

In vitro systems to study glycogen storage disease type 1 (GSD1)



PoLiMeR

Polymers in the Liver - Metabolism and Regulation

PhD Student: Maria Rodríguez Peiris

Supervisor: Prof. Dr. Kathrin Thedieck

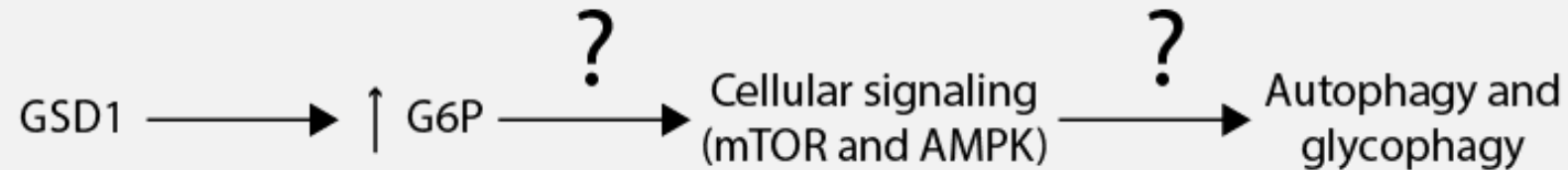
Daily supervisors: Dr. Alexander Heberle and Dr. José Miguel Ramos Pittol

Co-supervisor: Prof. Dr. Mathias Ziegler

My main aim in the PoLiMeR consortium



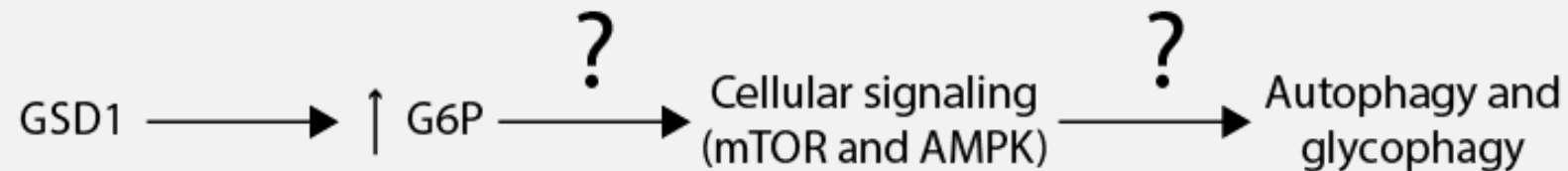
- Study mTOR and AMPK signaling and the downstream processes autophagy and glycolysis in glycogen storage disease type 1 (GSD1)



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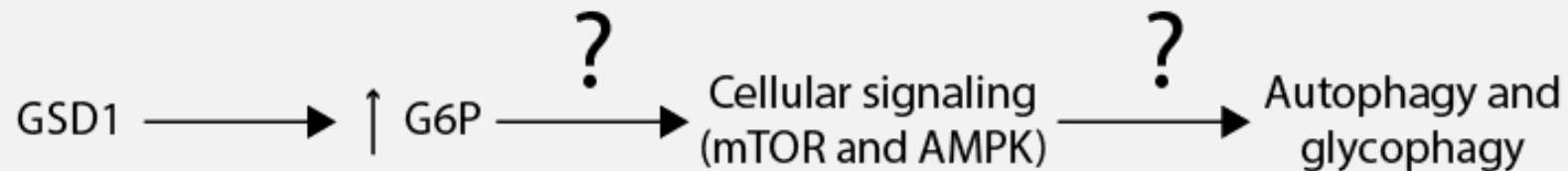


We cannot study the disease in patients → need for GSD1 models

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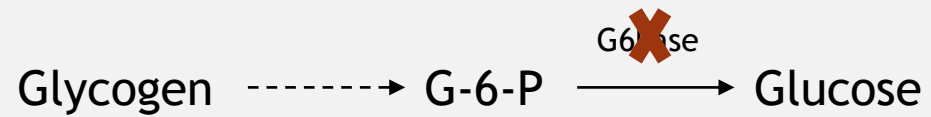
In vivo systems

In vitro systems

Glycogen storage disease type 1 (GSD1)



