



SAPIENZA
UNIVERSITÀ DI ROMA

**LMNA-related Dilated
Cardiomyopathy therapy:
Silencing LMNA mRNA and restoring
wild type lamin proteins**

Gene Therapy

Prof. I. Saggio

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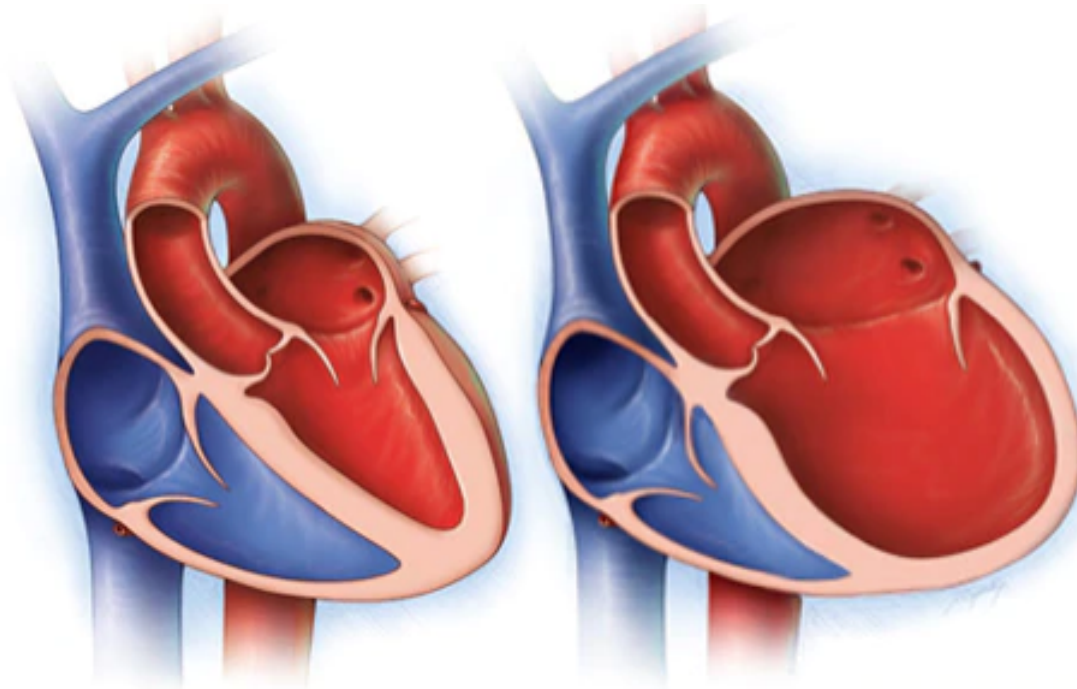
1.

The disease

LMNA-related dilated cardiomyopathy

LMNA-related dilated cardiomyopathy

- Autosomal **dominant** disorder
- **6-8%** of DCM cases in humans
- Symptoms between **20** and **60** years old



NORMAL HEART

DILATED HEART

LMNA-related dilated cardiomyopathy

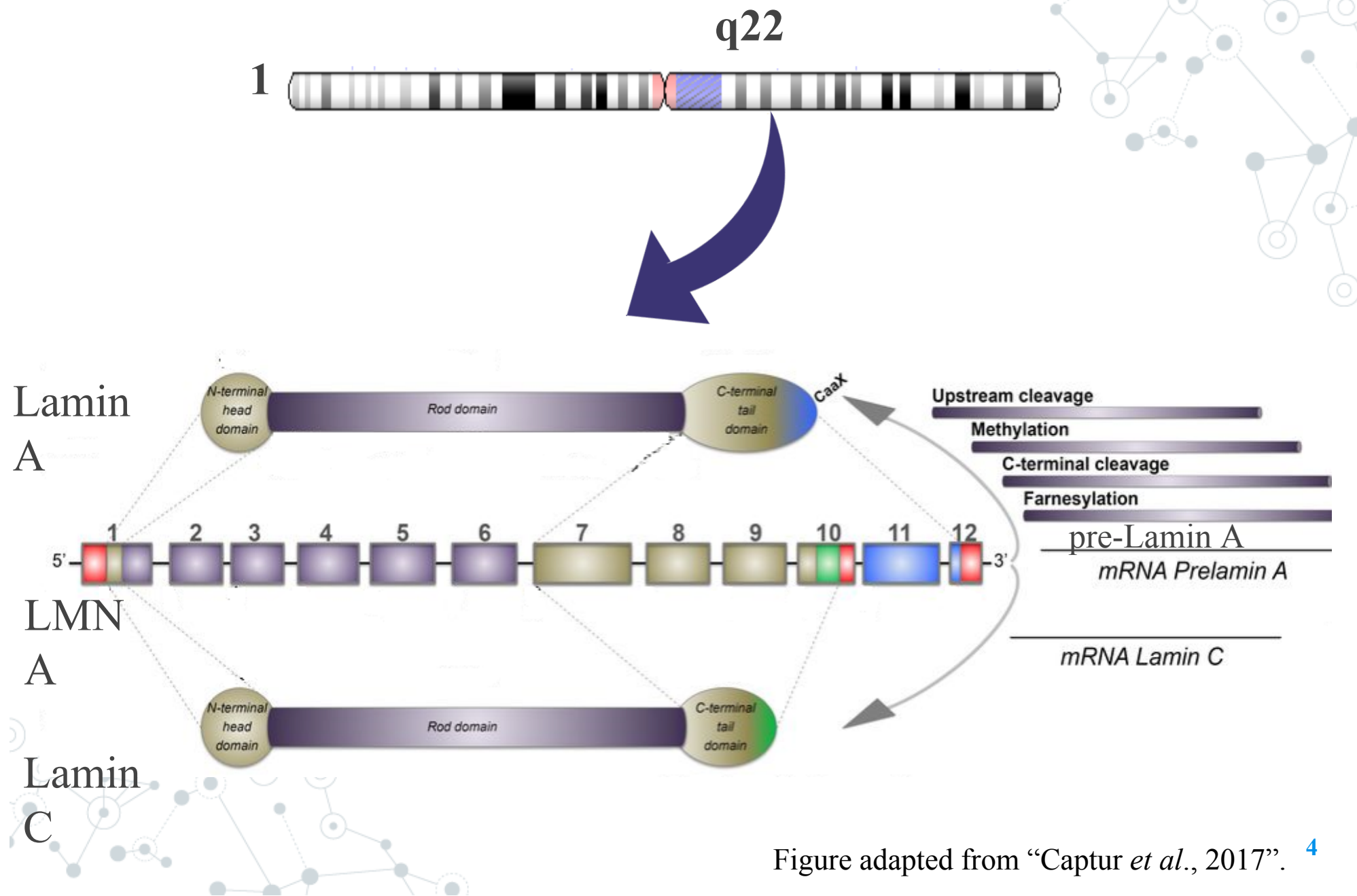


Figure adapted from "Captur *et al.*, 2017". ⁴

The background of the slide features a light blue-grey color with a repeating pattern of interconnected nodes and lines. The nodes are represented by small circles, some solid and some hollow, connected by thin lines, creating a network-like structure.

