Chicken Analysis Reports

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Organism: *Gallus gallus*
Tissues: Intestine, Meat, Reproductive Tissues, Macrophage and Monocyte
Total Samples: 2 replicates for each tissue

Differentially expressed (DE) analysis

Method:

For each pairwise analysis, we filtered out non-expressed and low-expressed genes.
Note: rm = rowMeans(mRNA2_meat) == 0

The surrogate variables (batch effects) were estimated by the SVA package (Leek JT et al., 2022) and adopted in the DE analysis model. The DE analysis and normalization data were performed using (Ritchie ME et al., 2015). We considered a DE significant gene showed FDR < 0.05.
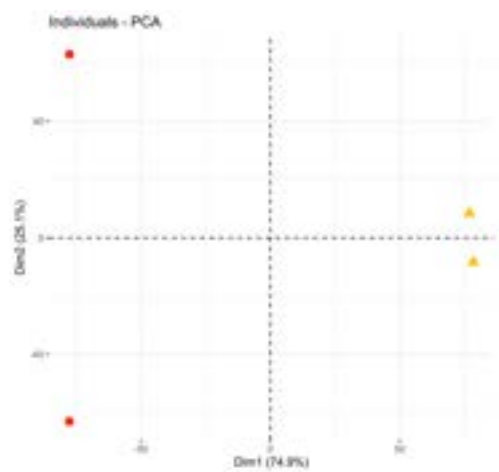
Note:
- **Surrogate variables** are covariates constructed directly from high-dimensional data (like gene expression/RNA sequencing/methylation/brain imaging data) that can be used in subsequent analyses to adjust for unknown, unmodeled, or latent sources of noise.
- **n.sv** is the number of latent factors that need to be estimated (number of surrogate variables)
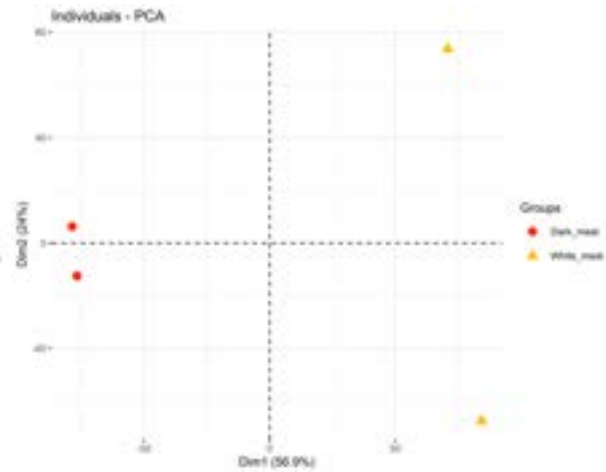
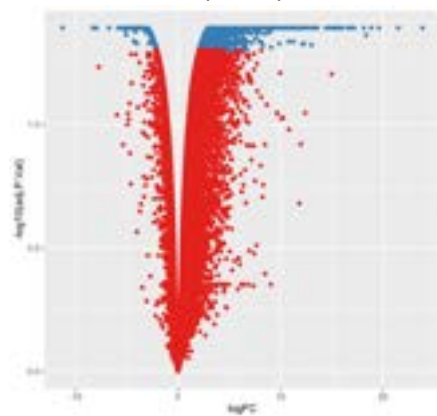**DE analysis: meat tissues**
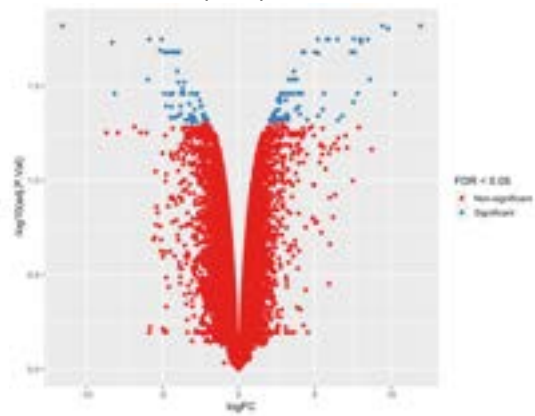Note: total n.sv=2

