

DELIVERABLE 2.4

Report on the Generation and Breeding of Double Transgenic Model



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W: gemstoneproject.eu
SM: [@gemstonehorizon](https://twitter.com/gemstonehorizon)
E: gemstone@acibadem.edu.tr

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1. THE DOUBLE TRANSGENIC MODEL: SNCA MICE

The *SNCA* gene encodes the alpha-synuclein protein, one of the hallmarks of Parkinson's disease pathophysiology. There is no consensus on the origin of alpha-synuclein in the body in the scientific literature. Task 2.5, "Generation of Double Transgenic Model: SNCA Mice," within Work Package (WP) 2 aims to create a novel experimental animal model targeting this significant gap in the literature. The double transgenic model "SNCA mice" will be generated to enable conditional deletion of the *snca* gene (Ninkina et al., 2015). The hypothesized double transgenic model will have the deletion of the *snca* gene specifically as neural cell targeted. Following the successful generation of this double transgenic model, the model will be validated using PCR, ELISA, and immunohistochemical assays in the frame of Task 2.6, "Validation of SNCA Deletion in Double Transgenic Model."

2. THE TRANSGENIC MICE STRAINS TO GENERATE THE DOUBLE TRANSGENIC MODEL

Two different transgenic mice strains will be used to generate the double transgenic model:

- Nestin-Cre
- SNCAflox delta neo

2.1. Nestin-Cre Mice

The nestin-Cre mice strain, its scientific nomenclature B6.Cg-Tg(Nes-cre)1Kln/J, express Cre recombinase driven with nestin promoter. Since nestin is a neural stem/progenitor cell marker, Cre recombinase is expressed in central and peripheral nervous system in this mice strain. In the presence of cre-loxP recombination, this strain will enable the specification of gene modification only in neural cells. More detailed information regarding this mice strain can be found at: <https://www.jax.org/strain/003771>.

2.2. SNCAflox Delta Neo Mice

The SNCAflox delta neo mice strain, its scientific nomenclature B6(Cg)-*Snca*^{tm1.1Vlb}/J, possess loxP sites on either side of the first coding exon (exon II) of the *snca* gene. This strain is useful for generating conditional gene modifications applicable in alpha-synuclein targeted Parkinson's disease research studies. More detailed information regarding this mouse strain can be found at: <https://www.jax.org/strain/025636>.

3. TRANSFER OF NESTIN-CRE AND SNCAFLOX DELTA NEO STRAINS TO ACU DEHAM

Nestin-Cre and SNCAflox delta neo mice strains have been purchased from The Jackson Laboratory (JaxLab). Purchased mice were transferred to Acibadem Mehmet Ali Aydinlar University's (ACU) Transgenic Animal Research and Biosafety Laboratory (DEHAM) from JaxLab on 9 January 2023. Following a one-week quarantine period, each mouse was checked for their health status by Talat Taygun Turan, a member of the GEMSTONE Project.

