

#### The Education, Scholarships, Apprenticeships and Youth Entrepreneurship

"Targeting transcriptional addictions in cancer" - "Generation of TP53 knockouts using CRISPR/Cas9 technology".

5-26.09.2022

Strategic interuniversity cooperation to improve research abilities for Ph.D. Students For Higher Educational Quality- QUALITAS- SEE-21-COP-0049

This book was realised with the EEA Financial Mechanism 2014-2021 financial support. Its content (text, photos) does not reflect the official opinion of the Programme Operator, the National Contact Point and the Financial Mechanism Office. Responsibility for the information and views expressed therein lies entirely with the authors.

# Crispr-Cas9 experiment

## The protocol

#### Step 1

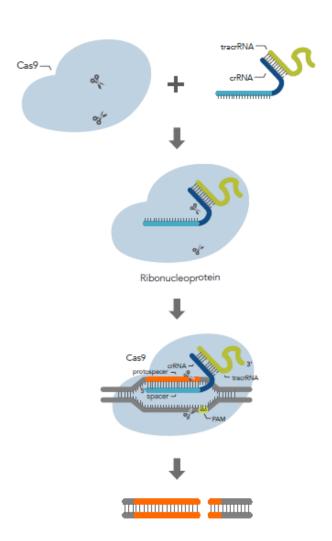
gRNA and enzyme combine to form RNP

#### Step 2

gRNA directs RNP to genome target

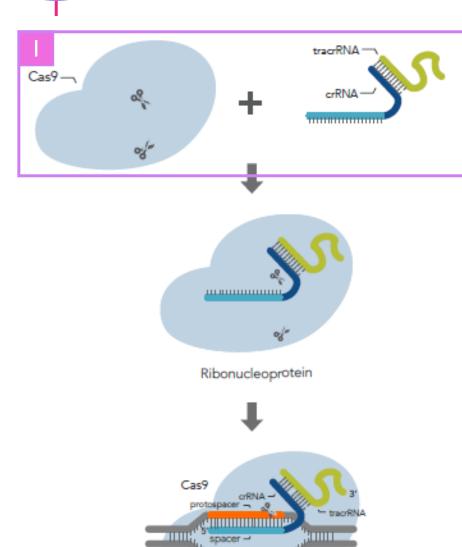
#### Step 3

Enzyme cleaves target

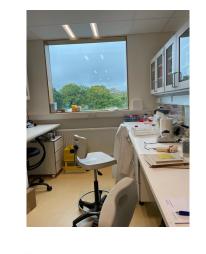


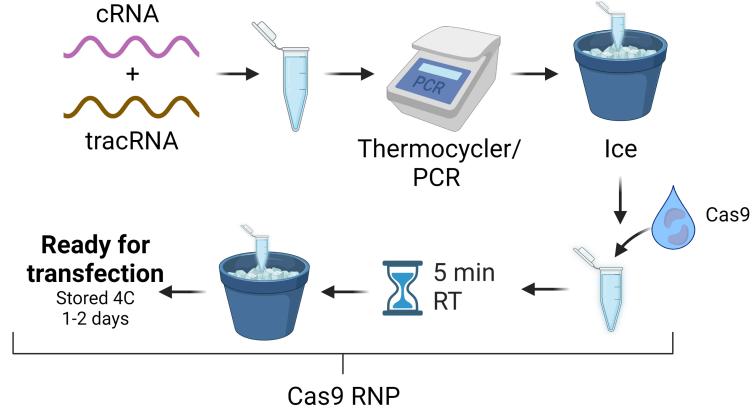


# Crispr-Cas9 experiment



## The experiment:





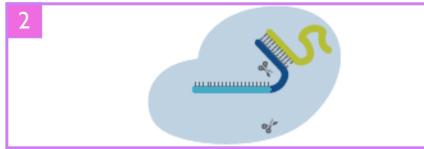


