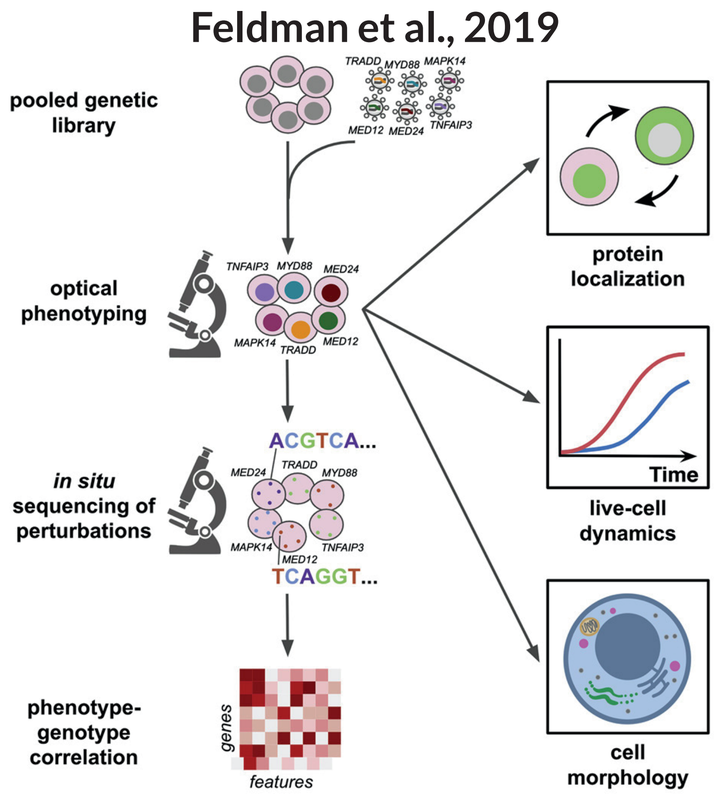
SciLifeLab **CRISPR Functional Genomics TDP 2022-2023**

Enabling high-content phenotyping in pooled CRISPR screens by in situ guide RNA readout

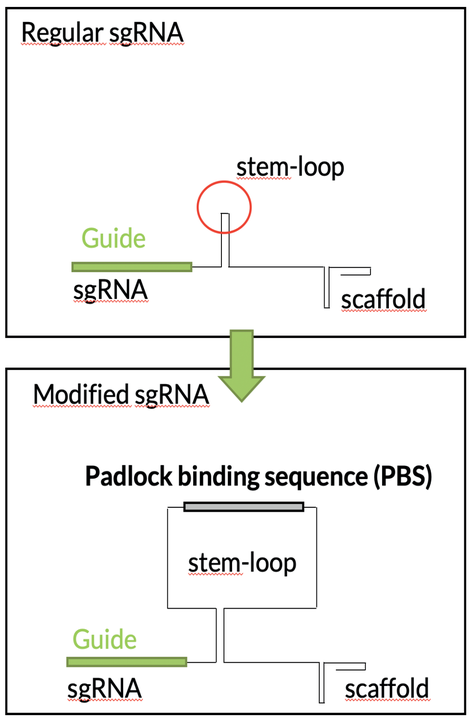
Why?

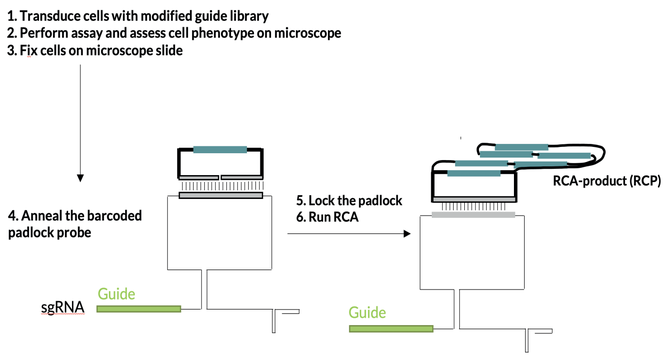
Pooled CRISPR screens are a powerful tool to assign genes to phenotypes in a massively parallel fashion. Such screens are however limited to isolatable phenotypes (fitness, cell-sorting). Complex microsopic phenotypes in 2D or 3D cell cultures as well as tissue sections cannot be interrogated. This TDP is aimed at bringing such complex phenotyping into pooled screens.

