Chromosomal barcoding as a tool for multiplexed phenotypic characterization of laboratory evolved lineages

Adaptive laboratory evolution is an important tool to evolve organisms to increased tolerance towards different physical and chemical stress. It is applied to study the evolution of antibiotic resistance as well as genetic mechanisms underlying improvements in production strains. Adaptive evolution experiments can be automated in a high-throughput fashion. However, the characterization of the resulting lineages can become a time consuming task, when the performance of each lineage is evaluated individually. Here, we present a novel method for the markerless insertion of randomized genetic barcodes into the genome of *Escherichia coli* using a novel dual-auxotrophic selection approach. The barcoded *E. coli* library allows multiplexed phenotyping of evolved strains in pooled competition experiments. We use the barcoded library in an adaptive evolution experiment; evolving resistance towards three common antibiotics. Comparing this multiplexed phenotyping with conventional susceptibility testing and growth-rate measurements we can show a significant positive correlation between the two approaches. Use of barcoded bacterial strain libraries for individual adaptive evolution experiments drastically reduces the workload of characterizing the resulting phenotypes and enables prioritization of lineages for in-depth characterization. In addition, barcoded clones open up new ways to profile community dynamics or to track lineages in vivo or situ.
Dependency of Heterochromatin Domains on Replication Factors

Chromatin structure regulates both genome expression and dynamics in eukaryotes, where large heterochromatic regions are epigenetically silenced through the methylation of histone H3K9, histone deacetylation, and the assembly of repressive complexes. Previous genetic screens with the fission yeast *Schizosaccharomyces pombe* have led to the identification of key enzymatic activities and structural constituents of heterochromatin. We report here on additional factors discovered by screening a library of deletion mutants for silencing defects at the edge of a heterochromatic domain bound by its natural boundary the IR-R⁺ element or by ectopic boundaries. We found that several components of the DNA replication progression complex (RPC), including Mcr1/Claspin, Mcf1/Ctf4, Swi1/Timeless, Swi3/Tipin, and the FACT subunit Pob3, are essential for robust heterochromatic silencing, as are the ubiquitin ligase components Pof3 and Def1, which have been implicated in the removal of stalled DNA and RNA polymerases from chromatin. Moreover, the search identified the cohesin release factor Wpl1 and the forkhead protein Fkh2, both likely to function through genome organization, the Ssz1 chaperone, the Fkbp39 proline cis-trans isomerase, which acts on histone H3P30 and P38 in *Saccharomyces cerevisiae*, and the chromatin remodeler Frt3. In addition to their effects in the mating-type region, to varying extents, these factors take part in heterochromatic silencing in pericentromeric regions and telomeres, revealing for many a general effect in heterochromatin. This list of factors provides precious new clues with which to study the spatiotemporal organization and dynamics of heterochromatic regions in connection with DNA replication.

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**Pharmacogenomics of GPCR Drug Targets**
Natural genetic variation in the human genome is a cause of individual differences in responses to medications and is an underappreciated burden on public health. Although 108 G-protein-coupled receptors (GPCRs) are the targets of 475 (∼34%) Food and Drug Administration (FDA)-approved drugs and account for a global sales volume of over 180 billion US dollars annually, the prevalence of genetic variation among GPCRs targeted by drugs is unknown. By analyzing data from 68,496 individuals, we find that GPCRs targeted by drugs show genetic variation within functional regions such as drug- and effector-binding sites in the human population. We experimentally show that certain variants of μ-opioid and Cholecystokinin-A receptors could lead to altered or adverse drug response. By analyzing UK National Health Service
drug prescription and sales data, we suggest that characterizing GPCR variants could increase prescription precision, improving patients' quality of life, and relieve the economic and societal burden due to variable drug responsiveness.

