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Intracellular trehalose via transporter TRET1 as a method to cryoprotect CHO-K1 cells

(Supplement file)

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Plasmid map of the trehalose transporter (TRET1)

Figure S1 shows the plasmid map of the trehalose transporter (TRET1) expression vector used in the present study (pcDNA5/FRT-PvTret1-AcGFP1).

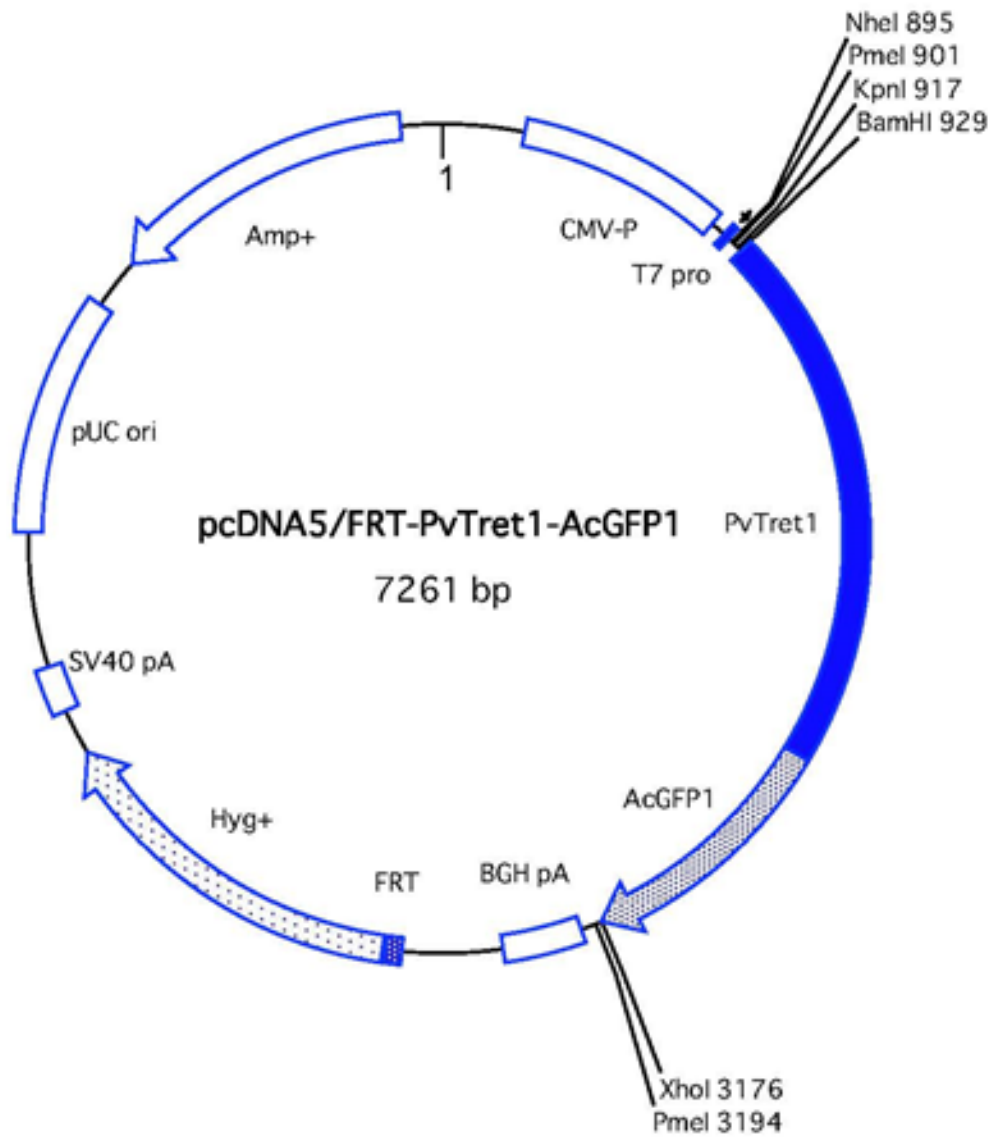


Fig. S1. Plasmid map of the trehalose transporter (TRET1) expression vector used in the present study (pcDNA5/FRT-PvTret1-AcGFP1).

Estimation of the cooling rate V_f at various experimental conditions

In the experiments, we obtained the temperature profile during the sample cooling. Figure S2 shows a typical profile of cooling on 1-mL, 500-mM trehalose-containing medium.

