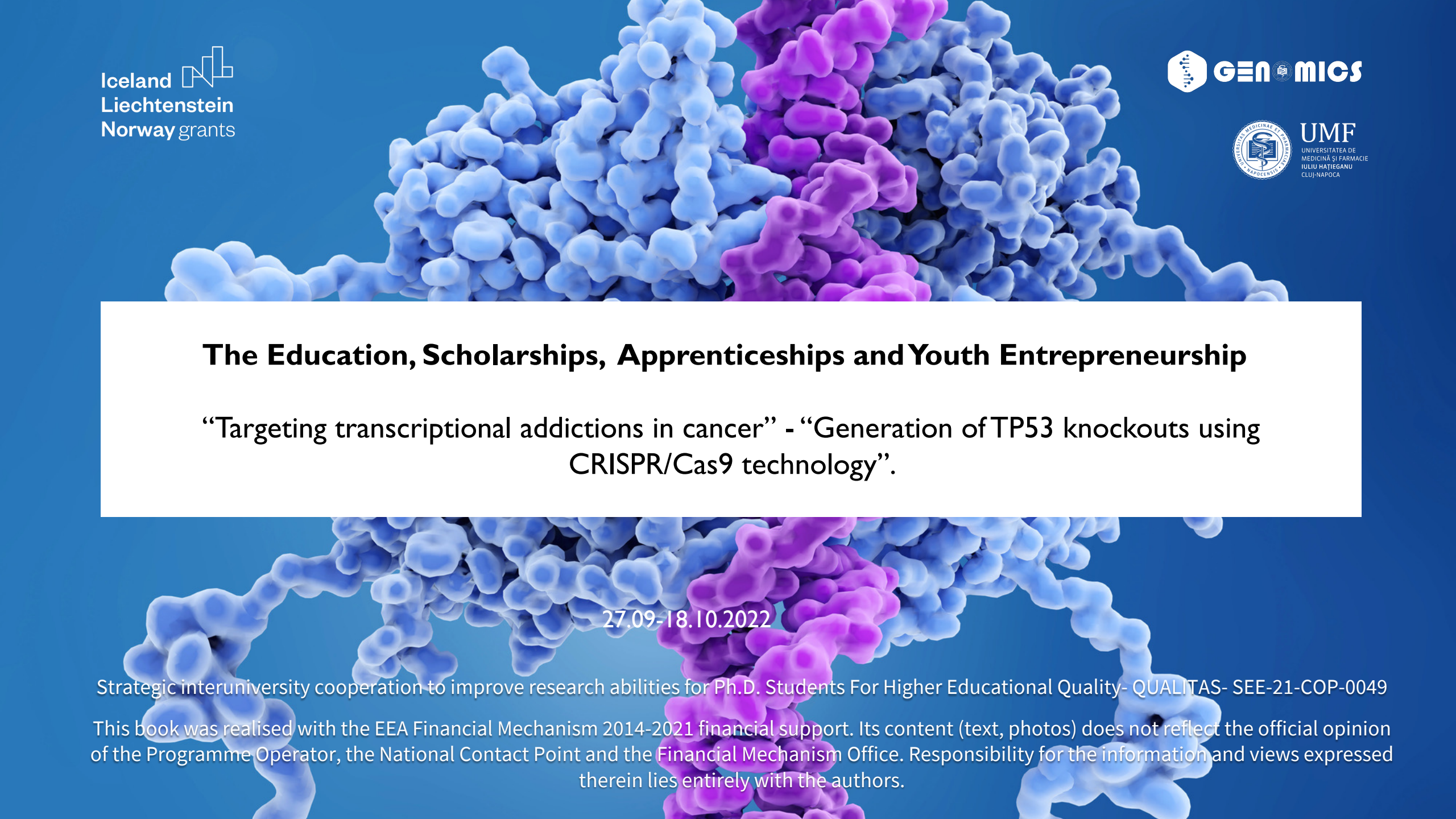

Iceland Liechtenstein Norway grants

GENOMICS

UMF UNIVERSITATEA DE MEDICINĂ ȘI FARMACIE IULIU HAȚIEGANU CLUJ-NAPOCA

The Education, Scholarships, Apprenticeships and Youth Entrepreneurship

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

27.09-18.10.2022

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

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STMS2 – Dr. Sergiu Chira and Dr. Radu Pirlog

Aim of the mobility:

1. Learn gene editing protocol using CRISPR-Cas9 approach and nucleofection
2. Engage in intercultural communication and exchanges in good practices regarding research, teaching and scientific results dissemination.