Adaptive Laboratory Evolution of Antibiotic Resistance Using Different Selection Regimes Lead to Similar Phenotypes and Genotypes

Antibiotic resistance is a global threat to human health, wherefore it is crucial to study the mechanisms of antibiotic resistance as well as its emergence and dissemination. One way to analyze the acquisition of de novo mutations conferring antibiotic resistance is adaptive laboratory evolution. However, various evolution methods exist that utilize different population sizes, selection strengths, and bottlenecks. While evolution in increasing drug gradients guarantees high-level antibiotic resistance promising to identify the most potent resistance conferring mutations, other selection regimes are simpler to implement and therefore allow higher throughput. The specific regimen of adaptive evolution may have a profound impact on the adapted cell state. Indeed, substantial effects of the selection regime on the resulting geno- and phenotypes have been reported in the literature. In this study we compare the geno- and phenotypes of Escherichia coli after evolution to Amikacin, Piperacillin, and Tetracycline under four different selection regimes. Interestingly, key mutations that confer antibiotic resistance as well as phenotypic changes like collateral sensitivity and cross-resistance emerge independently of the selection regime. Yet, lineages that underwent evolution under mild selection displayed a growth advantage independently of the acquired level of antibiotic resistance compared to lineages adapted under maximal selection in a drug gradient. Our data suggests that even though different selection regimens result in subtle genotypic and phenotypic differences key adaptations appear independently of the selection regime.
Two Histone Deacetylases, FfHda1 and FfHda2, Are Important for Fusarium fujikuroi Secondary Metabolism and Virulence

Histone modifications are crucial for the regulation of secondary metabolism in various filamentous fungi. Here we studied the involvement of histone deacetylases (HDACs) in secondary metabolism in the phytopathogenic fungus Fusarium fujikuroi, a known producer of several secondary metabolites, including phytohormones, pigments, and mycotoxins. Deletion of three Zn2+-dependent HDAC-encoding genes, ffhda1, ffhda2, and ffhda4, indicated that FfHda1 and FfHda2 regulate secondary metabolism, whereas FfHda4 is involved in developmental processes but is dispensable for secondary-metabolite production in F. fujikuroi. Single deletions of ffhda1 and ffhda2 resulted not only in an increase or decrease but also in derepression of metabolite biosynthesis under normally repressing conditions. Moreover, double deletion of both the ffhda1 and ffhda2 genes showed additive but also distinct phenotypes with regard to secondary-metabolite biosynthesis, and both genes are required for gibberellic acid (GA)-induced bakanae disease on the preferred host plant rice, as Δ ffhda1 Δ ffhda2 mutants resemble the uninfected control plant. Microarray analysis with a Δ ffhda1 mutant that has lost the major HDAC revealed differential expression of secondary-metabolite gene clusters, which was subsequently verified by a combination of chemical and biological approaches. These results indicate that HDACs are involved not only in gene silencing but also in the activation of some genes. Chromatin immunoprecipitation with the Δ ffhda1 mutant revealed significant alterations in the acetylation state of secondary-metabolite gene clusters compared to the wild type, thereby providing insights into the regulatory mechanism at the chromatin level. Altogether, manipulation of HDAC-encoding genes constitutes a powerful tool to control secondary metabolism in filamentous fungi.
Projects:

**Characterisation of product yield heterogeneity of cell factories during fermentation**
Jahn, L. J., PhD Student, Novo Nordisk Foundation Center for Biosustainability
Sommer, M. O. A., Main Supervisor
Heinemann, M., Supervisor
Weber, T., Examiner
Ingmer, H., Examiner
Johnsen, P. J., Examiner
Marie Curie (EU-stipendium)
01/07/2015 → 04/05/2019
Award relations: Characterisation of product yield heterogeneity of cell factories during fermentation
Project: PhD

**MetaRNA: RNA-based Technologies for single-cell metabolite analysis**
Sommer, M. O. A., Project Participant, Department of Systems Biology, Drug Resistance and Community Dynamics, Novo Nordisk Foundation Center for Biosustainability, Research Groups, Bacterial Cell Factories
Vazquez-Uribe, R., Project Participant, Novo Nordisk Foundation Center for Biosustainability
Jahn, L. J., Project Participant, Novo Nordisk Foundation Center for Biosustainability
FP7 Contract ID: 642738
Project ID: 53110
01/01/2015 → 31/12/2018
Project: Research

Activities:

**Wenner-Gren Foundations International Symposium**
Period: 30 May 2018 → 2 Jun 2018
Leonie Johanna Jahn (Participant)
Novo Nordisk Foundation Center for Biosustainability
Bacterial Synthetic Biology
Degree of recognition: International

Related event

**Wenner-Gren Foundations International Symposium: Antibiotic resistance: Evolutionary concepts versus clinical realities**
30/05/2018 → 02/06/2018
Stockholm, Sweden
Activity: Attending an event › Participating in or organising a conference

**Wenner-Gren Foundations International Symposium**
Period: 30 May 2018 → 2 Jun 2018
Leonie Johanna Jahn (Participant)
Novo Nordisk Foundation Center for Biosustainability
Bacterial Synthetic Biology

Related event

**Wenner-Gren Foundations International Symposium: Antibiotic resistance: Evolutionary concepts versus clinical realities**
30/05/2018 → 02/06/2018
Stockholm, Sweden
Activity: Attending an event › Participating in or organising a conference

**Challenges and new concepts in antibiotics research 2018**
Period: 19 Mar 2018 → 22 Mar 2018
Leonie Johanna Jahn (Participant)
Novo Nordisk Foundation Center for Biosustainability
Bacterial Synthetic Biology
Degree of recognition: International

**Related event**

**Challenges and new concepts in antibiotics research 2018**
19/03/2018 → 22/03/2018
Paris, France
Activity: Attending an event › Participating in or organising a conference

**The Annual Congress of The Danish Microbiological Society (DMS)**
Period: 13 Nov 2017
Leonie Johanna Jahn (Participant)
Novo Nordisk Foundation Center for Biosustainability
Bacterial Synthetic Biology
Degree of recognition: National

**Related event**

**The Annual Congress of The Danish Microbiological Society (DMS)**
13/11/2017 → 13/11/2017
Copenhagen, Denmark
Activity: Attending an event › Participating in or organising a conference

**Bioinformatics for microbiologists**
Period: 6 Nov 2017 → 15 Nov 2017
Leonie Johanna Jahn (Organizer)
Novo Nordisk Foundation Center for Biosustainability
Bacterial Synthetic Biology
Degree of recognition: Local

**Related event**

**Bioinformatics for microbiologists**
06/11/2017 → 15/12/2017
Copenhagen, Denmark
Activity: Attending an event › Participating in or organising workshops, courses, seminars etc.

**Prokaryotic metabolism**
Period: 7 Nov 2016 → 11 Nov 2016
Leonie Johanna Jahn (Participant)
Novo Nordisk Foundation Center for Biosustainability
Bacterial Synthetic Biology
Degree of recognition: Local

**Related event**

**Prokaryotic metabolism**
Groningen, Netherlands
Activity: Attending an event › Participating in or organising workshops, courses, seminars etc.

**Riboswitch design**
Leonie Johanna Jahn (Participant)
Novo Nordisk Foundation Center for Biosustainability
Bacterial Synthetic Biology

**Related event**

**Riboswitch design**
29/02/2016 → 04/03/2016
Darmstadt, Germany
Activity: Attending an event › Participating in or organising workshops, courses, seminars etc.

**Introduction to aptamer selection and characterisation**
Leonie Johanna Jahn (Participant)
Novo Nordisk Foundation Center for Biosustainability

Bacterial Synthetic Biology

**Related event**

**Introduction to aptamer selection and characterisation**
12/10/2015 → 16/10/2015
Bordeaux, France
Activity: Attending an event › Participating in or organising workshops, courses, seminars etc.

**Prizes:**

**1st price in the Wikipedia competition organized by the International Society for Computational Biology**
Leonie Johanna Jahn (Recipient)
Novo Nordisk Foundation Center for Biosustainability, Bacterial Synthetic Biology

**Description**
Leonie Jahn and Alexander Hauser got the 1st price for improving the Wikipedia article about molecular docking.

**Details**
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Degree of recognition: International
Granting Organisations: International Society for Computational Biology
event: ISMB 2016
Prize: Prizes, scholarships, distinctions