# Crispr-Cas9 experiment





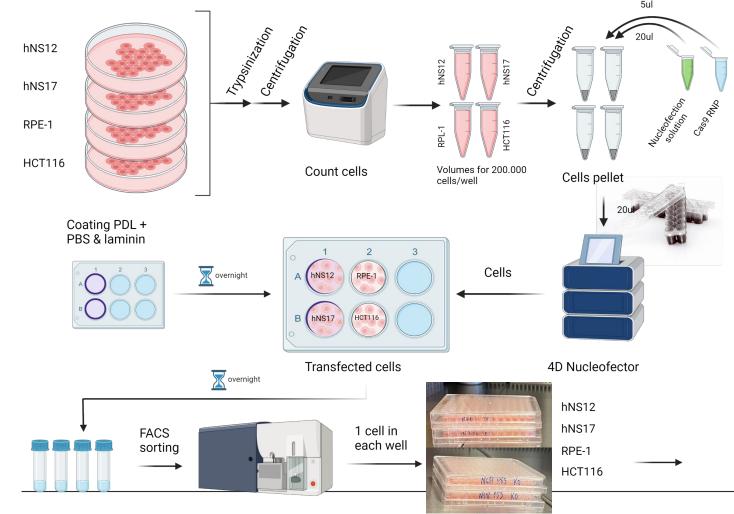




Cas9
protospacer — 29
tracrRNA

Ribonucleoprotein

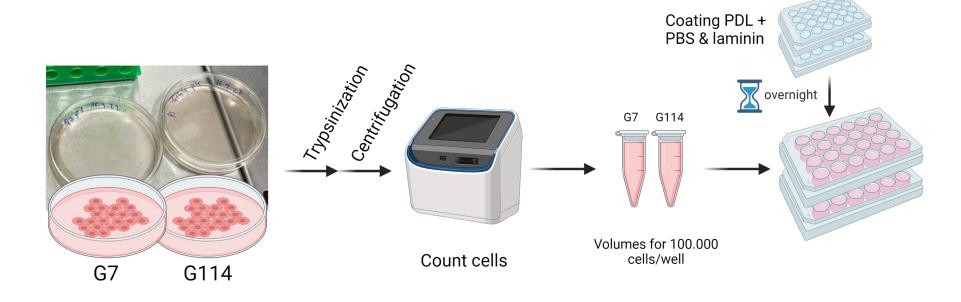
#### The experiment - Nucleofection



- I.G7 and GII4 was plated.
- 2. The cells was treated with a drug.
- 3. RNA extraction from the cells.
- 4. Nanodrop and TapeStation.
- 5. cDNA.

## I. G7 and G114 was plated.



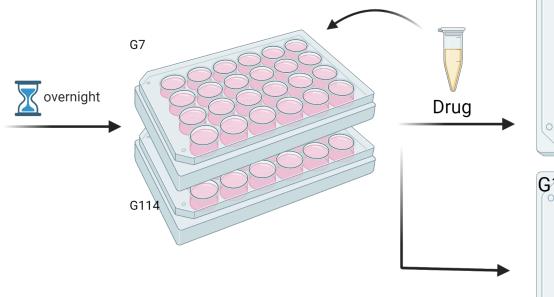




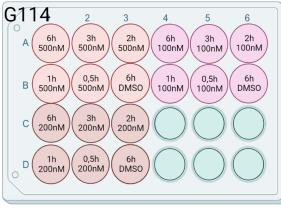
I. G7 and G114 was plated.

#### 2. The cells was treated with a drug.

- RNA extraction from the cells.
- 4. Nanodrop and TapeStation.
- 5. cDNA.

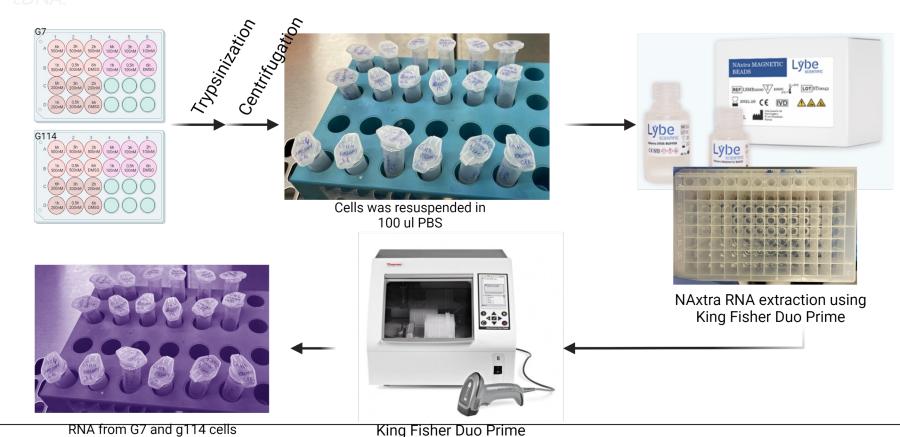


G7  A SoonM
B 0,5h 0,5h 0MSO 1h 100nM 0,5h 6h 0MSO 6h 100nM 0MSO
C 200nM 200nM 200nM 200nM
D 1h 200nM 200nM 6h DMSO



- I. G7 and G114 was plated.
- 2. The cells was treated with a drug.
- 3. RNA extraction from the cells.
- 4. Nanodrop and TapeStation.
- 5. cDNA.

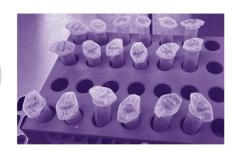




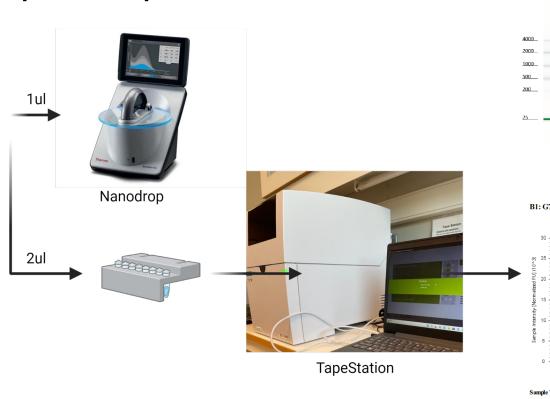
- I. G7 and G114 was plated
- 2. The cells was treated with a drug
- 3. RNA extraction from the cells.