2.

Objectives and strategy

Our goals and what we are going to do



GOALS

Blocking the expression of mutated LMNA gene

Restoring the WT phenotype of lamin proteins A and C

Assess the lentiviral **specificity** for cardiomyocytes



STRATEGY

Stable and long-term **expression** of Antisense Oligonucleotide (ASO) through liposome infection

Designing **circRNA** constructs encoding for the WT lamin proteins delivered through lentivectors

Lentiviral vector with a **specific promoter** against the cardiomyocytes



3.

Delivery systems

RNAiMAX™ Reagent GapmeR ASO
delivery and

3rd generation lentiviral vectors mediated
circRNA delivery

Delivering GapmeR ASO with liposomes

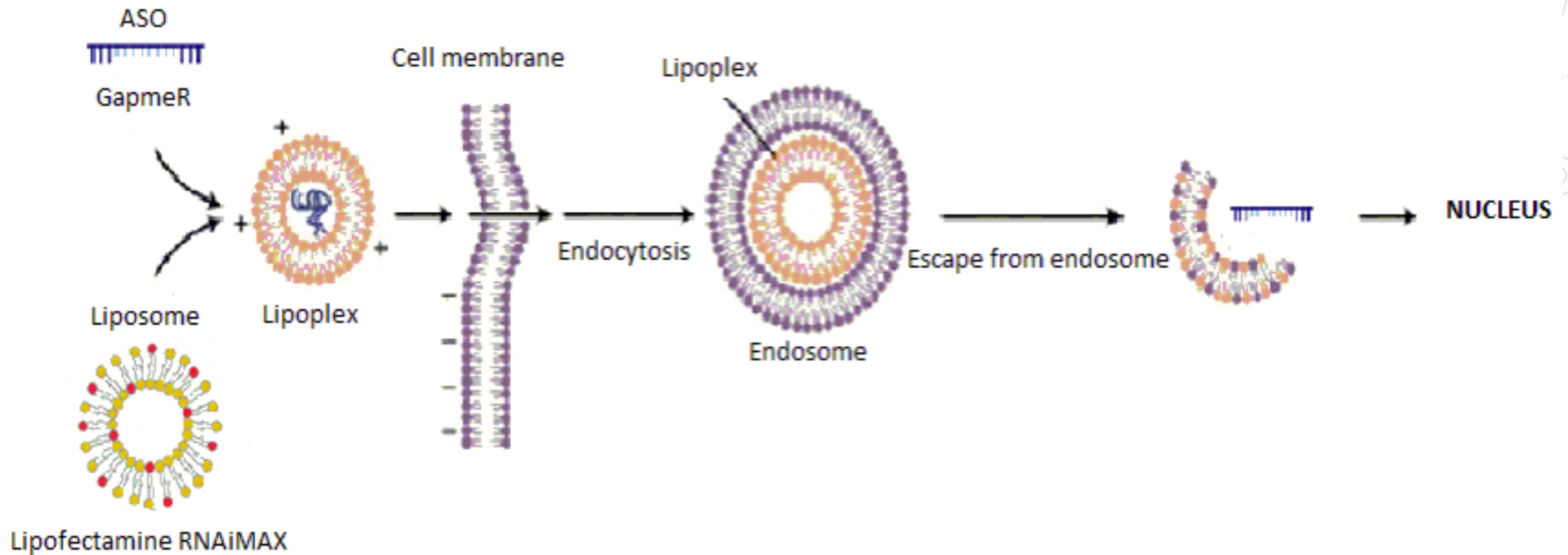
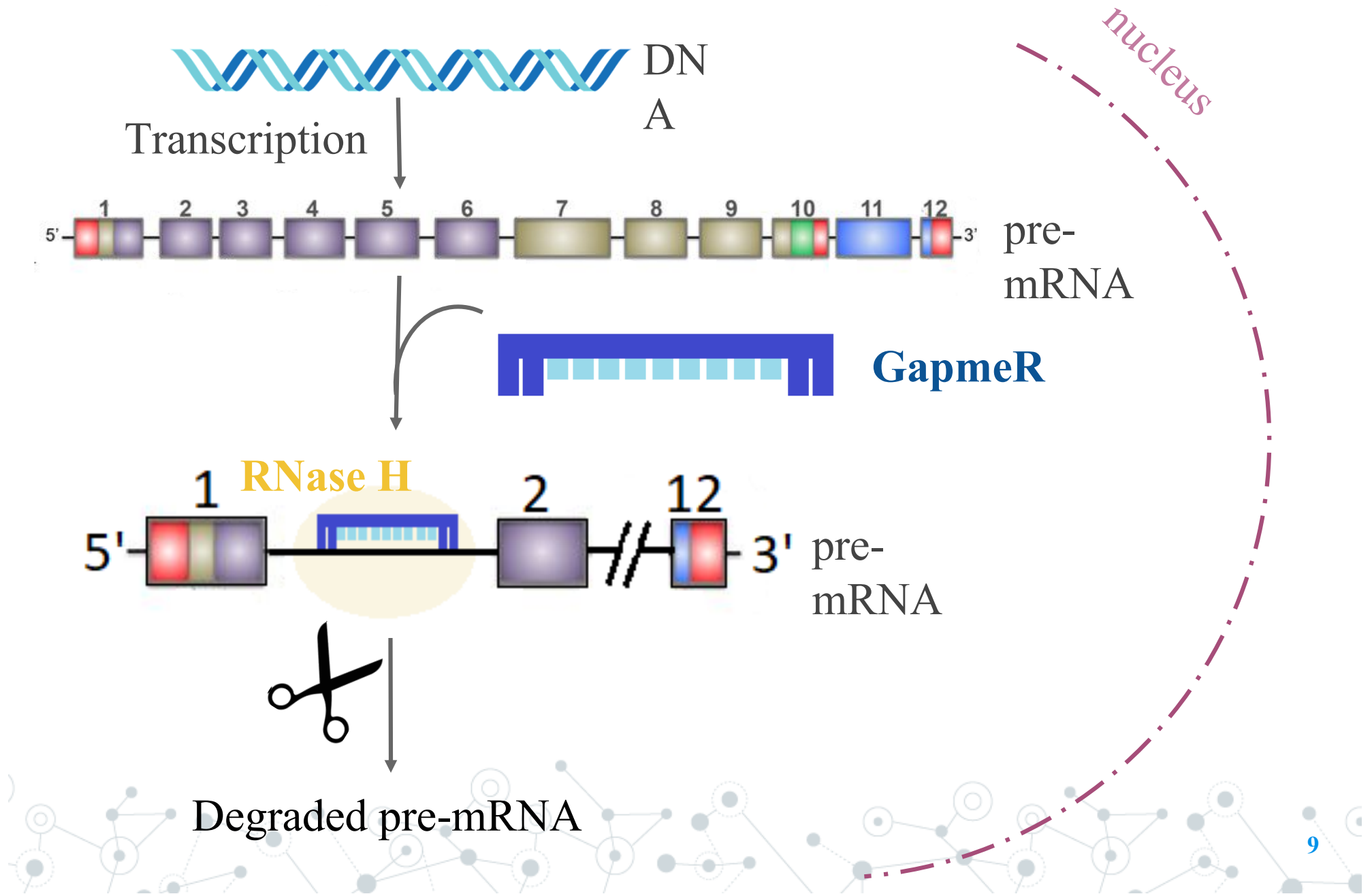
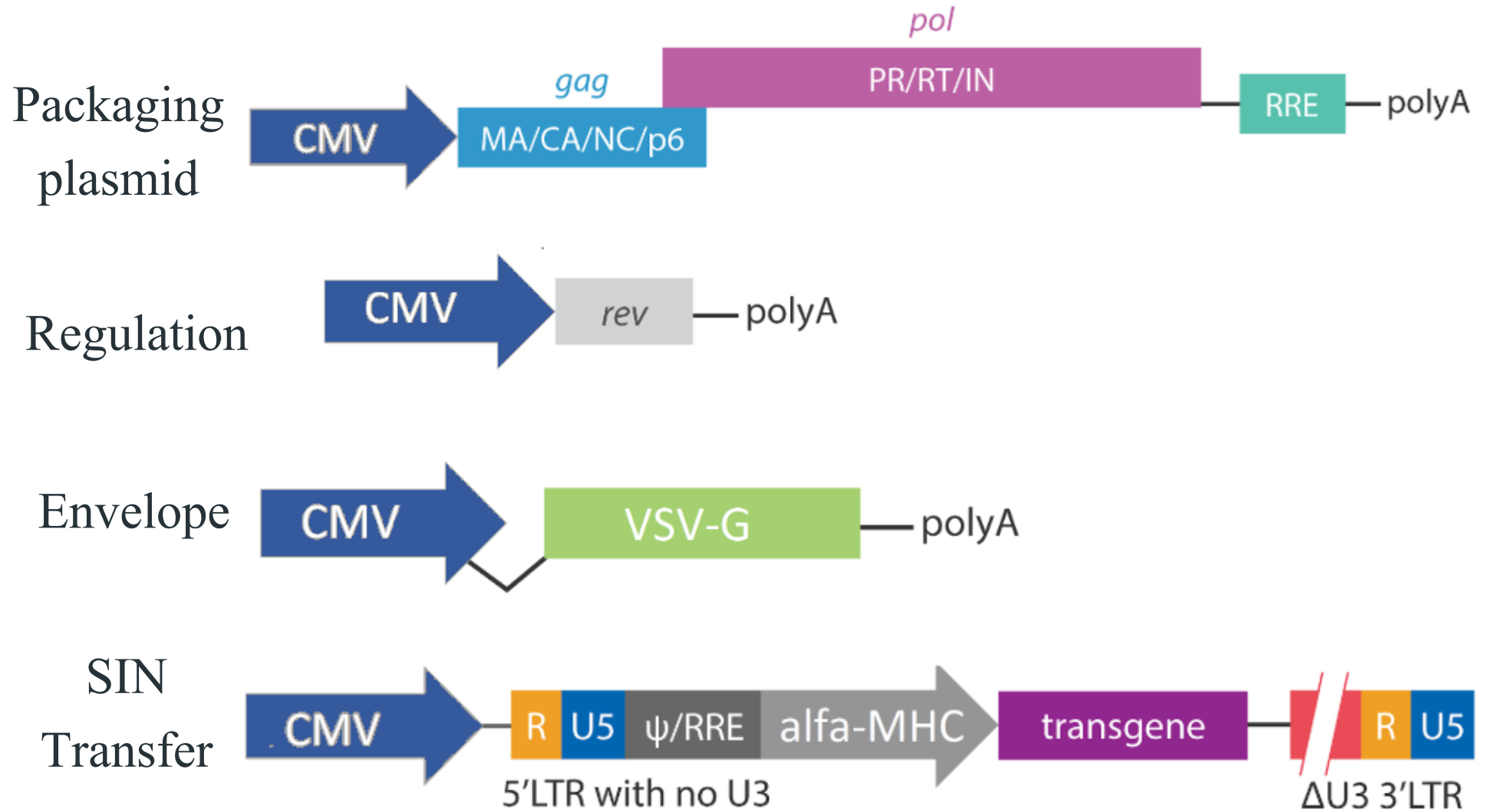
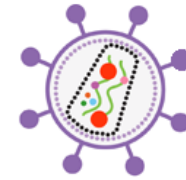


