Complete absence of thebaine biosynthesis under home-brew fermentation conditions

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The aptabot: an inducibly affinity-switching, minimally invasive *in vivo* contrast agent

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Abstract

The ambitions of current neuroscience—understanding neurological disease progression and mapping the connectome—demonstrate a need for safe *in vivo* tools for creating intricate maps of brain circuitry. Present *in vivo* contrast agents are often limited by their specificity, uptake, resolvability, and/or clearance.

We describe an aptamer-functionalized sensor for high-resolution imaging that can switch imaging targets by an induced multi-stage aptamer reaction. Included are synthetic methods as well as calculations of sensor efficacy based on known kinetics. Calculations show that 10 distinct targets may be imaged in a living brain at the submicron scale within 42 hours.

Introduction

In order to map fine details of brain circuitry, agents must be developed that both bind specific targets of interest and provide highly resolvable contrast. MRI contrast agents have long been used to image brain activity and structure, and in recent years, specifically targeted PET, MRI, and radio imaging agents have been developed for expressed cell surface markers (Xue 2009). Current whole brain contrast agents are often constrained either by radiolabels that provide inadequate resolution to identify individual receptors and emit radiation in their environment, or metal nanoparticles with inefficient clearance or degradation that impedes imaging of subsequent targets. A multi-functionalized contrast agent that attaches to successive targets can provide the key to more intricate brain maps.

Rapidly evolving homing CRISPR barcodes

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Keywords: DNA barcodes, lineage tracing, CRISPR, Cas9, homing CRISPR, homing guide RNA, brain mapping, fluorescent in situ sequencing, FISSEQ, genome engineering.

Abstract: We present here an approach for engineering evolving DNA barcodes in living cells. The methodology entails use of a homing guide RNA (hgRNA) scaffold that directs the Cas9-hgRNA complex to target the DNA locus of the hgRNA itself. We show this homing CRISPR-Cas9 system acts as an expressed evolving genetic barcode, and corresponding small RNAs can be assayed as single molecules *in situ*. This integrated approach will have wide ranging applications, such as in deep lineage tracing, cellular barcoding, molecular recording, dissecting cancer biology, and connectome mapping.

Main Text

A single totipotent zygote has the remarkable ability to generate an entire multicellular organism. Methodologies to comprehensively map and modulate the parameters that govern this transformation will have far ranging impact on our understanding of human development and our ability to restore normal function in damaged or diseased tissues. Precise lineage history of cells during development is one of the parameters that can shed important insights into developmental processes (Sulston et al. 1983; Kretzschmar and Watt 2012). Contemporary lineage-tracing approaches, however, do not scale readily to the model organisms, such as mice, that are most relevant to human development (Weisblat, Sawyer, and Stent 1978; Dymecki and Tomasiewicz 1998; Walsh and Cepko 1992; Porter et al. 2014; Lu et al. 2011). Precise mapping of lineage history in these organisms may be facilitated by combining modern genome engineering and DNA sequencing technologies (Mali, Esvelt, and Church 2013; Lee et al. 2014; Church, Marblestone, and Kalhor, 2015.): if every cell in an organism contained a

Biologically plausible learning in recurrent neural networks reproduces neural dynamics observed during cognitive tasks

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Abstract

Neural activity during cognitive tasks exhibits complex dynamics that flexibly encode task-relevant variables. Chaotic recurrent networks, which spontaneously generate rich dynamics, have been proposed as a model of cortical computation during cognitive tasks. However, existing methods for training these networks are either biologically implausible, and/or require a continuous, real-time error signal to guide learning. Here we show that a biologically plausible learning rule can train such recurrent networks, guided solely by delayed, phasic rewards at the end of each trial. Networks endowed with this learning rule can successfully learn nontrivial tasks requiring flexible (context-dependent) associations, memory maintenance, nonlinear mixed selectivities, and coordination among multiple outputs. The resulting networks replicate complex dynamics previously observed in animal cortex, such as dynamic encoding of task features and selective integration of sensory inputs. We conclude that recurrent neural networks offer a plausible model of cortical dynamics during both learning and performance of flexible behavior.

Suite2p: beyond 10,000 neurons with standard two-photon microscopy

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Abstract

Two-photon microscopy of calcium-dependent sensors has enabled unprecedented recordings from vast populations of neurons. While the sensors and microscopes have matured over several generations of development, computational methods to process the resulting movies remain inefficient and can give results that are hard to interpret. Here we introduce Suite2p: a fast, accurate and complete pipeline that registers raw movies, detects active cells, extracts their calcium traces and infers their spike times. Suite2p runs on standard workstations, operates faster than real time, and recovers ~2 times more cells than the previous state-of-the-art method. Its low computational load allows routine detection of ~10,000 cells simultaneously with standard two-photon resonant-scanning microscopes. Recordings at this scale promise to reveal the fine structure of activity in large populations of neurons or large populations of subcellular structures such as synaptic boutons.

Introduction

Standard resonance-scanning two-photon microscopes readily image the activity of large numbers of neurons, but pipelines for processing the resulting data still suffer from significant limitations. Ideally, such a pipeline should satisfy several criteria. First, it should be fast, to keep up with ever-larger data sets produced by next-generation microscopes [1,2]. Second, the pipeline should be transparent and its results interpretable, so that the original data undergo minimal processing, and a human curator can recognize mistakes or biases in the pipeline's output. Third, the pipeline should be accurate, so that its results require only brief curation by a human operator. Fourth, the pipeline should generalize to recordings of multiple cell types, subcellular structures, and brain regions, which can exhibit widely different activity patterns. Fifth, the pipeline should appropriately model and handle experimental confounds such as neuropil contamination [3]. Finally, it would be ideal if the pipeline could run on inexpensive workstations rather than requiring a cluster of servers, as some current software packages do [4].

To fulfil these criteria, we developed Suite2p, an end-to-end pipeline made of fast and accurate algorithms (Figure 1a). The pipeline involves four independent stages: 1) image registration; 2) region-of-interest (ROI) detection; 3) ROI labelling and quality control; 4) activity extraction with neuropil correction and spike deconvolution (Figure 1b-e). Each stage can be separately adapted

A Rationally Designed Aminoacyl-tRNA Synthetase for Genetically Encoded Fluorescent Amino Acids

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Abstract: The incorporation of non-canonical amino acids into proteins has emerged as a promising strategy to manipulate and study protein structure-function relationships with superior precision *in vitro* and *in vivo*. To date, fluorescent non-canonical amino acids (f-ncAA) have been successfully incorporated in proteins expressed in bacterial systems, *Xenopus* oocytes, and HEK-293T cells. Here, we describe the rational generation of an orthogonal aminoacyl-tRNA synthetase based on the *E. coli* tyrosine synthetase that is capable of encoding the f-ncAA tyr-coumarin in HEK-293T cells.

Keywords: Unnatural amino acids, aminoacyl-tRNA synthetase, coumarin

INTRODUCTION

The emergence of chemical conjugation and genetic encoding techniques to label proteins with fluorescent probes has enabled significant advances in the mechanistic understanding of proteins in biochemical and cellular environments [1]. Encoding large fluorescent proteins (e.g. GFP) as fusion protein products is experimentally straightforward, however, the relative size of the probes can alter the function and biology of the protein being studied. Alternatively, chemical conjugation of an expressed protein requires the labeling sites are solvent accessible and labeling of cytoplasmic sites often comes with significant background reactivity [2]. A possible solution to these issues is the use of genetic code expansion to introduce a relatively compact fluorescent side chain as a non-canonical amino acid directly into the target protein in a site-specific fashion [3]. Indeed, the rapid development of genetically encoded fluorophores as a non-canonical amino acids (ncAA) is emerging as a promising strategy to describe protein function under minimal perturbations in eukaryotic cells [3,4]. The experimental strategy employs an orthogonal suppressor tRNA and an evolved aminoacyl tRNA synthetase (RS), often based upon the tyrosine pair, which can be used to encode the ncAA at virtually any site in the reading frame of the target gene. This pair is orthogonal to the translation system employed, meaning that an evolved TyrRs cannot acylate endogenous Tyr-tRNA molecules and the suppressor tRNAtyr is minimally acylated by host cell synthetases [5]. The orthogonal tRNA has the appropriate anticodon to suppress the nonsense codon, thus allowing for an introduced amber codon of target genes in both prokaryote and eukaryotic systems [3,5,6]. This approach has been successfully used for site-specific incorporation of f-ncAA into a soluble proteins in prokaryote cells (dansyl and hydroxycoumarin) [4,7], membrane proteins expressed in *Xenopus leavis* oocytes (bodipy and anap) [8,9], and proteins expressed in mammalian cells (dansyl and anap) [10,11]. Hydroxycoumarin is notable because of its small size and high environmental sensitivity [12,13], however, no system yet exists for its incorporation via expanding genetic code in mammalian cells.

Genome Editing With Targeted Deaminases

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Easi-CRISPR: Efficient germline modification with long ssDNA donors

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Abstract: CRISPR/Cas9 technology efficiently produces short insertions or deletions (*indels*) and can insert short exogenous sequences at Cas9 cut sites. However, targeting long inserts is still a major technical challenge. To overcome this challenge, we developed *Easi*-CRISPR (*E*fficient *a*dditions with *s*sDNA *i*nserts-CRISPR), a method that uses long, *in vitro*-synthesized, single-stranded DNAs with 50-100 base homology arms as repair templates. We demonstrate that *Easi*-CRISPR can generate knock-in and floxed alleles in mice with an efficiency at many loci as high as 100%. The simple design requirements for donor DNAs and the reproducibly high-efficiency of *Easi*-CRISPR enables rapid development of many types of commonly used animal and cell models.

Tractography-based connectomes are dominated by false-positive connections

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Structure of the *Legionella* Dot/Icm type IV secretion system *in situ* by electron cryotomography

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In vivo magnetic recording of neuronal activity

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Magnetic fields, magnetoencephalography, MEG, spin electronics, magnetic sensors.

Using high-throughput barcode sequencing to efficiently map connectomes

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Abstract

The function of a neural circuit is determined by the details of its synaptic connections. At present, the only available method for determining a neural wiring diagram with single synapse precision—a "connectome"—is based on imaging methods that are slow, labor-intensive and expensive. Here we present SYNseq, a method for converting the connectome into a form that can exploit the speed and low cost of modern high-throughput DNA sequencing. In SYNseq, each neuron is labeled with a unique random nucleotide sequence—an RNA "barcode"—which is targeted to the synapse using engineered proteins. Barcodes in pre- and postsynaptic neurons are then associated through protein-protein crosslinking across the synapse, extracted from the

A systematic comparison of error correction enzymes by next-generation sequencing

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Abstract

Gene synthesis, the process of assembling gene-length fragments from shorter groups of oligonucleotides (oligos), is becoming an increasingly important tool in molecular and synthetic biology. The length, quality, and cost of gene synthesis is limited by errors produced during oligo synthesis and subsequent assembly. Enzymatic error correction methods are cost-effective means to ameliorate errors in gene synthesis. Previous analyses of these methods relied on cloning and Sanger sequencing to evaluate their efficiencies, limiting quantitative assessment and throughput. Here we develop a method to quantify errors in synthetic DNA by next-generation sequencing. We analyzed errors in a model gene assembly and systematically compared six different error correction enzymes across 11 conditions. We find that ErrASE and T7 Endonuclease I are the most effective at decreasing average error rates (up to 5.8-fold relative to the input), whereas MutS is the best for increasing the number of perfect assemblies (up to 25.2-fold). We are able to quantify differential specificities such as ErrASE preferentially corrects C/G → G/C transversions whereas T7 Endonuclease I preferentially corrects A/T → T/A transversions. More generally, this experimental and computational pipeline is a fast, scalable, and extensible way to analyze errors in gene assemblies, to profile error correction methods, and to benchmark DNA synthesis methods.

