Designing sgRNAs with CRISPy web

Tips and tricks of the trade

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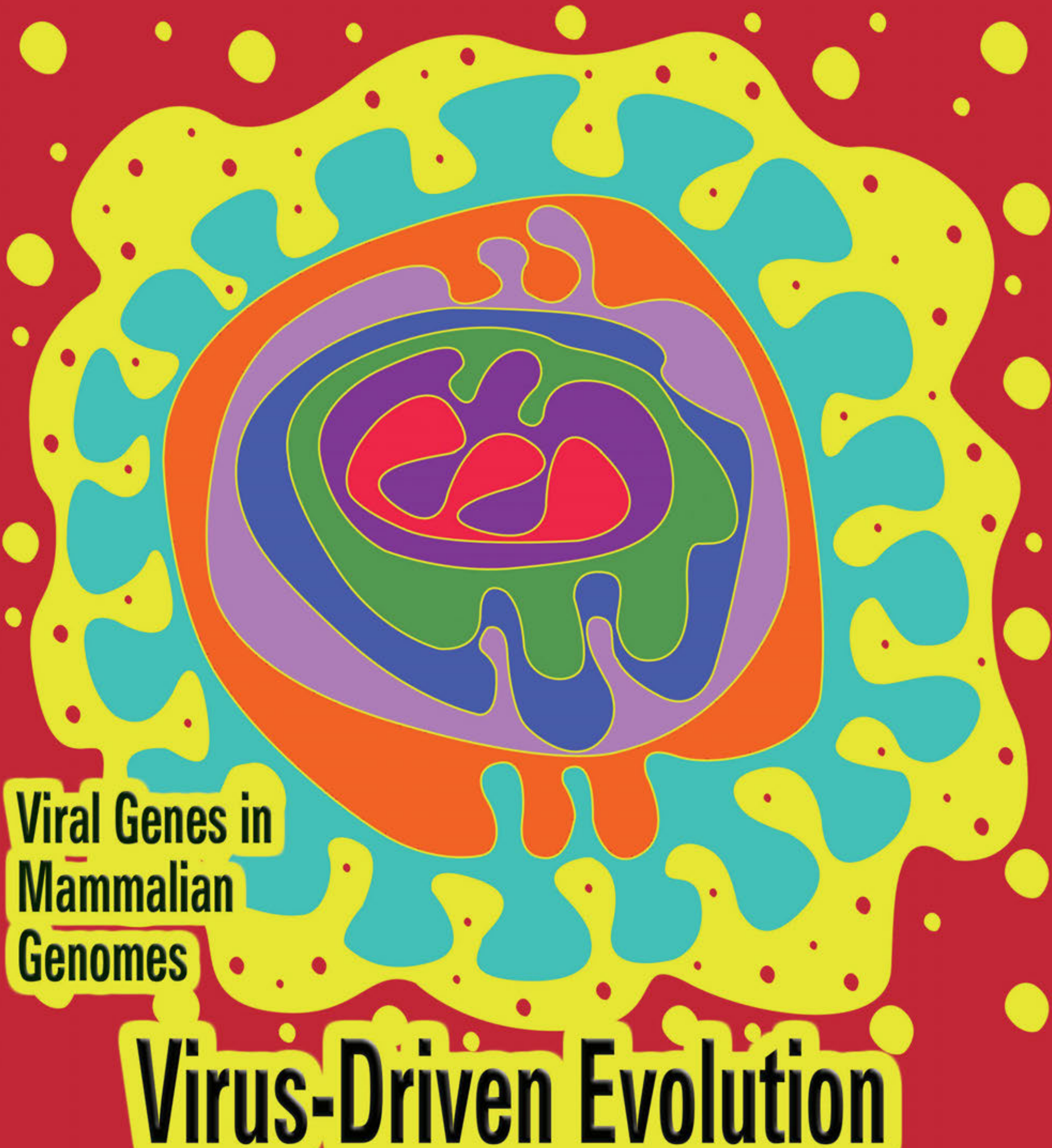

Viral Genes in Mammalian Genomes

Virus-Driven Evolution

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Product survey: Manual Micropipettes

Not Dead Yet

Manual pipettes are under pressure by faster and more ergonomic electric pipettes and liquid handlers. But they may spend a future life in robots, utilising manual pipettes instead of pipetting heads for liquid dispensing.

Though some experts, such as pipette calibration specialist and head of pipette calibration company Calibrate-It, Michel Bryce, have already proclaimed the end of manual pipetting, manual micropipettes are still the most prevalent pipetting tools in life science laboratories – in spite of liquid handlers and electronic pipettes, which are not only faster but also reduce the risk of muscle and strain injuries.

Bullet-proof construction

A major argument, besides the lower price, still speaking in favour of manual pipettes, is their simple and easy use. Lab technicians and researchers are trained on manual pipettes from day one in the lab – operating a manual pipette has almost become second nature to them. The rugged and almost bullet-proofed construction is another advantage of the manual pipette. The mechanics that drive plunger and piston inside the shaft is very robust and may be easily disassembled for cleaning. Even autoclaving at 121°C, to prevent contaminations, doesn't bother manual pipettes – in contrast to most electronic pipettes that quit their work after autoclaving.

