



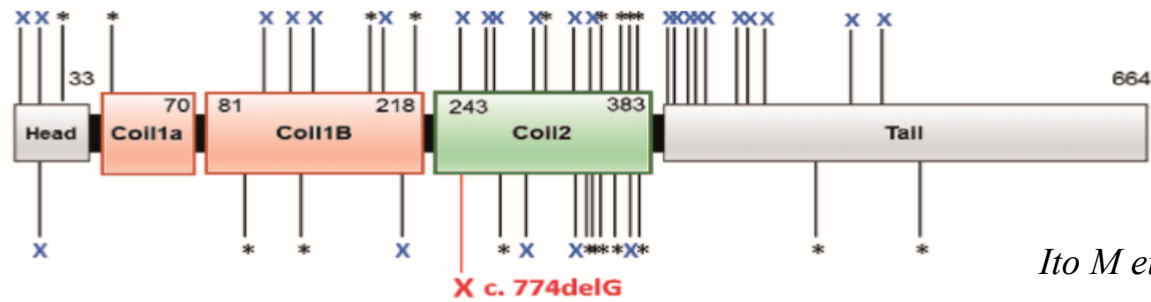
SAPIENZA
UNIVERSITÀ DI ROMA

*Use of ciRNA
in LMNA-RELATED DILATED CARDIOMIOPATHIES therapy*

CdL Biologia e Tecnologie cellulari
Anno 2018/2019

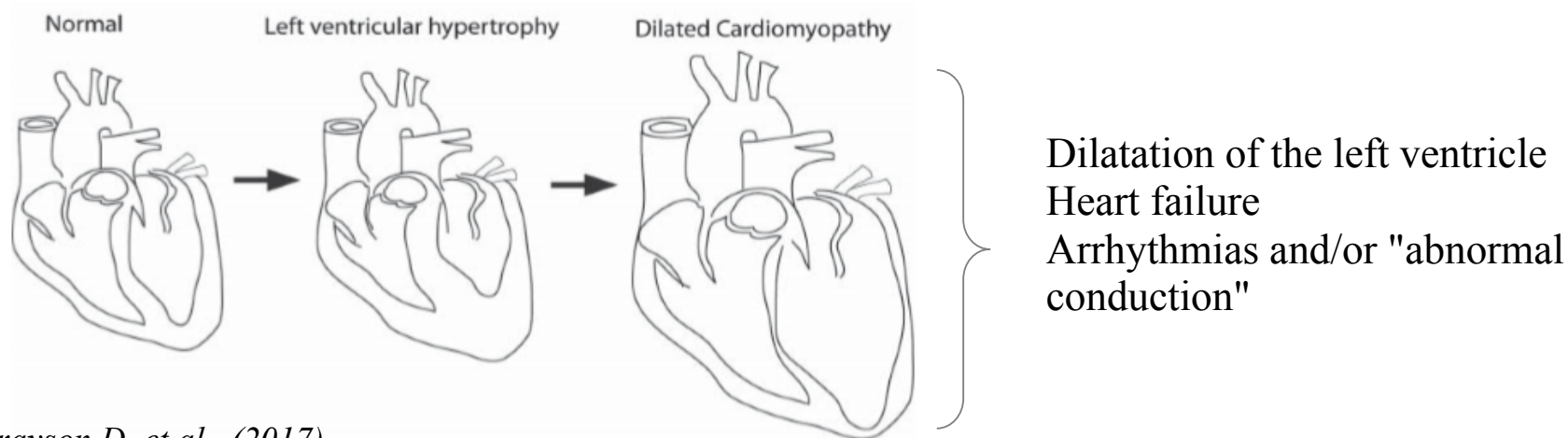
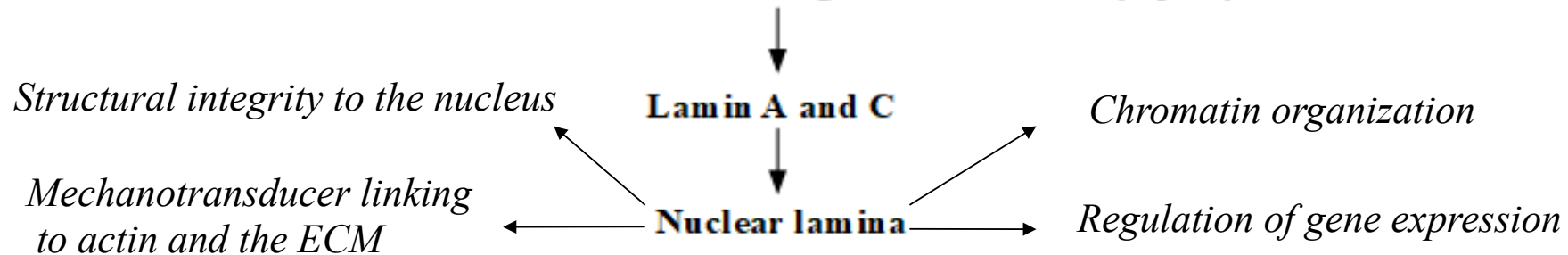
Giulietti Sarah
Chemsì Isa
Rotili Arianna

LMNA- RELATED DILATED CARDIOMYOPATHY (DCM)