Introduction

Synthetic DNA is a central tool for biological research [1]. Notably, the initial development of nucleic acid synthesis led directly to the cracking of the genetic code [2]. Today, progress in biology is often limited by the difficulty in producing long, high-quality synthetic DNA [3, 4]. This bottleneck is particularly apparent in the assembly of gene-sized fragments of DNA known as gene synthesis [5].

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Novel Abundant Oceanic Viruses of Uncultured Marine Group II Euryarchaeota Identified by Genome-Centric Metagenomics

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An inducible CRISPR-ON system for controllable gene activation in human pluripotent stem cells

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Running title: CRISPR-ON gene activation system in human pluripotent stem cells

Keywords: CRISPR, transcription activation, human pluripotent stem cells, NANOG, pluripotency

Engineered shell proteins confer improved encapsulated pathway behavior in a bacterial microcompartment

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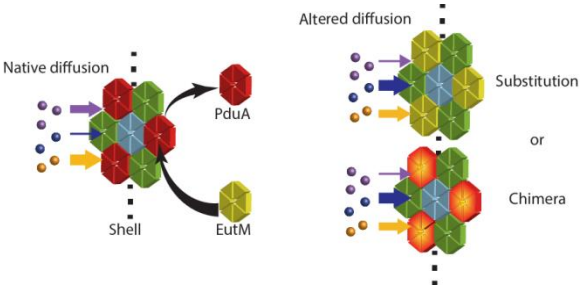
TOC Abstract Graphic



Abstract: Bacterial microcompartments are a class of proteinaceous organelles comprising a characteristic protein shell enclosing a set of enzymes. Compartmentalization can prevent escape of volatile or toxic intermediates, prevent off-pathway reactions, and create private cofactor pools. Encapsulation in synthetic microcompartment organelles will enhance the function of heterologous pathways, but to do so, it is critical to understand how to control diffusion in and out of the microcompartment organelle. To this end, we explored how small differences in the shell protein structure result in changes in the diffusion of metabolites through the shell. We found that the ethanolamine utilization (Eut) protein EutM properly incorporates into the 1,2-propanediol utilization (Pdu) microcompartment, altering native metabolite accumulation and the resulting growth on 1,2-propanediol as the sole carbon source. Further, we identified a single pore-lining residue mutation that confers the same phenotype as substitution of the full EutM protein, indicating that small molecule diffusion through the shell is the cause of growth enhancement. Finally, we show that the hydropathy index and charge of pore amino acids are important indicators to predict how pore mutations will affect growth on 1,2-

Multidimensional encoding of brain connectomes

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The ability to map brain networks at the macroscale in living individuals is fundamental in efforts to chart the relation between human behavior, health and disease. We present a framework to encode structural brain connectomes and diffusion-weighted magnetic resonance data into multidimensional arrays (tensors). The framework overcomes current limitations in building connectomes; it prevents information loss by integrating the relation between connectome nodes, edges, fascicles and diffusion data. We demonstrate the utility of the framework for *in vivo* white matter mapping and anatomical computing. The framework reduces dramatically storage requirements for connectome evaluation methods, with up to 40x compression factors. We apply the framework to evaluate 1,980 connectomes, thirteen tractography methods, and three data sets. We describe a general equation to predicts connectome resolution (number of fascicles) given data quality and tractography model parameters. Finally, we provide open-source software implementing the method and data to reproduce the results.

INTRODUCTION

A fundamental goal of neuroscience is to develop methods to understand how brain networks support function and behavior in individuals across human populations [1–3]. The recent increase in availability of neuroimaging data and large scale projects has the potential to empower new ways of discovery by studying large populations of human brains [4–22]. Exploiting these large-scale data sets will require advances in measurement, computational approaches and theories [23].

Innovation in measurement and computational methods for human brain mapping is shifting the *in vivo* study of the white matter and large-scale brain networks beyond qualitative characterization (such as *camera lucida* drawings), toward structural and functional quantification [24–30]. Tractography and diffusion-weighted magnetic resonance imaging (dMRI) are the primary methods for mapping structural brain connectivity and white matter tissue properties in living human brains. These *in vivo* investigations have shown that there is much to learn about the macrostructural organization of the human brain such that network neuroscience has become one of the fastest-growing fields [3,25,28,29,31–39].

Tractography algorithms use dMRI data to estimate the three-dimensional trajectory of neuronal axons bundles wrapped by myelin sheaths – the white matter fascicles. Fascicles are normally represented as sets of brain coordinates, with coordinates segments spanning anything between 0.01 to 1 mm in length (**Fig. 1a top**). Fascicles have historically been clustered into anatomically cohesive groups called white matter tracts. The largest tracts in the human brain are relatively well characterized and associated with names – such as the corticospinal tract (CST) and the arcuate fasciculus (**Fig. 1b top** [40,41]). White matter tracts communicate between cytoarchitectonically and functionally distinct areas – such as Broca's or Wernicke's areas involved in human language processing (**Fig. 1c top** [42–44]). White matter tracts and brain areas together compose a large-scale network called the connectome [45]. Within this network, white-matter tracts represent communication pathways (the edges; **Fig. 1b top**) and brain areas units of information processing (the nodes; **Fig. 1c-top**).

Title: Individual nucleotide resolution UV cross-linking and immunoprecipitation (iCLIP) to determine protein-RNA interactions.

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Uncharacterized bacterial structures revealed by electron cryotomography

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Running title: ECT of novel bacterial structures

Keywords: bacteria, electron cryotomography, cryo-EM, bacterial ultrastructure, uncharacterized structures

In vivo structures of an intact type VI secretion system revealed by electron cryotomography

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Broadband spectral responses in visual cortex revealed by a new MEG denoising algorithm

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Whole exome sequencing reveals a mutation in *ARMC9* as a cause of mental retardation, ptosis and polydactyly

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Running title: Exome sequencing reveals cause of mental retardation, ptosis and polydactyly syndrome.

Key Words: Intellectual disability, ARMC9, Exome sequencing, Homozygosity mapping, Splice site assay.

ABSTRACT

Intellectual disability (ID) refers to deficits in mental abilities, social behaviour and motor skills to perform activities of daily living as compared to peers. Numerous genetic and environmental factors may be responsible for ID. We report on identification of a novel gene for syndromic ID, using homozygosity mapping followed by exome sequencing in a family with mental retardation, ptosis and polydactyly. The analysis revealed a synonymous mutation c.879G>A which leads to a splicing defect in *ARMC9* gene. The variant is present in conserved region of ARM domain of ARMC9 protein which is predicted to form a platform for protein interaction. This domain is likely to be altered in patients due to splicing defect caused by this synonymous mutation. Our study was helpful in elucidation of molecular basis of mental retardation, ptosis and polydactyly phenotype and addition of *ARMC9* to group of genes leading to syndromic ID.

INTRODUCTION

Intellectual disability has a prevalence of 1-3% in population and can result from heterogeneous causes like environmental/nutritional effect, chromosomal or monogenic causes[1]. More than 230 genes have been reported to be involved in causation of syndromes of intellectual disability. Mental retardation, ptosis and polydactyly [2] are a distinctive combination of clinical features reported by us. This is a rare type of intellectual disability syndrome, reported only in a single consanguineous Muslim family from India, where three individuals were affected[2].

Intellectual disability, short stature and polydactyly was suggestive of possibility of Bardet Biedl syndrome (BBS, MIM: 209900), but absence of obesity, renal abnormality, retinopathy and normal sexual development were some of the phenotypes that were different from BBS. Another possibility was 3MC syndrome, which was formally known as Carnevale syndrome (MIM: 265050), however patients did not had hip dysplasia, cryptorchidism and abdominal muscle defect. For identification of candidate gene in this family we have employed homozygosity mapping followed by exome sequencing in all the three affected siblings with mental retardation, ptosis and polydactyly phenotype.

MATERIALS AND METHODS

Patients Details

We described the clinical features in three affected siblings born out of consanguineous union, characterized as mental retardation, ptosis and polydactyly phenotype[2]. The proband showed severe intellectual disability (ID), bilateral ptosis, downslanting palpebral fissures, hypertelorism, round face, high arched palate, clinodactyly, tapering of fingers and

Patterns of shared signatures of recent positive selection across human populations

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Toxicity and applications of internalised magnetite nanoparticles within live *Paramecium caudatum* cells

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Abstract The nanotechnology revolution has allowed us to speculate on the possibilities of hybridising nanoscale materials with live substrates, yet significant doubt still remains pertaining to the effects of nanomaterials on biological matter. In this investigation we cultivate the ciliated protistic pond-dwelling microorganism *Paramecium caudatum* in the presence of excessive quantities of magnetite nanoparticles in order to assess both potential beneficial applications for this technique as well as any deleterious effects on the organisms' health. Our findings indicate that these nanoparticles are well-tolerated by paramecia, who were observed to consume in quantities exceeding 10% of their body volume: cultivation in the presence of magnetite nanoparticles does not alter *P. caudatum* cell volume, swim speed, growth rate or peak colony density and cultures may persist in nanoparticle-contaminated medium for many weeks. We demonstrate that *P. caudatum* cells ingest starch coated magnetite nanoparticles which facilitates their being magnetically immobilised whilst maintaining apparently normal ciliary dynamics, thus demonstrating that nanoparticle biohybridisation is a viable alternative to conventional forms of ciliate quieting. Ingested magnetite nanoparticle deposits appear to aggregate, suggesting that (a) the process of being internalised concentrates and therefore detoxifies nanomaterial suspensions in aquatic environments and (b) *P. caudatum* is a candidate organism for programmable nanomaterial manipulation and delivery.

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Harnessing optogenetics to probe sub-cellular mechanics

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Modeling allele-specific gene expression by single-cell RNA sequencing

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Holocene selection for variants associated with cognitive ability: Comparing ancient and modern genomes.

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Repressing Integrase attachment site operation with CRISPR-Cas9 in *E. coli*

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Abstract

Serine integrases are bacteriophage proteins responsible for integrating the phage genome into that of the host. Synthetic biologists have co-opted these proteins into useful tools for permanent DNA logic, utilizing their specific DNA recombination abilities to build synthetic cell differentiation and genetic memory systems. Each integrase has a specific pair of DNA sequences (attP/attB sites) that it recombines, but multiple identical sites can result in unpredictable recombination. We have developed a way to control integrase activity on identical attP/attB sites by using catalytically dead Cas9 (dCas9) as a programmable binding protein that can compete with integrase for binding to specific attachment sites. Utilizing a plasmid that contains two identical Bxb1 attP sites, integration can be repressed up to 8 fold at either one of the two attP sites when guide RNA and dCas9 are present. Guide RNA sequences that bind specifically to attB, or either of two attP sites, have been developed. Future goals are to utilize this technology to construct larger and more complex integrase logic circuits.

viruses

MDPI

Communication

How to name and classify your phage: an informal guide

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Abstract: With this informal guide, we try to assist both new and experienced phage researchers through two important stages that follow phage discovery, i.e. naming and classification. Providing an appropriate name for a bacteriophage is not as trivial as it sounds and the effects might be long-lasting in databases and in official taxon names. Phage classification is the responsibility of the Bacterial and Archaeal Viruses Subcommittee (BAVS) of the International Committee on the Taxonomy of Viruses (ICTV). While the BAVS aims at providing a holistic approach to phage taxonomy, for individual researchers who have isolated and sequenced a new phage, this can be a little overwhelming. We are now providing these researchers with an informal guide to phage naming and classification, taking a "bottom-up" approach from the phage isolate level.

