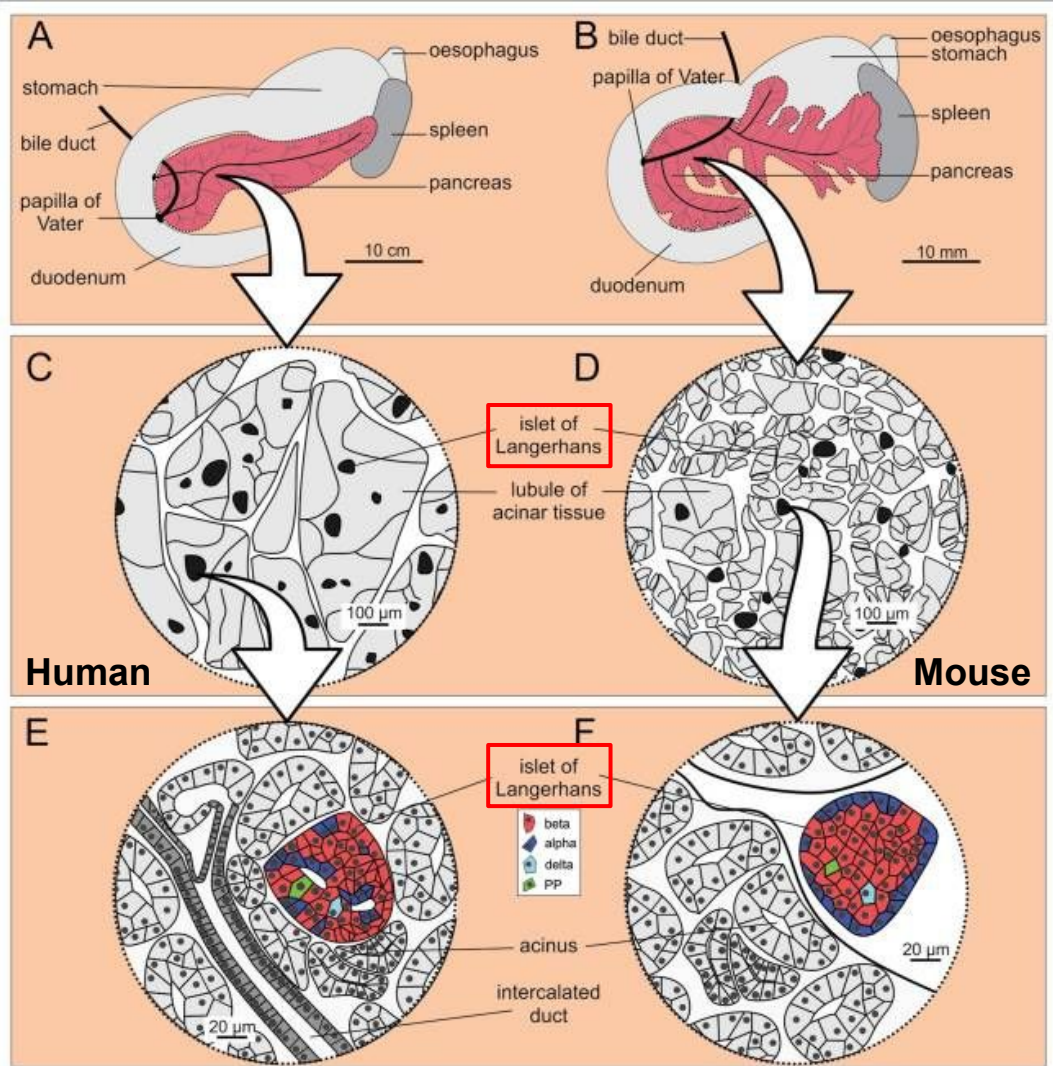
β -cell - Synthesize and secrete insulin in order to maintain circulating glucose levels within physiological range.

α -cell - Synthesize and secrete the peptide hormone glucagon, which elevates the glucose levels in the blood.

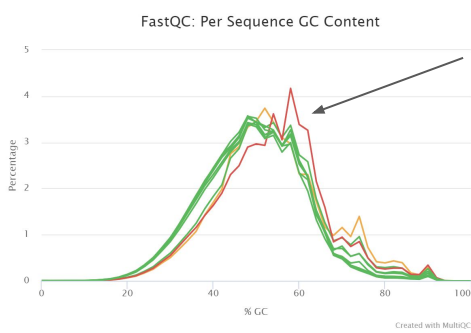
δ -cell - Secrete somatostatin which regulates the endocrine system and affects neurotransmission and cell proliferation.