Figure adapted from “Molecular Medicine, 2003”

Silencing pre-mRNA with GapmeR ASO



3rd generation lentiviral vectors



Circular RNA

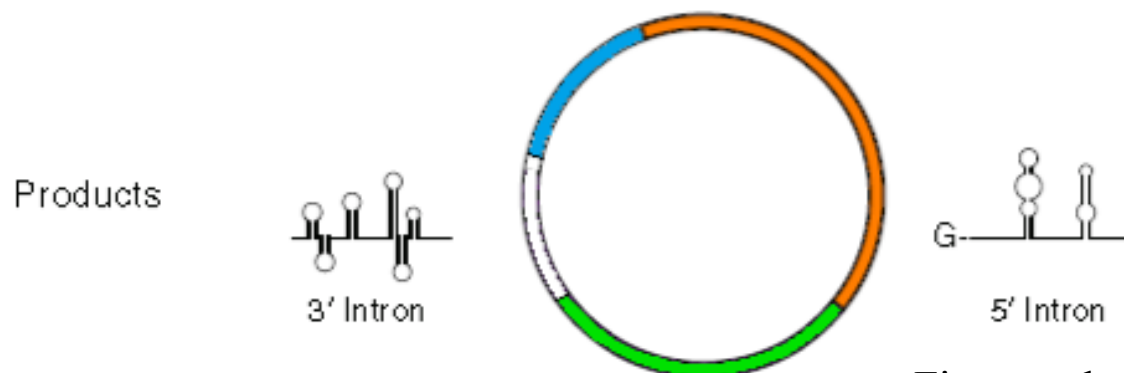
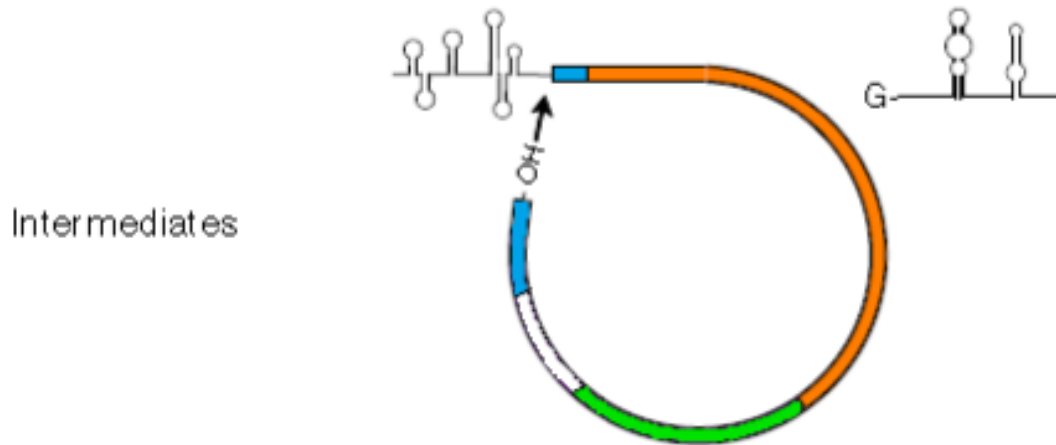
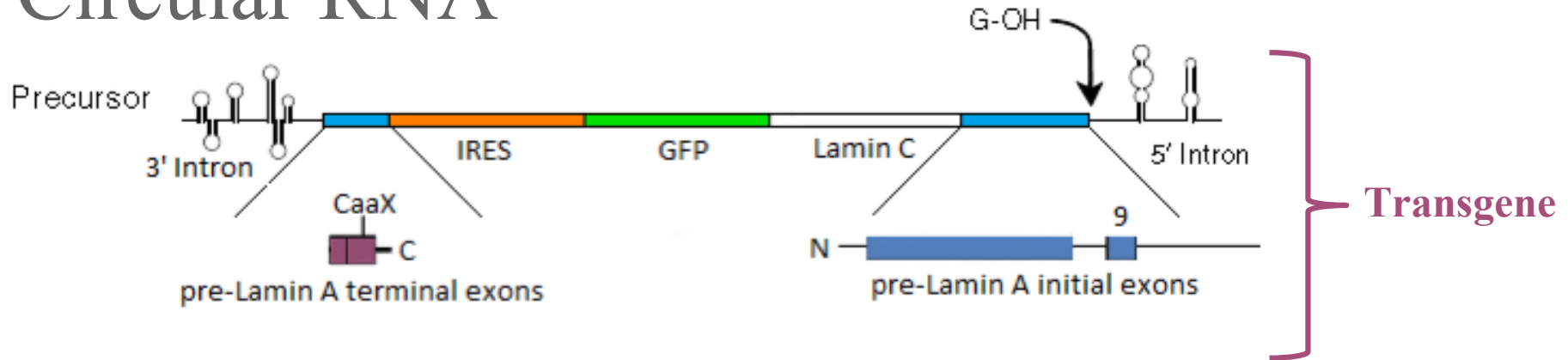
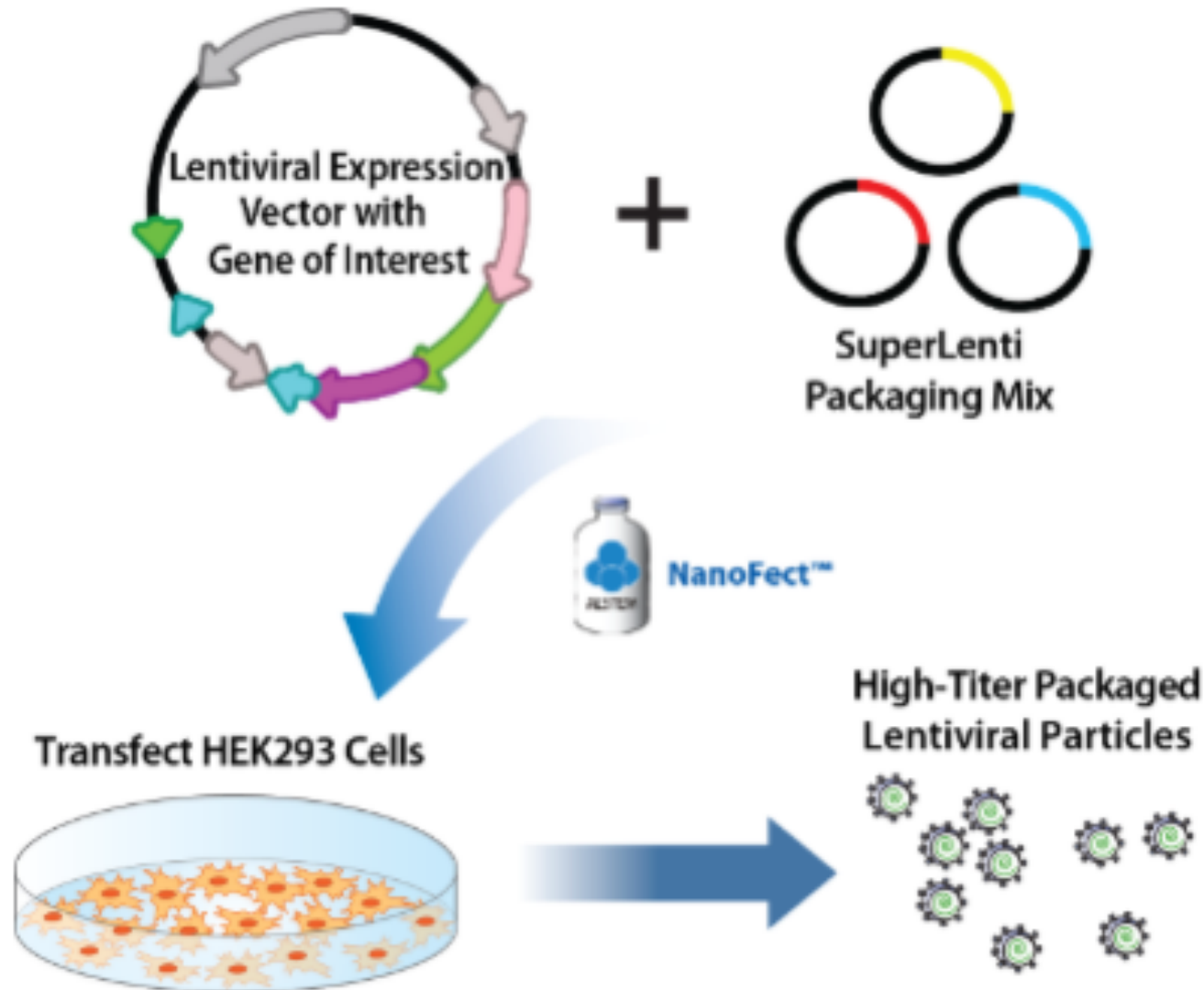


Figure adapted from "Wesselhoeft *et al.*, 2018"

Lentivectors production



The background of the slide is a light blue-grey color with a repeating pattern of interconnected nodes and lines. The nodes are represented by small circles, some of which are solid and some are hollow, connected by thin lines. The overall effect is a complex, network-like structure that suggests a scientific or technological theme.