Using sva with n.sv=1

Not using sva

Using sva with n.sv=1
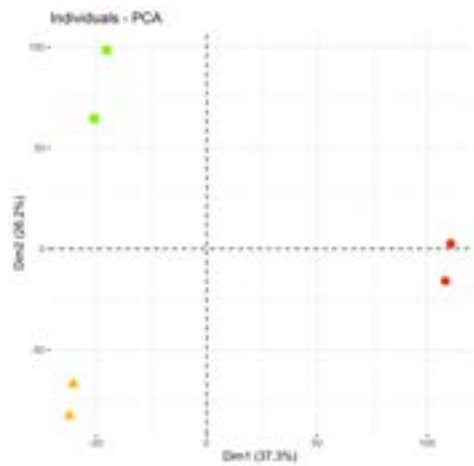
Not using sva

(3000)

(147)

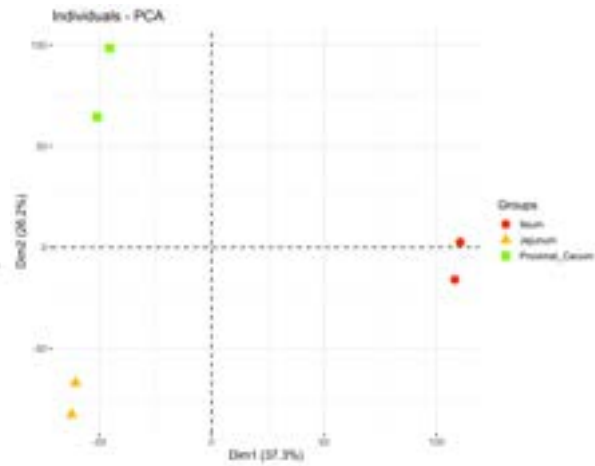Note: the values in brackets are the number of significant DE

DE analysis: intestine tissues
Note: total n.sv=3, but only n.sv=1 can work in svaseq() function

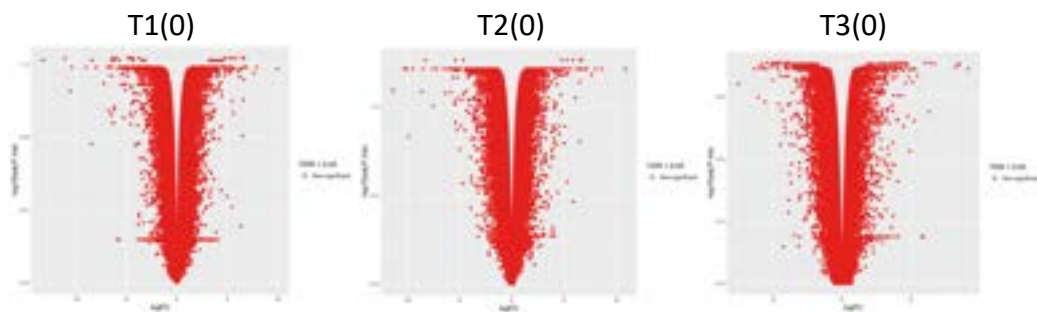**Using sva with n.sv=1**   **Not using sva**
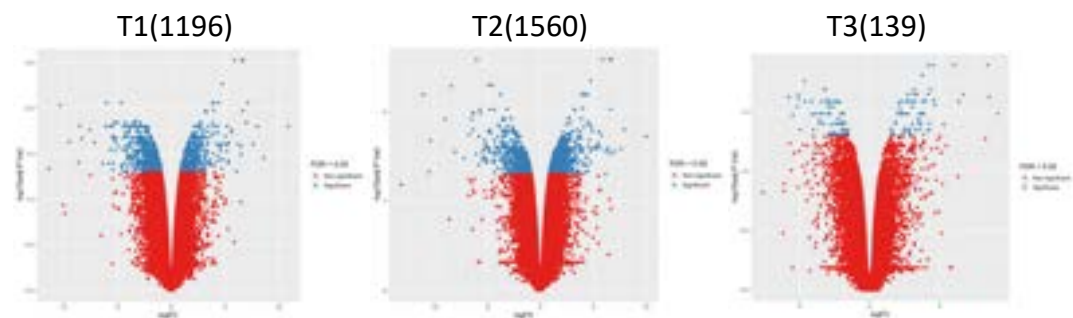

Note: The two PCA are the same.