Pancreatic polypeptide cells - Self-regulate pancreatic secretion activities.

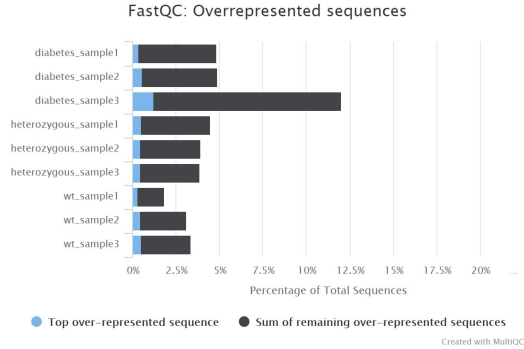
2015. Dolenšek J, Rupnik MS, Stožer A. Structural similarities and differences between the human and the mouse pancreas.



Methods - Potential Problems From FastQC



db/db -
sample 3

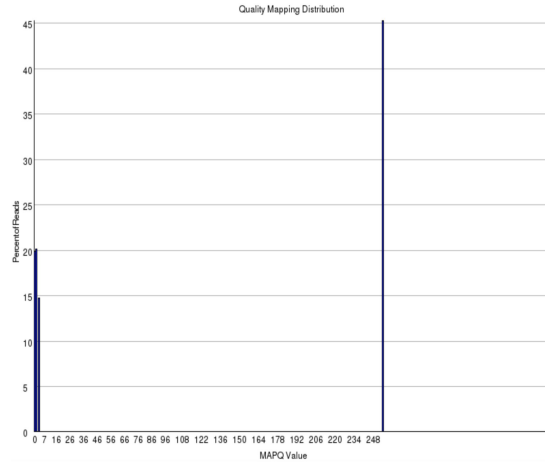


Possible Explanation:
Diabetes sample 3 was run for more PCR cycles than the other samples, resulting in the duplication of more sequences and higher bias for GC content.

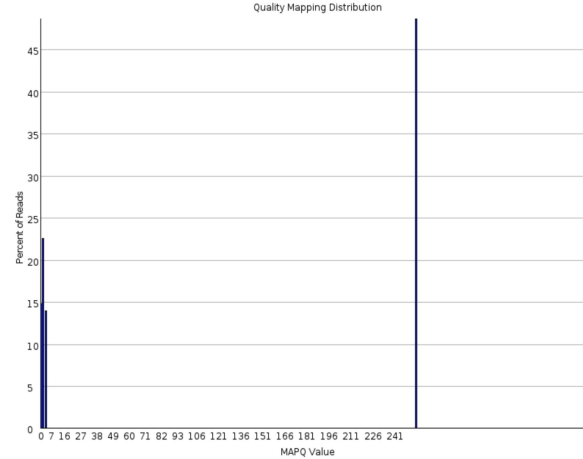
Gain insight into problem by using BLAST to find most overrepresented sequences from diabetes sample 3 - top hit shown below (Rn7s2 gene)

Sequence	Count	Percentage
CCTTAGGCAACCTGGTGGTCCCCCGCTCCCGGGAGGTCACCATATTGATG	269364	1.197866

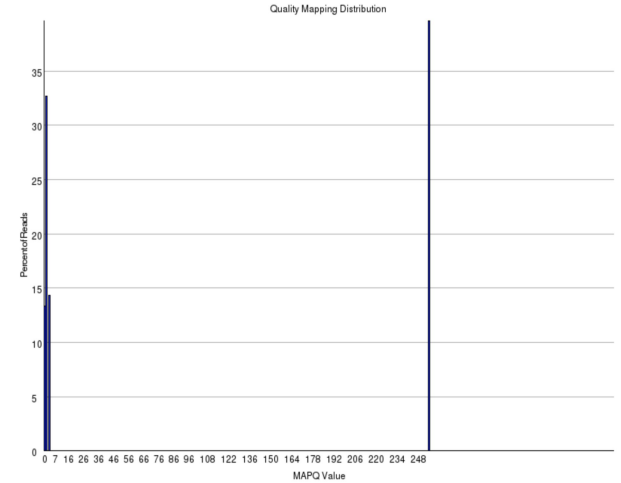
Methods - Potential Problems From bamQC



Diabetes Sample 1



Diabetes Sample 2



Diabetes Sample 3

Implication: Large proportion of multi-mapped reads can lead to lack of significant genes found by featureCounts later on.

