PhD Open Days

A dual-fluorescent reporter platform to unravel X chromosome inactivation reversal and maintenance in naïve pluripotency

PhD Program in Bioengineering and Biosciences

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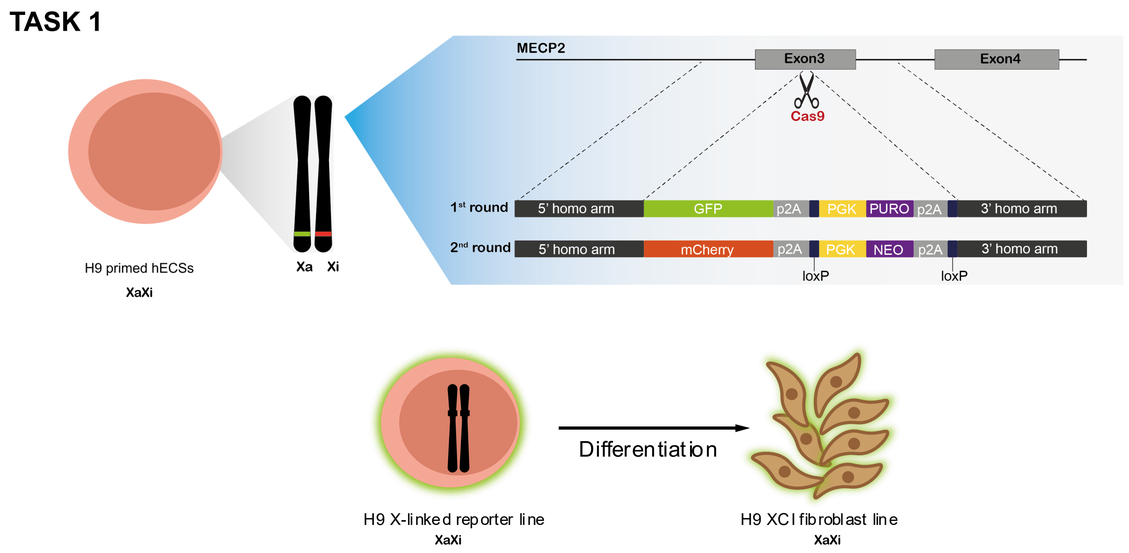
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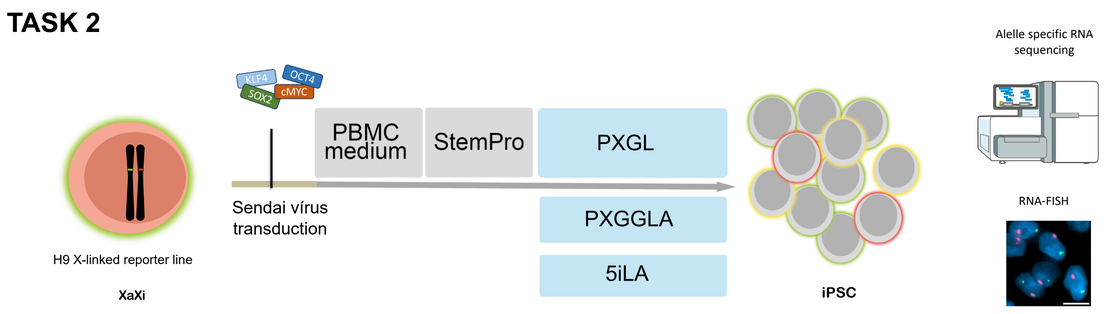
Reprogramming somatic cells into induced pluripotent stem cells (iPSCs) holds vast potential for developmental biology and regenerative medicine. However, conventional reprogramming protocols generate primed iPSCs (resembling the post-implantation epiblast) that retain somatic epigenetic memory. In female XX cells, where one X chromosome is transcriptionally silenced via X-Chromosome Inactivation (XCI) to ensure dosage compensation, primed iPSCs maintain an active and an inactive X chromosome (XaXi). Over time in culture, these cells frequently suffer from XCI erosion (XaXe), leading to heterogeneous gene reactivation that persists through differentiation. Conversely, naïve iPSCs capture the pre-implantation ground-state of pluripotency, offering reduced epigenetic memory and XCI reversal into two active X chromosomes (XaXa). Despite these advantages, current naïve reprogramming protocols face major bottlenecks: morphological heterogeneity, karyotypic abnormalities, unstable XaXa maintenance, and an inability to restore natural random XCI upon differentiation.
Here, we aim to develop a dual-fluorescent reporter platform to track XCI dynamics in real time and optimize naïve culture conditions to generate reliable, stable XaXa human iPSCs.