Keywords: Bacteriophages; phage taxonomy; phage classification; naming guide; classification guide

1. Introduction

Virus taxonomy is currently the responsibility of the International Committee on the Taxonomy of Viruses (ICTV, [1]), which published its first report in 1971. The Bacterial and Archaeal Viruses Subcommittee (BAVS) within ICTV holds the responsibility of classifying new prokaryotic viruses. New proposals can be submitted year round by the public. These Taxonomy Proposals (TaxoProps) are evaluated by relevant Study Groups (SGs) and the BAVS [2], and are then discussed and voted on by the Executive Committee (EC) during the yearly meeting. All ICTV-accepted proposals are finally ratified by the members of the IUMS (International Union of Microbiological Societies) Virology Division through an email vote.

Bacterial virus taxonomy has undergone a number of changes since the discovery of bacteriophages in the early 20th century. Electron microscopy, which lead to the recognition of different phage morphologies, and nucleic acid content provided the basis for the first classification scheme [3,4]. Ever since, genome composition and morphology have been the major criterion for classification at the family rank, with the current taxonomy comprising 22 families grouping bacterial or archaeal viruses.

For many years, the grouping of prokaryotic viruses in lower rank taxa such as genus and subfamily, happened at a minimal pace. Taking the tailed phage families as an example, the 5th Report of ICTV (1991) described one genus in each of the families *Myoviridae*, *Podoviridae* and *Siphoviridae* [5]. This increased to 16 genera spread over the three families by the 7th Report [6] and 18 genera by the 8th Report [7]. As nucleotide sequencing techniques improved the number of publically available

Multiplexed confocal and super-resolution fluorescence imaging of cytoskeletal and neuronal synapse proteins

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ABSTRACT

Neuronal synapses contain dozens of protein species whose expression levels and localizations are key determinants of synaptic transmission and plasticity. The spectral properties of fluorophores used in conventional microscopy limit the number of measured proteins to four species within a given sample. The ability to perform high-throughput confocal or super-resolution imaging of many proteins simultaneously without limitation in target number imposed by this spectral limit would enable large-scale characterization of synaptic protein networks in situ. Here, we introduce PRISM: Probe-based Imaging for Sequential Multiplexing, a method that sequentially utilizes either high affinity Locked Nucleic Acid (LNA) or low affinity DNA probes to enable diffraction-limited confocal and PAINT-based super-resolution imaging. High-affinity LNA probes offer high-throughput, confocal-based imaging compared with PAINT, which uses low affinity probes to realize localization-based super-resolution imaging. Simultaneous immunostaining of all targets is performed prior to imaging, followed by sequential LNA/DNA probe exchange that requires only minutes under mild wash conditions. We apply PRISM to quantify the co-expression levels and nanometer-scale organization of one dozen cytoskeletal and synaptic proteins within individual neuronal synapses. Our approach is scalable to dozens of target proteins and is compatible with high-content screening platforms commonly used to interrogate phenotypic changes associated with genetic and drug perturbations in a variety of cell types.

Rapid sequential *in situ* multiplexing with DNA-Exchange-Imaging

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Abstract

To decipher the molecular mechanism of biological function, it is critical to map the molecular composition of individual cells in the context of their biological environment *in situ*. Immunofluorescence (IF) provides specific labeling for molecular profiling. However, conventional IF methods have finite multiplexing capabilities due to spectral overlap of the fluorophores. Various sequential imaging methods have been developed to circumvent this spectral limit, but are not widely adopted due to the common limitation of requiring multi-rounds of slow (typically over 2 hours at room temperature to overnight at 4 °C in practice) immunostaining. DNA-Exchange-Imaging is a practical platform for rapid *in situ* spectrally-unlimited multiplexing. This technique overcomes speed restrictions by allowing for single-step immunostaining with DNA-barcoded antibodies, followed by rapid (less than 10 minutes) buffer exchange of fluorophore-bearing DNA imager strands. By eliminating the need for multiple rounds of immunostaining, DEI enables rapid spectrally unlimited sequential imaging. The programmability of DNA-Exchange-Imaging allows us to further adapt it to diverse microscopy platforms (with Exchange-Confocal, Exchange-SIM, Exchange-STED, and Exchange-PAINT demonstrated here), achieving highly multiplexed *in situ* protein visualization in diverse samples (including neuronal and tumor cells as well as fresh-frozen or paraffin-embedded tissue sections) and at multiple desired resolution scales (from ~300 nm down to sub-20-nm). Validation highlights include 8-target imaging using single-channel Exchange-Confocal in tens of micron thick retina tissue sections in 2-3 hours (as compared to days required in principle by previous methods using comparable equipment), and 8-target super-resolution imaging with ~20 nm resolution using Exchange-PAINT in primary neurons. These results collectively suggest DNA-Exchange as a versatile, practical platform for rapid, highly

orco mutagenesis causes loss of antennal lobe glomeruli and impaired social behavior in ants

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CRISPR/Cas9 screening using unique molecular identifiers

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Loss of function screening by CRISPR/Cas9 gene knockout with pooled, lentiviral guide libraries is a widely applicable method for systematic identification of genes contributing to diverse cellular phenotypes. Here, random sequence labels (RSLs) were incorporated into the guide-library. RSLs function as internal replicates for robust and reproducible hit calling, and act as unique molecular identifiers (UMIs) to allow massively parallel lineage tracing (MPLT) and true dropout screening.

Pooled CRISPR/Cas9 loss of function screening is a powerful approach to identify genes contributing to a wide range of phenotypes. Most commonly, a library of guide sequences is transduced lentivirally into a population of Cas9-expressing cells, which are then subjected to some form of selection pressure. Relative guide frequencies in the genome of the population before and after selection are quantified by next generation sequencing (NGS).

The approach has been applied successfully, but suffers from two major shortcomings: First, the presence of a guide in a cell does not necessarily lead to loss of function of the corresponding gene, as the total read count for a guide reflects cells with distinct genotypes, which are the result of mono- or bi-allelic frameshifts or in-frame deletions[1] (**Supplementary Fig. 1a**), as well as off target effects. These different genotypes also result in a range of phenotypes for the cells, and optimal identification of hit genes ideally requires a method that would individually track each clonal cell lineage derived from a single editing event. Secondly, identification of fitness genes whose guides are under negative selection can be statistically challenging, because of confounders such as random drift or undersampling.

Finally, creating a sufficient number of replicates in this type of experiment is labor intensive and costly. As a consequence, the read count variance in CRISPR/Cas9 pooled screening is commonly estimated globally from a single data set[2, 3], similar to statistical methods developed for RNA-Seq and ChIP-Seq[4, 5]. This is only valid if the vast majority of guides lack a detectable effect, which might not always be the case, for instance in smaller, targeted libraries. With an insufficient number of replicates, outliers tend to be called as hits and technical artefacts such as PCR bias or other random effects cannot be distinguished from real biological effects.

To address these issues, we have developed a method that allows tracing of individual virus-transduced cell lineages during a CRISPR/Cas9 screen. Depending on the kinetics of genome editing, we can either follow up single clones of identically edited cells, or small populations of sublineages with

A marker-free co-selection strategy for high efficiency human genome engineering

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Intracellular production of hydrogels and synthetic RNA granules by multivalent enhancers

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Light Sheet Theta Microscopy for High-resolution Quantitative Imaging of Large Biological Systems

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Two-photon calcium imaging of medial prefrontal cortex and hippocampus without cortical invasion

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Decoding directional genetic dependencies through orthogonal CRISPR/Cas screens

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Neuronal brain region-specific DNA methylation and chromatin accessibility are associated with neuropsychiatric disease heritability

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Anatomical and functional organization of the human substantia nigra and its connections

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REGULATION OF LIFE SPAN BY THE GUT MICROBIOTA IN THE SHORT-LIVED AFRICAN TURQUOISE KILLIFISH

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Motor-Like Properties of Non-Motor Enzymes

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Abstract: Biological molecular motors use chemical free energy to drive mechanical motion in a specific direction. This function appears to require high molecular complexity, and it is interesting to consider how the evolutionary leap from non-motor enzymes to molecular motors occurred. Here, atomistic simulations coupled with kinetic modeling show that conformational switching of non-motor enzymes, induced by substrate binding and catalysis, induces motor-like, directional torsional motions, as well as oar-like, reciprocating motions, which should be detectable experimentally. Such directional motions in the earliest enzymes would have been starting point for the evolution of motor proteins. Additionally, driven molecular motions in catalytically active enzymes may help explain why the apparent diffusion constants of some enzymes increase with enzyme velocity (*1-3*).

One Sentence Summary: Analysis of protein simulations shows that catalytically active non-motor enzymes can execute motor-like motions.

Main Text:

A biological molecular motor is an enzyme that uses the free energy of an out-of-equilibrium chemical reaction to drive mechanical motion. This motion must have a specific direction to fulfill the motor's functional role; for example, a helical flagellum must rotate in the appropriate sense to propel the organism. The ability to generate directional motion may appear to be a complex protein property, so it is interesting to consider how non-motor enzymes could have evolved to molecular motors. Here, we use computational and theoretical methods to test a hypothesis that essentially any enzyme catalyzing an out-of-equilibrium reaction executes directional motions. The results bear on motor evolution, the importance of chirality, and recent experimental observations of enzyme diffusion and motility.

Gamma and beta bursts during working memory read-out suggest roles in its volitional control

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Abstract

Working memory (WM) activity is not as stationary or sustained as previously thought. There are brief bursts of gamma (~55–120 Hz) and beta (~20–35 Hz) oscillations, the former linked to stimulus information in spiking. We examine these dynamics in relation to read-out from WM, which is still not well understood. Monkeys held a sequence of two objects and had to decide if they matched a subsequent sequence. Changes in the balance of beta/gamma suggested their role in WM control. In anticipation of having to use an object for the match decision, there was an increase in spiking information about that object along with an increase in gamma and a decrease in beta. When an object was no longer needed, beta increased and gamma as well as spiking information about that object decreased. Deviations from these dynamics predicted behavioral errors. Thus, turning up or down beta could regulate gamma and the information in working memory.

Introduction

Sustained spiking activity has been the dominant neural model of working memory (WM)[1-5]. The idea is that, once activated by a stimulus, neurons keep spiking, sustaining the representation of that stimulus. Recent closer examinations have revealed that complex dynamics underlie sustained activity, with brief, discrete narrow-band oscillatory bursts in the gamma and beta bands[6]. Gamma bursts (~55–120 Hz) were tied to spiking carrying information about the remembered items. Beta bursts (~20–35 Hz) were associated with suppression of both informative spiking and gamma. These data are consistent with a model in which gamma-associated spiking stores memories by short-term changes in synaptic weights[7]. Multiple items can be held in WM without mutual interference because different gamma bursts store different items.

Multiplex genome editing for synthetic biology in *Vibrio natriegens*

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Vibrio natriegens has recently emerged as an alternative to *Escherichia coli* for molecular biology and biotechnology, but low-efficiency genetic tools hamper its development. Here, we uncover how to induce natural competence in *V. natriegens* and describe methods for multiplex genome editing by natural transformation (MuGENT). MuGENT promotes integration of large genome edits at high-efficiency on unprecedented timescales, which will extend the utility of this species for diverse applications.

