Molecular cloning for babies:
Development of gRNA for CRISPRi in prairie voles

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Background:

Prairie voles are **monogamous**. This is a genetically driven behavior.

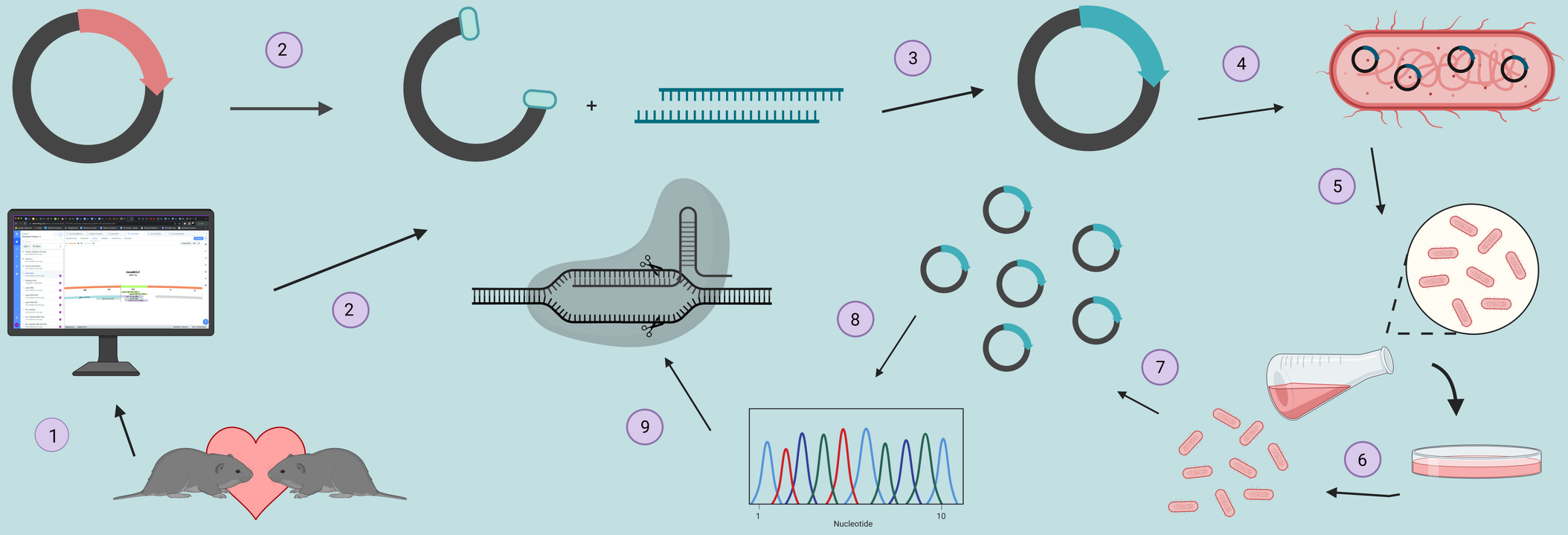
DRD4	(Dopamine receptor D4), member of G-protein coupled receptor family, is associated with novelty seeking behavior, with attention and with many psychiatric disorders.
CRYM	Protein binds thyroid hormone. It mediates effects of social isolation on reward behavior.
CREB1	(CAMP Responsive Element Binding Protein 1) encodes a transcription factor. Phosphorylated protein induces the transcription of genes in response to the hormonal stimulator of the cyclic AMP pathway. CREB1 is also very important in learning and memory.
HTR1A	(5-Hydroxytryptamine Receptor 1A) is associated with stress and social behaviour. When these gene is not expressed it can lead to anxiety and depression.
ESR2	(Estrogen Receptor 2) gene is responsible for parental care and affection in many organisms including humans and mice.
ERBB3	(erb-b2 receptor tyrosine kinase 3) upregulated after extended partner separation. It is possible that extended partner separation downregulates Erbb3-associated pathways resulting in reduced myelination by oligodendrocytes.

We think that these genes are responsible for their monogamous behavior.