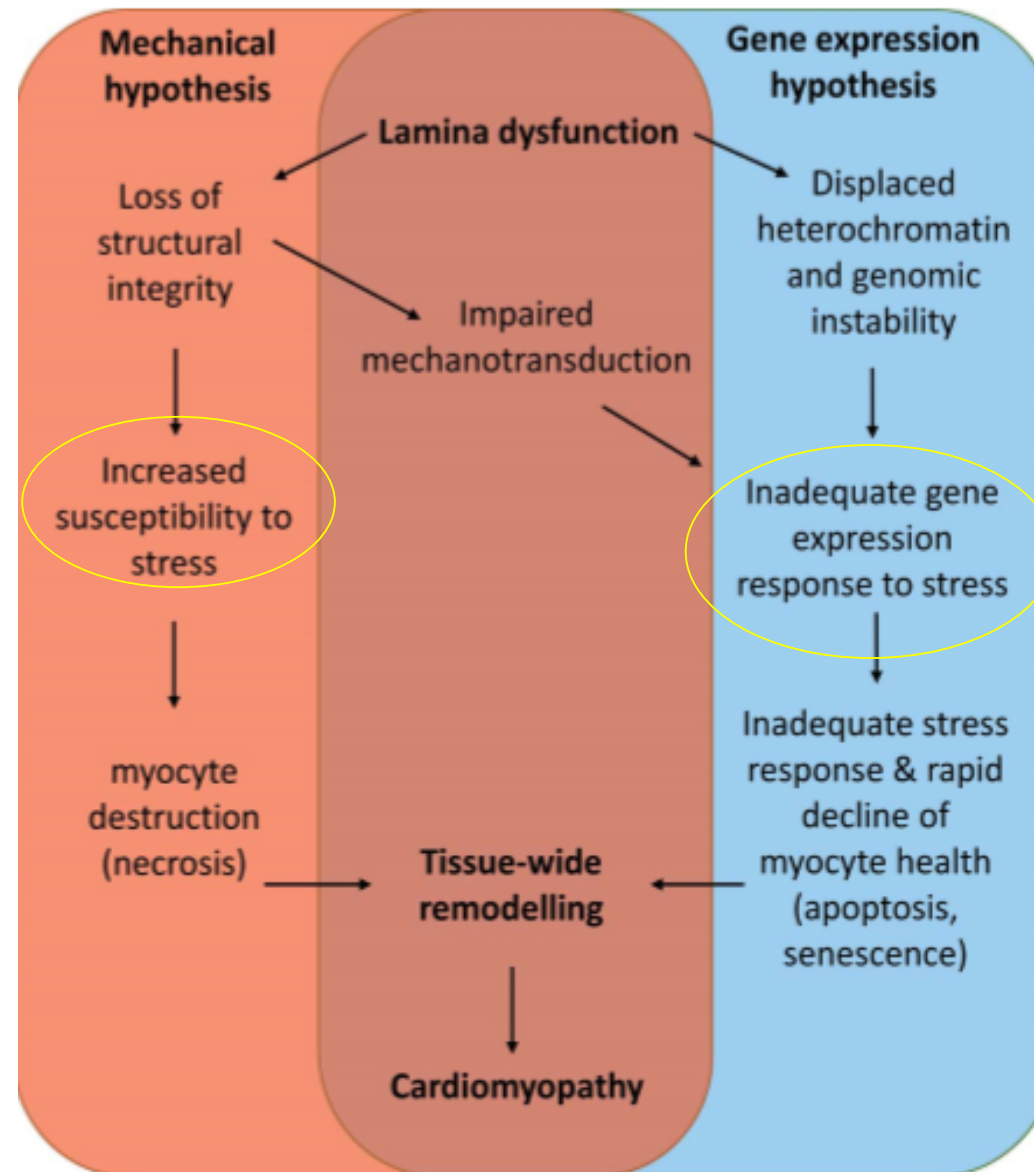
X =non-missense mutation (truncation, frameshift, in-del) * =missense mutation

More than 160 different mutations in the **LMNA** gene cause cardiomyopathy



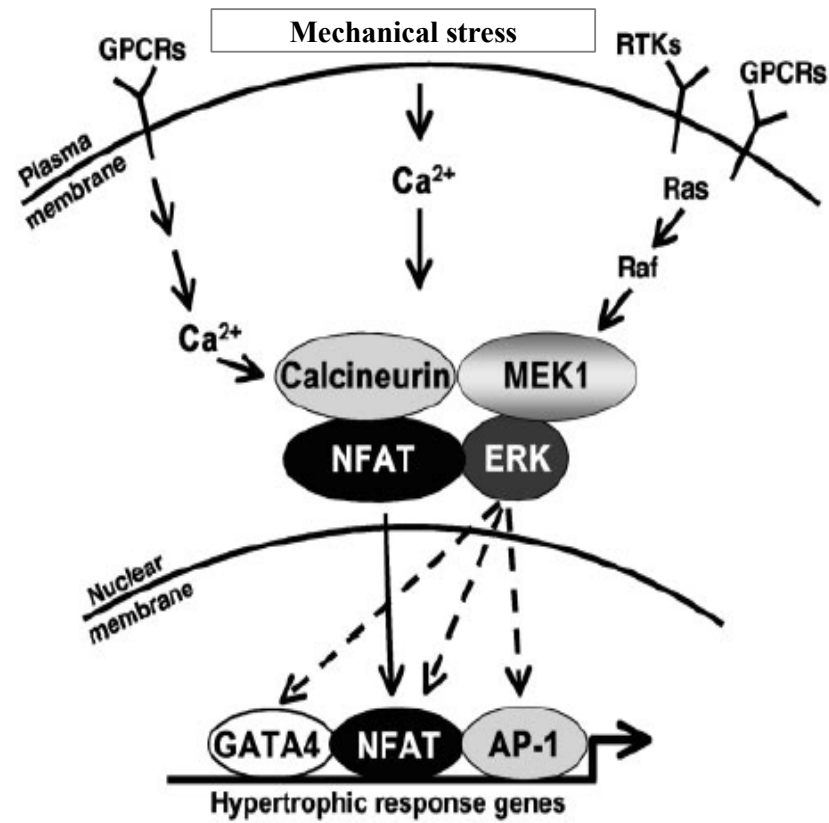
Brayson D. et al., (2017)