## 4. Nanodrop and TapeStation.

5. cDNA.



RNA from G7 and g114 cells

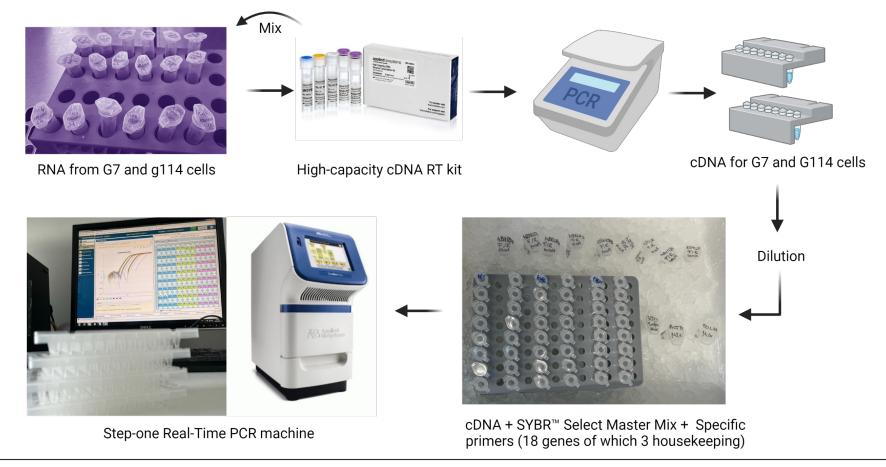


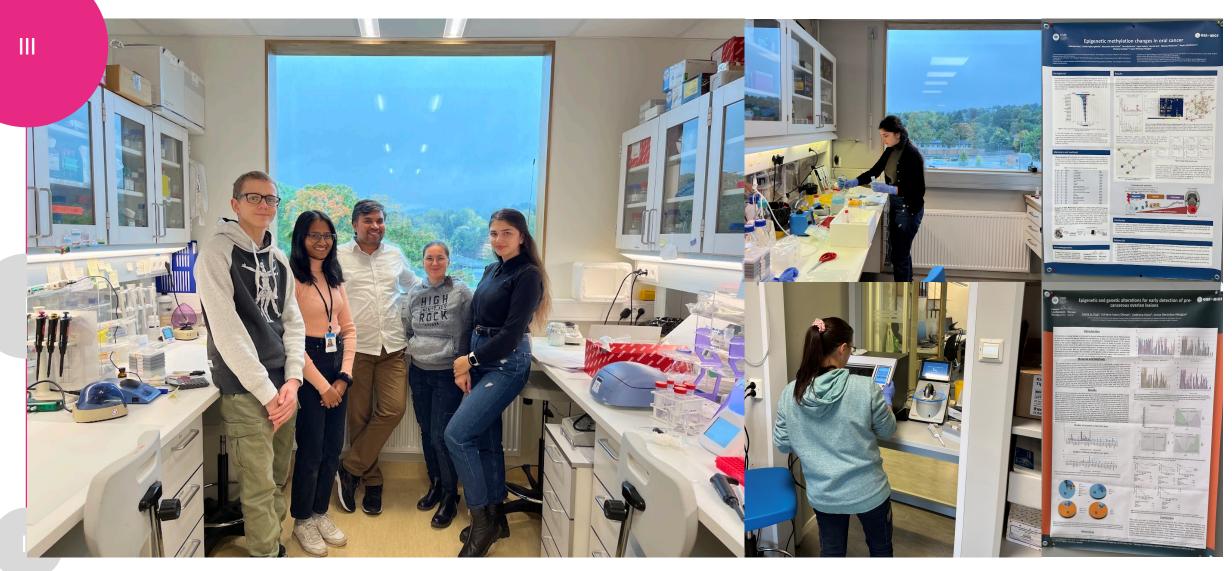
30 ¬	Compet		,85 185		
25					
20					
15					
10				\	
5			AJ-		

	Well	RINe	28S/18S (Area)	Conc. [pg/µl]	Sample Description	Alert	Observations
		0.6		20100	CT 500 N C		Sample concentration
	B1	8.5	3.4	20100	G7 500nM 6h	<u> </u>	outside recommended
							range

- I. G7 and G114 was plated.
- The cells was treated with a drug.
- 3. RNA extraction from the cells.
- 4. Nanodrop and TapeStation.

#### 5. cDNA.





Strategic interuniversity cooperation to improve research abilities for Ph.D. Students For Higher Educational Quality- QUALITAS- SEE-21-COP-0049

This book was realised with the EEA Financial Mechanism 2014-2021 financial support. Its content (text, photos) does not reflect the official opinion of the Programme Operator, the National Contact Point and the Financial Mechanism Office. Responsibility for the information and views expressed therein lies entirely with the authors.