GSD1



Clinical manifestations

Hypoglycaemia

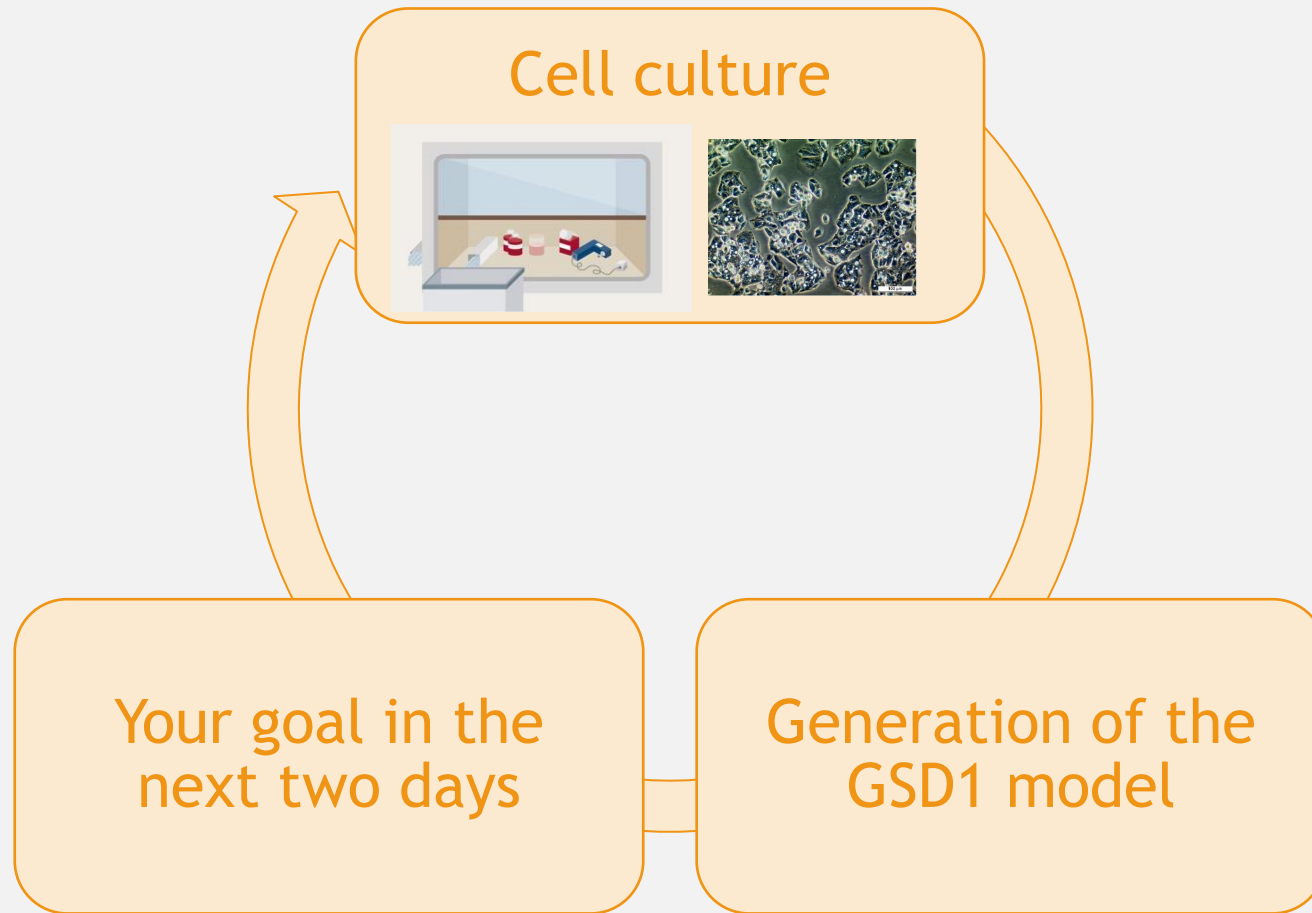
↑ hepatic glycogen

↑ hepatic lipids

Adenomas and carcinomas



Generation of the GSD1 *in vitro* system



Generation of the GSD1 *in vitro* system



Cell culture: growth of cells from an organism in an artificial environment.