KNOWN MECHANISMS OF LMNA-CARDIOMYOPATHY

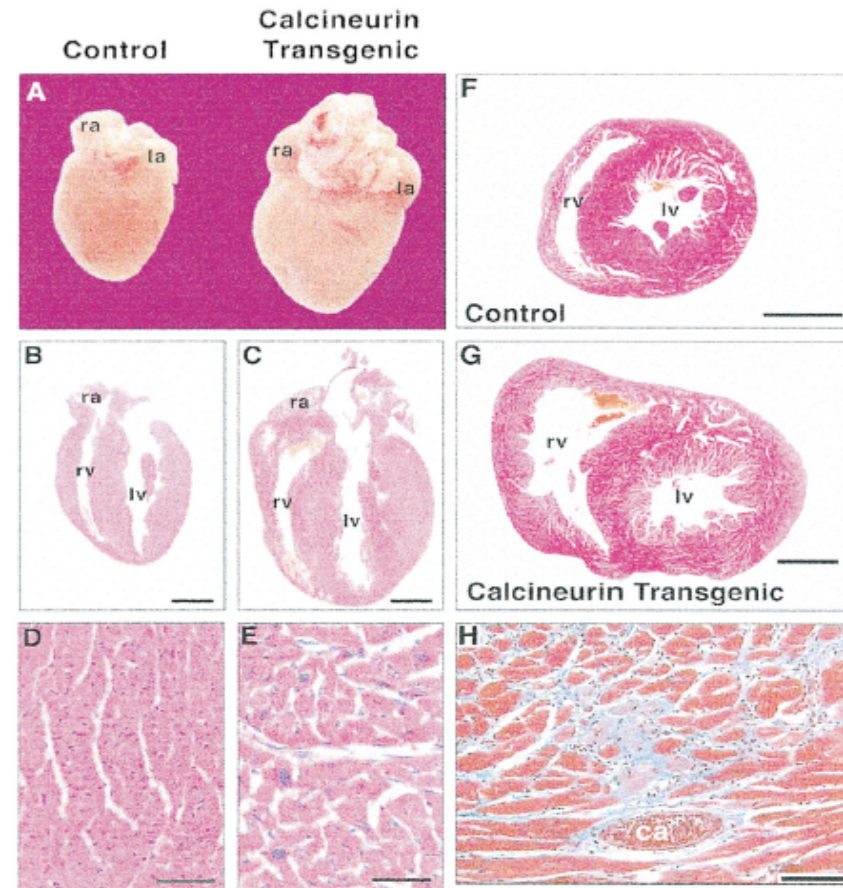


Brayson D. et al., (2017)

RESPONSE TO THE MECHANICAL STRESS: ROLE OF CALCINEURIN

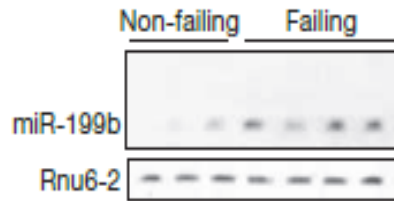


(Sanna B. et al., 2005)