Goals and expectations:

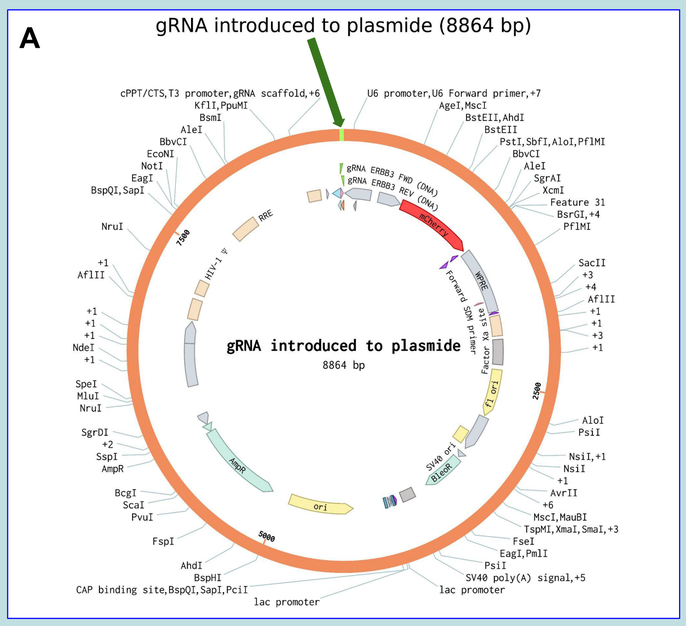
- **Find genes responsible for pair bonding**
- Develop molecular tools to **repress** these genes in specific brain regions

Methods:



Results:

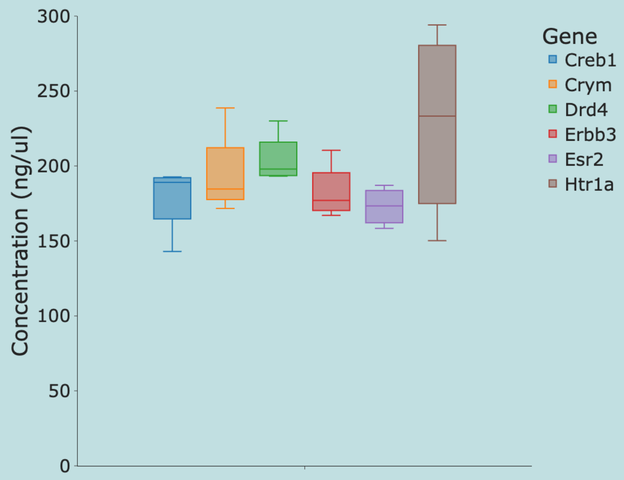
Building of gRNA



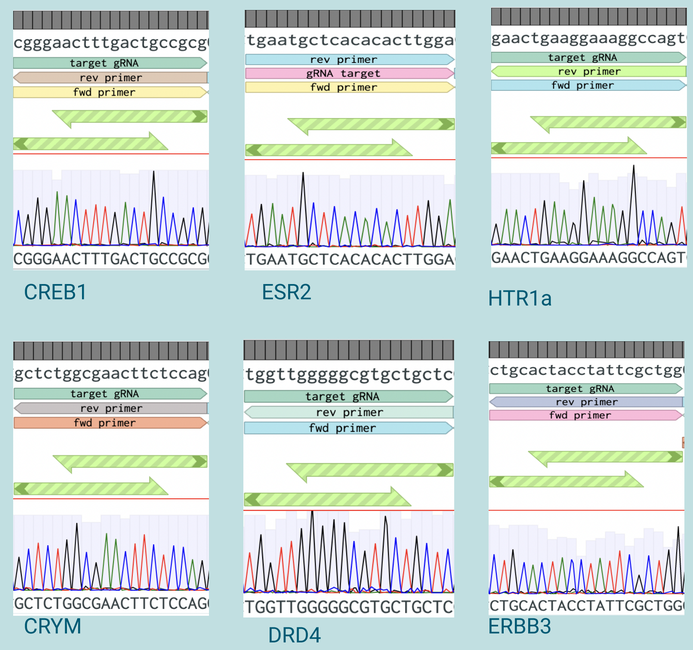
Picture A. Using bioinformatics tools to predict gRNA sequence and implement in bacterial plasmid.

Previous research data of CRISPRi use showed that the highest efficiency of gRNA bonding to the complemental part of needed DNA are achieved 50 nucleotide bases after the promoter of corresponding gene. There is a challenge of finding the sequence that demonstrate high selectivity in this area of DNA.

Concentration of extracted DNAs



Sequence data



Conclusions:

- Final results that we got from sequences of synthesized plasmids demonstrated relevance of the following protocol and advantages of further using.
- More than 91% of gRNAs were successfully introduced to the bacterial plasmids of *E.Coli*.
- We agree that there is still room to continue increasing effectiveness of methods used in gRNA producing.
- Making new ways of controlling gene expression is perspective and we agree that it is need to be studied more in the future.

What can we learn next?

- The social behavior of prairie voles (*Microtus ochrogaster*) is to study further using genome editing to understand what is the biological base of ability to form monogamous relationship.
- It is yet to check the role of the genes chosen for this experiment using CRISPRi with the gRNA obtained from the work in the lab.
- Further refinement of procedure is needed to provide increased selectivity and efficiency for the methods of genes expression regulating used in this lab.