Mol Biotechnol
DOI 10.1007/s12033-015-9905-y

ORIGINAL PAPER

# A Rapid and Cheap Methodology for CRISPR/Cas9 Zebrafish Mutant Screening

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**Abstract** The introduction of new genome editing tools such as ZFNs, TALENs and, more recently, the CRISPR/Cas9 system, has greatly expanded the ability to *knock-out* genes in different animal models, including zebrafish. However, time and costs required for the screening of a huge number of animals, aimed to identify first founder fishes (F0), and then carriers (F1) are still a bottleneck. Currently, high-resolution melting (HRM) analysis is the most efficient technology for large-scale InDels detection, but the very expensive equipment demanded for its application may represent a limitation for research laboratories. Here, we propose a rapid and cheap method for high-throughput genotyping that displays efficiency rate similar to the HRM. In fact, using a common ViiA$^{TM}$7 real-time PCR system and optimizing the parameters of the melting analysis, we demonstrated that it is possible to discriminate between the mutant and the wild type melting curves. Due to its simplicity, rapidity and cheapness, our method can be used as a preliminary one-step approach for massive screening, in order to restrict the scope at a limited number of embryos and to focus merely on them for the next sequencing step, necessary for the exact sequence identification of the induced mutation. Moreover, thanks to its versatility, this simple approach can be readily adapted to the detection of any kind of genome editing approach directed to genes or regulatory regions and can be applied to many other animal models.

**Keywords** Zebrafish *knock-out* mutants · CRISPR/Cas9 · qPCR · Derivative melting curve · Mutation screening

## Introduction

In the last 10 years, morpholino anti-sense oligonucleotides have been the most common *knock-down* technique used in zebrafish, as well as in many other organisms [1]. However, to better understand the function of a given gene, especially during adulthood, hereditable genetic mutations are desirable [2]. In order to induce site-specific mutations, genome editing tools have become fundamental for reverse genetics studies and loss-of-function approaches in different animal models, including zebrafish.

Zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) are the first examples, in which endonuclease catalytic domains are connected to DNA-binding proteins for the purpose of causing DNA double-stranded breaks (DSB) in a specific genomic locus [3]. Once the DSB is determined, the endogenous error-prone Non-Homologous End-Joining system (NHEJ) repairs the damage in absence of a template, leading to random insertion or deletion (InDels) at the cut site [4].

More recently, the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas9 system has been

**Electronic supplementary material** The online version of this article (doi:10.1007/s12033-015-9905-y) contains supplementary material, which is available to authorized users.

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Published online: 16 December 2015