- **Primary cell culture:** directly obtained from tissues or organs
- **Cell line:** obtained after subculturing the primary culture

Cell
culture

Your
goal in
the
next
two
days



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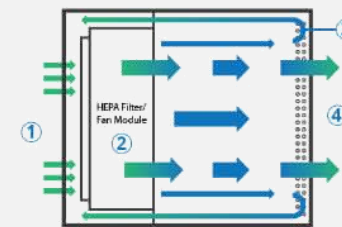
Your goal in the next two days



Laminar Flow hood / cabinet

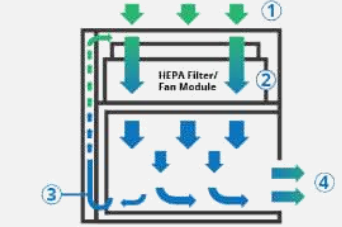


Horizontal Laminar Flow Hood Diagram (Cutaway Side View)



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Vertical Laminar Flow Hood Diagram (Cutaway Side View)



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Generation of the GSD1 *in vitro* system

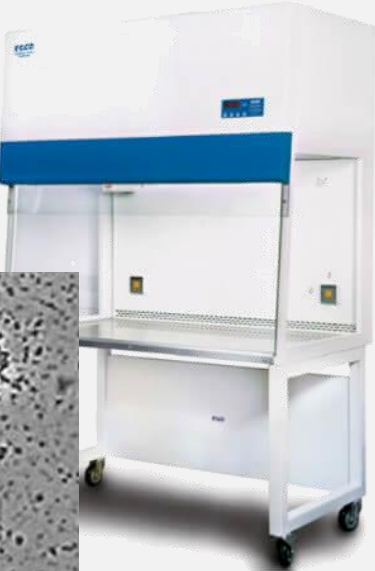
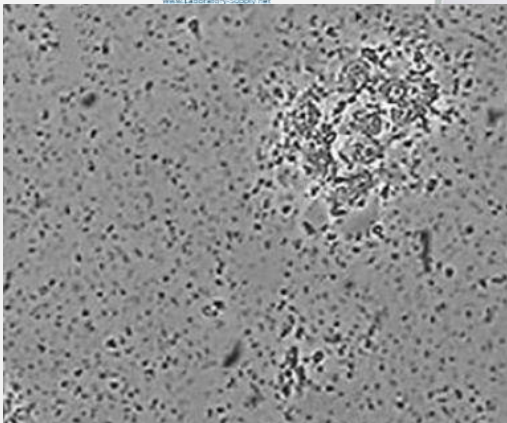
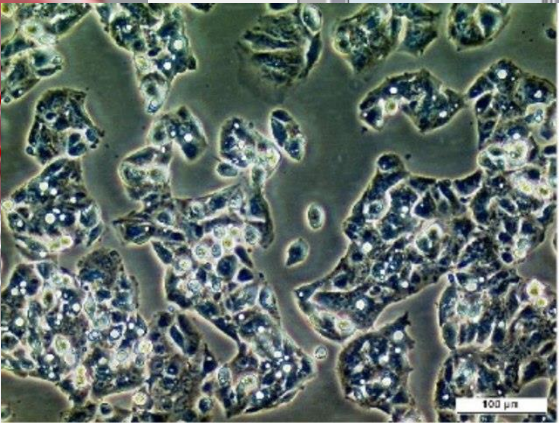
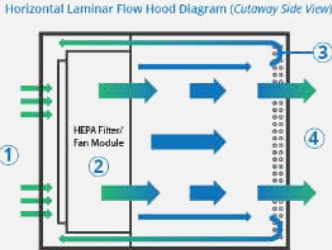


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Laminar Flow hood / cabinet



Cell culture

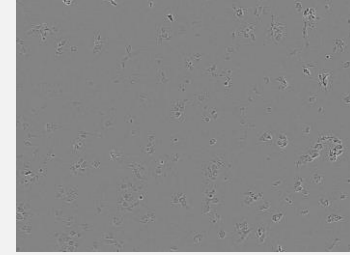
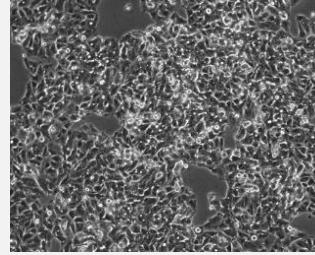
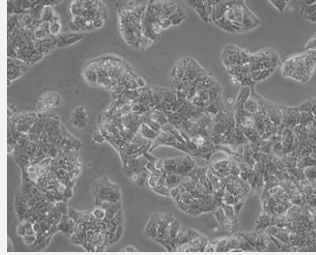
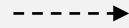
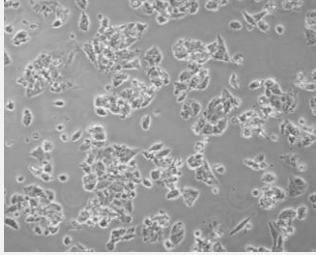
Your goal in the next two days



