Design of highly efficient sgRNA libraries through comprehensive feature analysis

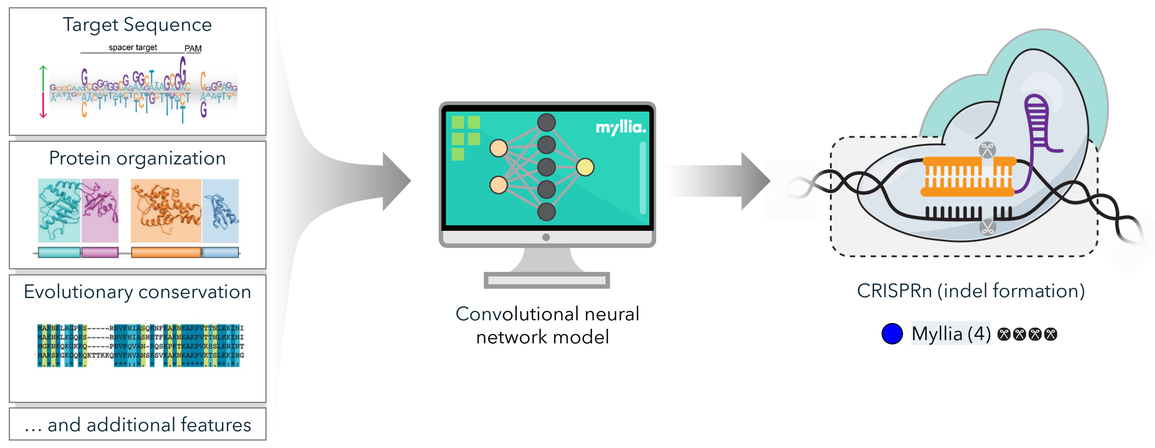
Stefanie Fellinger, Anatoly Vasilyev, Nicole Untermoser, Sumit Pawar, Henrik Schmidt, Rahul Sharma, Anke Loregger, Adam Krejci, Lukas Badertscher, and Tilmann Buerckstuemmer

Myllia Biotechnology GmbH | Vienna, Austria

About Myllia Biotechnology

Myllia Biotechnology combines CRISPR screening with single-cell RNA sequencing, leveraging two powerful technologies to enable detailed analysis of complex phenotypes. Using CROP-Seq alongside other high-content screening technologies, we systematically investigate the effects of thousands of genetic perturbations in primary human cells. Our platform delivers a comprehensive view of transcriptomic changes at single-cell resolution, revealing how genetic perturbations shape cellular behaviour and disease mechanisms. This innovative approach supports a wide range of applications, including the discovery of novel drug targets, elucidating the mechanism of action of drugs, and understanding genetic variants linked to disease risk.

1 The sgRNA design algorithm for CRISPRn screens



2 Commonly used genome-scale sgRNA libraries

GeCKO_v2

Correspondence | Published: 30 July 2014

Improved vectors and genome-wide libraries for CRISPR screening

Neville E Sanjana, Ophir Shalem & Feng Zhang

Behan

Prioritization of cancer therapeutic targets using CRISPR–Cas9 screens

Fiona M. Behan[1,2,12], Francesco Iorio[1,2,3,12], Gabriele Picco[1,12], Emanuel Gonçalves[1], Charlotte M. Beaver[1], Giorgia Migliardi[4,5], Rita Santos[6], Yanhua Rao[7], Francesco Sassi[8], Marika Pinnelli[4,5], Rizwan Ansari[1], Sarah Harper[1], David Adam Jackson[1], Rebecca McRae[1], Rachel Pooley[1], Piers Wilkinson[1], Dieudonne van der Meer[1], David Dow[2,9], Carolyn Buser-Doepner[2,7], Andrea Bertotti[4,5], Livio Trusolino[4,5], Euan A. Stronach[2,6], Julio Saez-Rodriguez[2,3,8,9,10], Kosuke Yusa[1,2,11,13]* & Mathew J. Garnett[1,2,13]*