V. natriegens is the fastest growing organism known, with a doubling time of <10 min[1,2]. With broad metabolic capabilities, lack of pathogenicity, and its rapid growth rate, it is an attractive alternative to *E. coli* for diverse molecular biology and biotechnology applications[3]. Methods for classical genetic techniques have been developed for *V. natriegens*, but these are relatively laborious, require multiple steps, and must be used sequentially to generate multiple genome edits[3]. The challenges of these techniques contrast with the ease of genetics in *Vibrio* species that are naturally transformable. Competent *Vibrios* can take up DNA from the environment and integrate it into their genome by homologous recombination; processes known as natural competence and natural transformation, respectively[4-7]. The inducing cue for natural transformation in competent *Vibrio*s is growth on the chitinous shells of crustacean zooplankton, which are commonly found in the aquatic environment where these microbes reside[4]. Chitin induces

The 100 € lab: A 3-D printable open source platform for fluorescence microscopy, optogenetics and accurate temperature control during behaviour of zebrafish, *Drosophila* and *C. elegans*.

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Hypocretin underlies the evolution of sleep loss in the Mexican cavefish

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Local Connectome Phenotypes Predict Social, Health, and Cognitive Factors

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Short Title: Local Connectome Phenotypes
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A Systematic Nomenclature for the *Drosophila* Ventral Nervous System

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Abstract

Insect nervous systems are proven and powerful model systems for neuroscience research with wide relevance in biology and medicine. However, descriptions of insect brains have suffered from a lack of a complete and uniform nomenclature. Recognising this problem the Insect Brain Name Working Group produced the first agreed hierarchical nomenclature system for the adult insect brain, using *Drosophila melanogaster* as the reference framework, with other insect taxa considered to ensure greater consistency and expandability (***Ito et al., 2014***). ***Ito et al.*** (***2014***) purposely focused on the gnathal regions that account for approximately 50% of the adult CNS. We extend this nomenclature system to the sub-gnathal regions of the adult *Drosophila* nervous system to provide a nomenclature of the so-called ventral nervous system (VNS), which includes the thoracic and abdominal neuromeres that was not included in the original work and contains the neurons that play critical roles underpinning most fly behaviours.

Background

Insect nervous systems are proven and powerful model systems for neuroscience research with wide relevance for biology and medicine. Although vast anatomical, physiological and molecular data are already available, integrating this information into a common analytical framework would generate an even more powerful resource. Computational analysis combined with digital microscopy now make it possible to consolidate data from multiple techniques and transform how we analyse nervous system function (***Jenett et al., 2006***; ***Dance, 2015***; ***Boettiger et al., 2016***). It is no longer sufficient to use 2D labelled diagrams and photomicrographs to identify and define anatomical structures, as it is now possible to use multilayer microscopy with computational reconstruction to precisely define and allocate boundaries and structures in 3D. This requires a systematic and consistent nomenclature to precisely define anatomical structures and boundaries. Furthermore the definitions and nomenclature need to be rationalised as multiple names can be used for the same structure. The precise inter relationships between structures also need to be specified. Once this is complete, new findings can be more easily added to this framework allowing

Systematic gene tagging using CRISPR/Cas9 in human stem cells to illuminate cell organization

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Rapid and Programmable Protein Mutagenesis Using Plasmid Recombineering

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Covalent protein labeling by SpyTag-SpyCatcher in fixed cells for super-resolution microscopy

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Integrated computational guide design, execution, and analysis of arrayed and pooled CRISPR genome editing experiments

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The Developing Human Connectome Project: a Minimal Processing Pipeline for Neonatal Cortical Surface Reconstruction

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Abstract

The Developing Human Connectome Project (dHCP) seeks to create the first 4-dimensional connectome of early life. Understanding this connectome in detail may provide insights into normal as well as abnormal patterns of brain development. Following established best practices adopted by the WU-MINN Human Connectome Project and pioneered by FreeSurfer, the project utilises cortical surface-based processing pipelines. In this paper, we propose a fully automated processing pipeline for the structural Magnetic Resonance Imaging (MRI) of the developing neonatal brain. This proposed pipeline consists of a refined framework for cortical and sub-cortical volume segmentation, cortical surface extraction and cortical surface inflation of neonatal subjects, which has been specifically designed to address considerable differences between adult and neonatal brains, as imaged using MRI. Using the proposed pipeline our results demonstrate that images collected from

Recording action potential propagation in single axons using multi-electrode arrays

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High Accuracy Base Calls in Nanopore Sequencing

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***Abstract*—Nanopore sequencing has introduced the ability to sequence long stretches of DNA, enabling the resolution of repeating segments, or paired SNPs across long stretches of DNA. Unfortunately significant error rates >15%, introduced through systematic and random noise inhibit downstream analysis. We propose a novel method, using unsupervised learning, to correct biologically amplified reads before downstream analysis proceeds. We also demonstrate that our method has performance comparable to existing techniques without limiting the detection of repeats, or the length of the input sequence.**

I. Introduction

DNA sequencing has become a critical part of most biological research, in tasks ranging from gene network identification, to biological engineering, to organism identification and the generation of phylogenies. Most of these applications have 2 primary foci: the length of DNA sequence reads, and the per-base error in those reads. Third generation sequencing technologies presented by Oxford Nanopore Technologies(ONT) and PacBio offer read lengths 10-100x what was possible with previous sequencing technologies, but at a per-base read accuracy near 85%, down from 99.99% using second generation technologies. While the increased read length enables many new biological applications [1], [2] the low read accuracy hinders others. Extensive research has gone into developing techniques that can robustly improve Nanopore read accuracy and analyzing the trade-offs [3]–[5].

We propose an approach to correcting errors that does not make assumptions about the presence of a reference genome and is robust to mixed biological populations where reads with significant overlap must be identified as different. To accomplish this task multiple copies of the same original DNA sequence must be read, resulting in a large number reads, of which a small number are high-error copies of one another. We then use the K-means algorithm to cluster reads based on an engineered feature space. This technique does not require replicated reads to be biologically attached [6], meaning that the individual sequence lengths are not constrained. Additionally our approach groups reads with their own replicates, removing the need for a reference genome. Our contributions are summarized below:

- We demonstrate that while some base calling error is random there is a significant component that is systematic and can be modeled. in section mbox IV-A:mbox we present a simple model for predicting easily identifiable k-mers and show that our model correlates well with data found empirically.
- We propose a feature space based on the easily identifiable k-mers that accurately groups copies of unique DNA strands. In section mbox V-A:mbox , we demonstrate comparable clustering performance to MinHash [7] with a number of simulated reads similar to biological experiments.
- We demonstrate that a simple K-means approach can replace a more complex biological process to group unique DNA strands together.
- The proposed method allows for the analysis of larger DNA strands compared to INC-Seq [6].

II. Related Works

Given the importance of sequence read accuracy, a variety of methods have been proposed for increasing read accuracy. These approaches centered around either detecting overlapping regions in genomic alignments to provide read corrections [4], [8], [9], or correcting reads before alignment [3], [6], [10], [11].

Read overlapping [9], and correction from genomic alignments [4], [8] has shown significant potential for read correction. These techniques have shown the ability to increase sequence accuracy into the high 90%'s, with relatively minimal requirements [4]. One major shortcoming is that they are limited by error rate and genomic similarity, with large genomes or high error rates resulting in excessive correction times or erroneous results [8]. Some of these shortcomings can be softened by the presence of a reasonable reference genome, as the error rate for read-genome alignments is approximately half of that seen in read-read alignments.

Read pre-correction has gained significant steam recently, and particular advantages have been seen with small genomes which contain more than 10x even within a single sequencing run. Early attempts at pre-correction focused on aligning high accuracy second generation reads to long nanopore reads [3], [11]. This provides the possibility to resolve many features but introduces systematic error within repeating regions of the reads. More recent attempts have focused on nanopore reads exclusively [6], trading a significantly reduced read length for increased read accuracy. To a large extent both of these

Modulation of Genome Editing Outcomes by Cell Cycle Control of Cas9 Expression

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Enhancing Multiplex Genome Editing by Natural Transformation (MuGENT) via inactivation of ssDNA exonucleases

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ABSTRACT

Recently, we described a method for multiplex genome editing by natural transformation (MuGENT). Mutant constructs for MuGENT require large arms of homology (>2000 bp) surrounding each genome edit, which necessitates laborious *in vitro* DNA splicing. In *Vibrio cholerae*, we uncover that this requirement is due to cytoplasmic ssDNA exonucleases, which inhibit natural transformation. In ssDNA exonuclease mutants, one arm of homology can be reduced to as little as 40 bp while still promoting integration of genome edits at rates of ~50% without selection *in cis*. Consequently, editing constructs are generated in a single PCR reaction where one homology arm is oligonucleotide encoded. To further enhance editing efficiencies, we also developed a strain for transient inactivation of the mismatch repair system. As a proof-of-concept, we used these advances to rapidly mutate 10 high-affinity binding sites for the nucleoid occlusion protein SlmA and generated a duodecuple mutant of 12 diguanylate cyclases in *V. cholerae*. Whole genome sequencing revealed little to no off-target mutations in these strains. Finally, we show that ssDNA exonucleases inhibit natural transformation in *Acinetobacter baylyi*. Thus, rational removal of ssDNA exonucleases may be broadly applicable for enhancing the efficacy and ease of MuGENT in diverse naturally transformable species.

A general method to fine-tune fluorophores for live-cell and *in vivo* imaging

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Nanopore sequencing and assembly of a human genome with ultra-long reads

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N.J.L, M.L, J.T.S, and J.R.T conceived the study, J.Q developed the long read protocol, A.D.B, M.J, M.L, H.M, S.M, T.N, J.O'G, J.Q, H.R, J.R.T and L.T prepared materials and/or performed sequencing, A.T.D, I.T.F, M.J, S.K, N.J.L, M.L, K.H.M, H.E.O, B.P, B.S.P, A.M.P, A.R.Q, A.C.R, A.R, T.A.S, J.T.S and J.R.T performed bioinformatics analysis and wrote or modified software, I.T.F, M.J, S.K, N.J.L, M.L, K.H.M, J.O'G, H.E.O, B.P, A.M.P, J.Q, A.R.Q, A.C.R, T.A.S, J.T.S, T.P.S and J.R.T wrote and edited the manuscript. All authors approved the manuscript and provided strategic oversight for the work.

Recombineering in *Vibrio natriegens*

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Abstract

Here, we show that λ-Red homologs found in the *Vibrio*-associated SXT mobile element potentiate allelic exchange in *V. natriegens* by ~10,000-fold. Specifically, we show SXT-Beta (s065), SXT-Exo (s066), and λ-Gam proteins are sufficient to enable recombination of single- and double- stranded DNA with episomal and genomic loci. We characterize and optimize episomal oligonucleotide-mediated recombineering and demonstrate recombineering at genomic loci. We further show targeted genomic deletion of the extracellular nuclease gene *dns* using a double-stranded DNA cassette. Continued development of this recombination technology will advance high-throughput and large-scale genetic engineering efforts to domesticate *V. natriegens* and to investigate its rapid growth rate.

Main text

We have previously proposed the fast growing marine bacterium *Vibrio natriegens* as a powerful bacterial host and reported foundational genomics resources for its utilization (*1*). While methods for genome modification of *V. natriegens* by homologous recombination have recently been reported, they are laborious and protract experimental time. Plasmid-based integration methods require extensive cloning, conjugation, and a strong negative selection for elimination of the plasmid backbone (*2*). Similarly, recombination of double-stranded DNA cassettes by natural competence, though attractive due to its efficiency, requires cloning of unwieldy homology arms up to 3 kb and extended incubation times (*3*). Development of one-step recombineering method which tolerates short homology arms, particularly with oligonucleotides, would be an attractive advancement for genomic manipulation of *V. natriegens*.