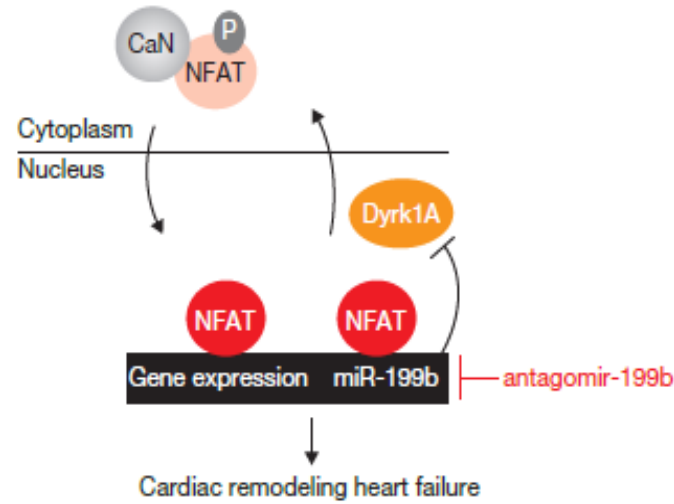


Molkentin J. D. et al., 1998

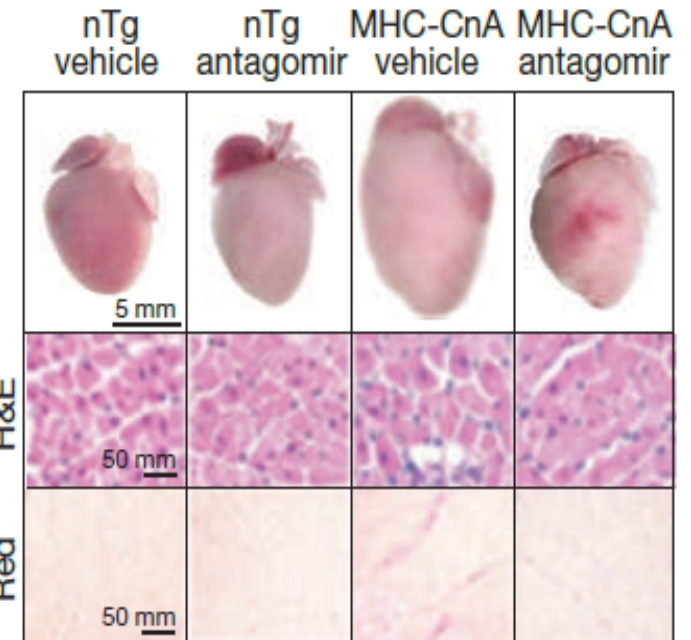
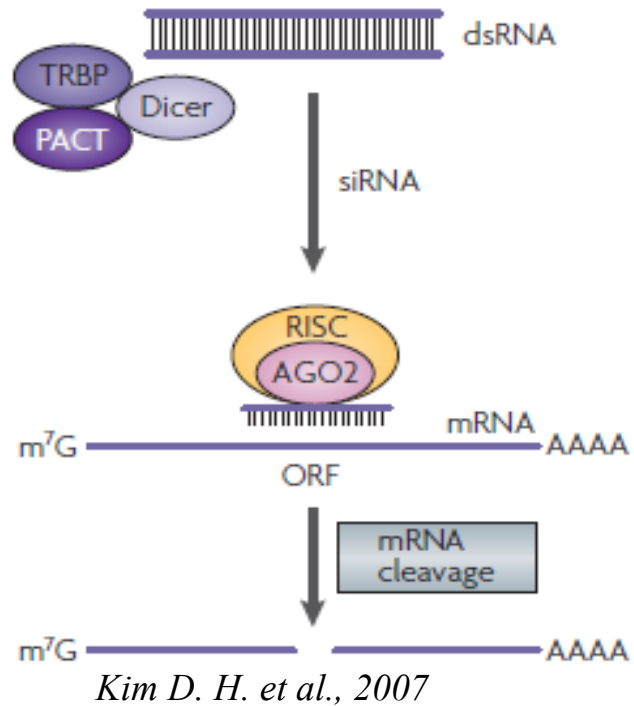
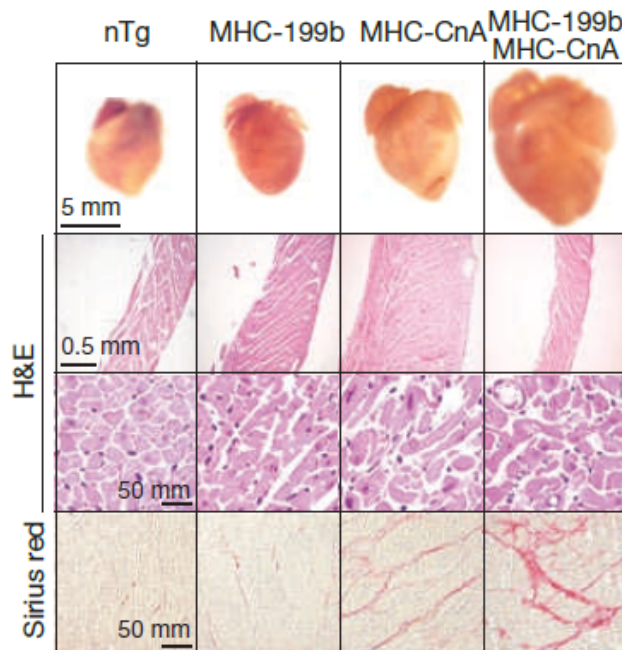
microRNA-199b and calcineurin/NFAT signalling in mouse model of heart failure (MHC-CnA)



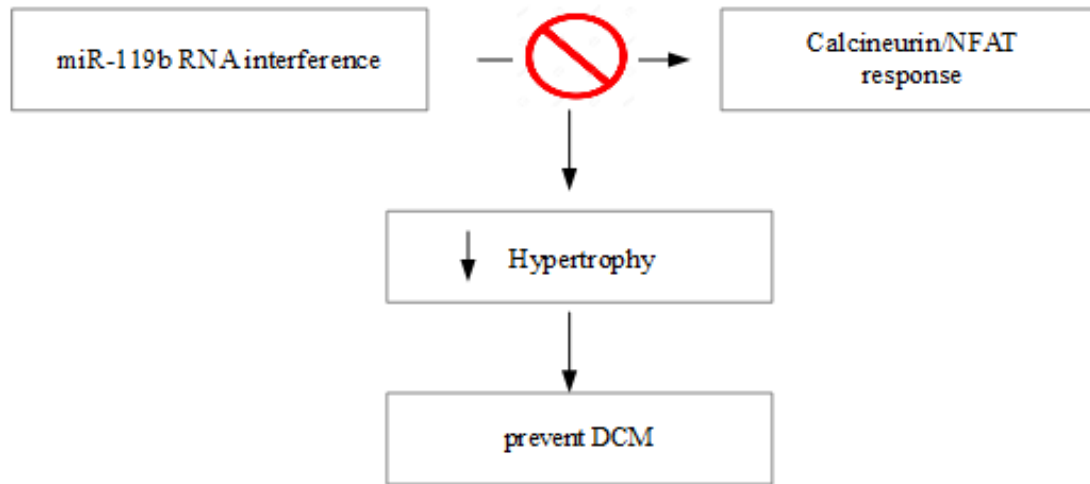
miR-199b is overexpressed in HF
da Costa Martins P. A. et al., 2010