TKO_v3

Evaluation and Design of Genome-Wide CRISPR/SpCas9 Knockout Screens

Traver Hart,[†,1] Amy Hin Yan Tong,[†] Katie Chan,[†] Jolanda Van Leeuwen,[†] Ashwin Seetharaman,[†] Michael Aregger,[†] Megha Chandrashekhar,[†] Nicole Hustedt,[‡] Sahil Seth,[§] Avery Noonan,[†] Andrea Habsid,[†] Olga Sizova,[†] Lyudmila Nedyalkova,[†] Ryan Climie,[†] Leanne Tworzyanski,[†] Keith Lawson,[†] Maria Augusta Sartori,[†] Sabriyeh Alibeh,[†] David Tieu,[†,**] Sanna Masud,[†,**] Patricia Mero,[†] Alexander Weiss,[†] Kevin R. Brown,[†] Matej Usaj,[†] Maximilian Billmann,[††] Mahfuzur Rahman,[††] Michael Costanzo,[††] Chad L. Myers,[††] Brenda J. Andrews,[†,**,‡‡] Charles Boone,[†,**,‡‡] Daniel Durocher,[‡,**] and Jason Moffat[†,**,‡‡,1]

VBC

Multilayered VBC score predicts sgRNAs that efficiently generate loss-of-function alleles

Georg Michlits[1,4], Julian Jude[2,4], Matthias Hinterndorfer[2], Melanie de Almeida[2], Gintautas Vainorius[1], Maria Hubmann[1], Tobias Neumann[2], Alexander Schleiffer[1,2], Thomas Rainer Burkard[1,2], Michaela Fellner[2], Max Gijsbertsen[1], Anna Traunbauer[2], Johannes Zuber[2,3]✉ and Ulrich Elling[1]✉

Brunello

Optimized libraries for CRISPR-Cas9 genetic screens with multiple modalities

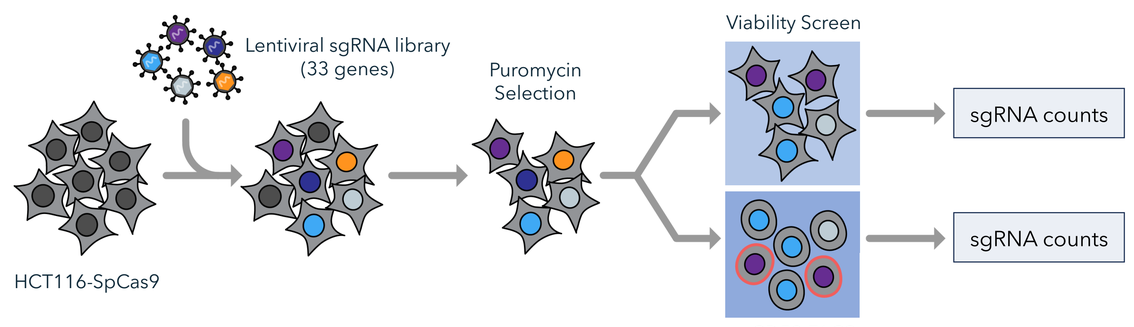
Kendall R. Sanson[1], Ruth E. Hanna[1], Mudra Hegde[1], Katherine F. Donovan[1], Christine Strand[1], Meagan E. Sullender[1], Emma W. Vaimberg[1], Amy Goodale[1], David E. Root[1], Federica Piccioni[1] & John G. Doench[1]

MinLibCas9

Minimal genome-wide human CRISPR-Cas9 library

Emanuel Gonçalves[1], Mark Thomas[1], Fiona M. Behan[1], Gabriele Picco[1], Clare Pacini[1,2], Felicity Allen[1], Alessandro Vinceti[3], Mamta Sharma[1], David A. Jackson[1], Stacey Price[1], Charlotte M. Beaver[1], Oliver Dovey David Parry-Smith[1], Francesco Iorio[1,3], Leopold Parts[1,4], Kosuke Yusa[5] and Mathew J. Garnett[1*]