> contrast.matrix_intestine

	T1	T2	T3
Ileum	1	1	0
Jejunum	-1	0	1
Proximal_Cecum	0	-1	-1

Using sva with n.sv=1

T1(0)   T2(0)   T3(0)

Not using sva

T1(1196)   T2(1560)   T3(139)

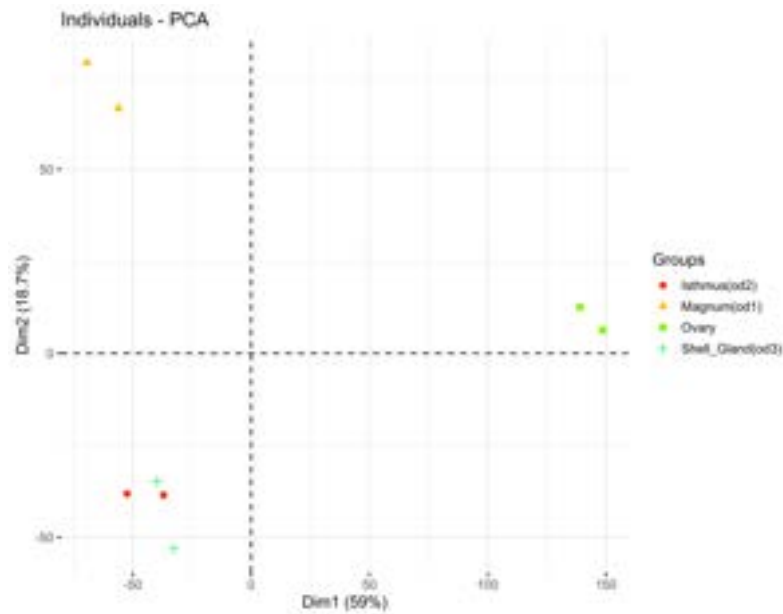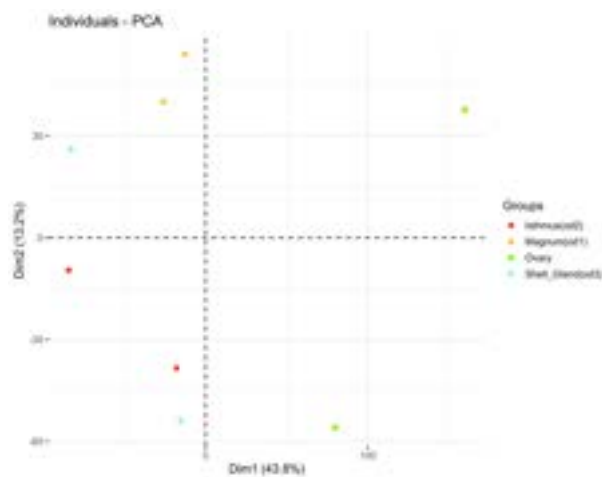**DE analysis: reproductive tissues**
Note: total n.sv=4, there are no significance DE among all tissue comparisons when n.sv=2 or 3