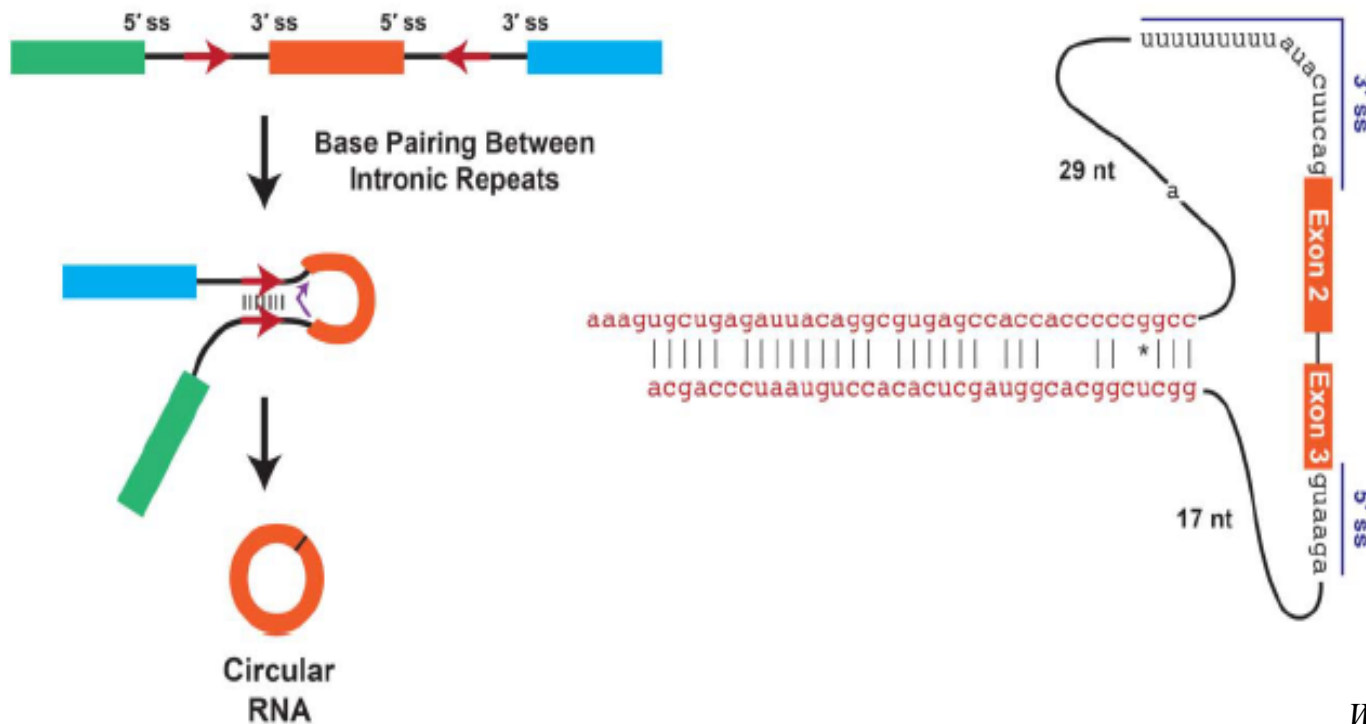
antamiR and RNA interference



AIM AND STRATEGY: miR-199b RNAi as a treatment for DCM



BUT HOW TO INCREASE THE STABILITY OF antamiR?



Mouse model: $Lmna^{H222P/H222P}$



Skeletal dystrophy: rigid posture

Dilatation left ventricle

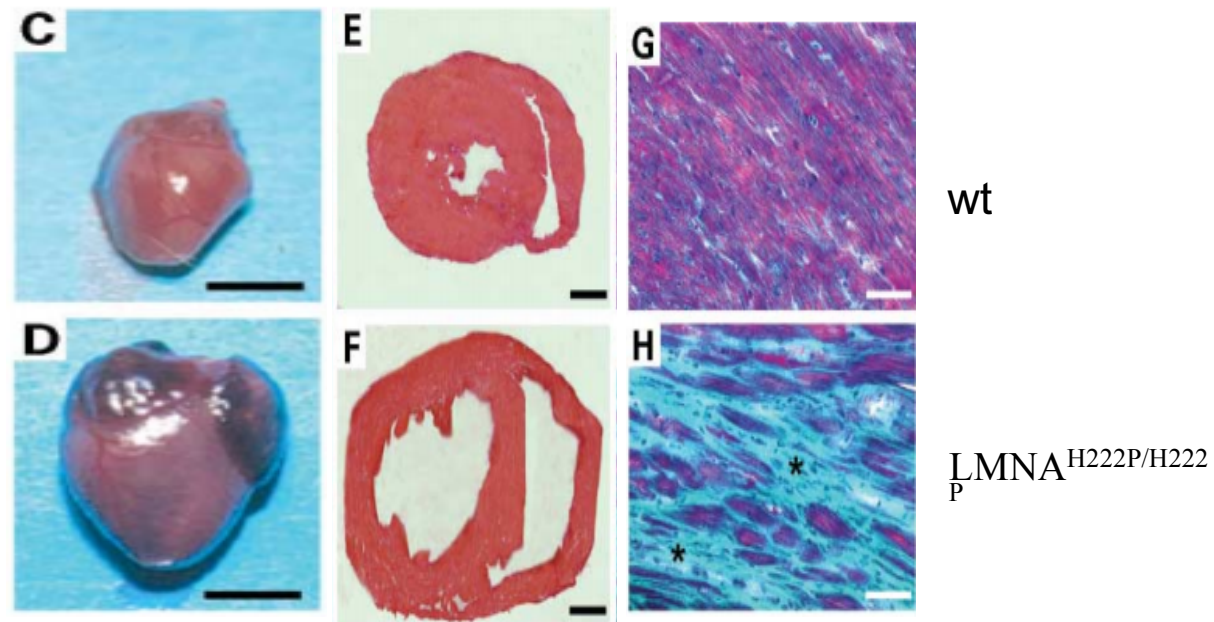
Hypokinesia

Abnormal conduction

Hypertrophy

Extensive degeneration of myocytes and massive fibrosis

(Arimur T. et al., 2007)