4.

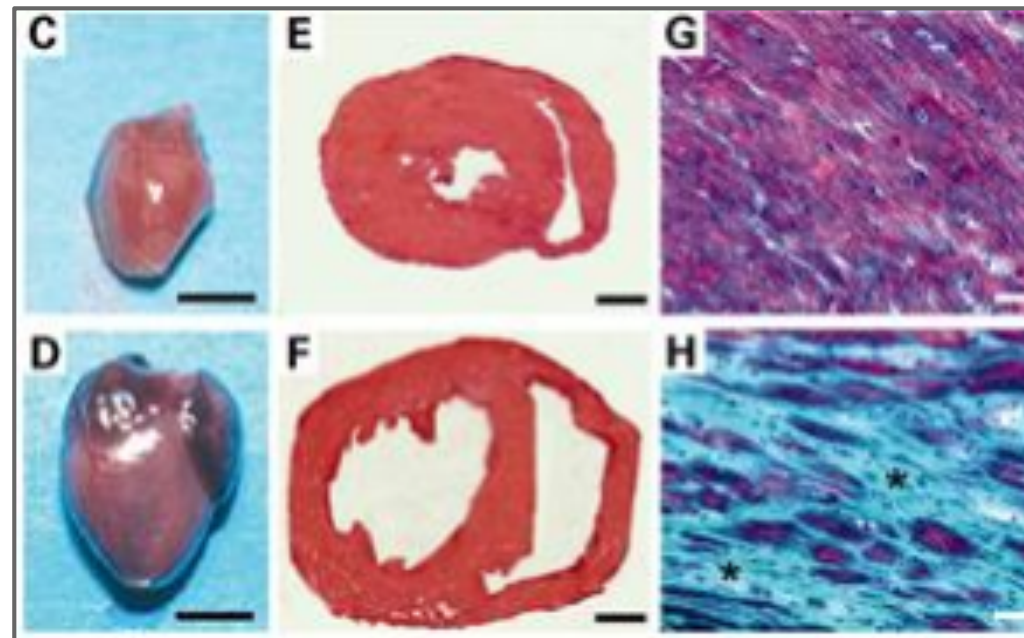
Experimental plan

Testing the therapy

Organism model → H222P Mouse model

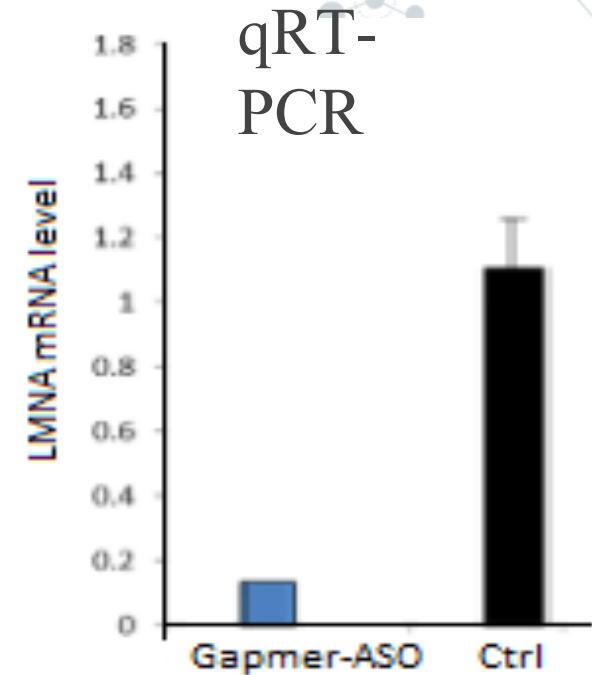
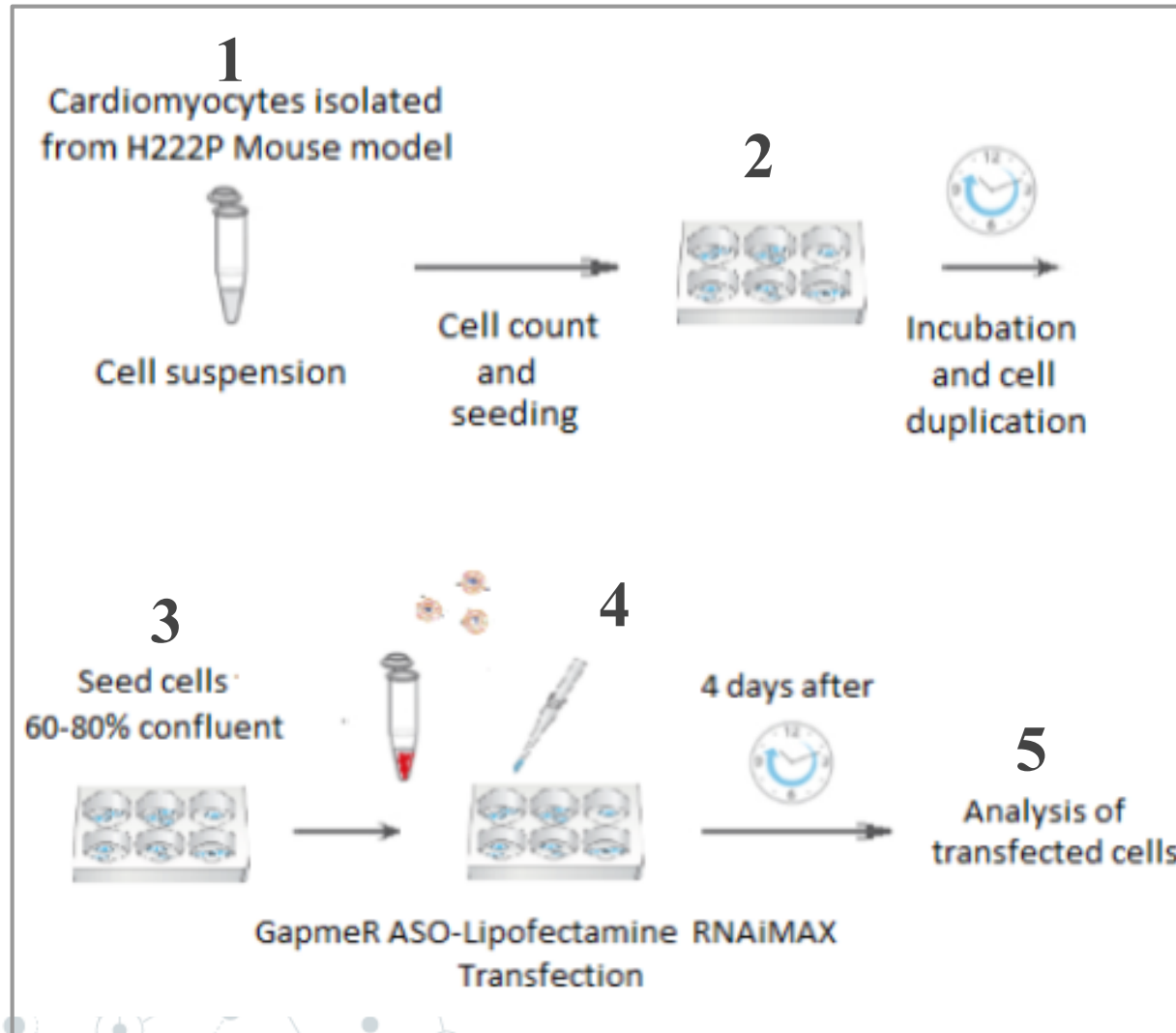