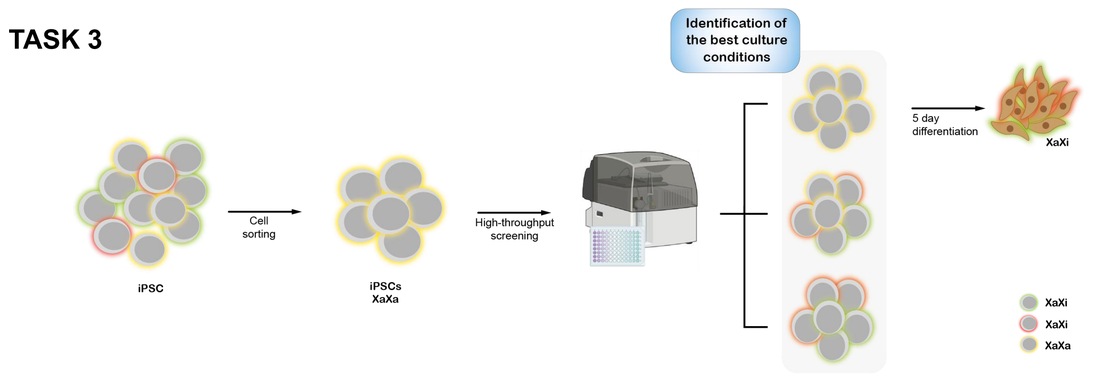
Experimental Strategy

To track XCI dynamics in real time, we design a CRISPR/Cas9 strategy to tag both alleles of the X-linked MECP2 gene (Exon 3) with distinct fluorescent reporters (GFP on Xa and mCherry on Xi) in female H9 human embryonic stem cells (hESCs) (Task 1).
Edited cells differentiated into XaXi fibroblasts undergo Sendai virus (SeV) reprogramming under different naïve media (e.g., PXGL, t2iLGo+Y, 5iLA) to identify the condition yielding the highest proportion of double-positive (XaXa) iPSCs, validated by allele-specific RNA-seq and RNA-FISH (Task 2).
Sorted XaXa iPSCs are then used in an automated high-throughput screening (HTS) platform to refine small molecule stoichiometry, followed by a 5-day differentiation assay to confirm random XCI restoration (Task 3).

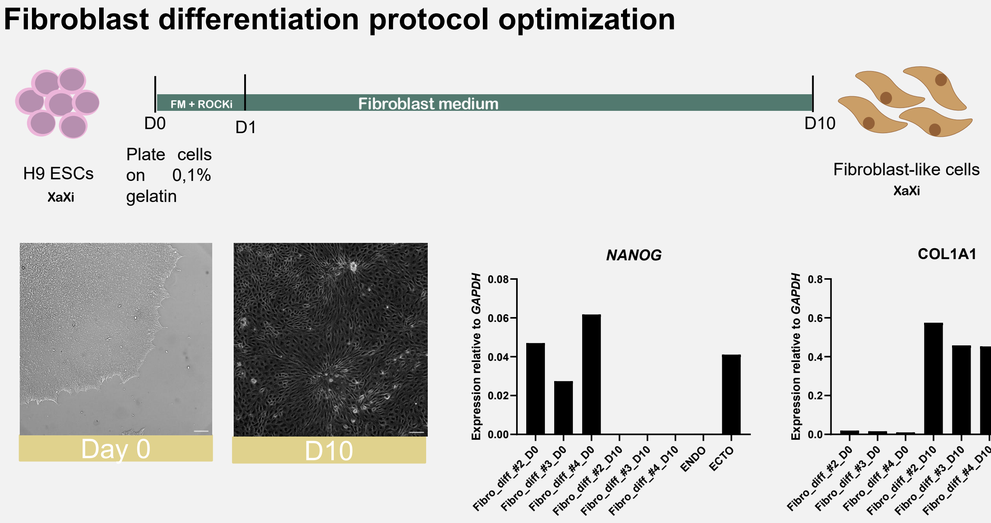
Finally, the optimized protocol is validated in primary female PBMCs (Task 4).







Preliminary Results



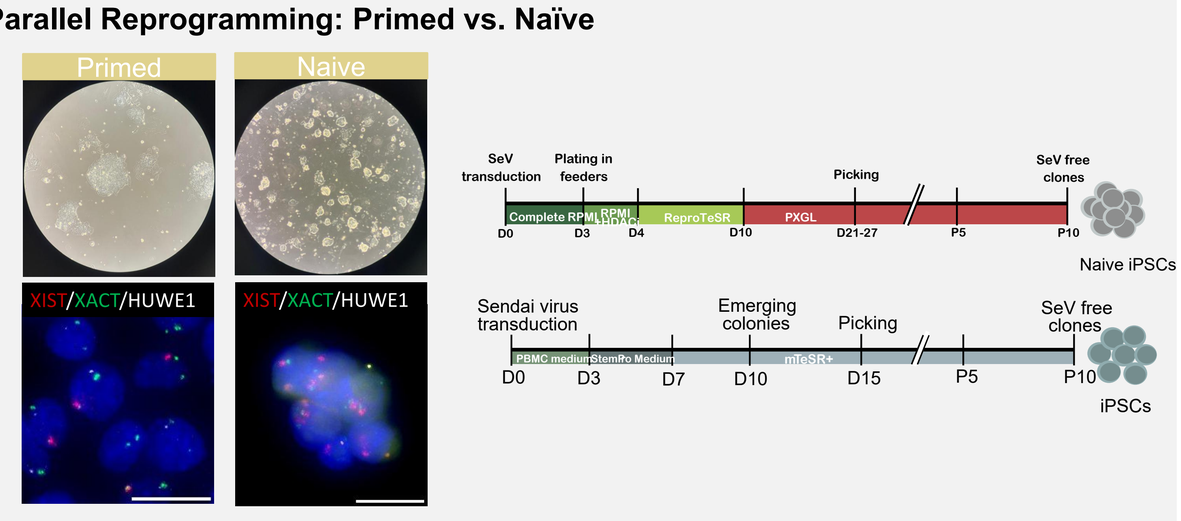


Fig1 - Preliminary Results: (Left) Optimization of the fibroblast differentiation protocol from female H9 hESCs (D0 to D10), showing the experimental timeline, brightfield microscopy, and gene expression analysis (*NANOG* down-regulation and *COL1A1* induction). **(Right)** Parallel reprogramming of H9-derived fibroblasts into Primed and Naïve states, displaying representative colony morphologies, timelines, and RNA-FISH evaluation of X-chromosome inactivation dynamics

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