TEST questions: Please answer all t
submit your answers.

1. You used a Gad67GFP knock-in al
approach compared to using a trans
promoter sequence?

2. Describe the Gad67-GFP express
(embryonic day)?

3. How would you determine the eml

4. How did you identify the serotoner

Name	Status	Grade	Last modified (submission)
Näide a	Submitted for grading Graded	3.00 / 5.00	Friday, 16 January 2026, 2:55 PM

Näide b	Submitted for grading Graded	5.00 / 5.00	Friday, 16 January 2026, 2:54 PM
Näide c	Submitted for grading	5.00 / 5.00	Friday, 16 January 2026, 2:56

	Graded		PM
Näide d	Submitted for grading	4.00 / 5.00	Friday, 16 January 2026, 2:49

	Graded		PM
Näide e	Submitted for grading	3.50 / 5.00	Friday, 16 January 2026, 3:07

	Graded		PM
Näide f	Submitted for grading	5.00 / 5.00	Friday, 16 January 2026, 2:52

	Graded		PM
Näide g	Submitted for grading	4.50 / 5.00	Friday, 16 January 2026, 3:15

Graded PM

he questions. Word limit applies (max 300 words). You have 45 minutes to

llele to visualize the Gad67 expressing regions. What are the advantages of this sgenic mouse strain, where GFP expression is driven by a piece of Gad67

ion pattern in the shown mouse embryo. What is the stage of development

bryonic stage without knowing the plug day from the mating?

rgic and the GABAergic cell clusters in the scRNAseq data?

Online text

(297 words)

Last modified (grade)
Wednesday, 21 January 2026, 3:39 PM

1.The advantage of using a knock-in allele to visualize the expression of Gad67 regions is that when compared to a transgenic mouse strain the GFP expression is driven by a promoter sequence, but with the knock-in allele GFP can be expressed in the cells or as a protein making it more likely to be visible. If the Gad67 is only driven by the promoter sequence it means that it is only expressed on a protein level and thus this expression could also be turned off due to for example mutations in the mouse strain.

2. The expression of Gad67-GFP in the embryo can be seen in the tail, spinal cord and all brain regions. This would make the embryo to be E11.5, as at this stage the GABAergic interneurons are generated starting from the tail and migrate dorsally to the cortical plate in the brain. In this picture the migration is currently happening, as GFP expression is seen visible from the tail to the brain.

3. In mouse embryos the differences between the stages of development per day in the embryos is visible to the eye. This means that just by comparing pictures of different stages of development you can determine at what stage the embryos are. For example, at stage E12 the brain regions are already developing and are more visible than in E6.

4.In the scRNAseq data we searched for serotonergic cell clusters by looking for serotonergic markers (Sert and Fev). These markers were then plotted (feature plot) so that we were able to see where these

(208 words)

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1. Using Gad67-GFP (knock-in) instead of transgenic mouse strain gives more accurate and endogenous expression of the Gad67. It is also expressed in the right place (locus), because there is a chance that in transgenic mouse the integration is at a random place in genome. This in turn can lead to altered expression or no expression at all because of the integration site effects. In Gad67-GFP knock-in all the regulatory elements are present, which is why the expression is endogenous.

2. A) spinal cord

B) hindbrain

C) midbrain

D) diencephalon

E) telencephalon

F) tail

3. The expression/signal is the highest in F and C. But there is also some signal in other parts as well, but not as much as in midbrain or tail. The stage of development/embryonic day is E10.5-11.0.

4. You look at the pictures of developing embryo (Theller stages of

(294 words)

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1. If the GFP would simply be expressed under a Gad67 promoter, the GFP protein might be expressed without Gad67 being expressed. If the GFP is within the Gad67 gene sequence it will only be expressed when the protein is being expressed. A transgene would most likely be too small to have all the regulatory sequences and if it only has one promoter sequence the GFP expression would not reflect Gad67 as well.

2. Gad67 is expressed in the forebrain (E), hypothalamus (D), midbrain (C), at the midbrain-hidbrain barrier (B), at the end of the tail (F) and the spinal cord (A). 10.5 embryos only had GFP in the tail. Based on the GFP expressing regions the stage is E11.5.