Generation of the GSD1 *in vitro* system



Cell culture



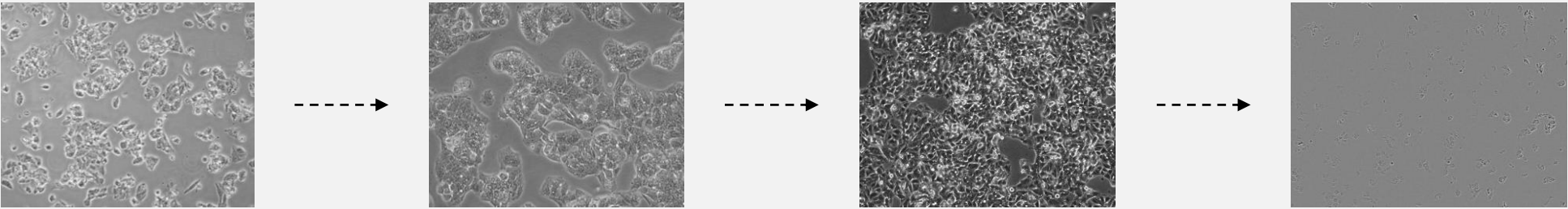
Your goal in the next two days



Generation of the GSD1 *in vitro* system

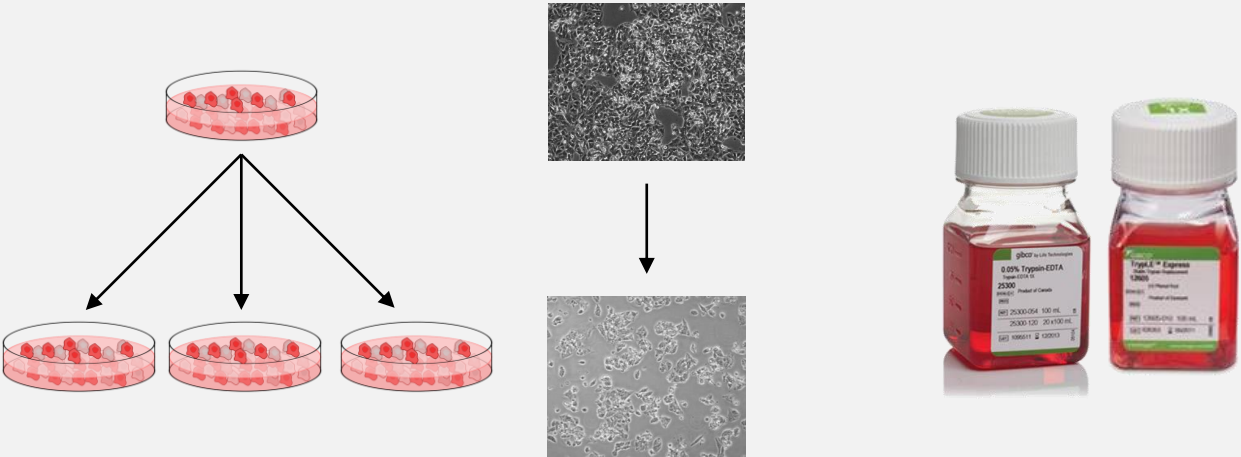


Cell culture



Subculture, cell passaging

Your goal in the next two days



ATCC; Ibdij, 2021; Gibco



Generation of the GSD1 *in vitro* system



Which cell line can we use to establish a GSD1 *in vitro* system?

- HepG2: human hepatoma

Cell
culture

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Generation of the GSD1 *in vitro* system

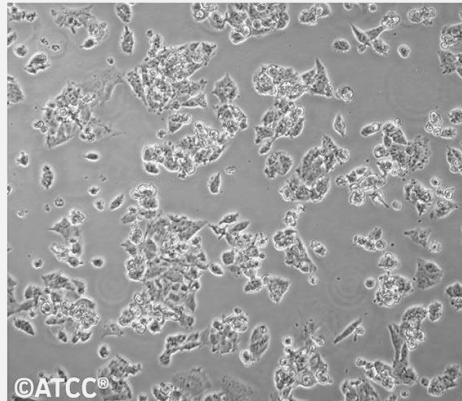


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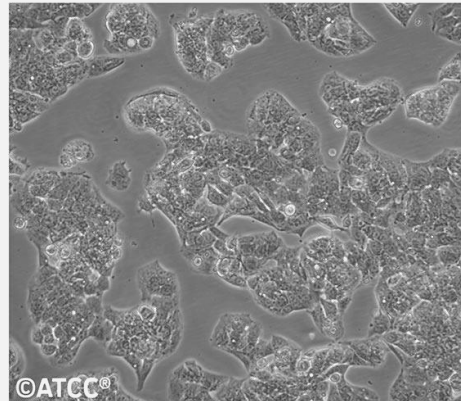
- **HepG2:** human hepatoma