Recombineering is a powerful method for precise DNA editing, enabling *in vivo* construction of mutant alleles and structural changes such as insertions and deletion of genes (*4–9*). These mutations can be introduced by allelic exchange between the target sequence and recombinant single- or double-stranded DNA, potentiated by expression of powerful homologous recombination (HR) proteins found in bacteriophages (*5*). λ-Beta, the most well-studied phage recombinase, has been shown to enhance HR in *E. coli* by ~10,000-fold over the basal mutation rate (*10*). Unfortunately, λ-phage Red Beta does not sustain this efficiency in diverse bacteria

A practical guide to CRISPR/Cas9 genome editing in Lepidoptera

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Abstract

CRISPR/Cas9 genome editing has revolutionized functional genetic work in many organisms and is having an especially strong impact in emerging model systems. Here we summarize recent advances in applying CRISPR/Cas9 methods in Lepidoptera, with a focus on providing practical advice on the entire process of genome editing from experimental design through to genotyping. We also describe successful targeted GFP knock-ins that we have achieved in butterflies. Finally, we provide a complete, detailed protocol for producing targeted long deletions in butterflies.

Introduction

The order Lepidoptera represents a tenth of the world's described species and contains many taxa of scientific and agricultural importance. Despite major interest in this group, however, there has been a frustrating lack of progress in developing routine approaches for manipulative genetic work. While the last decade has seen examples of transgenesis and targeted knockouts using methods like transposon insertion[1], zinc-finger nucleases[2,3], and TALENs[4,5], especially in the silk moth *Bombyx mori*, these approaches have resisted widespread application due to their laborious nature. We see two other main reasons manipulative genetics has failed to become routine in Lepidoptera. The first is that many lepidopterans are sensitive to inbreeding, and in some species it can be difficult to maintain experimental lines without special effort. The second is that lepidopterans appear to have an unusual resistance to RNAi[6,7], a method that has dramatically accelerated work in many other groups of insects.

Classification: Biological Sciences, Neuroscience

Arc restores juvenile plasticity in adult mouse visual cortex

Short title: Arc restores juvenile plasticity in adult mice

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Keywords: Arc, plasticity, visual cortex, ocular dominance plasticity, amblyopia, critical period

Title:

Fused dorsal-ventral cerebral organoids model human cortical interneuron migration.

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Rapid whole brain imaging of neural activities in freely behaving larval zebrafish

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A high resolution whole brain imaging using Oblique Light Sheet Tomography

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Deep Neural Networks in Computational Neuroscience

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Keywords: deep neural networks, deep learning, convolutional neural networks, objective functions, recurrence, black box, levels of abstraction, modelling the brain, input statistics, biological detail

Summary

The goal of computational neuroscience is to find mechanistic explanations of how the nervous system processes information to support cognitive function and behaviour. At the heart of the field are its models, i.e. mathematical and computational descriptions of the system being studied. These models typically map sensory stimuli to neural responses and/or neural to behavioural responses and range for simple to complex. Recently, deep neural networks (DNNs), using either feedforward and recurrent architectures, have come to dominate several domains of artificial intelligence (AI). As the term "neural network" suggests, these models are inspired by biological brains. However, current DNN models abstract from many details of biological neural networks. Their abstractions contribute to their computational efficiency, enabling to perform complex feats of intelligence, ranging from perceptual tasks (e.g. visual object and auditory speech recognition) to cognitive tasks (e.g. machine translation), and on to motor control tasks (e.g. playing computer games or controlling a robot arm). In addition to their ability to model complex intelligent behaviours, DNNs have been shown to predict neural responses to novel sensory stimuli that cannot be predicted with any other currently available type of model. DNNs can have millions of parameters (connection strengths), which are required to capture the domain knowledge needed for task performance. These parameters are often set by task training using stochastic gradient descent. The computational properties of the units are the result of four directly manipulable elements: input statistics, network structure, functional objective, and learning algorithm. The advances with neural nets in engineering provide the technological basis for building task-performing models of varying degrees of biological realism that promise substantial insights for computational neuroscience.

BioRxiv Preprint

A Survey of Genome Editing Activity for 16 Cpf1 orthologs

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Whole-brain serial-section electron microscopy in larval zebrafish

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Title: Intercellular mRNA trafficking via membrane nanotubes in mammalian cells

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Key words: β-actin mRNA, membrane nanotubes, MS2 aptamer, single-molecule FISH, mammalian cells

A thermostable Cas9 with increased lifetime in human plasma

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TITLE PAGE

Title: Removal of inhibition uncovers latent movement preparation dynamics

Abbreviated title: Motor potential during saccade preparation

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Dynamic blue light-inducible T7 RNA polymerases (Opto-T7RNAPs) for precise spatiotemporal gene expression control

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KEYWORDS: optogenetics, light control, photoreceptor, LOV domain, transcription, dynamic gene expression, T7 RNA polymerase, split protein

ABSTRACT: Light has emerged as control input for biological systems due to its precise spatiotemporal resolution. The limited toolset for light control in bacteria motivated us to develop a light-inducible transcription system that is independent from cellular regulation through the use of an orthogonal RNA polymerase. Here, we present our engineered blue light-responsive T7 RNA polymerases (Opto-T7RNAPs) that show properties such as low leakiness of gene expression in the dark-state, high expression strength when induced with blue light, or an inducible range of more than 300-fold. Following optimization of the system to reduce expression variability, we have created a variant, which returns to the inactive dark-state within minutes, once blue light is turned off. This allows for precise dynamic control of gene expression, which is a key aspect for most applications using optogenetic regulation. The regulators were developed and tested in the bacterium *Escherichia coli*, which is a crucial cell factory for biotechnology due to its fast and inexpensive cultivation and well understood physiology and genetics. However, minor alterations should be sufficient to allow their use in other species in which the T7 RNAP polymerase and the light-inducible Vivid regulator were shown to be functional, which comprises other bacterial species and eukaryotes such as mammalian cells or yeast. We anticipate that our approach will expand the applicability of using light as an inducer for gene expression independent from cellular regulation, and allow for a more reliable dynamic control of synthetic and natural gene networks.

INTRODUCTION

Small molecule induced gene expression systems are a key component in synthetic biology[1] and biotechnological applications[2]. However, chemical inducers are limited in their application in space and time. Spatiotemporal control is of increasing interest, as biological systems are regulated dynamically and respond to intracellular stimuli and changes in internal states[3]. Although static perturbations, such as growth media variation and gene knockouts, have been extensively and successfully used to elucidate gene network structure and function, approaches using dynamic perturbations are providing new insight into the organizing principles of biology and the study of gene networks[3]. Dynamic regulation is also starting to be explored by metabolic engineers[4–6]. However, very recent work addresses the problem that few broadly applicable tools are available for dynamic pathway regulation, and further show that dynamic regulation can significantly increase product titers through dynamic pathway regulation[7].

Light-based regulation is superior to conventional small-molecule inducers in this regard, displaying better temporal properties, as removal of small molecules might be challenging in scenarios such as batch or fed-batch processes. Further, it allows for spatial control of individual cells (whereas small molecules are diffusion-limited), and is minimally invasive[8], a desired feature for basic research. These distinguishing properties of light over small molecules led to the development of numerous light-controlled devices[9–13]. Light-inducible dimerization domains were successfully exploited in two-hybrid-like systems[14] to create optogenetic gene expression systems in eukaryotes[8–10,15], and for reconstitution of functional proteins from their split parts[16–19].

Dynamically light-inducible systems allow new regulation schemes, by moving the controller out of the cell and using light as input signal for control. Both biology and engineering make use of feedback control to achieve robust regulation, which in turn allows natural and engineered systems to function reliably in the face of disturbances or changing environmental conditions. However, the design of synthetic biological feedback controllers remains challenging due to the fact that biological parts do not behave as reproducibly as electronic ones. To overcome this obstacle, *in silico* feedback control was introduced by our group, which allows for electronic control of biological responses[20,21].

Another challenge for precise control is that depending on the growth phases, nutrient conditions, and other extrinsic factors, the concentration of RNA polymerase varies, ranging from 1,800 to 10,200 molecules per cell[22]. Along with fluctuations in the ribosome concentration, this can result in changes of expression levels[23–25] and reduce the performance of constitutive promoters. This poses a challenge to systems that require precise balances in expression levels[26], especially when media and growth conditions change such as during industrial scale-up[27]. To decouple expression of a gene of interest from cellular RNAP concentrations, the heterologous T7 DNA-dependent RNA polymerase (T7RNAP), originating from the T7 bacteriophage[28,29], is commonly used for protein overexpression. The polymerase shows high processivity, a high selectivity for the T7 promoter, and does not transcribe se-

A complete electron microscopy volume of the brain of adult *Drosophila melanogaster*

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An Efficient CRISPR protocol for generating Conditional and Knock-in mice using long single-stranded DNA donors.

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The CRISPR/Cas9 tool can easily generate knockout mouse models by disrupting the gene sequence, but its efficiency for creating models that require either insertion of exogenous DNA (knock-in) or replacement of genomic segments is very poor. The majority of mouse models used in research are knock-in (reporters or recombinases) or gene-replacement (for example, conditional knockout alleles containing *LoxP* sites flanked exons). A few methods for creating such models are reported using double-stranded DNA as donors, but their efficiency is typically 1-10% and therefore not suitable for routine use. We recently demonstrated that long single-stranded DNAs serve as very efficient donors, both for insertion and for gene replacement. We call this method *Easi*-CRISPR (efficient additions with ssDNA inserts-CRISPR), a highly efficient technology (typically 25%-50%, and up to 100% in some cases), one that has worked at over a dozen loci thus far. Here, we provide detailed protocols for *Easi*-CRISPR.

Genetically engineered orange petunias on the market

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Abstract: Genetic engineering of petunia was shown to lead to novel flower color some twenty years ago. Here we show that petunia lines with orange flowers, generated for scientific purposes, apparently found their way to petunia breeding programmes, intentionally or unintentionally. Today they are widely available, but have not been registered for commerce.

The pathway to the colored anthocyanins in the ornamental plant petunia (*Petunia hybrida*) is a well-known example of substrate specificity of one enzyme limiting the spectrum of possible products of the pathway[1]. Anthocyanins are water soluble pigments giving flowers, fruits and sometimes vegetative parts of plants colours ranging from orange and red to blue and purple[2]. Anthocyanins are extensively glycosylated and acylated, the molecular decoration affecting their spectral properties. At the aglycone level the three most common variants of the molecule are the anthocyanidins pelargonidin, cyanidin and delphinidin, differing by the number of hydroxyl groups (one, two or three, respectively) in the B-ring of the molecule. Hydroxylation takes place at the level of dihydroflavonols in the pathway (possibly earlier in some cases) by two enzymes, flavonoid 3'-hydroxylase (F3'H) and flavonoid 3'5'-hydroxylase (F3'5'H). The enzyme dihydroflavonol reductase (DFR) converts dihydroflavonols to corresponding leucoanthocyanidins, which then are oxidized to anthocyanidins by anthocyanidin synthase (syn. leucoanthocyanidin oxidase). In petunia, the DFR enzyme does not react with the simplest precursor (dihydrokaempferol), therefore the natural range of petunia flower colours lack orange hues typical to pelargonidin derivatives. Flowers of petunia cultivars that have mutations in the two hydroxylases are therefore white.