3. Whether the embryo it is 3 mm or 5 mm long would give some indication. The limb formation happens at a spescific time. I could look at the mouse atlas and see if the embryo is forming limbs, fingers, whisker rows, eyes etc. There are many markers one should look for and cross reference to be more accurate.

4. The serotonergic clusters were identified based on the expression level of Fev and Slc6a genes which are known to be expressed in serotonergic precursors. By projecting these genes on the UMAP, it was easy to pick out the cluster 19, which had cells expressing both. The GABAergic clusters were identified by drawing a violin plot of Gad1 and Gad2 gene expression levels per cell across all 0-20 clusters. There were many clusters that had cells expressing both

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(220 words)

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1.In knock-in the gene insertion is targeted versus in transgenesis the insertion can happen anywhere in the gene. Targeted insertion is less likely to disrupt the gene or its regulatory regions, which could affect GFP/Gad67 expression.
2. GFP expression can be seen in the fore- and midbrain (telencephalon, ventrolateral diencephalon), spinal cord and tail bud. E11.5
3.Certain genes are expressed at certain stages of development, so I would stain for that gene product to see the expression pattern and from that determine the stage of development.
4.The sequencing data is first normalised and processed to exclude too high or too low RNA count and mitochondrial RNA percentage so that only healthy single cells remain. PCA and UMAP are done to reduce dimensionality and make data more readable for the human eye. Data is clustered based on relative gene expression differences and plotted. Cells in the same cluster have similar gene expression patterns, clusters closer together indicate more similar gene expression differences than those far from each other. This doesn't tell anything about the amount of gene expression so we must identify interesting clusters with marker genes. The gene expression pattern of the marker (e.g. Fev for serotonergic and Nkx2-2 for GABAergic neurons) is compared to the clusters patterns and plotted to show what cluster has similar patterns as the marker gene.

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(289 words)

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1.Advantages of using Gad67GFP knock-in is its specificity. The EGFP can be added to the specific place in Gad67 allele, and therefore can use the same promoter and regulator elements. Then the GFP is a very good marker for Gad67 expression, when they use the same promoter and regulator elements. If a transgenic mouse strain was used, the Gad67 expression wouldn't be that easy to detect from GFP, because it's not that specific where the EGFP is attached.
2.We can see that the Gad67-GFP is expressed especially in hindbrain, but also as lighter in forebrain, midbrain and spinal cord. In hindbrain, the Gad-gene is active, which means that Gad67 enzyme is expressed. Gad67 enzyme is included in GABA production. These GABAergic cells show fluorescence signal, because GFP is active. Also, there is strong expression in tail bud, because there is also GABAergic cells during embryo development. But the cells in the tail bud are not neurons, instead there are stem cells. The embryonic day seems to be E11.5, according to showing GFP pattern and embryo's structure.
3.I would look at the GFP fluorescence signal and determine the embryonic stage from there. Earlier embryos do not have that strong fluorescence signal in the brain. In early stages there is stronger fluorescence in the tail bud, because of stem cells. When time goes by, the brain fluorescence increases, due to Gad-gene expression increasing, and the tail bud signal decreases.
4.I did the UMAP plot of the clusters, where I saw the different clusters. And to indentify the serotonergic/GABA clusters, I used spesific markergenes for serotonergic and GABAergic cells, which showed the right clusters, where these serotonergic or GABAergic cells were. The

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(164 words)

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1. Knock-in produces results that mirror the in vivo biology more faithfully. The transgene is likely integrated to the genome multiple times or it could integrate into the middle of another gene breaking its function. Expression from the true Gad67 locus is also likely more accurate because gene regulation is quite complex and impacted by the 3D chromatin organization.

2. There is faint expression all over the neural tube. Stronger expression is seen in the tail bud, telencephalon, midbrain and hindbrain. Stage is E11.5.

3. There are certain landmarks that emerge at each embryonic day. The tail bud is one of them as well as vasculature, limbs and digits. By looking at the table in the slides. The developmental schedule of the GABAergic neurons is also known, so the fluorescence images will also be helpful in our case.

4. We plotted marker gene expression (serotonergic: Sert, Fev, GABAergic: Gad1, Gad2) of each cell on top of the uncolored UMAP plot using the FeaturePlot() function.