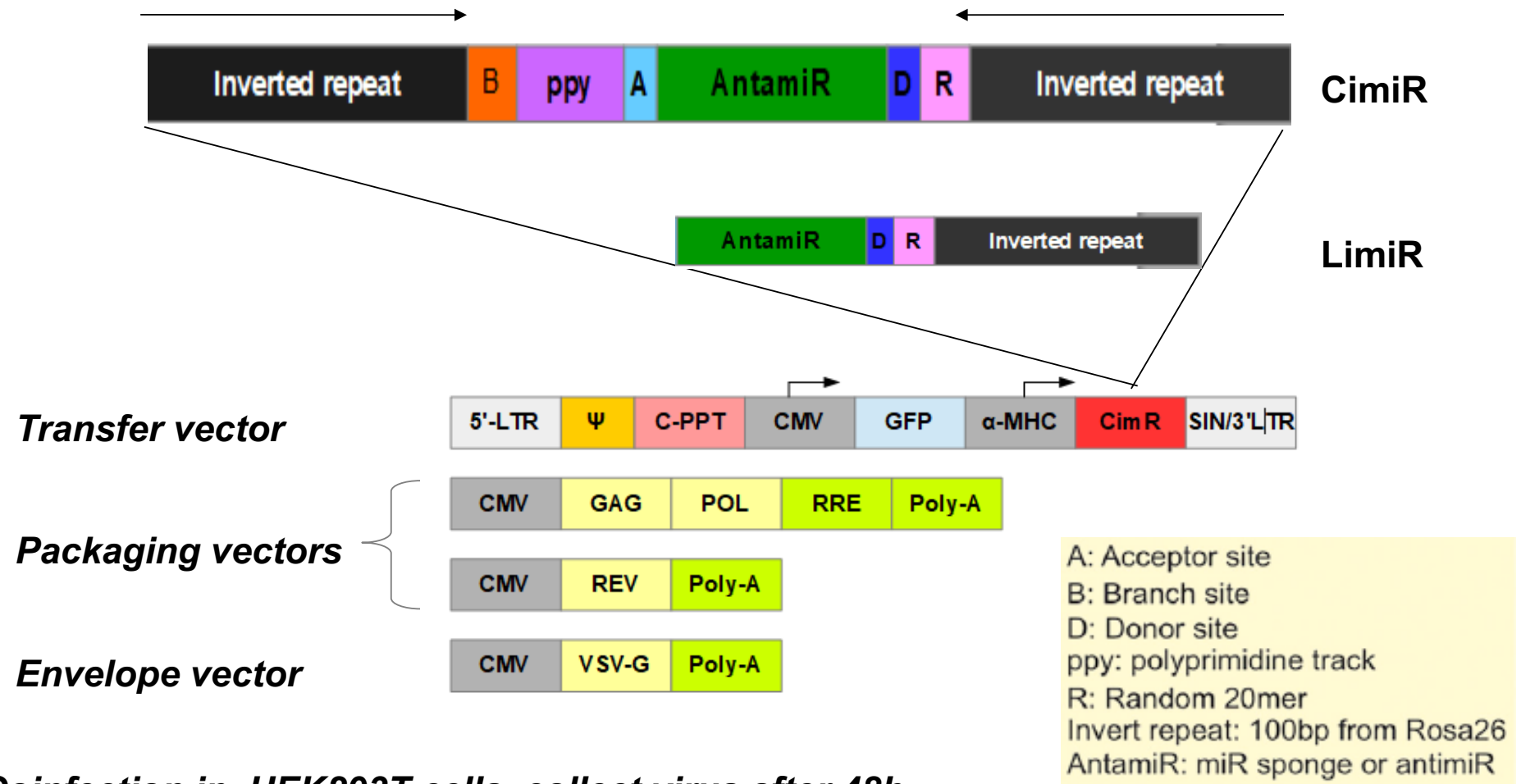


histopathological analysis

AntamiR vector construction

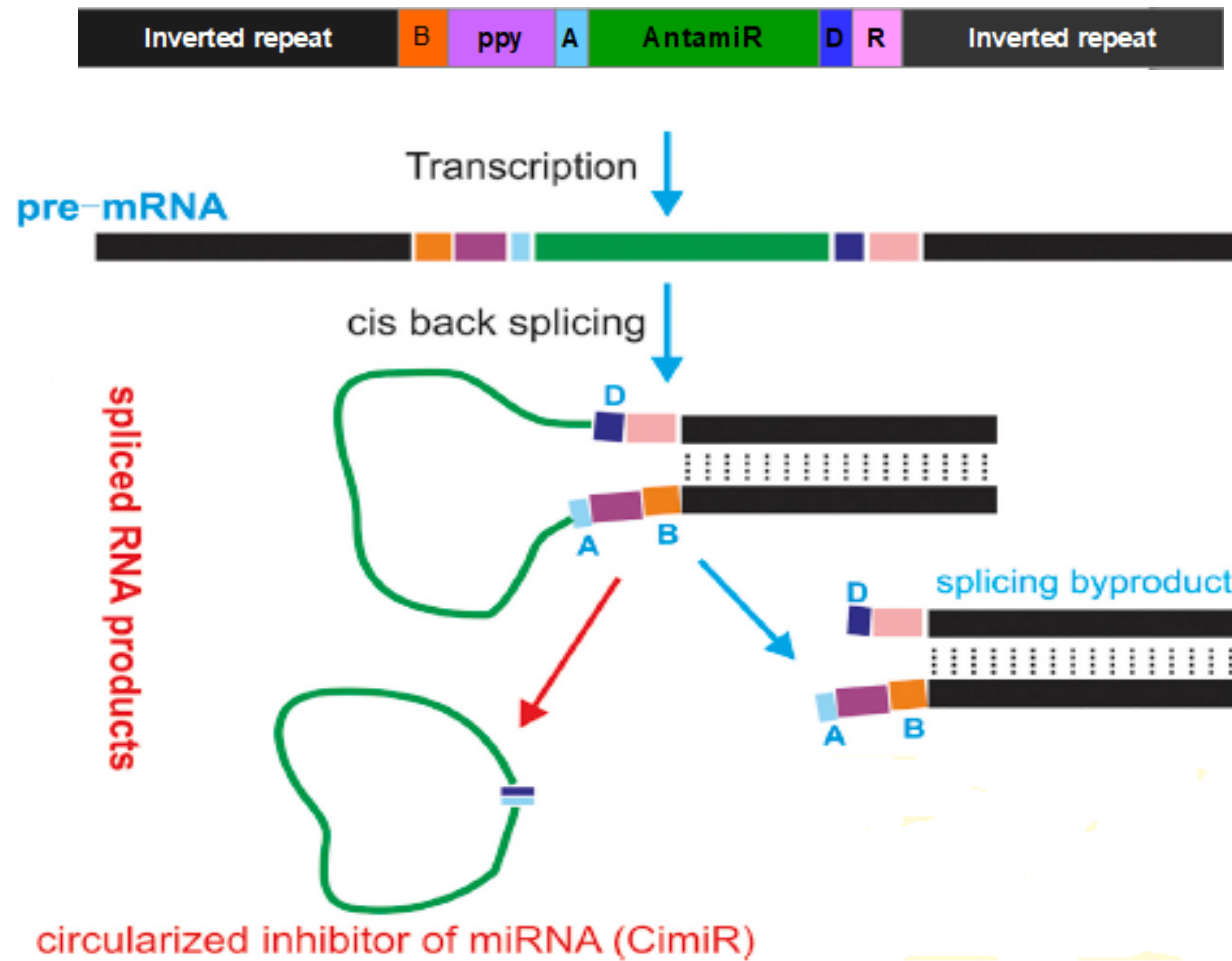
Third-generation SIN-HIV1 lentivirus:

- Capacity 8-10 kb
- Low immunogenicity
- Efficient integration into cells not in active proliferation



Coinfection in HEK293T cells, collect virus after 48h

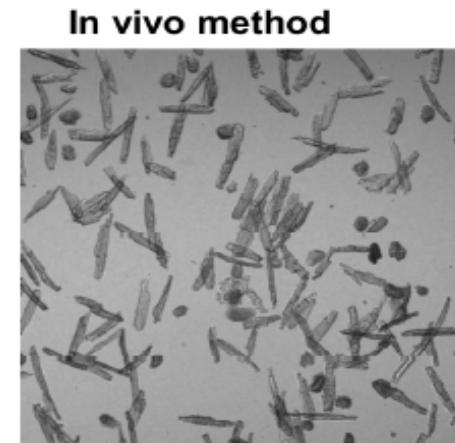
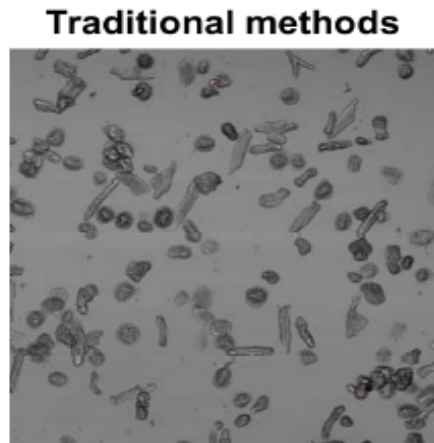
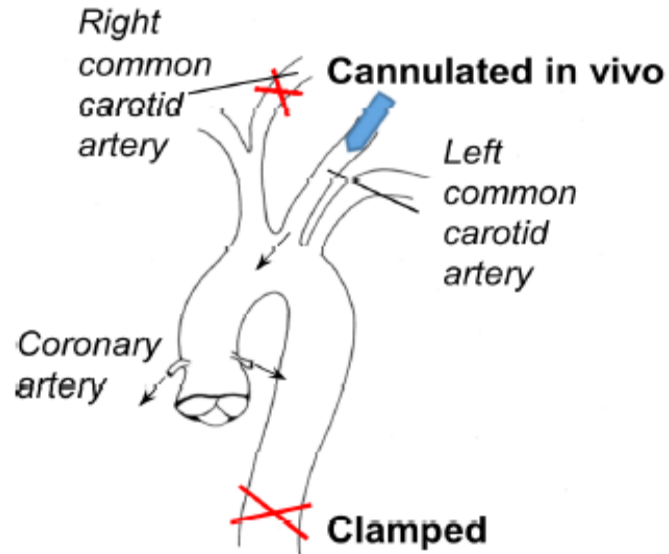
Back splicing mechanism



(Shu Y et al., 2018)

In vivo cannulation

Obtained high yield and high quality cardiomyocytes from both wild-type and heart disease models.



Jian Z. et al. 2016

- Arrest heart by injecting KCl solution via vena cava
- Infusion of enzyme digestion solution: (300 u/ml Collagenase type II, 0.04 mg/ml protease type XIV, 12.5 $\mu\text{mol/L}$ Ca)
- Dissect out the ventricular tissue
- Store cells in incubation solution with 1 mM Ca^{2+} , at 18–22°C

In vitro experiments

Is the vector integrated?