WT



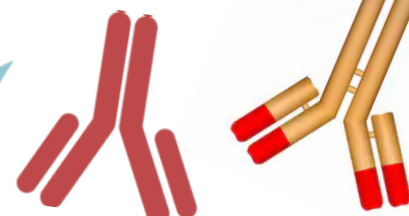
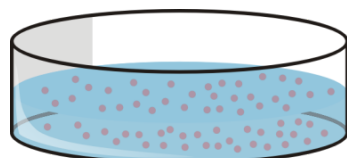
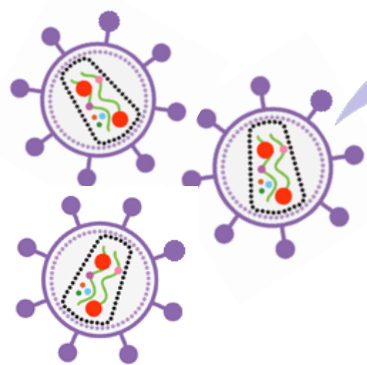
In vitro experiment- Part 1

GapmeR ASO-Liposome transfection for silencing the endogenous LMNA gene expression.

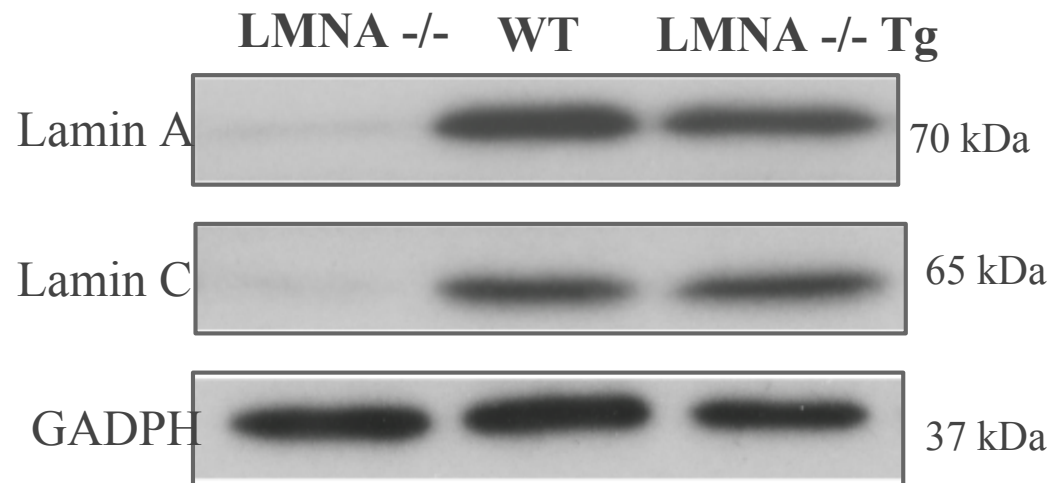
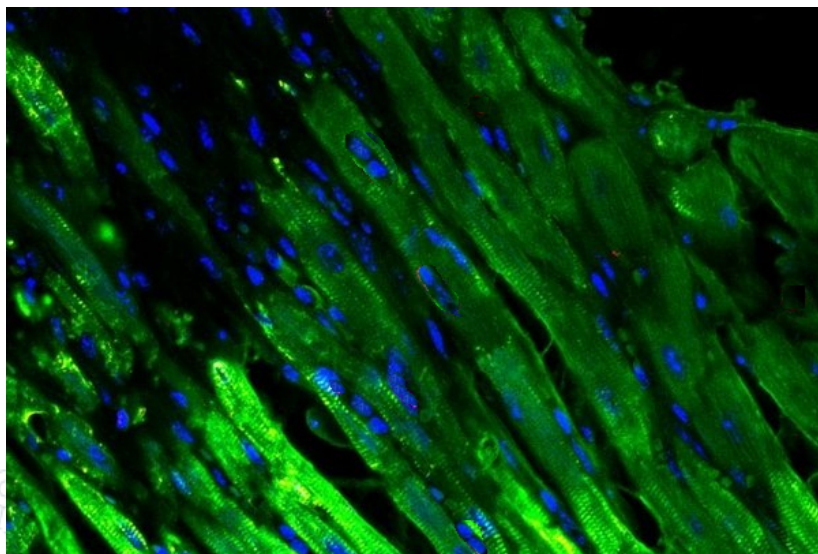