Results - Similar Number Of Significant Genes Found

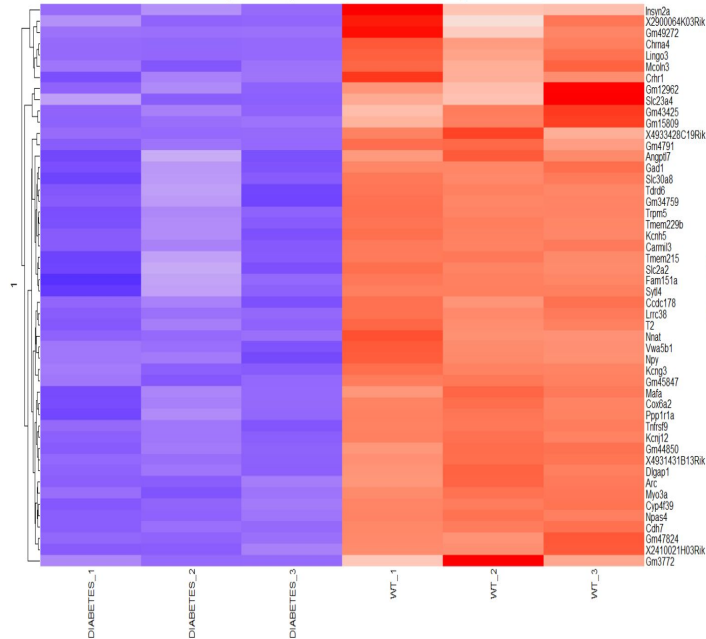
Main Takeaway: Similar number of significant genes received using counts produced from my pipeline and counts produced by publication for db/db and WT comparison.

Source Of Counts Used	Samples Compared	Number of Significant Genes By P-value	Top 3 Significant Genes By P-value	Number of Significant Genes By P-value and Log2 FC	Number of Upregulated/Downregulated Genes Significant By P-value and Log2 FC
Our Counts	HET vs. WT	672	Lars2, ENSMUSG00000106106 (CT010467.1), Gm15564	35	27/8
Our Counts	HET vs. DB	5993	Aldh1a3, Serpina7, Gc	1995	1492/503
Our Counts	DB vs. WT	5948	Aldh1a3, Serpina7, Gc	2092	1619/473
Publication Counts	DB vs. WT	5575	Serpina7, Aldh1a3, Npas4	1827	1354/473

Results - Comparison of Top 50 Downregulated Genes

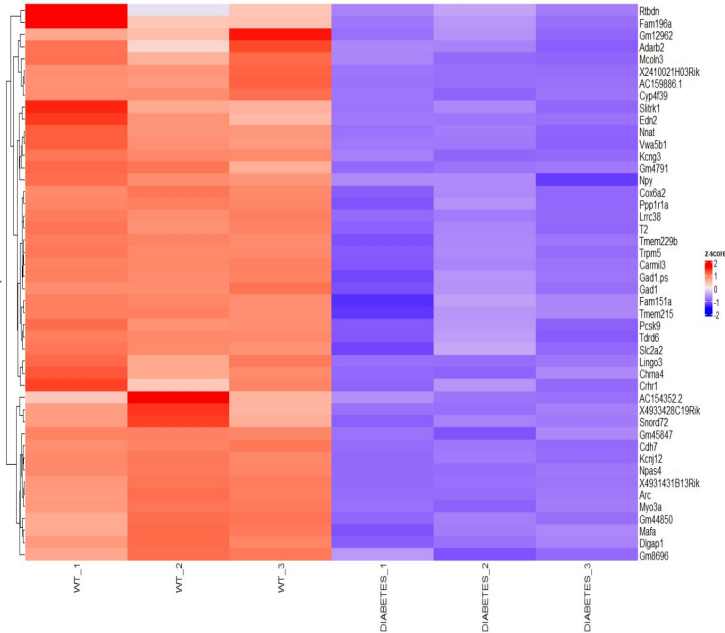
Takeaway: All three results give mostly different top 50 downregulated genes