Cell
culture

ATCC Number: **HB-8065**
Designation: **Hep G2**



©ATCC®
Low Density



©ATCC®
High Density



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Cell
culture

Assess the expression of G6PC in HepG2 cells by qPCR (mRNA) and western blot (protein levels)



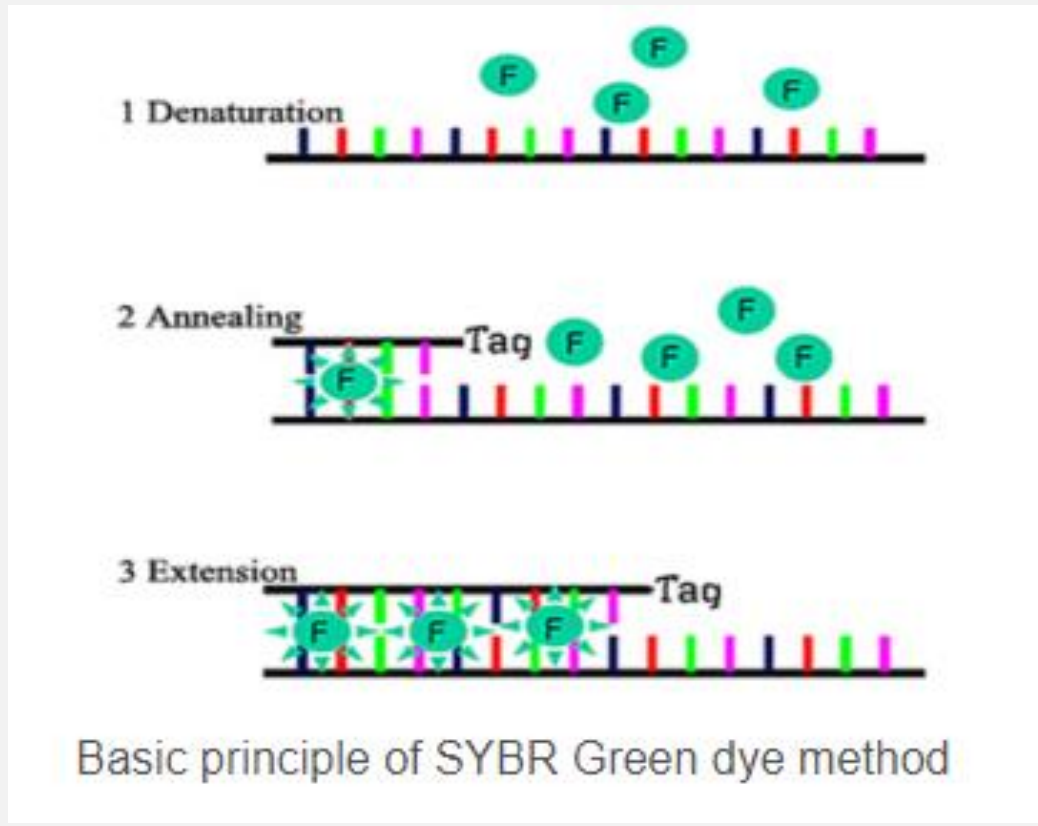
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Quantitative polymerase chain reaction (qPCR) using SYBR Green