In vitro experiment- Part 2

circRNA delivery with lentivectors into cardiomyocytes from H222P

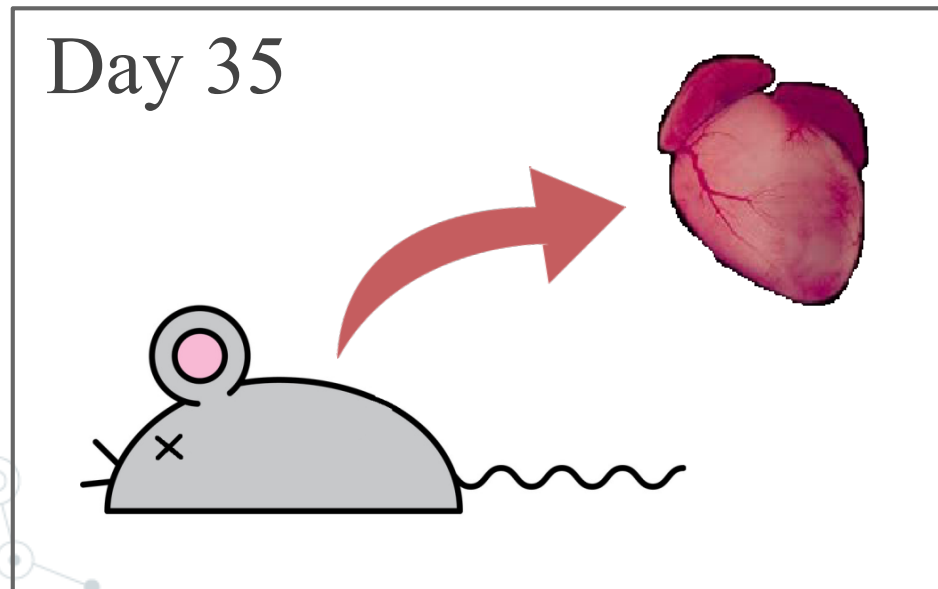
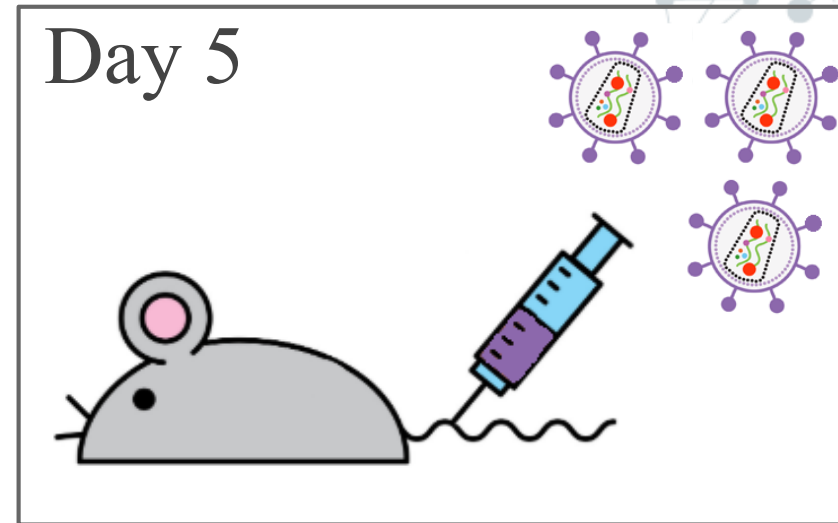
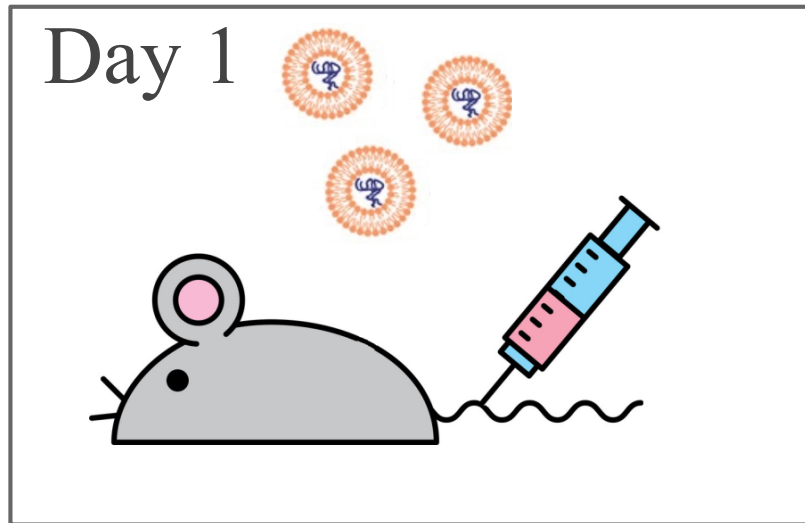


GFP expression



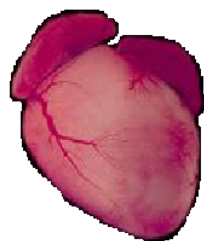
In vivo experiment

Lmna^{H222P/H222P} neonatal mice transfection

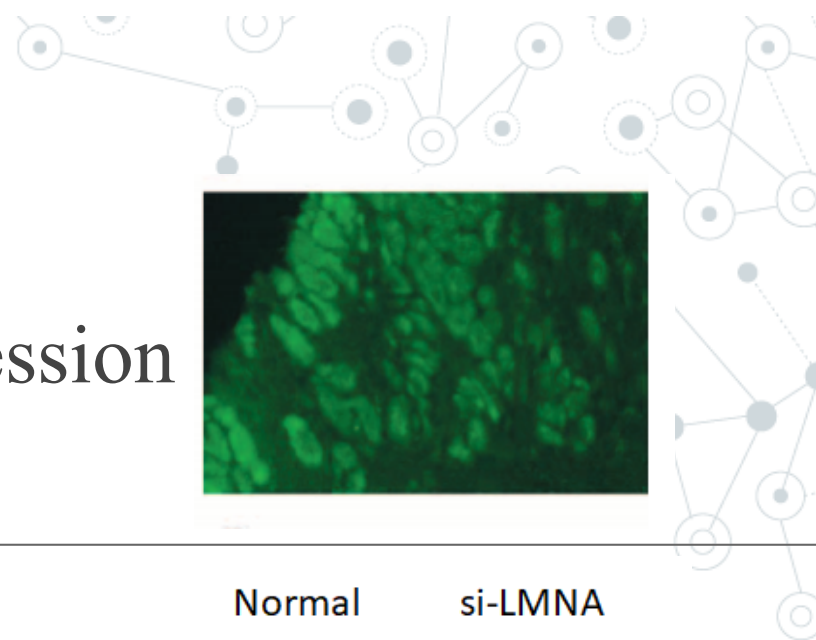
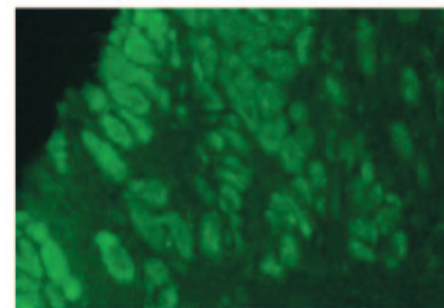