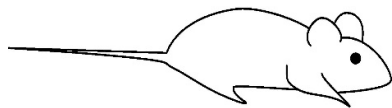
- GFP analysis
- Cellular count

Is the CimiR more stable than the linear form?

- Atc D treatment of transformed cells
- RT-PCR

Is miR-199b degraded?

- Cardiomyocyte transduction with Gluc-miR-199b

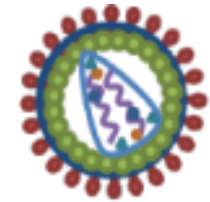


WT and
LMNA^{H222P/H222P}

Cardiomyocytes
isolation



Transduction



Are hypertrophic genes expressed?

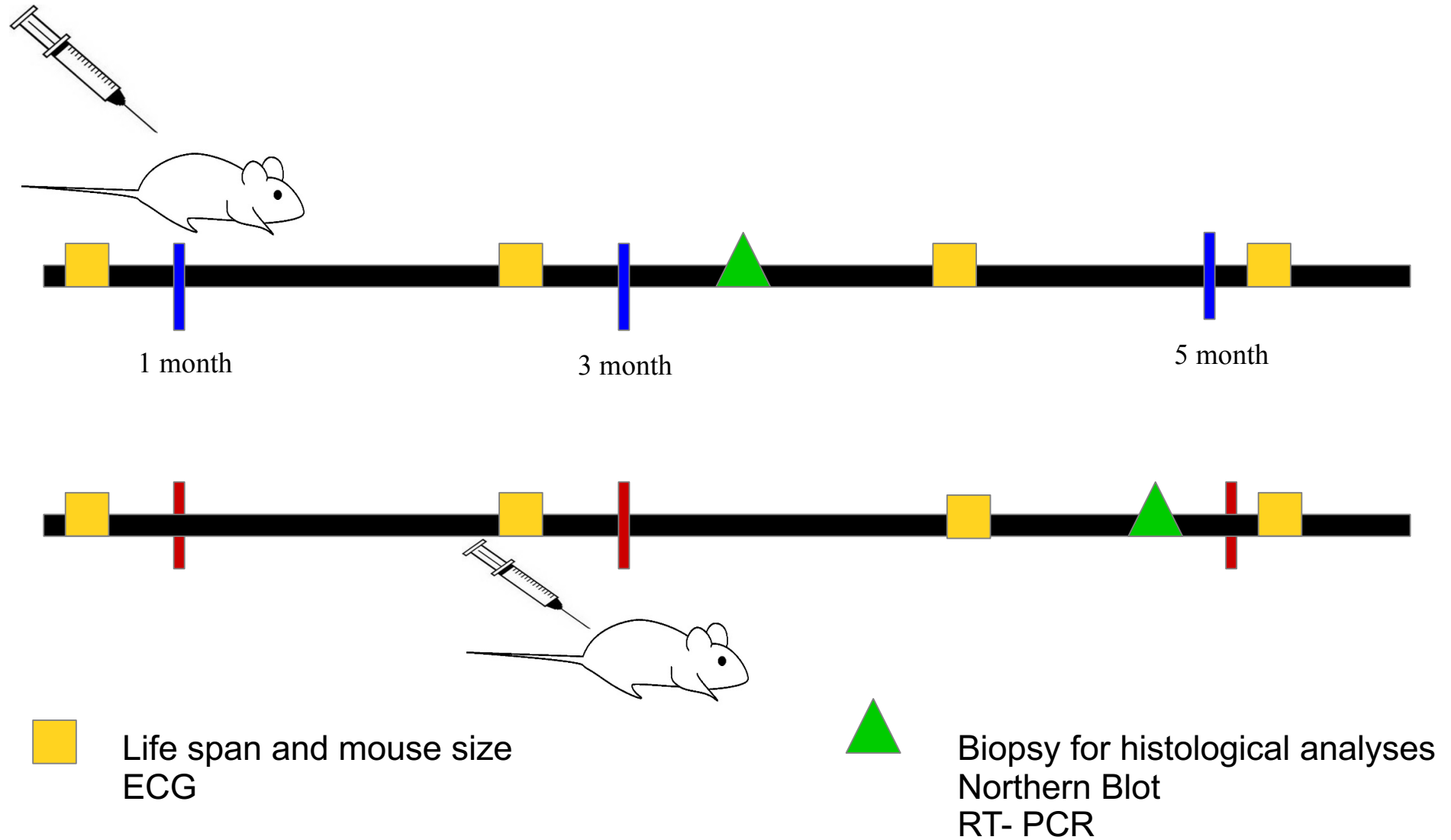
- RT-PCR
- Northern blot

Do I have an improvement in the responsiveness of cardiomyocytes?

- Mechanical stress test

In vivo experiment

On WT, LMNA^{H222P/H222P} non treated, LMNA^{H222P/H222P} treated with LimiR, CimiR, empty vector



Time and costs

Vector production In vitro experiments	In vivo experiments	Data analyses Publication results
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3 years

- 4 wt mouse (2 female, 2 male): \$87,46
- 12 LMNA^{H222P/H222P} (5 female, 5 male): \$1900,00
- QIAGEN Multiplex PCR Kit (100): \$284,00
- Northern blot Kit Thermofisher: \$247,00
- 2xViraPower™ Lentiviral Gateway™ Expression Kit: \$3150,00
- Luciferase Reporter Gene Assay Kit: \$285,00
- Animal facilities: \$30000,00
- Cell culture: \$2000,00
- Molecular biology equiped laboratory: \$8000,00
- Salary of researchers: \$50000,00

Total= \$90000,00

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