When calibrated properly, manual pipettes are very precise instruments, matching the accuracy and precision of electronic pipettes and liquid handlers. Let's face it: the errors in manual pipetting are largely produced by the operator – not by the pipette. A group led by Giuseppe Lippi from the University Hospital of Verona, Italy, recently analysed the intra- and inter-individual imprecision of manual pipetting (*Clin Chem Lab Med*: Doi 10.1515).

Individual pipetting techniques

They randomly chose twenty laboratory operators and let them dispense 1 ml, 100 µl or 10 µl water for ten consecutive times with three recently calibrated, certified

Different pipetting styles may lead to intra- and inter-individual imprecision of manual pipetting.

Tips and tricks of the trade

Designing sgRNAs with CRISPy-web

Photo: Tilmann Weber

Tilmann Weber's group at the Novo Nordisk Foundation Center for Biosustainability developed a user-friendly, web server implementation of the sgRNA prediction software, CRISPy, for non-computer scientists.

Lab Hint

The development of CRISPR/Cas9, which originates from a bacterial plasmid/phage defense system, into a powerful, genome-editing tool has been one of the major breakthrough technologies in biotechnology within the last few years. With the RNA-guided endonuclease Cas9 from the *Streptococcus pyogenes* CRISPR system, currently the most widely used enzyme, it is nowadays feasible to highly efficiently edit DNA in a broad variety of organisms. The method works in most organisms that allow the expression of the different components.

Simplified, Cas9 can be regarded as a programmable, blunt-cutting restriction endonuclease that recognises its target DNA sequence by Watson-Crick base-pairing with the ~20bp protospacer (crRNA) that is bound to the Cas9/tracerRNA complex and cleaves the target DNA within this protospacer region, i.e., is complementary to the target region. Cas9 and the RNAs may be used *in vitro* but may also be expressed within the cell.

Soon, it became evident that the genes encoding the tracer RNA and crRNA, which in the native system bind Cas9 as individual RNAs, can artificially be linked to a single-guide RNA (sgRNA), which still efficiently directs the Cas9 endonuclease to its target, while at the same time being easily cloned and expressed (*Science* 337:816–821). Another prerequisite for cleavage by Cas9 is the presence of a Protospacer Adjacent Motif (PAM) that has to directly follow the DNA sequence, to be targeted by the protospacer. In the case of the *S. pyogenes* Cas9, this PAM is NGG.

For a biotechnological application of this CRISPR-system it is, therefore, essential to design the target sequence for the sgRNAs in a way that ensures its placement directly in front of a PAM and, on the other hand, is unique within the genome to avoid that Cas9 cleaves at other positions in the genome than desired. In addition, Cas9 unfortunately also displays "off-target" effects, i.e., cleavage activity at positions not 100% identical to the protospacer sequence. These happen mostly at sequences, which still have some similarity to the protospacer but no 100% match.

Avoiding these sequences is quite challenging, when designing the sgRNAs by hand – computational tools are highly recommended to be used for finding suitable sequences.

CRISPR tool for non-model organisms

If you are working with model organisms, there are many different programmes and websites available that offer such functionality, for example, CHOPCHOP (*Nucleic Acids Res* 42: 401-07), or CCTop (*PLoS ONE*, 10:e0124633). However, when we started our work with developing the CRISPR technology for the organisms we work in our lab, no easy usable tool existed, to enable designing sgRNAs for such non-model organisms, i.e., tools that allow the users to provide an arbitrary genome sequence against which may be searched.

Therefore, we have developed CRISpy-web, a web-based tool to design sgRNAs for non-model microorganisms (*Synth Syst Biotechnol* 1:118-21). CRISpy-web is based on the software CRISpy, a web-tool to design sgRNAs for use with Chinese Hamster Ovary (CHO) cells that was previously developed at our institute (*Biotechnol Bioeng* 111:1604-16). CRISPy-web is freely accessible at: *http://crispy.secondarymetabolites.org*.

The first step to use CRISpy-web is to upload the genome sequence of the microorganism of interest in Genbank format. Alternatively, sequences can be directly transferred from the antiSMASH secondary metabolite genome mining platform (*Nucleic Acids Res* 43: 237-43), by entering the antiSMASH job ID instead. After selecting and uploading the genome to be analysed, in the next screen, the target region to predict protospacer sequences can be specified. It can be defined either by entering the positions as a range (e.g., 1234-5678), by providing the locus tag, gene name or protein ID (if annotated in the Genbank sequence), or – if the data was pre-analysed with antiSMASH – the gene cluster number. In this case, the antiSMASH detected gene cluster can also be directly selected by clicking on the respective line in the displayed table.

When pressing the "Find Targets" button, suitable protospacer sequences are identified in the selected region of the genome of interest. Depending on the size of the genome sequence to analyse, this step can take a few minutes to complete. On the top line of the screen, genes encoded within the selected region of the genome are displayed as arrows; in the case that the region contains several genes, the user can zoom in on individual genes by clicking on the gene of interest and selecting "show results for this gene only".

Potential protospacer motifs are indicated as little red boxes, depending on their DNA strand orientation (forward strand on top, reverse strand on bottom) and listed in the table – sorted by quality (uniqueness). To select a potential protospacer sequence for export, either click on the red box or click the shopping basket in the list view.

On clicking the "checkout" button at top right of the screen, a table containing all selected protospacer sequences is displayed and ready for export as a CSV file, and ready to be used in the individual sgRNA cloning workflows.

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