It was shown few decades ago that by introducing a gene encoding DFR from a species where the enzyme does not show substrate specificity into a petunia line that lacks F3'H and F3'5'H activity, one can open up the pathway to pelargonidin. Using the maize gene *A1* Meyer and colleagues generated brick red colored flowers in petunia[3] and using the gene from the ornamental plant *Gerbera hybrida*, our own laboratory generated petunia lines with bright orange flowers[4].

These petunia flowers were investigated concerning factors relating to stability of the transgene (and therefore the novel colour)[5,6], but they were never commercialized. The list of registered genetically modified petunia plants is very short and includes a single line transgenic for a chalcone synthase encoding gene approved for cultivation in China (http://www.isaaa.org/gmapprovaldatabase/). Therefore, it was a great surprise and a delight from the point of view of maybe gaining insight in the ways petunia germplasm changes under breeding, when we encountered bright orange coloured petunias in flower boxes decorating the Helsinki railway station during summers of 2015 and 2016 (Figure 1). Indeed, orange petunias are widely on

Tandem histone-binding domains enhance the activity of a synthetic chromatin effector

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Keywords: chromatin, histones, transcription factor, protein engineering, epigenetics

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ABSTRACT

Fusion proteins that specifically interact with biochemical marks on chromosomes represent a new class of synthetic transcriptional regulators that decode cell state information rather than DNA sequences. In multicellular organisms, information relevant to cell state, tissue identity, and oncogenesis is often encoded as biochemical modifications of histones, which are bound to DNA in eukaryotic nuclei and regulate gene expression states. We have previously reported the development and validation of the “Polycomb-based transcription factor” (PcTF), a fusion protein that recognizes histone modifications through a protein-protein interaction between its polycomb chromodomain (PCD) motif and trimethylated lysine 27 of histone H3 (H3K27me3) at genomic sites. We demonstrated that PcTF activates genes at methyl-histone-enriched loci in cancer-derived cell lines. However, PcTF induces modest activation of a methyl-histone associated reporter compared to a DNA-binding activator. Therefore, we modified PcTF to enhance its target affinity. Here, we demonstrate the activity of a modified regulator called Pc_2TF, which has two tandem copies of the H3K27me3-binding PCD at the N-terminus. Pc_2TF shows higher affinity for H3K27me3 *in vitro* and shows enhanced gene activation in HEK293 cells compared to PcTF. These results provide compelling evidence that the intrinsic histone-binding activity of the PCD motif can be used to tune the activity of synthetic histone-binding transcriptional regulators.

Highly parallel genome variant engineering with CRISPR/Cas9 in eukaryotic cells

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Abstract:

Direct measurement of functional effects of DNA sequence variants throughout a genome is a major challenge. We developed a method that uses CRISPR/Cas9 to engineer many specific variants of interest in parallel in the budding yeast *Saccharomyces cerevisiae*, and to screen them for functional effects. We used the method to examine the functional consequences of premature termination codons (PTCs) at different locations within all annotated essential genes in yeast. We found

Chimeric CRISPR guides enhance Cas9 target specificity

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Oligonucleotide-guided nucleases (OGNs) have enabled rapid advances in precision genome engineering. Though much effort has gone into characterizing and mitigating mismatch tolerance for the most widely adopted OGN, *Streptococcus pyogenes* Cas9 (SpCas9), potential off-target interactions may still limit applications where on-target specificity is critical. Here we present a new axis to control mismatch sensitivity along the recognition-conferring spacer sequence of SpCas9's guide RNA (gRNA). We introduce mismatch-evading lowered-thermostability guides (melt-guides) and exhibit how nucleotide-type substitutions in the spacer can reduce cleavage of sequences mismatched by as few as a single base pair. Co-transfecting melt-guides into human cell culture with an exonuclease involved in DNA repair, we observe indel formation on a standard genomic target at approximately 70% the rate of canonical gRNA and undetectable on off-target data.

The recent discoveries, characterizations, and modifications of natural oligonucleotide-guided nucleases associated with CRISPR and RNAi have empowered a genome-editing revolution [1–4]. Low barriers for OGNs' cost and design drive their widespread adoption over alternatives, including modular base-recognition domains (i.e., transcription activator like effector, zinc finger, and pumilio assemblies), which can be hard to synthesize, or meganucleases, which are difficult

A single-cell anatomical blueprint for intracortical information transfer from primary visual cortex

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The wiring diagram of the neocortex determines how information is processed across dozens of cortical areas. Each area communicates with multiple others via extensive long-range axonal projections[1–5], but the logic of inter-area information transfer remains unresolved. In sensory neocortex, previous work suggests that neurons typically innervate single cortical areas[3,5,6], implying that information is distributed via ensembles of dedicated pathways. Alternatively, single neurons could broadcast information to multiple cortical targets[6–9]. Distinguishing between these models has been challenging because the projection patterns of only a few individual neurons have been reconstructed. Here we map the projection patterns of axonal arbors from 595 individual neurons in mouse primary visual cortex (V1) using two complementary methods: whole-brain fluorescence-based axonal tracing[10,11] and high-throughput DNA sequencing of genetically barcoded neurons (MAPseq)[12]. Although our results confirm the existence of dedicated projections to certain cortical areas, we find these are the exception, and that the majority of V1 neurons broadcast information to multiple cortical targets. Furthermore, broadcasting cells do not project to all targets randomly, but rather comprise subpopulations that either avoid or preferentially innervate specific subsets of cortical areas. Our data argue against a model of dedicated lines of inter-areal information transfer via "one neuron – one target area" mapping. Instead, long-range communication between a sensory cortical area and its targets is based on a principle whereby individual neurons copy information to, and potentially coordinate activity across, specific subsets of cortical areas.

Correlated Light-Serial Scanning Electron Microscopy (CoLSSEM) for ultrastructural visualization of single neurons *in vivo*

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Inducible, tunable and multiplex human gene regulation using CRISPR-Cpf1-based transcription factors

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Impact of fluorescent protein fusions on the bacterial flagellar motor

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The experimental design and data interpretation in "Unexpected mutations after CRISPR–Cas9 editing *in vivo*" by Schaefer et al. are insufficient to support the conclusions drawn by the authors

The recent correspondence to the Editor of Nature Methods by Schaefer et. al.[1] has garnered significant attention since its publication as a result of its strong conclusions contradicting numerous publications in the field using similar analytical approaches and methods[2-4]. The authors suggest that the CRISPR-Cas9 system is highly mutagenic in genomic regions not expected to be targeted by the gRNA. We believe that the conclusions drawn from this study are unsubstantiated by the disclosed experiments as they were designed and carried out. Further, it is impossible to ascribe the observed differences in the subject mice to the effects of CRISPR *per se*. The genetic differences seen in this comparative analysis were likely present prior to editing with CRISPR.

In our view, the experiments, observations, and subsequent assertions in Schaefer et al.[1] can be summarized as follows. Two mice created using CRISPR-based genome editing in the zygote stage, when compared to a single "co-housed FVB/NJ mouse without CRISPR-mediated correction", showed a significant number of single nucleotide variants (SNVs) and insertions and deletions (indels) across the genome. The number of mutations common to the two independently generated CRISPR edited mice was 1,397 SNVs and 117 indels. Surprisingly, these apparent mutations all arose from sequences in the genome that contain poor homology to the gRNA (between 5% – 65%). Furthermore, none of the 50 closest, predicted off-target sites (based on gRNA sequence homology) had any observed activity (SNVs or indels). The authors speculate that there is an unreported activity where "certain sgRNAs may target loci independently of their target *in vivo*."

Our opinion is that the conclusions drawn from this study are unsubstantiated by the disclosed experiments and that it is impossible to ascribe the observed differences in the subject mice to the effects of CRISPR *per se* is based upon the following observations:

Firstly, the overall number of the study subjects is low (n = 2 treated mice and n = 1 untreated mouse) and the depth of sequencing applied to the treated and untreated mice is not equivalent. An underpowered study may prove limiting when attempting to understand statistical reproducibility and reliability of scientific observations.

Secondly, the selection of a co-housed mouse (as opposed to the parents or *bona fide* littermates) as the control is insufficient to attribute the observed differences between the treated mice and control mouse to CRISPR. The design of the experiment makes it impossible for the authors to rule out the possibility that the reported genomic differences between the experimental animals and the single control existed prior to experimental manipulation with CRISPR. In fact, published literature has shown that differences in the genomes of *littermates* analyzed by whole genome sequencing (WGS) can be significant (985 SNVs were identified by

Response to Editas: Unexpected mutations after CRISPR-Cas9 editing *in vivo*

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Fluidic microactuation of flexible electrodes for neural recording

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Mechanistic Implications of Enhanced Editing by a HyperTRIBE RNA-binding protein

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Variation in neuronal activity state, axonal projection target, and position principally define the transcriptional identity of individual neocortical projection neurons

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Questioning unexpected CRISPR off-target mutations in vivo

To the Editor: Recently, Schaefer et al.[1] reported that whole genome sequencing (WGS) of two Cas9-treated, gene-corrected mice and a wild-type control mouse unveiled 1,397 single-nucleotide variations (SNVs) and 117 small insertions and deletions (indels) present commonly in the two Cas9-treated mice "but absent in the uncorrected control" and from a database of mouse SNVs and indels. There was essentially no sequence homology between the on-target site and these SNVs and indel sites, most of which lacked a protospacer-adjacent motif (PAM) sequence, suggesting that these variations were both small guide RNA (sgRNA)-independent and Cas9-independent, respectively. Nevertheless, the authors made a bold claim that these variations were caused by CRISPR-Cas9 without validating these unexpected off-target effects even at a single SNV or indel site by performing an independent experiment in vitro or in vivo. Another major concern in Schaefer et al. is the absence of analysis of variants that are present in the control mice but absent in the two gene-edited mice.

Target specificities of CRISPR systems have been extensively studied in animals and cell lines. For example, we showed that certain CRISPR-Cas9 nucleases did not induce detectable off-target mutations at sites with just two or three-nucleotide mismatches in human cells, first using T7 endonuclease I assays[2] and then using targeted deep sequencing[3]. We and others also performed WGS to show that Cas9 rarely induced off-target indels in a clonal population of cells[4-7] or a gene-edited animal[8]. Note that Schaefer et al. did not find any off-target mutations in the two Cas9-treated mice at the top 50 most likely off-target sites with 3- to 4-nucleotide mismatches, in line with these previous reports. Given the remarkable specificity of CRISPR-Cas9, it is difficult to believe that Cas9 can cleave sites that differ by > 10 nucleotides from the on-target sequence, as suggested in Schaefer et al.

The authors did not articulate whether the unexpected off-target effects were limited to the particular target site or their method or FVB/NJ zygotes used in their experiments. In silico off-target predicting algorithms and cell-based, genome-wide off-target profiling methods such as GUIDE-seq[9] and HTGTS[10] cannot identify off-target sites with no sequence homology. However, Digenome-seq, an in vitro method of capturing in vitro cleavage sites using Cas9-digested, cell-free genomic DNA via WGS, does not rely on sequence homology[4], because DNA double-strand break ends remain intact in vitro and are protected from digestion by endogenous exonucleases in vivo. Note that we did not find any unusual, non-homologous off-target sites in human genomic DNA using Digenome-seq[4], showing that such sites cannot

CRISPR/Cas9-APEX-mediated proximity labeling enables discovery of proteins associated with a predefined genomic locus in living cells

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Abstract

The activation or repression of a gene's expression is primarily controlled by changes in the proteins that occupy its regulatory elements. The most common method to identify proteins associated with genomic loci is chromatin immunoprecipitation (ChIP). While having greatly advanced our understanding of gene expression regulation, ChIP requires specific, high quality, IP-competent antibodies against nominated proteins, which can limit its utility and scope for discovery. Thus, a method able to discover and identify proteins associated with a particular genomic locus within the native cellular context would be extremely valuable. Here, we present a novel technology combining recent advances in chemical biology, genome targeting, and quantitative mass spectrometry to develop genomic locus proteomics, a method able to identify proteins which occupy a specific genomic locus.