(273 words)

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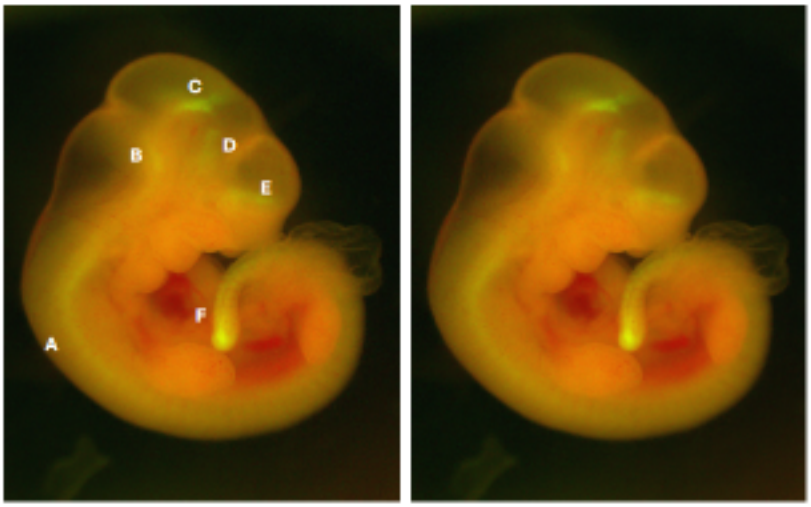
1. By knocking in Gad67GFP, we insert the EGFP sequence in the glutamic acid decarboxylase 67 kDa (Gad67 or Gad1) locus so that we have the true endogenous Gad67 expression. This avoids random insertion of the gene so that all the regulatory elements will be maintained, and thereby the expression level will be more accurate. Also, if the promoter of the Gad67 is used, there is the possibility of mosaic expression, which cannot happen in the knock-in method.

2. Since the expression of GFP can be observed in different regions containing the midbrain (C), forebrain (D), hindbrain (B), and also in the spinal cord (A) and tail buds (F), the embryo should be in approximately the E10.5 stage. Because GABAergic cells, which are marked by GFP in the GAD67 locus, are post-mitotic cells and only appear in later stages of the development.

3. There are different ways; for example, different morphology of the embryo can be taken into account. For example, in E10.5, embryos' eyes are getting formed and lenses are getting deeper; also, umbilical hernias begin to appear, while in E11.5, auditory hillocks appear and retina pigmentation becomes visible. In E12, retina pigmentation becomes visible, and foot plates, both anterior and posterior, appear with fingers.

4. I identified serotonergic and GABAergic cells using R programming by specifically using these cells' markers; serotonergic cells were

Test



Feedback comments

The GFP gene will produce GFP protein in the Gad67-FP mouse. The main advantage of the knock-in lines is the regulatory control from endogenous locus: the reporter gene (GFP) will be regulated by the promoter and enhancer elements of the gene of interest (Gad67).

The GFP expression pattern is recognized, but the neuron migration aspect should be clarified: The GABAergic neurons born in different anterior-posterior compartments remain in the same compartments, or migrate short distances. The cortical interneurons migrate from ventrolateral forebrain to dorsal forebrain.

Well done with learning the standards staging table and the scRNAseq analysis.

Very good!

Very good!

Overall very good, but could be more detailed about the GFP expression pattern in Q2. Also, the stage of shown embryo was younger than E11.5. The dissected E11.5 embryos showed much more GFP expression, especially in forebrain and diencephalon.
The GABAergic neuron markers in scRNAseq analysis were also Gad1 and Gad2. Nkx2-2 is expressed in serotonergic neuron progenitors in ventral hindbrain and in early serotonergic neuron precursors.

These answers are not wrong, but could be more specific.
The answer Q3 applies to staging of the Gad67-GFP embryos. Which other ways are there for determining the embryonic stage? What could you use to stage the embryos before the GFP can be seen?
In shown embryo, the GFP expression was brightest in the tail bud (F) ad midbrain (C).
Fev was used as marker gene to identify the serotonergic neurons and Gad1 and Gad2 were used as markers for the GABAergic neurons.

Very good!

Very good answers, with few minor mistakes. Fev was used as a marker of serotonergic cells. And it should be noted that the retina pigmentation is absent in many albino strains of lab mice, including the NMRI that we used here.