Journal of
Data Mining in Genomics & Proteomics

Avşar-Ban et al., J Data Mining Genomics Proteomics 2013, 4:3
DOI: 10.4172/2153-0602.1000137

Review Article Open Access

High-Throughput Injection System for Zebrafish Fertilized Eggs

Eriko Avşar-Ban[1], Hideo Miyake[1], Masaru Obata[2], Masatoshi Hashimoto[2] and Yutaka Tamaru[1]*

[1]*Graduate School of Bioresources, Mie University, 1577 Kurimamachiya, Tsu, Mie 514-8507, Japan*
[2]*Hashimoto Electronic Industry Co., Ltd. 3866-12 Takasu, Matsusaka, Mie 510-0104, Japan*

Abstract

The zebrafish have been successfully become a popular vertebrate model for studying gene function by large-scale genetic screens due to the see-through embryos and their accessibility to various experimental techniques throughout development. Furthermore, whole genomic sequence of the zebrafish genome has completely finished and its relationship to the human genomic data became clearer. The request of technological development in experiment using zebrafish must be accelerated with the increasing demand of zebrafish for understanding of the roles of specific genes involving human diseases. Here, we introduced a high-throughput injection system for zebrafish fertilized eggs to overcome the problems caused by manual injection and proposed a unique utilization of zebrafish as a host vector system of protein expression, not as a vertebrate models.

Keywords: Zebrafish fertilized egg; Protein expression; High-throughput injection system

Review

As an important animal model, the zebrafish (*Danio rerio*) has been used around the world for the basic research such as vertebrate development. This popular aquarium fish spawns hundreds of eggs in each egg laying and fertilized eggs and embryos are transparent. The see-through embryos develop rapidly outside their mother, all major organs appear within 48 hours post-fertilization (hpf), and the approximate generation time is three months. The development and function of zebrafish organs, such as gonads, liver, gut, heart, muscle, brain, and so on, are well characterized and significantly similar to those of humans. Therefore the zebrafish is a useful model for not only classical vertebrate developmental studies but also researches on toxicology, human congenital diseases, and so on. Most recently, genomic sequence in zebrafish was fully unveiled. As a result, the zebrafish has 26,000 genes and about 70% of them are common in human genes [1]. Furthermore, there are 84% of the orthologue genes in zebrafish which involved in human disease [1], so that the zebrafish must become more useful for researching many human genes implicated in diseases. Due to recent technological advancements including transgenic strains using Tol2 system [2], reverse genetics using RNAi like anti-sense morpholino mediated knockdown or targeted mutagenesis by zinc finger nucleases (ZFNs) [3], TALENs [4], and CRISPR [5] technologies, the attractiveness of the zebrafish as a vertebrate model is enhanced by improving methodologies for functional characterization of genes.

Injection of foreign materials such as DNA, RNAi, protein, and chemical compounds into zebrafish fertilized eggs is always attached to studies on genetics, transgenics, and drug discovery using zebrafish. However, the problems are caused by manual injection, such as variations in success rates and the results without reproducibility induced by individual proficiency or human fatigue. In addition, manual injection is very slow. Although it takes time to inject into a large number of fertilized eggs, it takes more time to collect them from aquatic tanks by pipetting and to set them on agarose gel. To overcome these problems, we have developed the automated high-throughput injection system consisting of three units; fully automatic egg-collecting unit, egg-arraying unit, and injection unit with multiple capillary syringes.

Since cell division during zebrafish embryogenesis occurs astonishingly fast for every 15 to 20 minutes, it is necessary to carry out the microinjection into thousands of fertilized eggs within a short time for obtaining statistically accurate data. In general, the processes of microinjection with zebrafish are composed of two steps, i.e., recovering fertilized eggs from water tanks and arraying them on the injection plate made by agarose gel. Furthermore, since these processes are ordinarily performed by manually pipetting, it is time-consuming even though it seems quite simple work. Therefore, we first approached the research and development of water tank system for automatically collecting zebrafish fertilized eggs (Figure 1). Water tank system consists of 6 rooms (Figure 1A) that can be arranged 10 small book-shaped tanks that have a rectangular-shaped upper part and a cone-shaped lower part (Figure 1B). Spawned eggs go down and flow outward of each tank through a tube connected to the bottom of tank. Each room has an independent drain and is lighted up in turn with a predetermined interval so that fertilized eggs are spawned at differential time and are gathered in a sifter set under the drain tube. Next, the collected eggs are manually poured into an egg-arraying apparatus which is one of the components in the automated injection system (Figure 2). In the egg-arraying unit, there are three parts: (1) a plate supplier, (2) a water supplier, and (3) an egg-arraying plate (308-well slides). Subsequently, zebrafish fertilized eggs are pulled into each well by a power of circulation water in the egg-arraying plate. To remove the extra eggs on the plate, after closing 308 wells by a sliding shutter which is one of the components of egg-arraying plate, the plate is inclined and the extra eggs poured off from the sliding shutter. The speed of egg-arraying process is approximately a 308-well plate per a minute. The egg-arraying plate filled with fertilized eggs moves onto a multiple-injection unit containing 14 capillary syringes. Injection into the fertilized eggs starts after the shutter is opened and finally