Body

Transcriptional regulation is a highly-coordinated process largely controlled by changes in protein occupancy at regulatory elements of the modulated genes. Chromatin immunoprecipitation (ChIP) followed by quantitative polymerase chain reaction (-qPCR), microarrays (-chip), or massively parallel next-generation sequencing (-seq) has been invaluable for our understanding of transcriptional regulation and chromatin structure, both at the individual locus and genome-wide levels (*1-6*). However, because ChIP requires the use of antibodies, its utility can often be limited by the presupposition of a suspected protein's occupancy, and lack of highly specific and high affinity reagents. Previously developed "reverse ChIP" type methods suffer from several drawbacks including loss of cellular and/or chromatin context, extensive engineering and locus disruption, reliance on repetitive DNA sequences, and the need for chemical crosslinking, which reduces sensitivity for mass spectrometric-based approaches (*7-11*). Therefore, we sought to develop a method to identify proteins associated with a specific, non-repetitive genomic locus in the native cellular context without the need for crosslinking or genomic alterations. Here, we utilized recent advances in sequence-specific DNA targeting and affinity labeling in cells to develop genomic locus proteomics (GLoPro) to characterize proteins associated with a particular genomic locus.

"Unexpected mutations after CRISPR-Cas9 editing *in vivo*" are most likely pre-existing sequence variants and not nuclease-induced mutations

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Competition for a limited supply of synaptic building blocks predicts multiplicative synaptic normalization and heterosynaptic plasticity

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Abstract

We present a mathematical model of synaptic normalization and heterosynaptic plasticity based on competition for limited synaptic resources. In the model, afferent synapses on a part of the dendritic tree of a neuron compete for a limited supply of synaptic building blocks such as AMPA receptors or other postsynaptic components, which are distributed across the dendritic tree. These building blocks form a pool of parts that are ready for incorporation into synapses. Using minimal assumptions, the model produces fast multiplicative normalization behavior and leads to a homeostatic form of heterosynaptic plasticity. It therefore supports the use of such rules in neural network models. Furthermore, the model predicts that the amount of heterosynaptic plasticity is small when many building blocks are available in the pool. The model also suggests that local production and/or assembly of postsynaptic building blocks across the dendritic tree may be necessary to maintain a neuron's proper function, because it facilitates their homogeneous distribution across the dendritic tree. Because of its simplicity and analytical tractability, the model provides a convenient starting point for the development of more detailed models of the molecular mechanisms underlying different forms of synaptic plasticity.

Author Summary

Changes in the efficacies of synapses are thought to be the neurobiological basis of learning and memory. When a synapse is strengthened, new neurotransmitter receptors are added to the postsynaptic membrane. Recent experiments have shown that the behavior of these receptors is highly dynamic, with receptors moving back and forth between synapses on time scales of seconds and minutes, reflecting a competition of synapses for available receptors. Here we propose a mathematical model of this competition of synapses for neurotransmitter receptors or other synaptic building blocks. Using minimal assumptions the model produces a multiplicative normalization behavior of synapses and it predicts a well-known form of so-called heterosynaptic plasticity, where changes in stimulated synaptic pathways induce changes of opposite sign in neighboring, unstimulated pathways. Thus, the model offers a parsimonious mechanistic explanation of these forms of synaptic plasticity.

Introduction

Simple mathematical models of Hebbian learning exhibit an unconstrained growth of synaptic efficacies. To avoid runaway dynamics, some mechanism for limiting weight growth needs to

Rapid and scalable characterization of CRISPR technologies using an *E. coli* cell-free transcription-translation system

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Key words: Cas9, Cascade, Cpf1, PAM, prototyping, synthetic biology, TXTL

Running title: Characterizing CRISPR with TXTL

Frequent lack of repressive capacity of promoter DNA methylation identified through genome-wide epigenomic manipulation

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Title: Functional consequences of genetic loci associated with intelligence in a meta-analysis of 87,740 individuals

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Running title: Secondary analysis of IQ GWAS

Conflict of Interest Statement

PF Sullivan reports the following potentially competing financial interests: Lundbeck (advisory committee), Pfizer (Scientific Advisory Board member), and Roche (grant recipient, speaker reimbursement). G Breen reports consultancy and speaker fees

Exploration and generalization in vast spaces

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ABSTRACT

Foraging for food, developing new medicines, and learning complex games are search problems with vast numbers of possible actions. Under time or resource constraints, optimal solutions are generally unobtainable. How do humans generalize and learn which actions to take when not all outcomes can be explored? We present two behavioural experiments and competitively test 27 models for predicting individual search decisions. We find that a Bayesian function learning model, combined with an optimistic sampling strategy, robustly captures how humans use generalization to guide search behaviour. Taken together, these two form a model of exploration and generalization that leads to reproducible and psychologically meaningful parameter estimates, providing novel insights into the nature of human search in vast spaces. Importantly, our modelling results and parameter estimates are recoverable, and can be used to simulate human-like performance, bridging a critical gap between human and machine learning.

Introduction

From engineering proteins for medical treatment[1] to mastering a game like Go[2], many complex tasks can be described as search problems[3]. Frequently, these tasks come with a vast space of possible actions, each corresponding to some reward that can only be observed through experience. In such problems, one must learn to balance the dual goals of exploring unknown options, while also exploiting existing knowledge for immediate returns. This frames the *exploration-exploitation dilemma*, typically studied using the multi-armed bandit framework[*4,5], with the assumption that each option has its own reward distribution to be learned independently. Yet under real-world constraints of limited time or resources, it is not enough to know *when* to explore, but also *where*. How could an intelligent agent, biological or machine, learn which actions to take when not all outcomes can be explored?

There is an intriguing gap between human and machine learning, since humans are able to quickly learn and adapt to unfamiliar environments, where the same situation is rarely encountered twice[6,7]. This contrasts with traditional approaches to reinforcement learning, which learn about the distribution of rewards for each state independently[8]. Such an approach falls short in more realistic scenarios where it is

[*]The multi-armed bandit is a metaphor for a row of slot machines in a casino, where each slot machine has an independent payoff distribution. Solutions to the problem propose different policies for how to learn about which arms are better to play (exploration), while also playing known high-value arms to maximize reward (exploitation).

DeepATAC: A deep-learning method to predict regulatory factor binding activity from ATAC-seq signals

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Abstract

Determining the binding locations of *regulatory factors*, such as transcription factors and histone modifications, is essential to both basic biology research and many clinical applications. Obtaining such genome-wide location maps directly is often invasive and resource-intensive, so it is common to impute binding locations from DNA sequence or measures of chromatin accessibility. We introduce DeepATAC, a deep-learning approach for imputing binding locations that uses both DNA sequence and chromatin accessibility as measured by ATAC-seq. DeepATAC significantly outperforms current approaches such as FIMO motif predictions overlapped with ATAC-seq peaks, and models based only on DNA sequence, such as DeepSEA. Visualizing the input importances for the DeepATAC model reveals DNA sequence motifs and ATAC-seq signal patterns that are important for predicting binding events. The Keras implementation and analysis pipelines of DeepATAC are available at https://github.com/hiranumn/deepatac.

1 Introduction

Knowing when and where proteins such as transcription factors interact with DNA is important for both clinical and research purposes. Biological assays such as ChIP-seq [5] are designed to directly measure these interactions, but they require significant resources and a large biological sample. To address these limitations several methods have been proposed to impute these binding locations from raw DNA sequence. The FIMO software [4] from the MEME suite, and deep-learning approaches such as DeepSea [11] and Basset [6] have been successful in this regard. However, DNA sequence alone does not contain any cell-type specific information, which is important for making more accurate predictions. This motivates combining DNA based predictions with predictions from cell type specific data sources.

There are several biological assays that measure cell type specific organization of genome, namely, DNase-seq, MNase-seq, HiC, and ATAC-seq [2, 7, 1, 3]. ATAC-seq is the most recent method and is rapidly gaining popularity due to its cost-efficiency and simplicity. In particular, ATAC-seq only requires 500 to 50,000 cells to measure in-vivo open chromatin signal, while other assays require millions of cells. This is a particularly important feature in clinical settings where you cannot sample a large number of cells from patients when performing personal level analysis. Traditionally, researchers have used putative binding locations predicted by motif finding algorithms (e.g FIMO) overlapped with ATAC-seq peaks to determine where transcription factors are bound. DeepATAC, a deep-learning model that is jointly trained on both ATAC-seq and DNA sequence, significantly outperforms this traditional approach.

Title: Neural entrainment determines the words we hear

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Ninety-nine independent genetic loci influencing general cognitive function include genes associated with brain health and structure (N = 280,360)

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Chemical control of a CRISPR-Cas9 acetyltransferase

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Abstract:
Lysine acetyltransferases (KATs) play a critical role in the regulation of transcription and other genomic functions. However, a persistent challenge is the development of assays capable of defining KAT activity directly in living cells. Towards this goal, here we report the application of a previously reported dCas9-p300 fusion as a transcriptional reporter of KAT activity. First we benchmark the activity of dCas9-p300 relative to other dCas9-based transcriptional activators, and demonstrate its compatibility with second generation short guide RNA architectures. Next, we repurpose this technology to rapidly identify small molecule inhibitors of acetylation-dependent gene expression. These studies validate a recently reported p300 inhibitor chemotype, and reveal a role for p300's bromodomain in dCas9-p300-mediated transcriptional activation. Comparison with other CRISPR-Cas9 transcriptional activators highlights the inherent ligand tuneable nature of dCas9-p300 fusions, suggesting new opportunities for orthogonal gene expression control. Overall, our studies highlight dCas9-p300 as a powerful tool for studying gene expression mechanisms in which acetylation plays a causal role, and provide a foundation for future applications requiring spatiotemporal control over acetylation at specific genomic loci.

Introduction

Lysine acetyltransferases (KATs) catalyze protein acetylation, a reversible posttranslational modification (PTM) that plays a critical role in many processes, including gene expression.[1] Two of the most well-studied KATs are EP300 and its homolog CREBBP (commonly referred to jointly as p300/CBP). These two KATs possess a versatile substrate scope which includes histones, transcription factors, and members of the transcriptional regulatory apparatus itself.[2] Accordingly, disruption of p300/CBP is associated with substantial changes in gene expression, and has been linked to several diseases.[3-4] Besides its KAT domain, p300 and CBP additionally contain several non-catalytic modules including zinc fingers, acetylysine readers (bromodomain, BRD), methyllysine readers (PHD domain), and protein-protein interaction domains.[2] Thus, a significant challenge in the study of p300/CBP lies in defining the specific role of the KAT domain in gene expression, as well as its targetable role in disease.