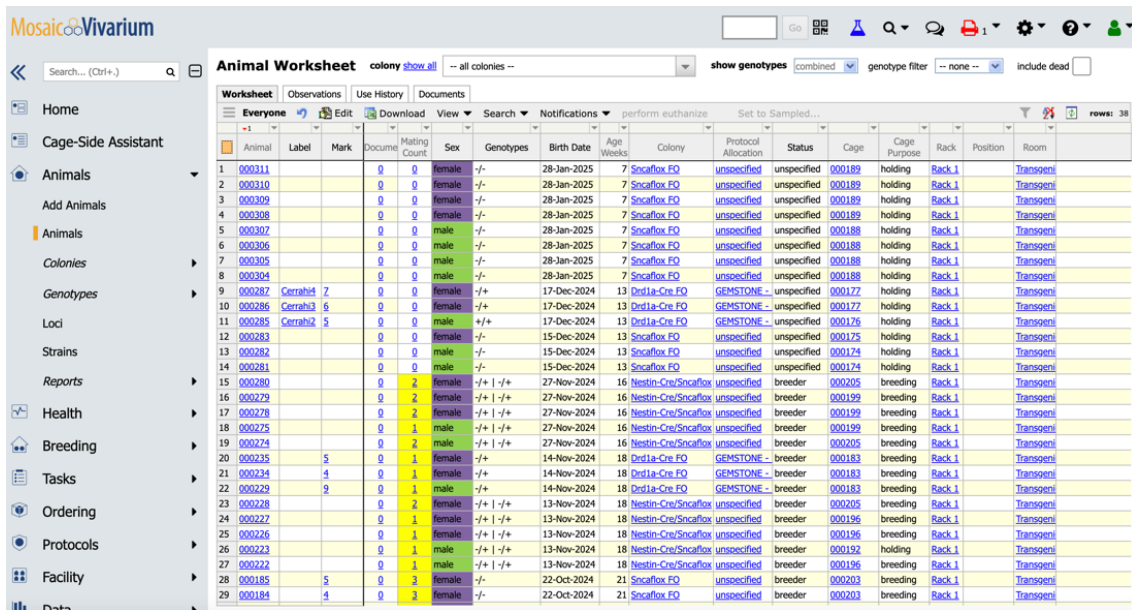


Figure 1: Photograph from the transfer of nestin-Cre and Sncaflox delta neo strains

4. COLONY MANAGEMENT: MOSAIC VIVARIUM

Mosaic Vivarium is a web-based laboratory animal management software. This system provides data-based and cost-effective solutions applicable to several processes in laboratory animal management. These processes vary from animal handling including registering the laboratory animals, tracking the breeding, and protocols, to financial issues such as tracking the orders and billing. As both a knowledge transfer and a technology transfer within the framework of the GEMSTONE Project, the Mosaic Vivarium software has been integrated into the ACU DEHAM.

Since the integration of Mosaic Vivarium software, it has been utilized for colony management of several transgenic mice colonies such as nestin-Cre, SNCAflox delta neo and Drd1a-Cre. In accordance with the Data Management Plan, data regarding the transgenic animal colonies are also produced and collected through Mosaic Vivarium software. As a result of these processes, the essential role of Mosaic Vivarium in ACU DEHAM marks another milestone in the capacity enhancement of ACU in the scope of GEMSTONE Project.



