

## Cardiac fibrosis assay

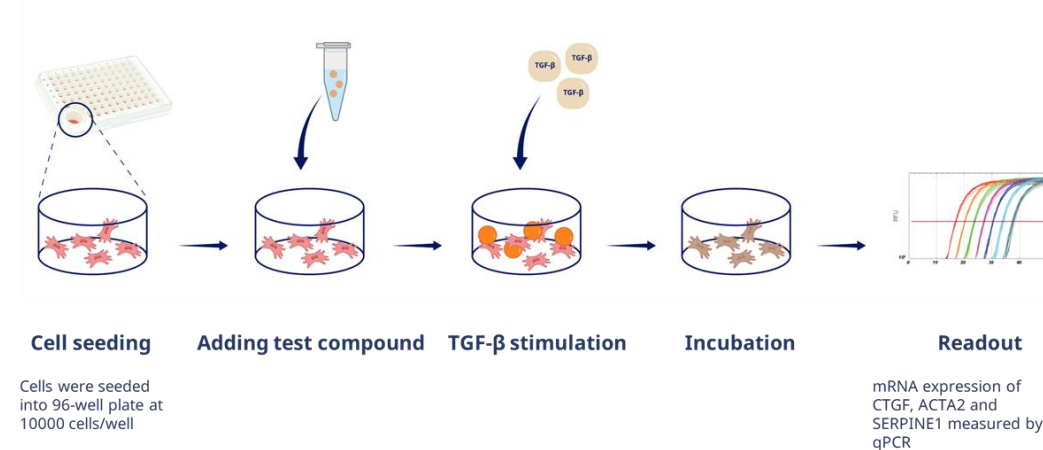
### Disease relevance

Cardiac fibrosis refers to the excess deposition of extracellular matrix in the heart tissue due to the proliferation and activation of cardiac fibroblasts. Cardiac fibrosis increases the stiffness and decreases the compliance of the heart, which has been recognized as one of the key mechanisms contributing to the development of heart failure.

### Assay set-up

Primary human cardiac ventricular fibroblasts are cultured in 96-well plate in basal state or stimulated by TGF- $\beta$  to induce fibrosis.

We will test your compounds for its ability to promote or inhibit fibrosis in basal and in fibrotic condition. And the readout will be mRNA regulation of fibrotic genes (CTGF, ACTA2 and SERPINE1)



### Experimental protocol

At day 1, primary human cardiac fibroblasts will be seeded into 96-well plate at 10000 cells/well in culture medium plus 10% FBS.

At day 2, Cells may be transfected with the tested compounds and the corresponding control (only when the test compounds are siRNA / ASO compounds) by Lipofectamine® RNAiMAX according to the manual in two different concentration (2, 10 nM for siRNA, 100, 250 nM for ASO)

At day 3, cell culture medium is replaced with starvation medium (0.5% FBS) for a 24-hour incubation

At day 4, cells are treated with or without TGF- $\beta$  in fresh starvation medium for another 24 hours. And cells may be co-treated with the test compounds (only when the test compounds are proteins / peptides / small molecules) in titrated concentration. Vehicle of the test compounds will be used as negative control. Cells will be incubated for another 24 hours

At day 5, cells will be lysed, total RNA will be extracted and mRNA level of fibrotic markers (CTGF, ACTA2 and SERPINE1) will be measured by qPCR.