Outcomes:

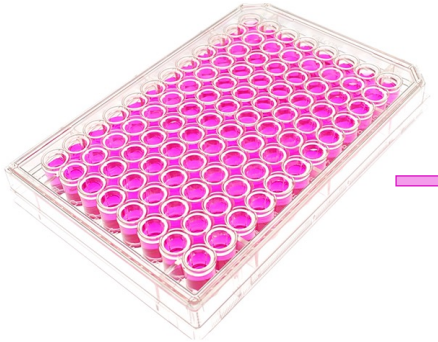
1. Performed gene editing protocol on 2 cell lines using Crispr-Cas9 and nucleofection.
2. Selected clones and expanded the subpopulations
3. Optimized Western Blot protocols for p53
4. Characterized selected clones using Western Blot

Outcomes – 1 Gene editing

- 2 cell lines – HCT116 – colorectal cancer / RPE-1 – normal retinal pigment epithelial cell line
- Cultured 600 000 cells/cell line
- Performed CRISPR-Cas9 TP53 KO genome editing by nucleofection with RNP Cas9(GFP)+crRNA/tracrRNA
- 2nd day – FACS single cell sorting for GFP positive cells – 4x96-well plates.
- Incubated plates at 37^{o}C for colony formation – 2 weeks.
- Screen plates for single colonies per well,

Outcome 2 - Selected clones and expanded the subpopulations

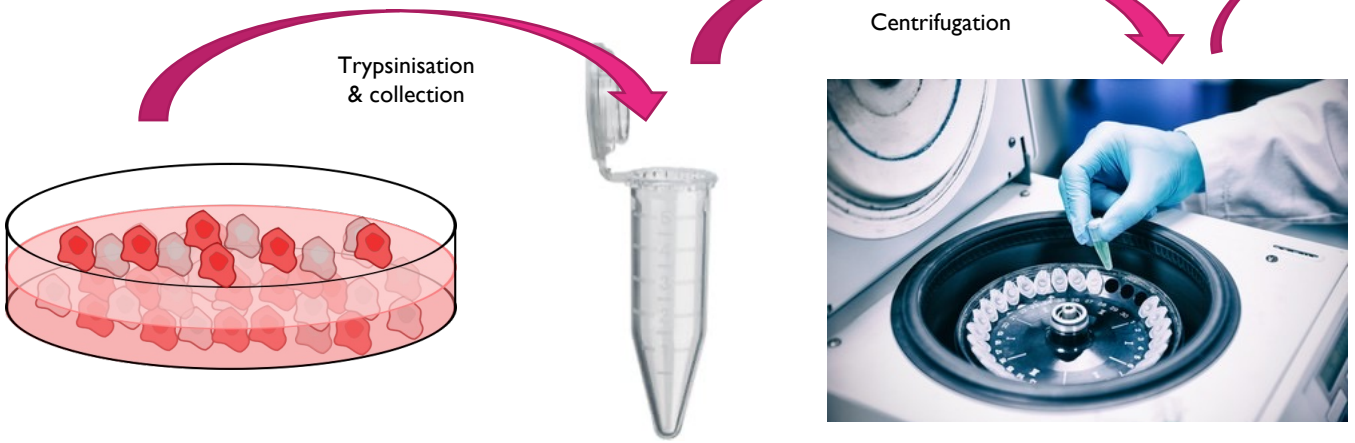
- Continued the experiment started by Laura and Andreea
- Selected 12 clones for each of the HCT116 and RPE-1 cell lines
- Expanded clones in 12 well plates – 3-5 days.