Animal	Label	Mark	Sex	Genotypes	Birth Date	Age Weeks	Colony	Protocol Allocation	Status	Cage	Cage Purpose	Rack	Position	Room
1	000311		female	-/-	28-Jan-2025	7	Snca1lox_FO	unspecified	unspecified	000189	holding	Rack_1		Transgeni
2	000310		female	-/-	28-Jan-2025	7	Snca1lox_FO	unspecified	unspecified	000189	holding	Rack_1		Transgeni
3	000309		female	-/-	28-Jan-2025	7	Snca1lox_FO	unspecified	unspecified	000189	holding	Rack_1		Transgeni
4	000308		female	-/-	28-Jan-2025	7	Snca1lox_FO	unspecified	unspecified	000189	holding	Rack_1		Transgeni
5	000307		male	-/-	28-Jan-2025	7	Snca1lox_FO	unspecified	unspecified	000188	holding	Rack_1		Transgeni
6	000306		male	-/-	28-Jan-2025	7	Snca1lox_FO	unspecified	unspecified	000188	holding	Rack_1		Transgeni
7	000305		male	-/-	28-Jan-2025	7	Snca1lox_FO	unspecified	unspecified	000188	holding	Rack_1		Transgeni
8	000304		female	-/-	28-Jan-2025	7	Snca1lox_FO	unspecified	unspecified	000188	holding	Rack_1		Transgeni
9	000287	Cerrah14	female	+/+	17-Dec-2024	13	Drd1a-Cre_FO	GEMSTONE	unspecified	000172	holding	Rack_1		Transgeni
10	000286	Cerrah13	female	+/+	17-Dec-2024	13	Drd1a-Cre_FO	GEMSTONE	unspecified	000172	holding	Rack_1		Transgeni
11	000285	Cerrah12	male	+/+	17-Dec-2024	13	Drd1a-Cre_FO	GEMSTONE	unspecified	000176	holding	Rack_1		Transgeni
12	000283		female	-/-	15-Dec-2024	13	Snca1lox_FO	unspecified	unspecified	000175	holding	Rack_1		Transgeni
13	000282		female	-/-	15-Dec-2024	13	Snca1lox_FO	unspecified	unspecified	000174	holding	Rack_1		Transgeni
14	000281		male	-/-	15-Dec-2024	13	Snca1lox_FO	unspecified	unspecified	000174	holding	Rack_1		Transgeni
15	000280		female	+/+ 1 +/+	27-Nov-2024	16	Nestin-Cre/Snca1lox	unspecified	breeder	000205	breeding	Rack_1		Transgeni
16	000279		female	+/+ 1 +/+	27-Nov-2024	16	Nestin-Cre/Snca1lox	unspecified	breeder	000199	breeding	Rack_1		Transgeni
17	000278		female	+/+ 1 +/+	27-Nov-2024	16	Nestin-Cre/Snca1lox	unspecified	breeder	000199	breeding	Rack_1		Transgeni
18	000275		male	+/+ 1 +/+	27-Nov-2024	16	Nestin-Cre/Snca1lox	unspecified	breeder	000199	breeding	Rack_1		Transgeni
19	000274		male	+/+ 1 +/+	27-Nov-2024	16	Nestin-Cre/Snca1lox	unspecified	breeder	000205	breeding	Rack_1		Transgeni
20	000235	5	female	+/+	14-Nov-2024	18	Drd1a-Cre_FO	GEMSTONE	breeder	000183	breeding	Rack_1		Transgeni
21	000234	4	female	+/+	14-Nov-2024	18	Drd1a-Cre_FO	GEMSTONE	breeder	000183	breeding	Rack_1		Transgeni
22	000229	9	male	+/+	14-Nov-2024	18	Drd1a-Cre_FO	GEMSTONE	breeder	000183	breeding	Rack_1		Transgeni
23	000228		female	+/+ 1 +/+	13-Nov-2024	18	Nestin-Cre/Snca1lox	unspecified	breeder	000205	breeding	Rack_1		Transgeni
24	000227		female	+/+ 1 +/+	13-Nov-2024	18	Nestin-Cre/Snca1lox	unspecified	breeder	000196	breeding	Rack_1		Transgeni
25	000226		female	+/+ 1 +/+	13-Nov-2024	18	Nestin-Cre/Snca1lox	unspecified	breeder	000196	breeding	Rack_1		Transgeni
26	000223		male	+/+ 1 +/+	13-Nov-2024	18	Nestin-Cre/Snca1lox	unspecified	breeder	000192	holding	Rack_1		Transgeni
27	000222		male	+/+ 1 +/+	13-Nov-2024	18	Nestin-Cre/Snca1lox	unspecified	breeder	000196	breeding	Rack_1		Transgeni
28	000185	5	female	-/-	22-Oct-2024	21	Snca1lox_FO	unspecified	breeder	000203	breeding	Rack_1		Transgeni
29	000184	4	female	-/-	22-Oct-2024	21	Snca1lox_FO	unspecified	breeder	000203	breeding	Rack_1		Transgeni

Figure 2: Screenshot from the Mosaic Vivarium System

Each transferred laboratory animal to ACU DEHAM has been logged into Mosaic Vivarium account of GEMSTONE following their register and health check. Each animal is registered with their data including sex, birth date, genotype, colony, etc. If permitted, any researcher within the group can access all data regarding the laboratory animals anytime, online and transparently. This easy and open-access feature of the software has also been another capacity enhancement of ACU DEHAM.

5. GENERATION OF THE DOUBLE TRANSGENIC MODEL

5.1. Generation of Nestin-Cre Colony

Nestin-Cre transgenic mice were purchased from JaxLab as homozygous males and females. Following the transfer of homozygous nestin-Cre mice to the ACU DEHAM laboratory, each mouse has been registered to the Mosaic Vivarium software under nestin-Cre colony. Then, the mice were put in breeding cages with the setting of two females and one male. The male was kept in the breeding cage during the whole period including breeding, birth and offspring care. Expected birth dates of the offspring are generated automatically through the Mosaic Vivarium software. Careful inspection of the expected offspring is crucial due to the high rates of infanticide among the transgenic mice. Researchers and/or the technicians responsible for colony management should prioritize checking the breeding cages daily and registering the litter if present. Approximately 3 weeks after the setting of the breeding cages, the expected offspring are born and approximately 4 weeks after their birth, the offspring can be weaned and moved to their cages according to their sexes.