***Corresponding author:** Yutaka Tamaru, Graduate School of Bioresources, Mie University, 1577 Kurimamachiya, Tsu, Mie 514-8507, Japan, Tel/Fax: +81 59 231 9560; E-mail: ytamaru@bio.mie-u.ac.jp

Received May 20, 2013; **Accepted** July 25, 2013; **Published** July 31, 2013

Citation: Avşar-Ban E, Miyake H, Obata M, Hashimoto M, Tamaru Y (2013) High-Throughput Injection System for Zebrafish Fertilized Eggs. J Data Mining Genomics Proteomics 4: 137. doi:10.4172/2153-0602.1000137

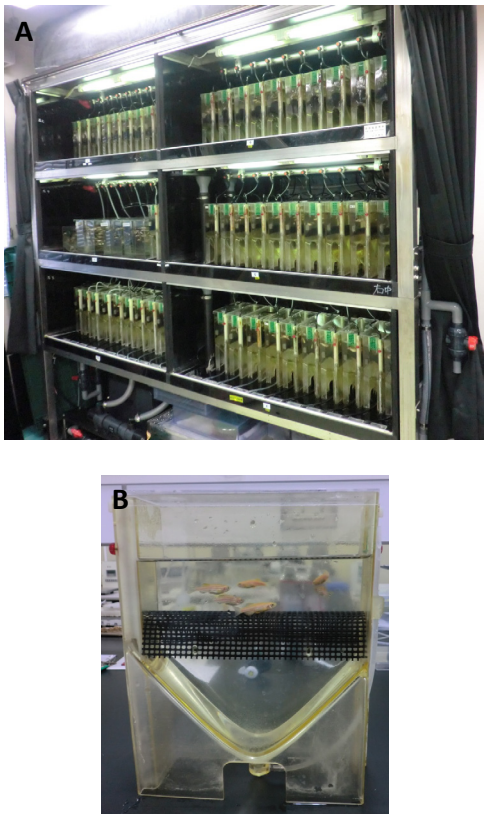

Figure 1: The water tank system automatically collecting zebrafish fertilized eggs. (A) Overview of water tanks system consisting of six rooms. Each room has an independent drain and is lighted up at the appointed time respectively. (B) A custom-made water tank which has a rectangular-shaped upper part and a cone-shaped lower part. It is capable to arrange ten water tanks per room.

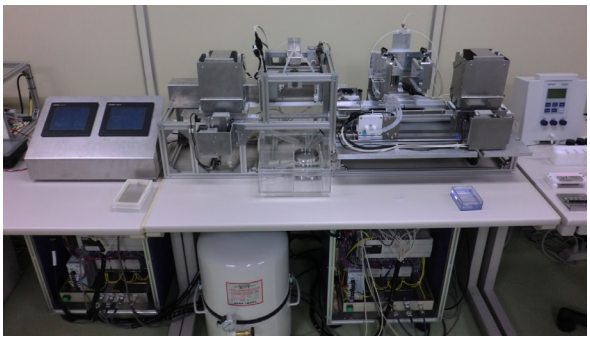

Figure 2: Whole image of the automated injection system. The injection system consists of 5 parts: control panel, plate supplier, egg arraying apparatus, injection apparatus, and plate receiver.

the plate slide into a plate receiver. The injection process is performed concurrently with egg-arraying and has been finished within two minutes. Finally, over 10,000 of zebrafish fertilized eggs per an hour are automatically injected by using the high-throughput injection system. Gene expression efficiency in zebrafish embryos depends on a construct of gene expression plasmid and the development-stage as described above. Therefore, we compared the injection efficiency with manual and machine as an indicator of EGFP expression (Table 1). As a result, the HTP injection system is either not inferior to or is not superior to manual injection. However, in case of the low expression efficiency, since we have to inject the target gene into large amounts of fertilized eggs to obtain more it's expressing embryos. Therefore, we propose the HTP injection system for zebrafish researchers.

Since 2006, in the research on solving membrane protein structures, the number of the recombinant membrane proteins has been increasing more than the proteins extracted from natural sources [6]. More than half of the recombinant membrane proteins were produced using prokaryotic cells, whereas the rest of them were produced using eukaryotic cells such as yeast, insect and mammalian. On the other hand, protein structure by the recombinant proteins produced by fish cells has never been solved so far. In the previous study, we revealed that the recombinant hPOMGnT1 was able to be expressed in the whole bodies of zebrafish embryos with its functional property [7]. We have been focusing on a potential of zebrafish host vector system to produce recombinant proteins which are difficult to be produced by the existing system such as Escherichia coli, yeast, and so on, and have developed the high-throughput injection system for zebrafish fertilized eggs. In order to construct the best use of our zebrafish expression system and high-throughput injection system, we are proposing a unique utilization of zebrafish not as a traditional vertebrate model, as a 'fish protein bank' (Figure 3). When a transgenic zebrafish line is stably produced, the recombinant proteins could be continuously supplied in large quantity just by spawning and incubating embryos for a couple of days. Or all you have to do is just injection of gene expression vector into zebrafish fertilized egg by the high-throughput (HTP) injection system; targeting protein can be produced in a few days, even if a transgenic