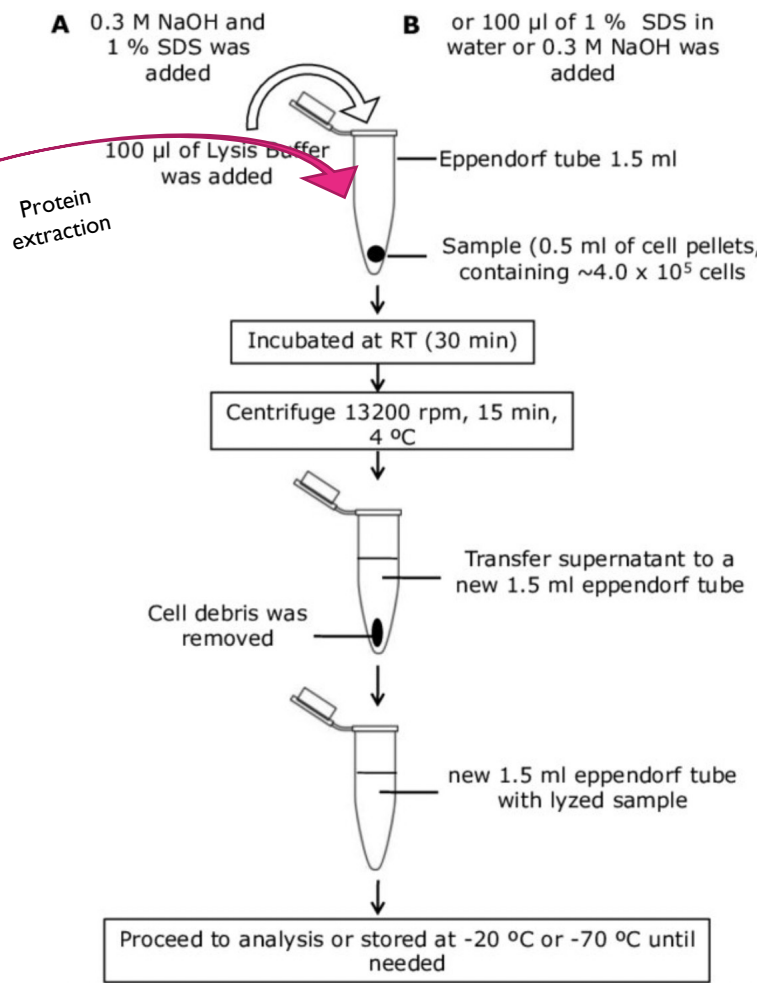


- Further expansion in 6 well plate and petri dishes.
- Long sterm storage of 2 cryotubes/cell line