DB vs. WT: Top 50 Significant and Downregulated Genes

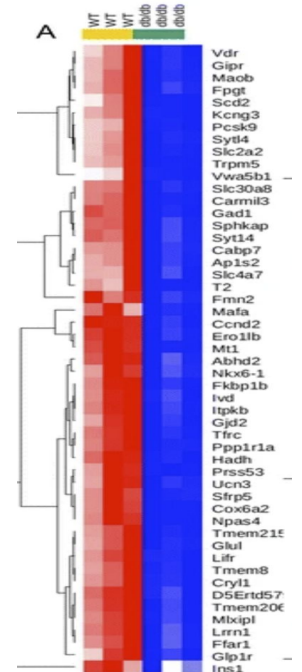


Using My Counts

DB vs. WT: Top 50 Significant and Downregulated Genes



Using counts from paper



Published Heatmap 8

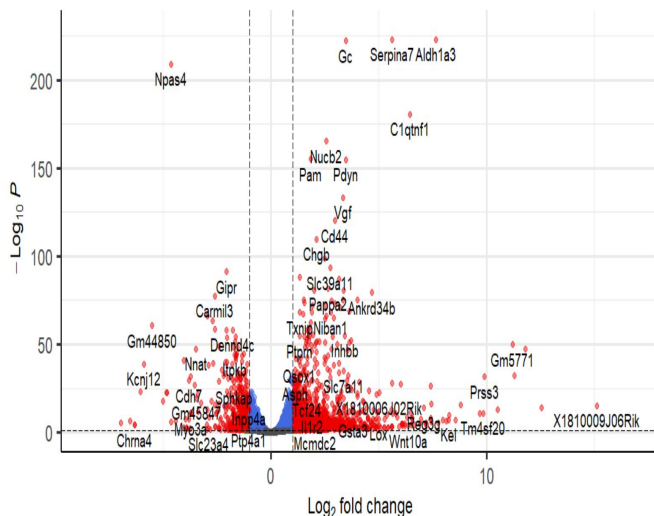
Results - Comparison of Volcano Plots

Takeaway: My counts create very similar volcano plot to that from the paper. Still inconsistencies between counts from paper and published volcano plots.

DB vs WT: with logFC shrinkage

EnhancedVolcano

● NS ● Log₂ FC ● p-value ● p-value and log₂ FC



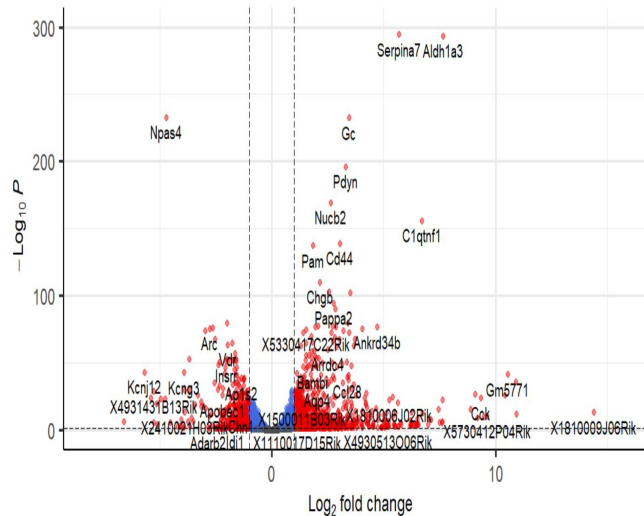
total = 28940 variables

Using My Counts

DB vs WT: with logFC shrinkage

EnhancedVolcano

● NS ● Log₂ FC ● p-value ● p-value and log₂ FC

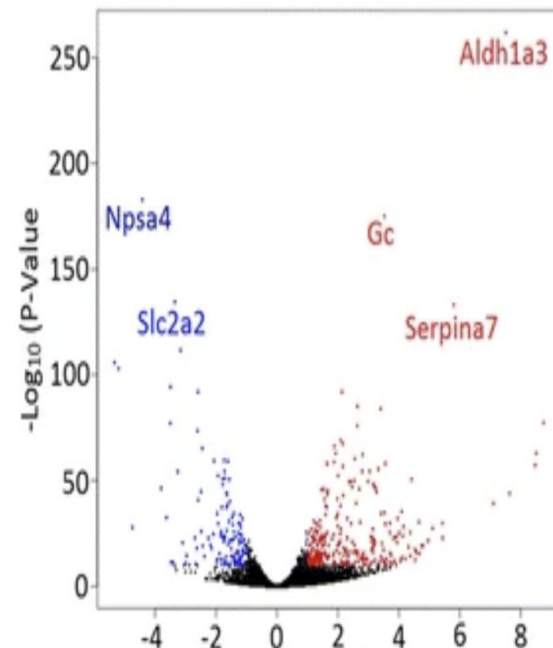


total = 28804 variables

Using counts from paper

C

Volcano Plot



Published Volcano Plot 9

Results - db/db Model Not Valid for Dedifferentiation

From Publication: “A reduction in the expression of Ins1, Ins2, Glut2, Pdx1 and MafA was indicative of dedifferentiation in db/db islets.”