In vivo experiment

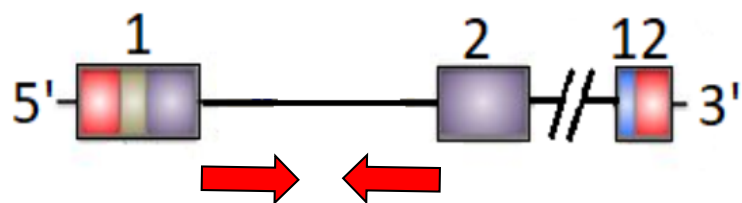
Results



GFP expression



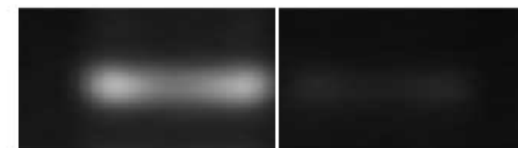
RT-PCR



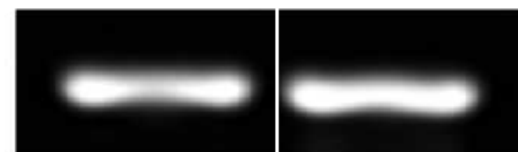
pre-mRNA LMNA

Normal

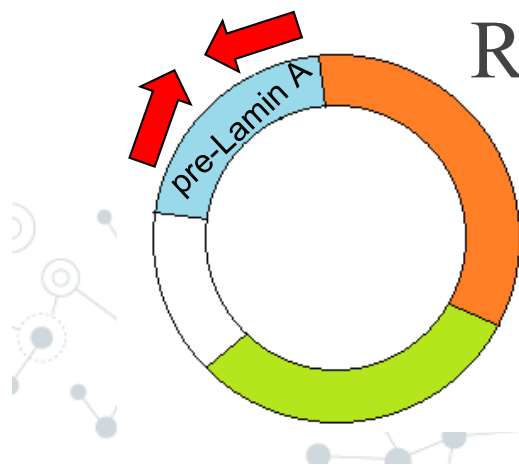
si-LMNA



GADPH



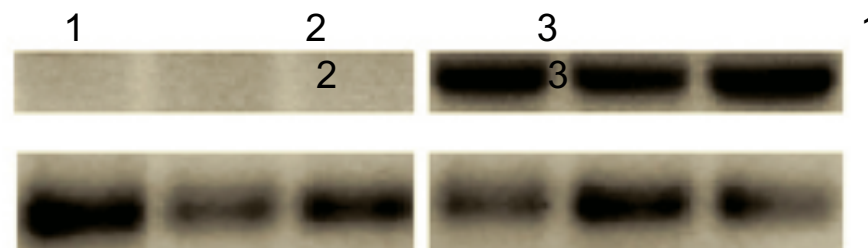
RT-PCR



pre-LaminA

linIRES pre-LaminA

split pre-LaminA



Figures adapted from "Frock *et al.*, 2012"



5.

Pitfalls and solutions

Pitfalls

1. Low expression of circRNAs and GapmeR ASO

1. Low regenerative power of cardiomyocytes

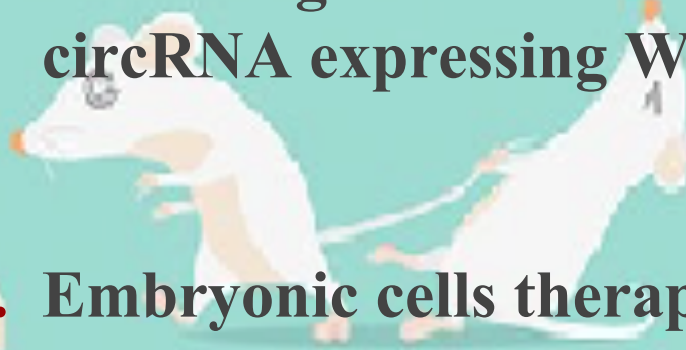
1. Liposomal specificity for cardiomyocytes and cytotoxicity

Solutions

1. Crispr/Cas9 lentiviral delivery system to knock-down the LMNA mutated gene and insertion of circRNA expressing WT protein

1. Embryonic cells therapy

1. Specific targeting of the nanoparticles: PCM and TAT co-modified liposome





6.

Materials and costs

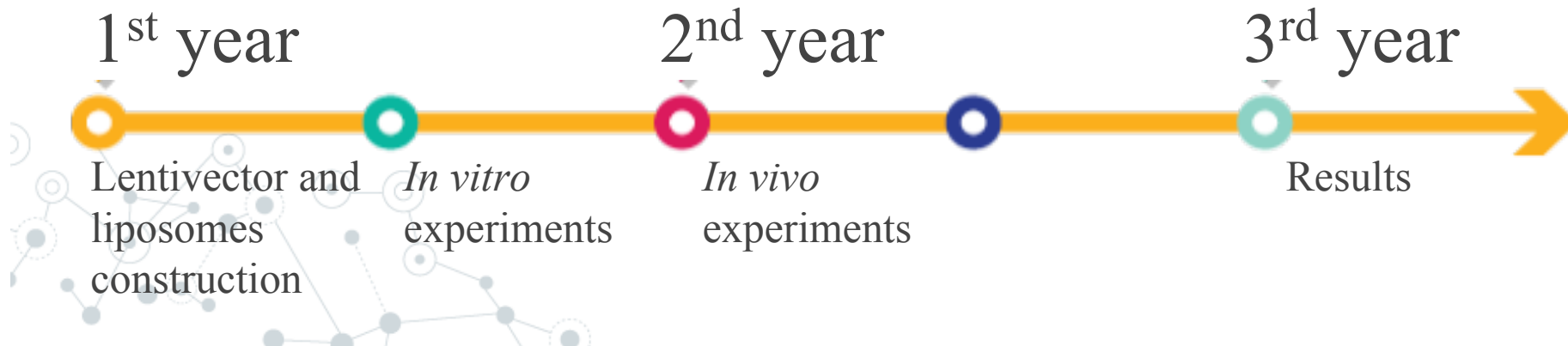
Timeline, materials and costs of production

- 3rd generation Lentivectors: € 1.500
- 5 x (WT) mice: € 500
- 10 x H222P mice: € 2.365/mouse
- Stabulation cost (each mouse): € 1.000 (x year)
- Western blot kit: € 200
- Western blot antibodies: € 300-400/antibody
- RT-PCR kit: € 500
- Cardiomyocytes cell culture: 500€
- Lipofectamine® RNAi max transfection reagent: € 964
- Molecular biology laboratory instruments: € 5.000
- GapmeR ASO: € 1.000



TOTAL COST: € 63.000

(without the salary cost of the researchers)





7.

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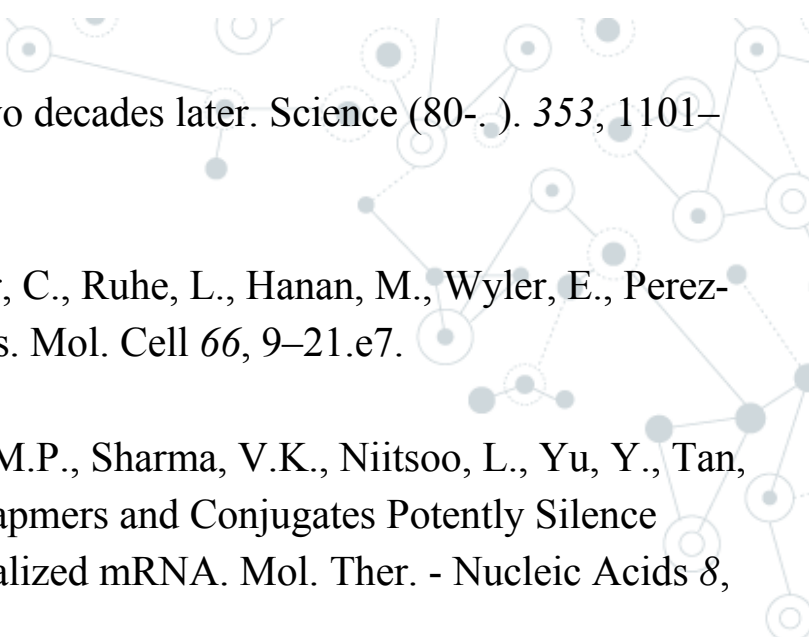
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Thank you!

Any questions?