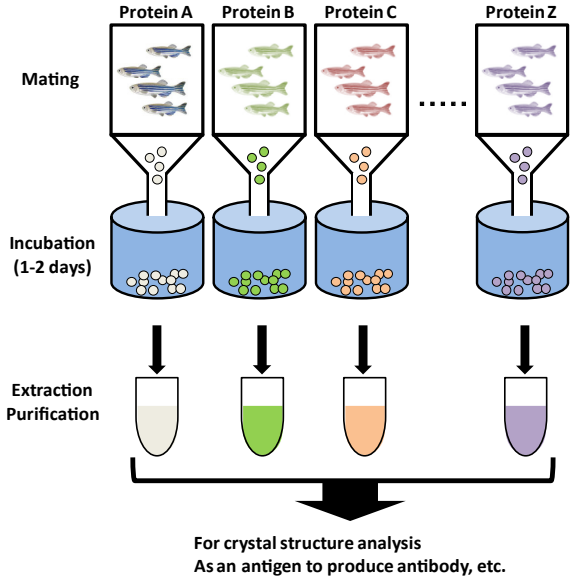

Figure 3: Concept of fish protein bank. The innovative utilization of zebrafish as host cells for the production of recombinant proteins, especially membrane proteins. The concept is 'Easy to produce proteins you need whenever you want'.

	Manual		Machine		No injection	
Dead	103	27.5%	220	27.7%	75	25.0%
EGFP(-)	140	37.4%	232	29.2%	225	75.0%
EGFP(+)	131	35.0%	343	43.1%	-	-
Total	374		795		300	

Injection solution: pXI-EGFP-His (50 ng/μl) and 100 μM (micro M) Rhodamine B

Table 1: Injection efficiency with manual and injection machine.

line is unable to be generated due to its characteristics. To quantify the injection efficacy by the completed injection system, a zebrafish expression vector, pZex-EGFP-pXI-hPOMGnT1 [7], was injected with a fluorescent dye, 100 μM Rhodamine B. By counting injected fertilized eggs, a success rate of injection was 85.7% as an indicator of Rhodamine B. Gene expression efficiency was 46.3% by counting EGFP-expressing embryos at 24 hpf. Eventually, by using the injection system, the efficiency of gene expression and survival rate of zebrafish embryos is as well as manual operation. Furthermore, we estimated the amount of expressed recombinant protein human protein *O*-linked mannose β-1, 2-*N*-acetylglucosaminyltransferase 1 (hPOMGnT1). After extraction of a membrane-protein fraction by ultracentrifugation from EGFP-expressing embryos, dot blot analysis revealed approximately 2.5 ng of hPOMGnT1 per a zebrafish embryo. The amount of expressed proteins may vary depending on targeting genes or expression vector. To increase the amount of target proteins, it is necessary to optimize the promoter region in the expression vector.

To realize this concept, we are carrying out the research and development of zebrafish expression vector with optimized promoter to increase the amount of target protein and trying to express multiple membrane protein (i.e., G protein-coupled receptor) which is difficult to express the full length form in the conventional expression host.

Acknowledgement

The research was supported by the Development of System and Technology for Advanced Measurement and Analysis, Japan Science and Technology Agency (JST -SENTAN).

References

1. Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, et al. (2013) The zebrafish reference genome sequence and its relationship to the human genome. Nature 496: 498-503.
2. Kawakami K (2004) Transgenesis and gene trap methods in zebrafish by using the Tol2 transposable element. Methods Cell Biol 77: 201-222.
3. Doyon Y, McCammon JM, Miller JC, Faraji F, Ngo C, et al. (2008) Heritable targeted gene disruption in zebrafish using designed zinc-finger nucleases. Nat Biothechnol 26: 702-708.
4. Huang P, Xiao A, Zhou M, Zhu Z, Lin S, et al. (2011) Heritable gene targeting in zebrafish using customized TALENs. Nat Biothechnol 29: 699-700.
5. Hwang WY, Fu Y, Reyon D, Maeder ML, Tsai SQ, et al. (2013) Efficient genome editing in zebrafish using a CRISPR-Cas system. Nat Biothechnol 31: 227-229.
6. Bill RM, Henderson PJF, Iwata S, Kunji ERS, Michel H, et al. (2011) Overcoming barriers to membrane protein structure determination. Nat Biotechnol 29: 335-340.
7. Avsar-Ban E, Ishikawa H, Akiyama S, Manya H, Endo T, et al. (2012) Functional and heterologous expression of human protein *O*-linked mannose β-1,2-*N*-acetylglucosaminyltransferase 1 in zebrafish. J Biosci Bioeng 114: 237-239.

This article was originally published in a special issue, **Bioinformatics for High-throughput Sequencing** handled by Editor: Dr. Heinz Ulli Weier, Lawrence Berkeley National Laboratory, USA