using sva with n.sv=3

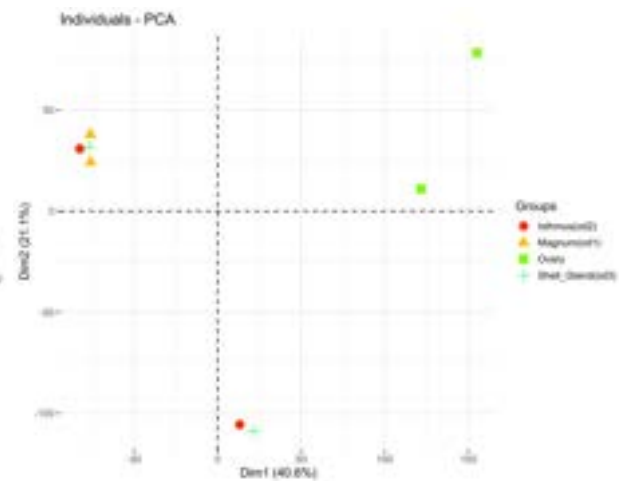

Using sva with n.sv=1   **not using sva**
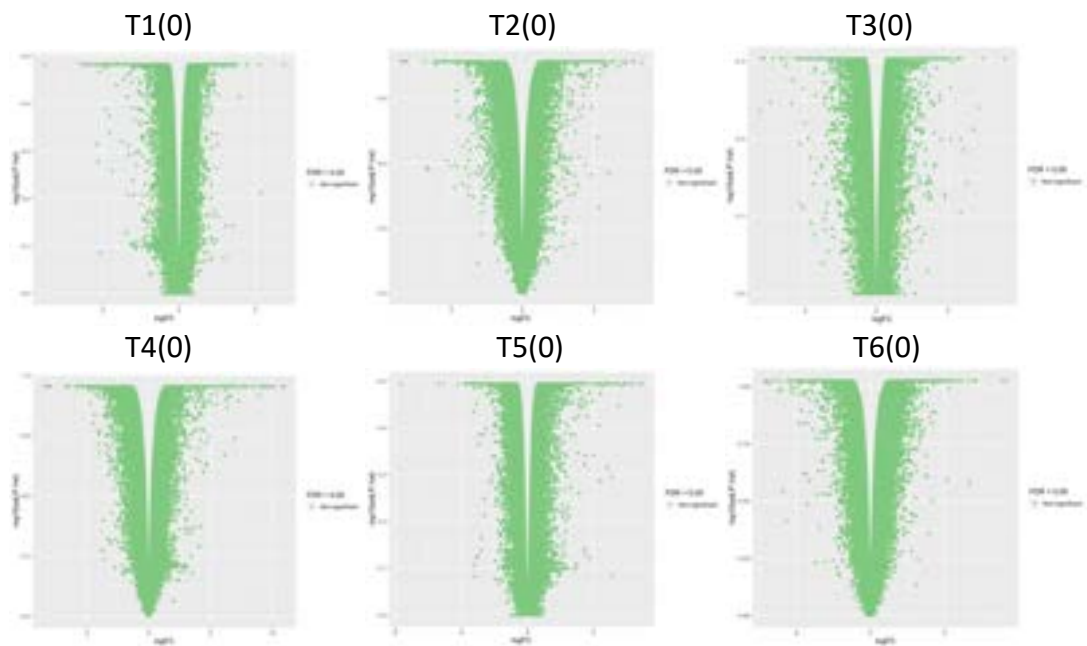
```
> contrast.matrix
                   T1  T2  T3  T4  T5  T6
Isthmus(od2)        1   1   1   0   0   0
Magnum(od1)        -1   0   0   1   1   0
Ovary               0  -1   0  -1   0   1
Shell_Gland(od3)    0   0  -1   0  -1  -1
```
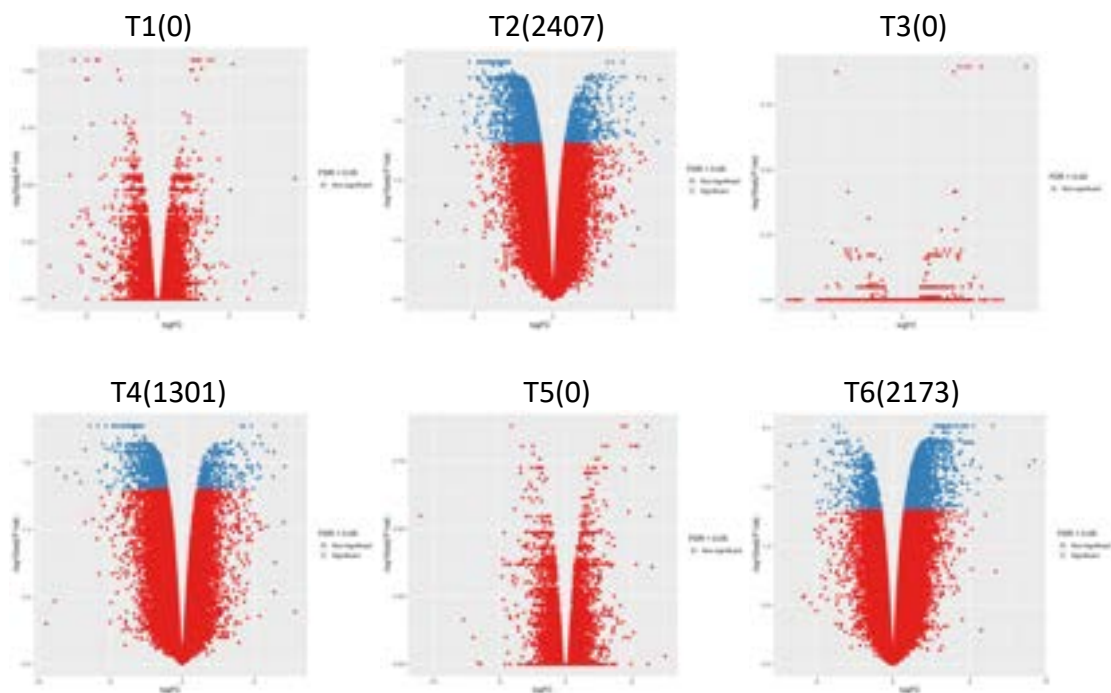
Using sva with n.sv=3