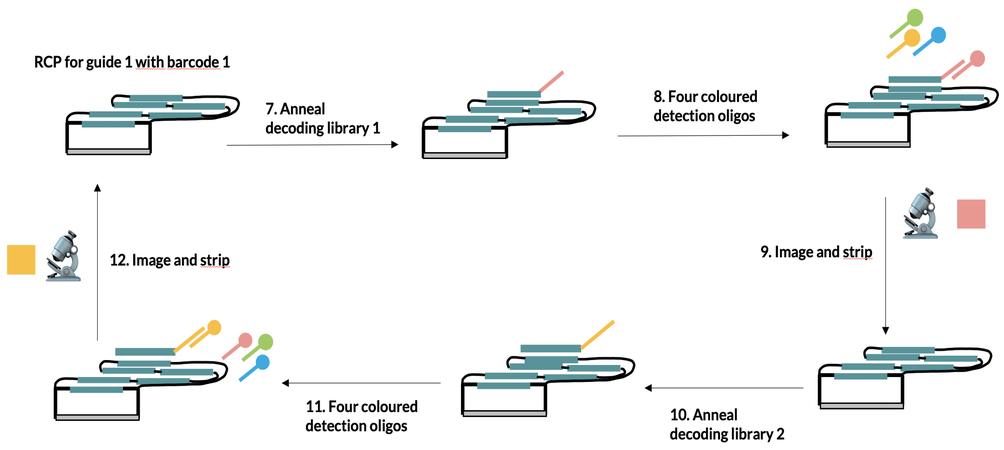


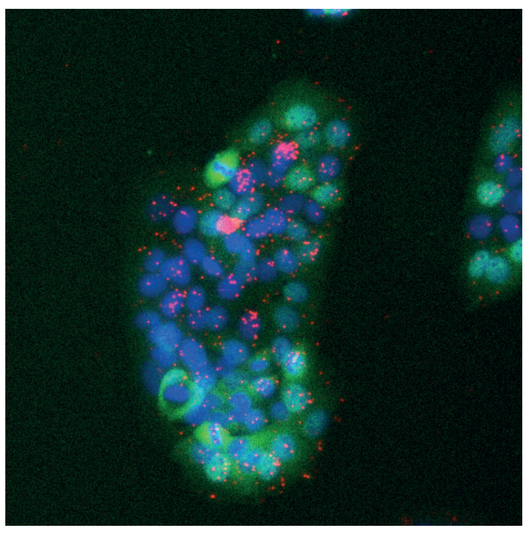
How?

Based on work pioneered in 2019 by Feldman et al., CFG is collaborating with Mats Nilsson's lab to implement an improved version of pooled CRISPR screens with microscopic readout. The method allows the readout of complex cellular phenotypes, and determination of the specific CRISPR perturbation each single cell harbours. The guide RNA is modified such that it contains two barcodes (padlock binding sequences, PBS) in the stem-loop of the guide RNA scaffold. Padlock probes containing new barcodes are attached to the PBS's, and rolling circle amplification (RCA) is used to multiply the padlock barcodes. Decoding libraries coupled to four fluorophores are then used to read out the barcodes by repeated cycles of hybridization and stripping. In this way, the identity of the guide is determined for each cell in the field of vision.



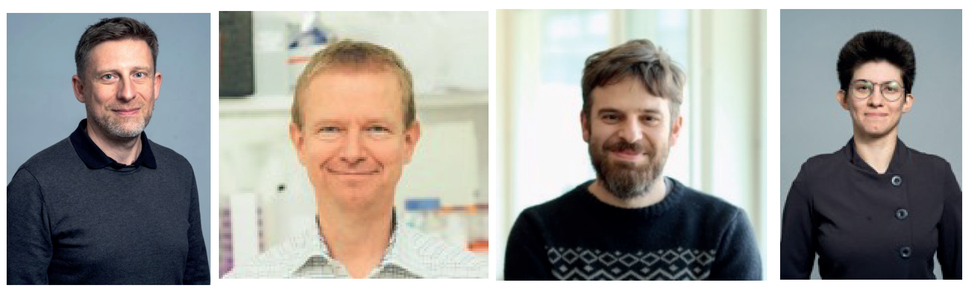






Human HaCaT cells
GFP-Reporter (Smad2-GFP)
DAPI
Rolling circle amplification products

Who?



Bernhard Schmierer, KI
Mats Nilsson, SU
Marco Grillo, SU
Miriam Selle, CFG, KI

Collaborators:

Andrew Bassett and Chun Hao Wong, Wellcome Sanger Institute, Cambridge