DB vs WT

Gene	Shrunken Log FC Value	Adjusted p-value
Ins1	-0.417222	0.251649
Ins2	0.0374102	0.874441
Glut2 (Slc2a2)	-2.86349	3.26316e-09
Pdx1	-1.13373	7.2644e-30
MafA	-3.5507	1.08515e-27

DB vs WT

Gene	Shrunken Log FC Value	Adjusted p-value
Ins1	-0.319718	0.35666
Ins2	0.0172055	0.944685
Glut2 (Slc2a2)	-2.80892	1.36774e-12
Pdx1	-1.02596	6.89333e-24
MafA	-3.15191	1.22448e-18

Using My Counts

Using Counts From Paper

Takeaway: In the analysis with both my counts and the publication counts, Ins1 and Ins2 (shown in the red box) were far from significant in adjusted p-value and Log FC (Not < -1). All other genes were significant with respect to both adjusted p-value and Log FC. However, authors show significance through qPCR

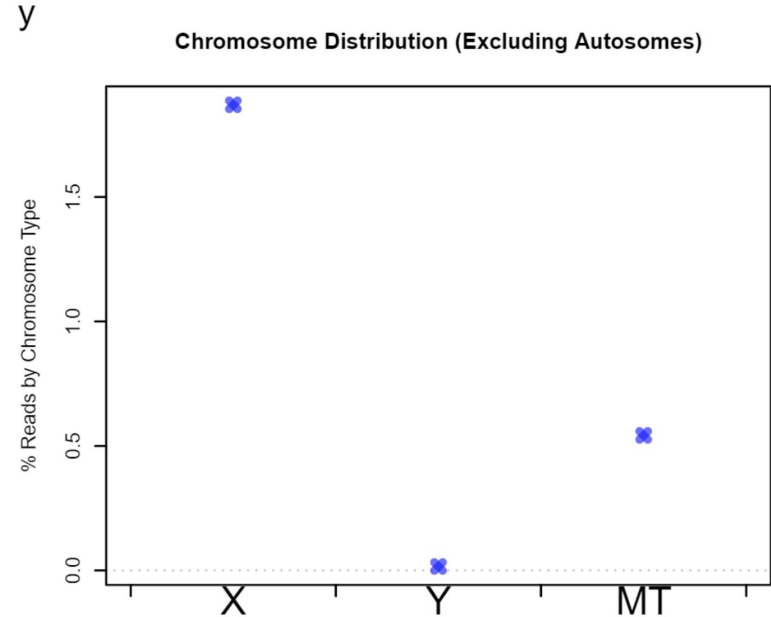
Limitations Of Experiment

1. All mice are females - shown by QoRTs

It has been shown that human sexes differ in their responses to diabetes. E.g. Women have greater increases of cardiovascular risk, myocardial infarction, and stroke mortality than men, compared with nondiabetic subjects.

No reads mapped to Y chromosome (Diabetes sample 1)

2. Mouse strain is monogenic and the most common types of diabetes are caused by multiple genes, and in T2D lifestyle factors such as obesity also influence diabetes progression



Conclusions

1. RNA-Seq analysis shows the db/db model is not as strong of an animal model for dedifferentiation as concluded by the paper, due to the lack of downregulation in *Ins1* and *Ins2* in diabetic samples demonstrated by the downstream analyses.
2. Reproducibility of final figures using count matrix from publication not possible due to lack of explanation in publication ---> IT IS EXTREMELY IMPORTANT TO INCLUDE ALL STEPS CONDUCTED IN PIPELINE, EXPLAIN DEFINITIONS OF SIGNIFICANCE FOR REPORTED VALUES, AND EXPLAIN ALL RESULTS FOR THE CREATION OF PROPER SCIENTIFIC RESEARCH

References

1. Dolenšek J, Rupnik MS, Stožer A. Structural similarities and differences between the human and the mouse pancreas. *Islets*. 2015;7(1):e1024405. doi: 10.1080/19382014.2015.1024405. PMID: 26030186; PMCID: PMC4589993.
2. Guest P.C., Rahmoune H. (2019) Characterization of the db/db Mouse Model of Type 2 Diabetes. In: Guest P. (eds) *Pre-Clinical Models. Methods in Molecular Biology*, vol 1916. Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-8994-2_18