A new technique for constitutive high-level production of heterologous proteins in eukaryotic systems using the CRISPR/Cas9 system was used to modify the genome of a Hybridoma cell line.

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Abstract

In several domains of biology, the CRISPR/Cas9 system's capability has revolutionised genome editing. In recent years, many applications, notably those using protein expression technology, have grown at an exponential rate. Because the CRISPR/Cas9 system prevents haphazard gene integration, it can be used to create a stable cell line for high-yield recombinant protein expression. We present a strategy for leveraging the CRISPR/Cas9 system to alter a hybridoma cell line for constitutive expression of proteins of interest. To begin, we substituted part of the light chain of immunoglobulin with the Green Fluorescent Protein (GFP) gene, resulting in a precise knock-in in the hybridoma genome with the goal of optimising the approach. GFP expression and secretion into the cells were confirmed. The gene encoding a protein of diagnostic relevance, the Bovine Herpesvirus 1 glycoprotein E, was then inserted in the donor DNA using the same method. We were able to isolate a viable clone that secreted gE protein fused to GFP into the culture medium. ELISA and Western Blot tests verified this result. This investigation supports the suitability of this cell line for the synthesis of diagnostically important proteins in a mammalian system through sustained gene expression. These tests will allow the technique to progress from proof-of-concept to more targeted applications in infectious illness detection.

Keywords: Heterologous proteins, Hybridoma cell line, CRISPR/Cas9 system, Eukaryotic systems.

Introduction

A surprisingly short time has passed from the initial discovery of the CRISPR / cas system as an RNA-programmed sequence-specific nuclease-based phage defense mechanism to the development of specific subtypes into versatile tools for genome editing. Did. Since then, it has been successfully applied to Saccharomyces cerevisiae, Oryza, zebrafish, Xenops, mice, rats, rabbits, bovines, cynomolgus monkeys and human cells, all in just over a year. There are few examples of equal success in new technologies. Wide applicability from yeast to plants and mammals has shown that this system is actually very efficient, although it has not evolved to cleave DNA associated with eukaryotic nucleosomes increase. Researchers are currently working to improve the cleavage specificity of the system and use programmable sequence-specific binding for purposes other than DNA cleavage [1].

Several of the abovecited studies already made use of CRISPR/cas9 induced DNA breaks to stimulate repair via an experimentally provided homologous recombination (HR) donor construct. These editing tools were either large plasmidbased constructs with long regions of sequence homology (kb range) or singlestranded oligonucleotides with a short region of sequence homology. The plasmid constructs usually template repair efficiently and can transfer large tag sequences, but their generation is timeconsuming. Singlestranded oligonucleotides are conveniently produced via chemical synthesis but due to size restraints their `coding capacity` is limited; they are most useful for specifying point mutations in active sites or introduction of e.g. loxP or attP sites. An approach that unites the convenience of oligonucleotide ordering with the capacity to introduce large tags has been developed in budding yeast: a series of plasmid templates combines e.g. epitope tags and a selectable marker, which are then amplified with flanking primers that specify the desired integration site via appended homology arms. Perhaps the lower integration efficiency of short homology arms is defeated by the very efficient choices possible with microorganisms [2].

CRISPR / cas9 technology and the simplicity of protein labelling in Saccharomyces cerevisiae, we sought to use polymerase chain reaction (PCR) -based homologous donors for genome editing in cultured Drosophila S2 cells. Efficient selection of stable transformed cells mediated by the blasts din resistance gene and stable expression of Cas9

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nucleases by targeted integration of donor constructs at the phosphoglycerate kinase (PGK) locus. I was able to rescue it easily. Repair via the non-homologous end joining pathway (NHEJ) is a competitive reaction for this purpose, ultimately causing mutations in the CRISPR target site without integrating the donor construct. Consistent with this concept, depletion of the lig4 gene by RNA interference prior to induction of DNA cleavage significantly increases the proportion of cells that retain the desired integration, reaching levels of up to 50% of blastisidin-resistant cell populations. I found that I did. Similar effects have been observed in lig4 mutant Drosophila embryos with Znfinger induced DNA double strand [3].

The system described here makes it easy to generate tagged proteins at chromosomal loci. This avoids the well-known problems inherent in plasmid cloning and transient transfection-based approaches, such as heterologous level overexpression and the presence of unlabelled endogenous proteins. However, some common restrictions on protein tags remain. First, adding tags can affect the function of the host protein. For plasmid transfection, this may be masked by the remaining pool of unlabelled endogenous protein. Therefore, the function of proteins tagged on chromosomes should be evaluated in cell clones tagged with all chromosomal alleles. Furthermore, appending a relatively stable protein such as GFP may influence the host protein`s stability, leading to altered expression levels despite an unaltered gene dose. Some fly genes show heterogeneity at the 3′end due to splice variants that involve the region coding for the protein Terminus. Fortunately, these cases are the exception rather than the rule. Here, introduction of a Terminal tag is not only impractical but in addition could influence the outcome of alternative splice decisions. It may be possible to introduce tags also internally with our method; however, the current template modules all contain a stop codon at the end of the tag and the FRT sequence, which remains after removal of the resistance cassette, will also introduce stop codons depending on the reading frame. Off target cleavage by the cas9 nuclease must be a concern. We have thus far employed CRISPR target sites that are very close to the stop codon of the host protein. Due to the limited sequence space available for DNA cleavage, the choice of CRISPR sites with minimal off target cleavage potential is essentially impossible in this situation. Further experiments should test whether the DNA cut may be set at a certain distance to the desired integration site, allowing for at least a minimal choice among potential CRISPR target sites. Finally, the use of truncated guide RNAs is not only efficient but has also reduced the off target cleavage rates in cultured human cells [4].