Considering methods to study cellular KAT activity, we were inspired by a recent report by Gersbach et al. which found that p300 could be delivered to specific genomic loci using the genomic-targeting methodology CRISPR-Cas9.[5] Specifically, this study engineered a catalytically inactive variant of *S. pyogenes* Cas9 (dCas9) fused to truncated p300 module containing the BRD and KAT domains (dCas9-300) (Figure 1). Expression of this fusion in combination with chimeric short guide RNAs (sgRNAs) targeted to promoter regions led to

Simulating extracted connectomes

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Connectomes derived from volume EM imaging of the brain can generate detailed physical models of every neuron, and simulators such as NEURON or GENESIS are designed to work with such models. In principal, combining these technologies, plus transmitter and channel models, should allow detailed and accurate simulation of real neural circuits. Here we experiment with this combination, using a well-studied system (motion detection in *Drosophila*). Since simulation requires both the physical geometry (which we have) and the models of the synapses (which are not currently available), we built approximate synapses corresponding to their known and estimated function. Once we did so, we reproduced direction selectivity in T4 cells, one of the main functions of this neural circuit. This verified the basic functionality of both extraction and simulations, and provided a biologically relevant computation we could use in further experiments. We then compared models with different degrees of physical realism, from full detailed models down to models consisting of a single node, to examine the tradeoff of simulation resources required versus accuracy achieved.

Our results show that much simpler models may be adequate, at least in the case of medulla neurons in *Drosophila*. Such models can be easily derived from fully detailed models, and result in simulations that are much smaller, much faster, and accurate enough for many purposes. Biologically, we show that a lumped neuron model reproduces the main motion detector operation, confirming the result of Gruntman[1], that dendritic compution is not required for this function.

INTRODUCTION

Connectomes, maps of biological neural networks in a computer, are derived from volume imaging of the brain and include very detailed physical models of each neuron, such as the portion of an extracted neuron shown in Fig. 1(a). Simulators such as NEURON[2] or GENESIS[3] are explicitly designed to work with physical models, and compute results that depend on physical parameters. Driving a simulator such as NEURON with the output of EM reconstruction should therefore be able to reproduce the operation of biological circuits. However, straightforward attempts to do this run into several obstacles.

First, in addition to geometry, concrete numerical models of synapse operation are required. EM reconstruction can give synapse locations, but does not tell how they operate (or in *Drosophila*, even the sign - inhibitory and excitory synapses look the same). Second, the use of detailed physical models brings additional concerns. The sheer number of nodes, plus the wide range of time constants between short and long segments, creates systems of equations that are hard to solve efficiently with numerical techniques. This not a problem unique to biological systems - circuit networks extracted from integrated circuits share the same concerns, and explicit techniques to avoid this problem have been used[4].

We investigated these problems by using the results from EM reconstruction to drive the simulator NEURON, to try to reproduce a known circuit operation - motion selectivity of the T4 cells in the medulla of *Drosophila*. We ran directly into the problems described above. First, we could not find in the literature detailed models for the graded synapses found in this circuit. Therefore we created analytic synapse models, tuned to get approximately the responses shown in the literature. Next, we found that if we used the fully detailed geometrical models, then the run times of the simulator were prohibitive. To proceed, we had to reduce the geometrical complexity of the extracted neurons. On a positive note, once we added plausible synapse models to our simplified geometrical models, we were able to reproduce major portions of the known network function.

To examine the tradeoff of geometrical complexity versus accuracy, we compared fully detailed simulations with several simpler models. These included both a simpler branched model and a model with a single lumped node. These simplified models are much smaller, much faster to simulate, and give nearly the same results for the neurons we consider here.

EXPERIMENTAL DESIGN

For this experiment, we chose a portion of the visual pathway of the *Drosophila* fly brain, since it has both a detailed connectome[5], and a wide variety of detailed experimental and theoretical data. In particular we decided to try to reproduce the motion selectivity of the T4 cells. These cells react strongly to motion in the sensitive direction, and less strongly to other stimuli, including motion in the opposing direction, motion at right angles to the sensitive direction, or a uniform flash across the visual field. The T4 circuit is complex, with at least 8 differing cell types providing input, and the operation is still not fully understood[6][7][8][9].

The physical structure of each neuron in the network was imported as an SWC file, generated by the reconstruction of Takemura, et al.[5]. This network contains

Efficient *in situ* barcode sequencing using padlock probe-based BaristaSeq

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Inter-homologue repair in fertilized human eggs?

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Many human diseases have an underlying genetic component. The development and application of methods to prevent the inheritance of damaging mutations through the human germline could have significant health benefits, and currently include preimplantation genetic diagnosis and carrier screening. Ma et al. take this a step further by attempting to remove a disease mutation from the human germline through gene editing[1]. They assert the following advances: (i) the correction of a pathogenic gene mutation responsible for hypertrophic cardiomyopathy in human embryos using CRISPR-Cas9 and (ii) the avoidance of mosaicism in edited embryos. In the case of correction, the authors conclude that repair using the homologous chromosome was as or more frequent than mutagenic nonhomologous end-joining (NHEJ). Their conclusion is significant, if validated, because such a "self-repair" mechanism would allow gene correction without the introduction of a repair template. While the authors' analyses relied on the failure to detect mutant alleles, here we suggest approaches to provide direct evidence for inter-homologue recombination and discuss other events consistent with the data. We also review the biological constraints on inter-homologue recombination in the early embryo.

In their first approach, Ma et al. used donor sperm from a patient heterozygous for the *MYBPC3*$^{\Delta GAGT}$ mutation to fertilize wild-type oocytes, such that half of the embryos started out as wild type at the *MYBPC3* locus and half heterozygous. Fertilized zygotes were injected with Cas9 and an sgRNA directed to create a double-strand break (DSB) in the mutant paternal allele. The authors report that 24% of the embryos at day 3 of development were mosaic, with some cells of the embryo containing the mutant paternal locus, either intact or modified by NHEJ, together with a wild-type locus. Remaining cells of the embryo contained only a detectable wild-type allele. While some zygotes were also co-injected with a wild-type, exogenous, single-stranded oligodeoxynucleotide template (ssODN) with two synonymous mutations, no mutations consistent with ssODN-templated repair were detected. Furthermore, 'wild-type only' cells were present at a similar frequency both in the presence and absence of the ssODN. The authors infer that these cells arose by homology-directed repair (HDR) of the mutant paternal allele using the wild-type maternal allele as a template, i.e., inter-homologue recombination, leading to gene correction.

In a second approach, earlier, MII-phase oocytes were coinjected with Cas9 complexes and donor sperm. In this case, mosaicism was not detected, except in a single embryo, which contained both 'wild-type only' cells and ones heterozygous for wild-type and ssODN-templated alleles. Although wild-type embryos were expected at 50% frequency, they appeared to comprise 72% of embryos. The authors

Reconstruction of developmental landscapes by optimal-transport analysis of single-cell gene expression sheds light on cellular reprogramming.

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WHOLE-BRAIN VASCULATURE RECONSTRUCTION AT THE SINGLE CAPILLARY LEVEL

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ABSTRACT

The distinct organization of the brain's vascular network ensures that it is adequately supplied with oxygen and nutrients. However, despite this fundamental role, a detailed reconstruction of the brain-wide vasculature at the capillary level remains elusive, due to insufficient image quality using the best available techniques. Here, we demonstrate a novel approach that improves vascular demarcation by combining CLARITY with a vascular staining approach that can fill the entire blood vessel lumen and imaging with light-sheet fluorescence microscopy. This method significantly improves image contrast, particularly in depth, thereby allowing reliable application of automatic segmentation algorithms, which play an increasingly important role in high-throughput imaging of the terabyte-sized datasets now routinely produced. Furthermore, our novel method is compatible with endogenous fluorescence, thus allowing simultaneous investigations of vasculature and genetically

Title: Large-scale screening of rare genetic variants in humans reveals frequent splicing disruptions

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Droplet-based microfluidic analysis and screening of single plant cells

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Abstract

Droplet-based microfluidics has been used to facilitate high throughput analysis of individual prokaryote and mammalian cells. However, there is a scarcity of similar workflows applicable to rapid phenotyping of plant systems. We report on-chip encapsulation and analysis of protoplasts isolated from the emergent plant model *Marchantia polymorpha* at processing rates of >100,000 protoplasts per hour. We use our microfluidic system to quantify the stochastic properties of a heat-inducible promoter across a population

Enzymatic synthesis of gene-length single-stranded DNA

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Single-stranded DNA (ssDNA) increases the likelihood of homology directed repair with reduced cellular toxicity, yet ssDNA synthesis strategies are limited by the maximum length attainable, as well control over nucleotide composition. Here, we apply purely enzymatic synthesis to generate ssDNA greater than 15 kb using asymmetric PCR, and illustrate the incorporation of diverse modified nucleotides for therapeutic and imaging applications.

Efficient ssDNA synthesis on the 10+ kb-scale is a major need for numerous biotechnology applications including templated homology directed repair for genome editing (1-4), systems-scale gene synthesis and cloning (5-9), and scaffolded DNA origami (10,11). Conventional ssDNA synthesis is performed using either chemical or enzymatic approaches. Chemical synthesis is currently limited to approximately 98% incorporation efficiency for each base addition and therefore limited to the production of ssDNA oligos up to only 200 bases(5). Standard enzymatic synthesis through ligation or polymerization yields double-stranded DNA (dsDNA) that requires additional steps to generate ssDNA. Commercially available ssDNA synthesis is limited to 2 kb from Integrated DNA Technologies, Inc. (IDT, Coralville, IA) or recommended up to 5 kb using a strandase enzyme-based approach from Takara Biosciences, Inc. (Mountain View, CA). Enzymatic or chemical approaches to denaturation of dsDNA to form ssDNA is an alternative approach to ssDNA production, but limited by low yield and high cost.

In contrast, asymmetric polymerase chain reaction (aPCR) offers the direct synthesis of ssDNA from an underlying dsDNA template and has been applied to generate ssDNA ranging from several hundred to several thousand nucleotides in length (12-15). aPCR differs from traditional PCR by having one primer (the forward primer) in molar excess over the second primer (the reverse primer). This approach has previously been applied to short ssDNA synthesis for aptamers and gene detection (12,15), and more recently to kb-scale ssDNA for scaffolded DNA origami (11). However, previous work was limited to 3.3 kb due to low enzyme processivity. Here, we overcome this limitation by using a highly-processive LongAmp Taq polymerase to achieve 15+ kb length ssDNA. Additionally, using a standardized protocol and rules-based primer design, we achieve pure product yields up to 690 ng per 50 µl reaction volume and demonstrate direct incorporation of chemically modified nucleotides for ssDNA applications in therapeutics and imaging that require base or backbone modifications.

High-fidelity polymerases such as Phusion® allow for long dsDNA synthesis in standard PCR, however, Phusion polymerase was unable to synthesize fragment large than 1kb ssDNA (**Fig. 1A**

Biological Insights Into Muscular Strength: Genetic Findings in the UK Biobank

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Short Title: Biological Insights Into Muscular Strength

Key words: Genome-wide association, genetics, grip strength, fitness

A cell-free synthetic biochemistry platform for raspberry ketone production

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Keywords

Synthetic biology, fine chemical, raspberry ketone, polyketide synthase, synthetic biochemistry

Efficient generation of targeted large insertions in mouse embryos using 2C-HR-CRISPR

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Rapid and efficient generation of large fragment targeted knock-in mouse models is still a major hurdle in mouse genetics. Here we developed 2C-HR-CRISPR, a highly efficient gene editing method based on introducing CRISPR reagents into mouse embryos at the 2-cell stage, taking advantage of the likely increase in HR efficiency during the long G2 phase and open chromatin structure of the 2-cell embryo. With 2C-HR-CRISPR and a modified biotin-streptavidin approach to localize repair templates to target sites, we rapidly targeted 20 endogenous genes that are expressed in mouse blastocysts with fluorescent reporters and generated reporter mouse lines. We showcase the