By the end of weaning, a small tissue sample was collected from each animal's ear cartilage (tissue sample collection site depends on the animal welfare and ethics regulations of the research center/university). Each animal's DNA was isolated from the collected tissue samples and genotypic verification of the offspring were done with PCR using *Tg(Nes-cre)1Kln-Chr12* targeted primer sets. More detailed technical information regarding the genotyping protocol of nestin-Cre mice can be found at: <https://www.jax.org/Protocol?stockNumber=003771&protocolID=31758>. The whole generation process should be repeated every 4-6 months for the maintenance of a healthy and fertile colony. Extra care and time are needed because it is very challenging to have the offspring ready for the research experiments.

5.2. Generation of SNCAflox Delta Neo Colony

SNCAflox delta neo transgenic mice were purchased from JaxLab as homozygous males and females. Following the transfer of homozygous nestin-Cre mice to ACU DEHAM laboratory, each mouse has been registered to the Mosaic Vivarium software under SNCAflox delta neo colony. Then, the mice were put in breeding cages with the setting of two females and one male. The male was kept in the breeding cage during the whole period including breeding, birth and offspring care. Expected birth dates of the offspring are generated automatically through the Mosaic Vivarium software. Careful inspection of the expected offspring is crucial due to the high rates of infanticide among the transgenic mice. Researchers and/or technicians responsible for colony management should prioritize checking the breeding cages daily and registering the litter if present. Approximately 3 weeks after the setting of the breeding cages, the expected offspring are born and approximately 4 weeks after their birth, the offspring can be weaned and moved to their cages according to their sexes.

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5.3. Cross-Breeding of Nestin-Cre and SNCAflox Delta Neo Mice

When nestin-Cre and SNCAflox delta neo colonies reached their optimal size, the first cross-breedings between nestin-Cre and SNCAflox delta neo mice have been planned and set up by Talat Taygun Turan. In these cross-breedings, the mice were put in breeding cages with the setting of one female and one male to maximize the offspring with a limited possible parent animal number. The male was kept in the breeding cage during the whole period including breeding, birth and offspring care. The cross-breedings were set as follows: 3 male homozygous nestin-Cre paired with 3 female homozygous SNCAflox delta neo mice, and 3 female homozygous nestin-Cre paired with 3 male homozygous SNCAflox delta neo mice.

Offspring was obtained with a double transgenic genotype of heterozygous nestin-Cre:SNCAflox delta neo as a result of these cross-breedings. Expected birth dates of the offspring are generated automatically through the Mosaic Vivarium software. Careful inspection of the expected offspring is crucial due to the high rates of infanticide among the transgenic mice. Researchers and/or technicians responsible for colony management should prioritize checking the breeding cages daily and registering the litter if present. Approximately 3 weeks after the setting of the breeding cages, the expected offspring are born and approximately 4 weeks after their birth, the offspring can be weaned and moved to their cages according to their sexes.

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6. BREEDING OF THE DOUBLE TRANSGENIC MODEL

When the expected heterozygous nestin-Cre:SNCAflox delta neo offspring from the cross-breedings between nestin-Cre and SNCAflox delta neo mice reached a sufficient number and adult age, the mice were put in breedings. The mice were put in breeding cages with the setting of two females and one male. The male was kept in the breeding cage during the whole period including breeding, birth and offspring care. Expected birth dates of the offspring are generated automatically through the Mosaic Vivarium software. Careful inspection of the expected offspring is crucial due to the high rates of infanticide among the transgenic mice. Researchers and/or technicians responsible for colony management should prioritize checking the breeding cages daily and registering the litter if present. Approximately 3 weeks after the setting of the breeding cages, the expected offspring are born and approximately 4 weeks after their birth, the offspring can be weaned and moved to their cages according to their sexes.

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Many different possible genotypes were expected as a result of these breedings:

- Nestin-cre (+): homozygous SNCAflox delta neo (+/+)
- Nestin-cre (+): heterozygous SNCAflox delta neo (+/-)
- Nestin-cre (+): no SNCAflox delta neo (-/-)
- Nestin-cre (-): homozygous SNCAflox delta neo (+/+)
- Nestin-cre (-): heterozygous SNCAflox delta neo (+/-)
- Nestin-cre (-): no SNCAflox delta neo (-/-)

In the framework of Task 2.6, immunohistochemical and biochemical assay experiments utilizing these mice with the possible genotypes mentioned above are currently in process by Talat Taygun Turan, who is currently at Lund University for his short-term visit within WP3. The results to be obtained from these experiments will also generate Deliverable 2.1, "Report on Distribution of α -syn in Different Regions in SNCA Transgenic Model."