Screening a transposon insertion bank in zebrafish for mutants

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ABSTRACT

Zebrafish (\textit{Danio rerio}) has been used in recent years as a model organism. Its small size, hardiness, together with its short generation time makes it a good model organism for studying vertebrate development. In many studies, transgenic fish displaying various different mutant phenotypes play an important in aiding the research. Chemical mutagens have traditionally been used to generate mutations in fish, but it is highly inefficient and mutations without a visible phenotype may be mistakenly discarded. The discovery of transposable DNA elements have given scientists a much more efficient tool to generate detectable mutations. In this project, a modified maize \textit{Ac/Ds} element was used in a large-scale screen to generate insertions for creation of mutant zebrafish with great efficiency.

INTRODUCTION

Zebrafish (\textit{Danio rerio}) has been a commonly used animal model for vertebrate development (Weinstein, 2008). Genetic manipulation of the embryo to create mutant zebrafish has given numerous researchers insight to functions of genes required in development (Amsterdam, 2006). Creation of additional mutants would give researchers more tools to investigate gene function (Vascotto, et al. 1997) and hence, there is interest for efficient and rapid mutagenesis methods in zebrafish. Traditionally in zebrafish, \textit{Tol2} and \textit{Sleeping Beauty} were used (Kawakami, et al)

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While in maize, rice and Arabidopsis, the Ac/Ds system has been widely used due to the high transgenesis rate and its capability to carry large cargo fragments (Parinov & Emelyanov, 2007) but it is only recently that Ac/Ds has begun to be used in vertebrates (Emelyanov, et al. 2006). In this study, a modified Ac/Ds transposon (DsDelGT4) construct was used for a large-scale mutagenesis screen.

**MATERIALS AND METHODS**

One-cell stage embryos were injected with the DsDelGT4) construct and Activator transposae mRNA. The embryos were then raised to adult hood and out-crossed to a wild type fish. The progeny (F$_1$) of the injected fish were visually screened for any visible phenotype. The F$_1$ fish were also analysed using PCR. To further verify the results, F1 fish were fin clipped and Thermal asymmetric interlaced (TAIL)-PCR was performed. DNA products obtained from the TAIL-PCR was extracted and sequenced to identify the flanking regions of the insertion. The sequences were blasted (Basic Local Alignment Search Tool) against databases to pinpoint the exact location of the insertion.

**RESULTS**

The visual screen did not show many fish with GFP and Cherry expression. However when PCR analysis was done, five out of twenty of the F0 fish had offspring that were positive for Ds insertion. The fin clip PCR analysis also showed similar high rates of transposition, with seven out of twelve analyzed fish were positive for Ds. When TAIL-PCR was conducted, the results varied. Some sequences were completely unspecified nucleotides while others had good sequences. The sequences were blasted against 3 databases. Similarly, the results ranged from good alignments to alignments that did not have any matches.
DISCUSSION

As the use of zebrafish as a model organism increases, more and more zebrafish mutants would be sought after (Weinstein 2008). A large genetic screen would be highly useful to create a database of useful mutants for research. As a result there is a need for an effective and easy method to generate and screen for many mutants. As shown above, the \textit{Ac/Ds} system, despite being of plant origin (Emelyanov, et al. 2006), has proven to be a very reliable and effective. \textit{Ac/Ds} produces a relatively high transgenic rate; ideal for a large scale screen. Thus far, the \textit{Ac/Ds} construct has proven to be a very valuable tool for genetic screens. From the above results, it can be seen that this large-scale genetic screen has been successful (Bao Ngoc Helen, unpublished data) and that much work still needs to be done to properly identify each mutant fish and classify their phenotype and function of each gene. Eventually, the results from this large-scale screen should be collected and placed in a centralized database where the data can be accessed easily. This resource would greatly aid the scientific community in the pursuit of knowledge. In addition, the successful use of the \textit{Ac/Ds} system for insertion mutagenesis in zebrafish indicates that the \textit{Ac/Ds} system is versatile enough for adaptation to other organisms. Additional studies can be conducted to access the full range of organisms that \textit{Ac/Ds} is capable of transposing into. This would provide yet another transposon insertion mutagenesis tool for researchers.

REFERENCES

Bao Ngoc Helen, Quach. “Unpublished Data.”