It is also important to consider when delivery of the guide RNA as a transcript or U6 promoter-based DNA template is more appropriate. Recent structural analyzes have shown that RNA binding is required for conformational activation of the Cas9 nuclease. This probably explains why apocas9 expression in cells and transgenic flies is nontoxic. Therefore, delivery of the guided RNA transcribed in vitro produces only a transient pulse of CRISPR/ cas9 cleavage activity. When using a DNA-based guided RNA template, this cassette can naturally integrate into the genome, resulting in permanent activation of cas9 and a high rate of off-target cleavage events. To provide the basis for a larger set of detailed analyzes, we suggest using in vitro transcribed guide RNA and clonal selection of labelled cells. In contrast, the U6promoterguide RNA-template fusion is a valuable tool for an automated high-throughput screening approach. A combination of lig4 RNAi, cas9-mediated DNA cleavage, PCR-generated HR donor introduction, and selection of blasts din ensures that more than 30% of cells have correctly targeted insertions at the genomic loci of interest. You can generate a cell population that you have on a daily basis. This is largely within the range that can be achieved by transient transfection, but has the advantage of preserving the endogenous promoter and gene mass, thereby avoiding overexpressing artefacts. Presumably, the yield of correctly inserted fragments is combined with optimizing the lig4 knockdown procedure and/or knockdown of other NHEJ factors, especially those involved in signalling pathways such as lig4-independent NHEJ.

This can be further improved. Labelling at endogenous expression levels clearly limits the scope of our technique to the set of proteins normally present in S2 cells. You can develop a similar template module for N-terminal tagging. They should provide a promoter to force expression of the fusion protein until the selectable marker is removed by FLP recombines. Prior to marker resection, the target gene is constitutively or conditionally expressed, depending on the promoter. Example: Interactive studies with proteins that are not normally present in cellular context are probably of limited value, and inexpression strategies are, for example, the Want signalling pathway in flies. Therefore, with well-designed readout, genome-scale overexpression can represent an effective and viable screening approach in S2 cells. Our system is also suitable for targeting luciferase reporters, for example, fused to or in place of the host gene. This allows for a convenient measurement of gene regulation in the context of chromosomes containing the 3'UTR when FLP-mediated excision of the resistant cassette is applied. It's easy to combine these reporters with a genome-wide RNA screen. In particular, transient cas9 expression may be sufficient to introduce the tag, so it can also be applied to other Drosophila cell lines that can be newly derived from flies with the genotype of interest [5].

Are PCR-based HR donors less efficient than established plasmid-based approaches with long homology? We have not attempted a parallel assessment and a quantitative comparison of flies and S2 cells is meaningless. Previous attempts by the Charkas lab to detect genomic modification of KC167 / M3 cells via HR used long homologous arms but failed to induce site-specific DNA cleavage. Therefore, it is not suitable for direct comparison with data. S2 cells showed approximately 1% Ago1 gene tagging efficiency without selection using a 1 kb homology arm. In fact, this is roughly comparable to the efficiency observed at the PGK locus without depleting lig4 prior to selection. After that, Eliminate only transfected cells and increase labelling frequency to 4-5% of selected cells. Our protocol selected stable integration of markers and therefore obtained a much higher percentage of cells expressing the labelled protein. Several publications have demonstrated that linear HR donors or circular HR donors with homology arms

of less than 200 nt in length are inefficient when microinjected into fly embryos. In contrast, work in cultured mammalian cells demonstrated comparable integration efficiency for long, cloned and short, PCR based homology arms. It therefore remains to be tested whether our PCR based HR donors can be used efficiently in vivo as well. In addition to the two supplementary figures mentioned in the text, the manuscript is accompanied by a detailed step by step protocol for our procedure that includes a collection of several figures giving technical detail on the constructs presented and information for primer design.

References

1. Tzur YB, Friedland AE, Nadarajan S, et al. Heritable Caenorhabditis elegans via a CRISPR-Cas9 system. Genetics. 2013;195:1181-85.
2. Kondo S, Ueda R. Highly improved gene targeting by germline-specific cas9 expression in Drosophila. Genetics. 2013;195:715-21.
3. Auer TO, Duroure K, De Cian A, et al. Highly efficient CRISPR/Cas9-mediated knock-in in zebrafish by homology-independent DNA repair. Genome Res. 2014;24:142-53.
4. Fu Y, Sander JD, Reyon D, et al. Improving CRISPR-Cas nuclease specificity using truncated guide RNAs. Nat Biotechnol. 2014:32.
5. Schertel C, Huang D, Bjorklund M, et al. Systematic screening of a Drosophila ORF library *in vivo* uncovers Wnt/Wg pathway components. Dev Cell. 2013;25:207-19.

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