T1(0)	T2(0)	T3(0)

T4(0)	T5(0)	T6(0)

Using sva with n.sv=1

T1(0)	T2(2407)	T3(0)

T4(1301)	T5(0)	T6(2173)

Not using sva

T1(8)

T2(2127)

T3(6)

T4(2798)

T5(1)

T6(2001)
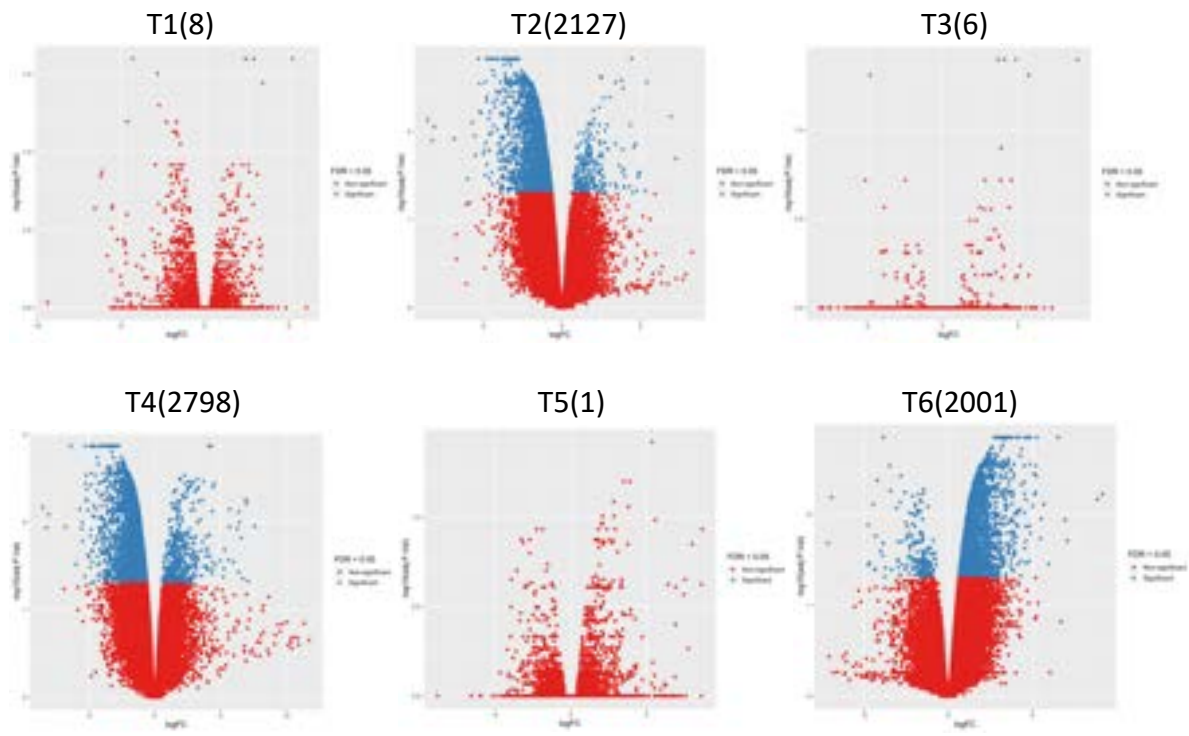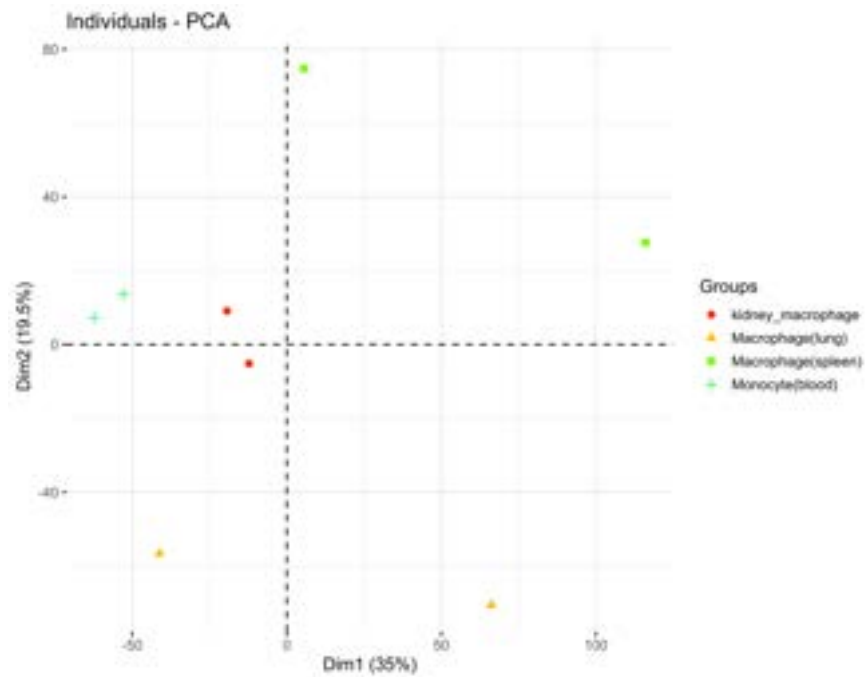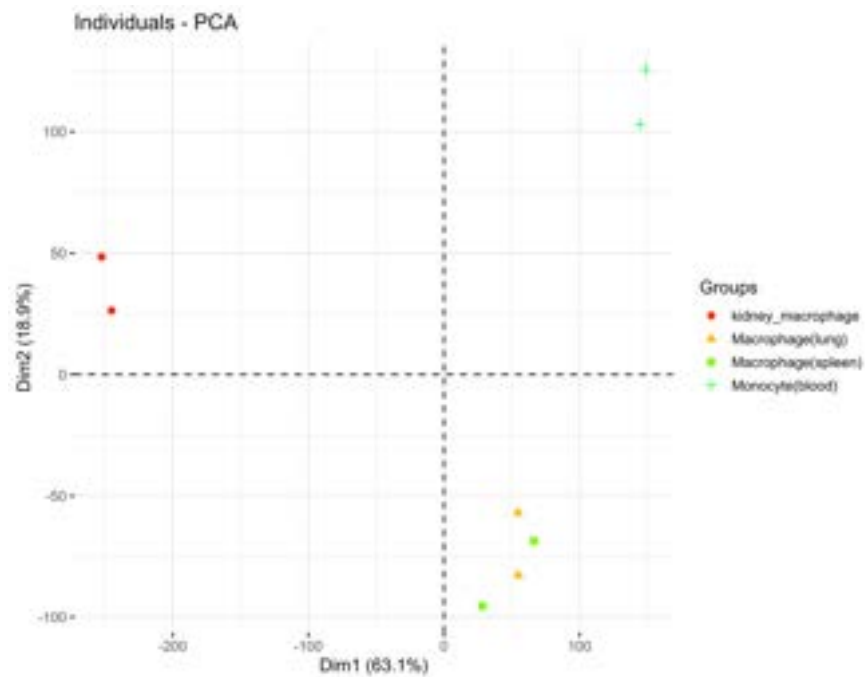
DE analysis: macrophage tissues and monocyte
Note: total n.sv=4, there are no significance DE among all tissue comparisons when n.sv=2 or 3

Using sva with n.sv=2

Using sva with n.sv=1

```
> contrast.matrix
                    T1   T2   T3   T4   T5   T6
macrophage_kidney    1    1    1    0    0    0
Macrophage_lung     -1    0    0    1    1    0
Macrophage_spleen    0   -1    0   -1    0    1
Monocyte             0    0   -1    0   -1   -1
```

Using sva with n.sv=1

T1 (30237)	T2(30932)	T3(36275)

T4(1301)	T5(5270)	T6(7172)