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Short Communications

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Unexpected Stability of Lentivirus Upon Freeze-Thaw Cycles

Shaoshuai Meng M[1*] and Wanting Yu[1]

School of Life Sciences, Peking University, Biomedical Pioneering Innovation Center, Peking University

[*]**Corresponding author**
Shaoshuai Meng M, School of Life Sciences, Peking University, Biomedical Pioneering Innovation Center, Peking, China.

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Characterizing virus stability at real-world conditions is crucial for infectious disease mitigation1,2. It is widely believed that virus are severely affected by repeated freeze-thaw cycles3,4. However, no studies have been conducted, to our knowledge, directly detailing this issue. Here, we present the effects on the infectivity of lentivirus upon multiple rounds of freeze-thaw cycles at different conditions. Surprisingly, we found that lentivirus is quite stable at freeze-thaw stress. It was able to keep up to about 40% activity after 10 freeze-thaw cycles.

We directly used GFP-expressing lentivirus for testing of its freeze-thaw stability. Briefly, clarified lentivirus containing DMEM supernatants was added to 293T cells cultured in 12-well plates (3.5x 105 cells per well) at 5μl per well directly or after 1, 3, 5, 10 freeze-thaw cycle(s) and GFP positive rate of transduced cells was measured afterwards. As for the freeze-thaw cycles, 30 μl lentivirus in EP tubes (Thermo Scientific™ 90410; 1.5 mL, low protein binding) was fast freezon in liquid nitrogen and then thawed on ice (completely thawed after about 40 mins) or in 37 ℃ water bath (completely thawed after about 1 min). After refreshing medium about 18 hours later, GFP positive rate was measured by flow cytometry (BD FACS Aria III) 40 hours post transduction.

We found that the decay rates of lentivirus infectivity were similar between quick (37 ℃ water bath) and slow (on ice) thawing conditions. About 83.5% (95%CI: 69.2%-97.9%), 67.0% (95%CI: 59.9%-74.1%), 51.2% (95%CI: 45.1%-57.3%), 38.4% (95%CI: 31.0%-45.8%) infectivity was kept at quick thaw condition and 83.2% (95%CI: 51.6%-114.9%), 54.4% (95%CI: 45.6%-63.1%), 48.3% (95%CI: 38.6%-58.1%), 40.1% (95%CI: 16.5%-63.8%) infectivity was kept at slow thaw condition after 1, 3, 5, 10 freeze-thaw cycles, respectively (Figure-1).

We acknowledge that it only represents lentivirus in culture medium supernatants. Nevertheless, our data demonstrated that virus could be quite resistant to freeze-thaw cycles. Our finding strongly suggests that life threatening high-impact virus should be further characterized for its stability at real-world conditions. Particularly, facing this pandemic challenge, we should carefully evaluate the possibility of transmission of virus through cold chain etc. And stringent usage of personal protective equipment and intensify virus surveillance should be implanted for cold chain related workers.

Contributors

SM conceived the study. SM designed the study and performed the experiments. SM and WY analyzed the data. SM wrote the manuscript. All authors approved the final version of the manuscript.

Declaration of Interests

All authors declare no competing interests. This study did not receive any external financial supports.

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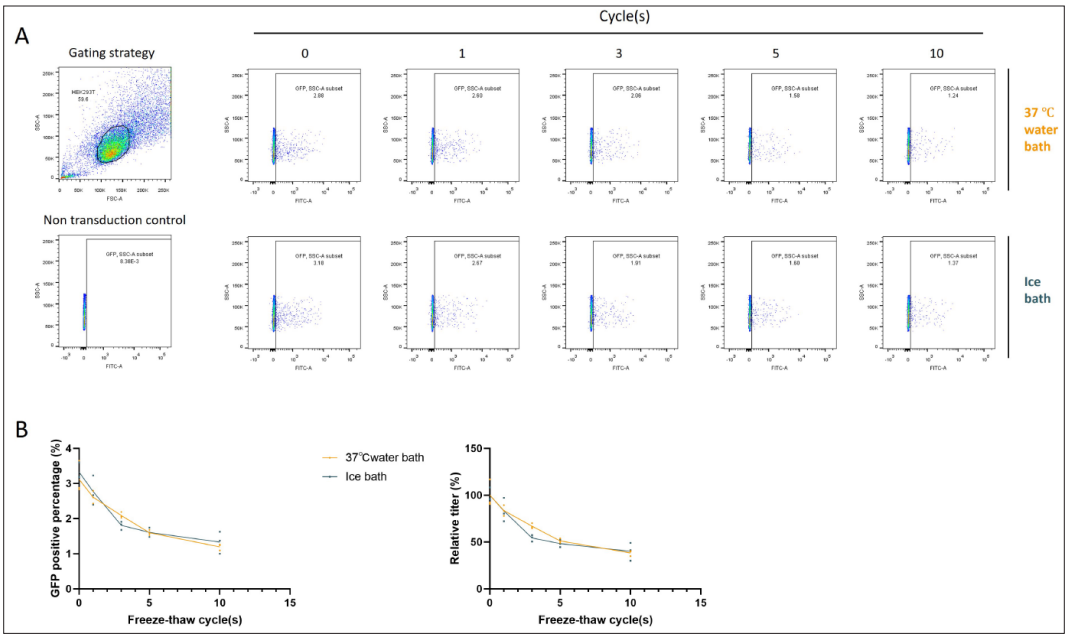

Figure-1: Lentivirus Remains Stable Upon Multiple Rounds of Freeze-Thaw Cycles at 37°C Water Bath or Ice Thaw Conditions. (A) Typical flow cytometry data. (B) Statistical data of 3 biological replicates

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