Outcome 2 - Selected clones and expanded the subpopulations

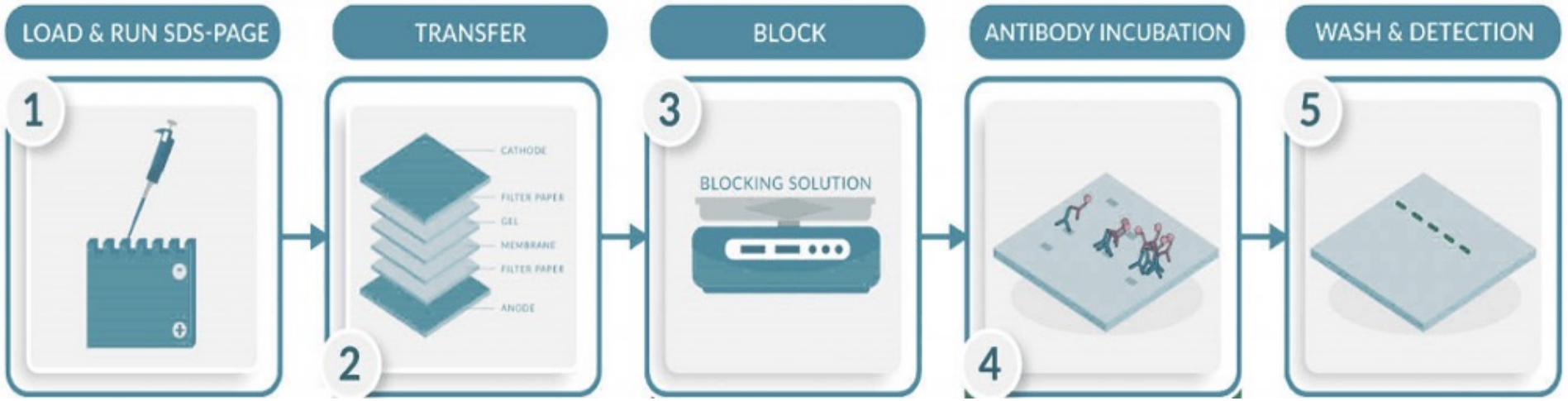
- Protein extraction

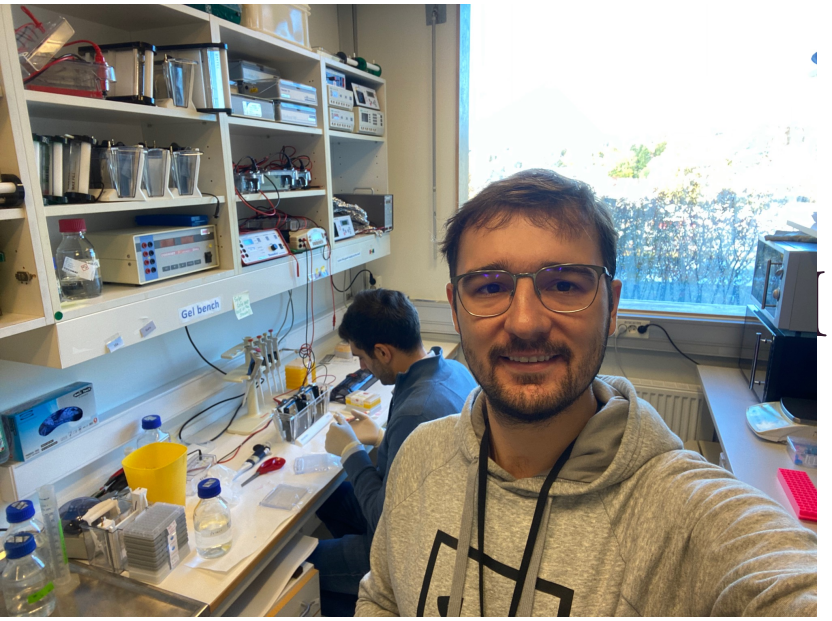




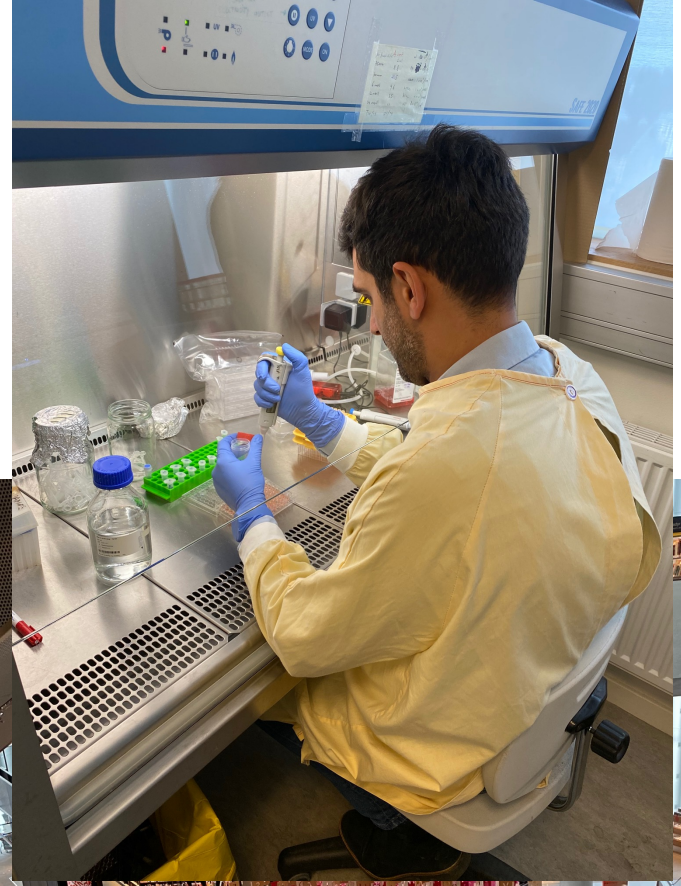
Outcome 3-4 – WB for p53 protein

- Aim of the study: Confirm the *TP53* KO using WB for p53 protein.




Gel bench


RISIKOAVFALL

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