Cell culture

Your goal in the next two days

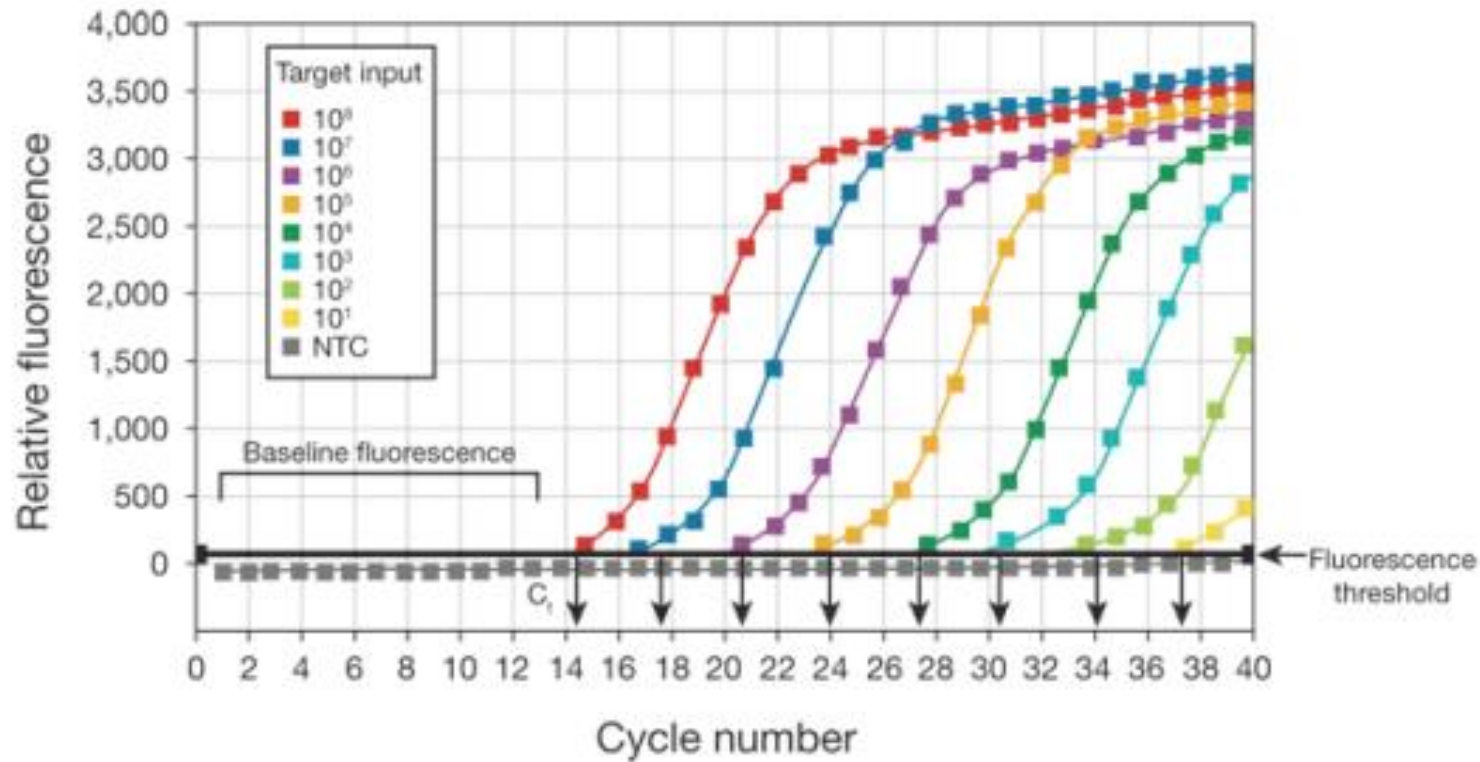


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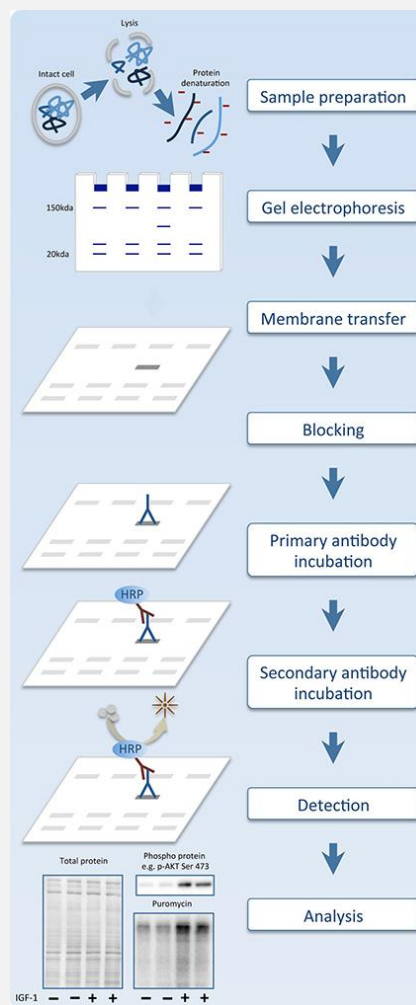


Western blot



Cell culture

Your goal in the next two days





Cell
culture

Protocols



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