3 Screens to study essential and non-essential genes

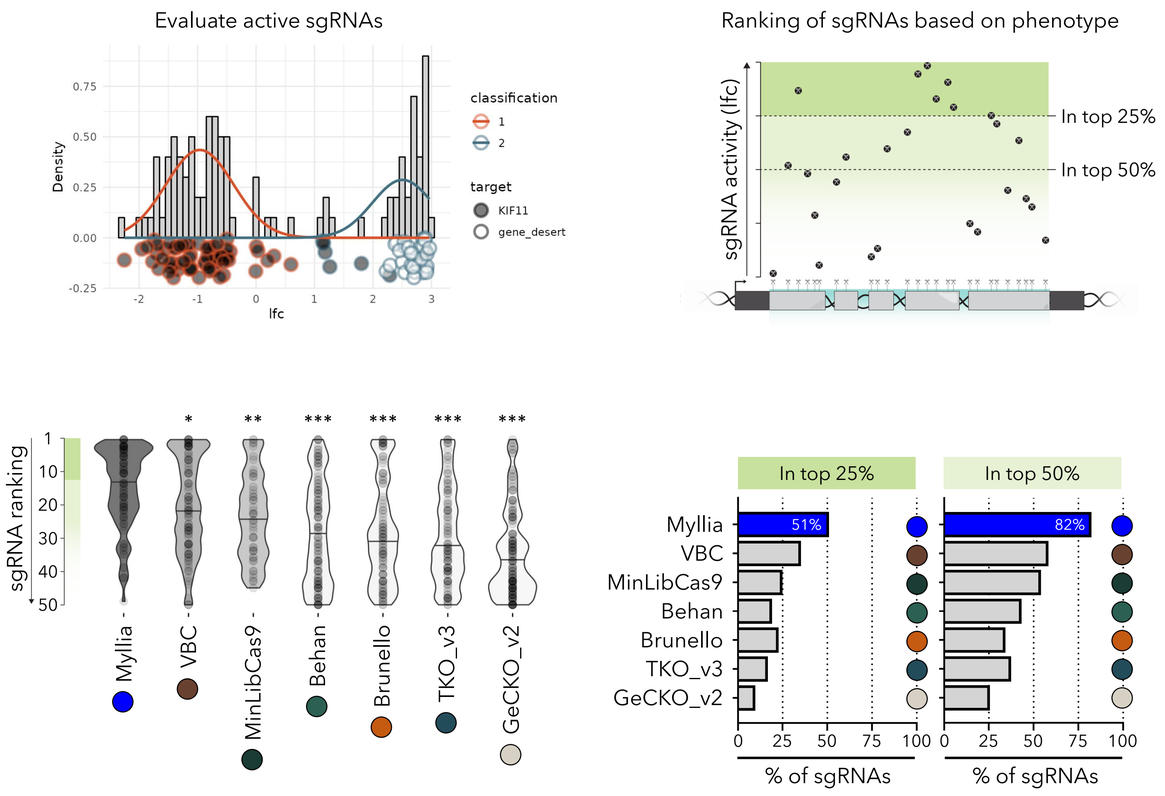


Dropout screen based on essentiality
- 24 genes, ~50 sgRNAs/gene

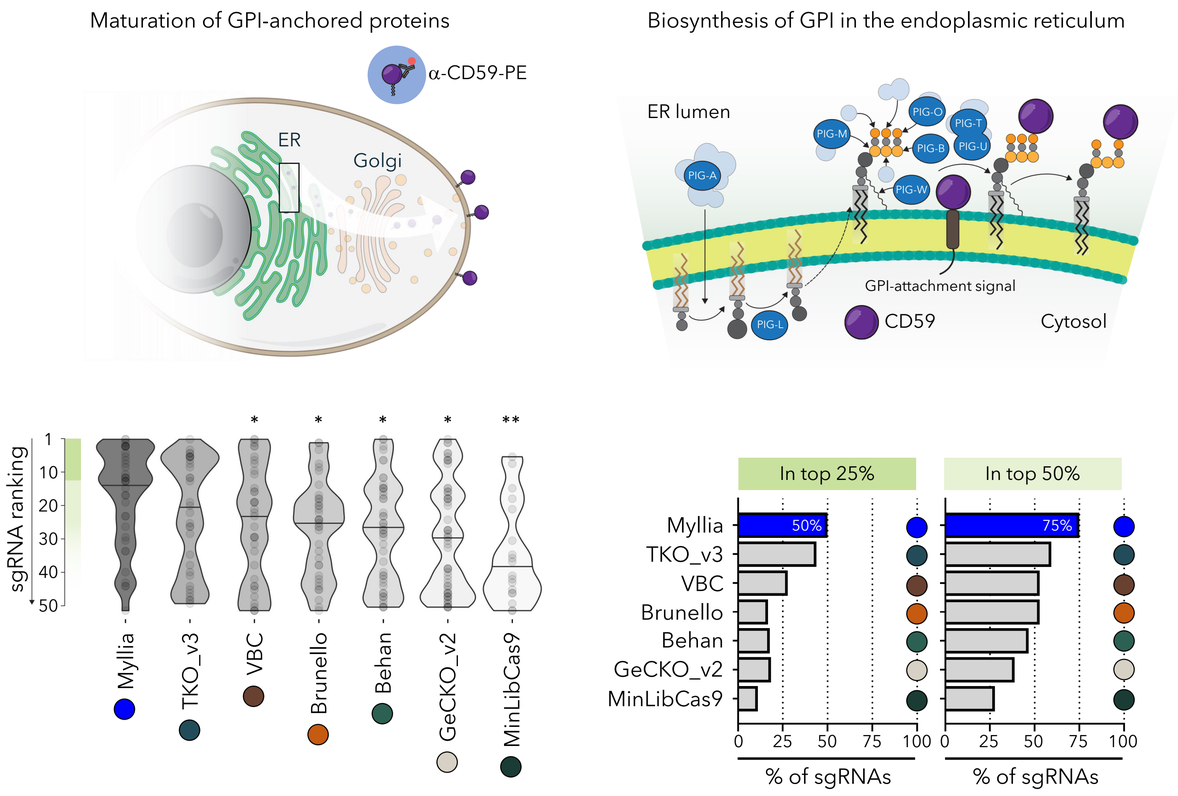
CD59 FACS readout
- 9 genes, ~50 sgRNAs/gene

HCT116 cells stably expressing SpCas9 were transduced with a lentiviral sgRNA library targeting 33 genes (~1600 sgRNAs) at an MOI of 0.4, maintaining at least 500x coverage per sgRNA sequence throughout the screen. The lentiviral vector contains an EF1a promoter for expression of EGFP as a fluorescent marker and a puromycin resistance gene for selection of transduced cells. After 21 days, cells were either pelleted for the viability screen or subjected to FACS to enrich for CD59-negative cells. From both experimental arms, gDNA was extracted, the sgRNA-expressing region was amplified by PCR and samples were analyzed by NGS. Experiments were performed in biological triplicate and sgRNA counts were analyzed using MAGeCK.

4 Viability phenotype to assess sgRNA activities



5 FACS-based screen to monitor CD59 localization



Conclusions and Outlook

- Myllia´s sgRNA design algorithm selects highly active sgRNA sequences
- Our sgRNA libraries contain optimal sgRNA sequences for both targeting essential and non-essential genes
- We will expand the screening campaign to target additional non-essential genes that could help to further improve sgRNA designs and increase editing rates
- CRISPRi libraries will be evaluated using a comparable workflow

Contact us

Myllia Biotechnology
www.myllia.com

E: info@myllia.com