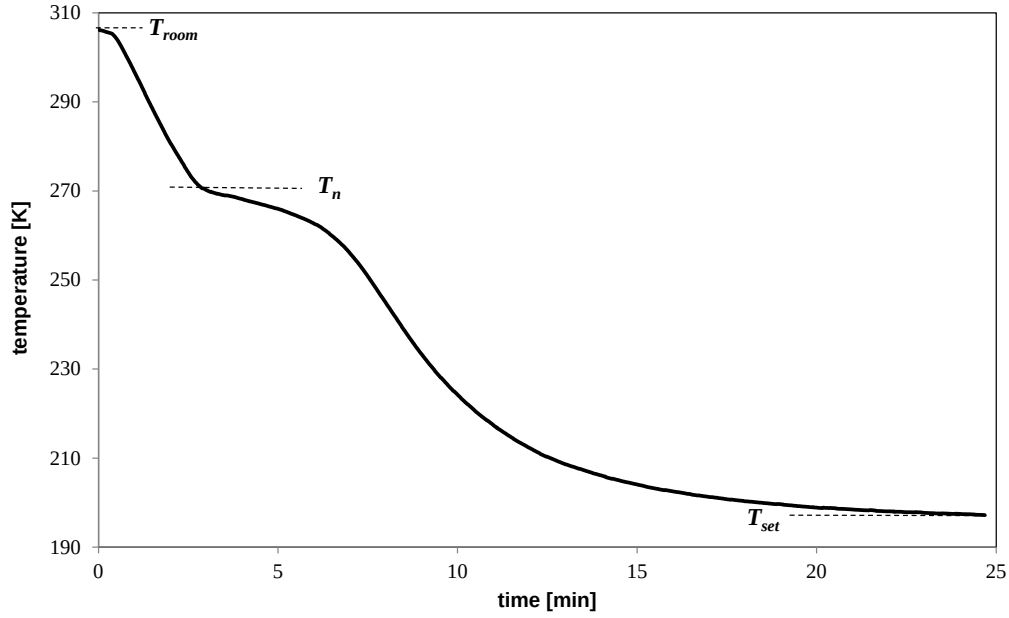


Fig. S2: Temperature profile of the freezing medium including 500-mM trehalose.

where T_{room} , T_n , and T_{set} are the room temperature, the ice nucleation temperature, and the setting temperature of the freezer, respectively. We then calculate the temperature gradient dT/dt [K/min] at each temperature, and plot the results in Fig. S3.

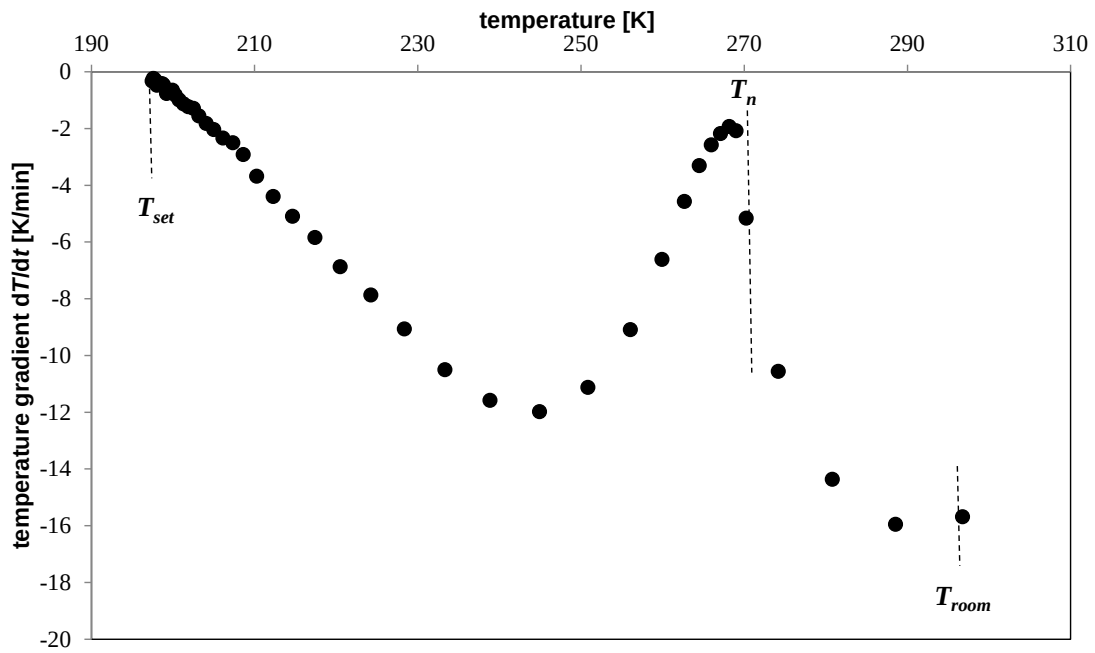


Fig. S3: Temperature gradient profile of the freezing process of the medium including 500-mM trehalose.

The cooling rate V_f is defined as the maximum value of $-dT/dt$ at $T < T_h$. For each T_{set} , we made 10 measurements of V_f , obtaining the average value and standard deviation (SD). Figure S4 shows the resulting V_f versus T_{set} diagram used for the conversion from Fig. 4 to Fig. 5.

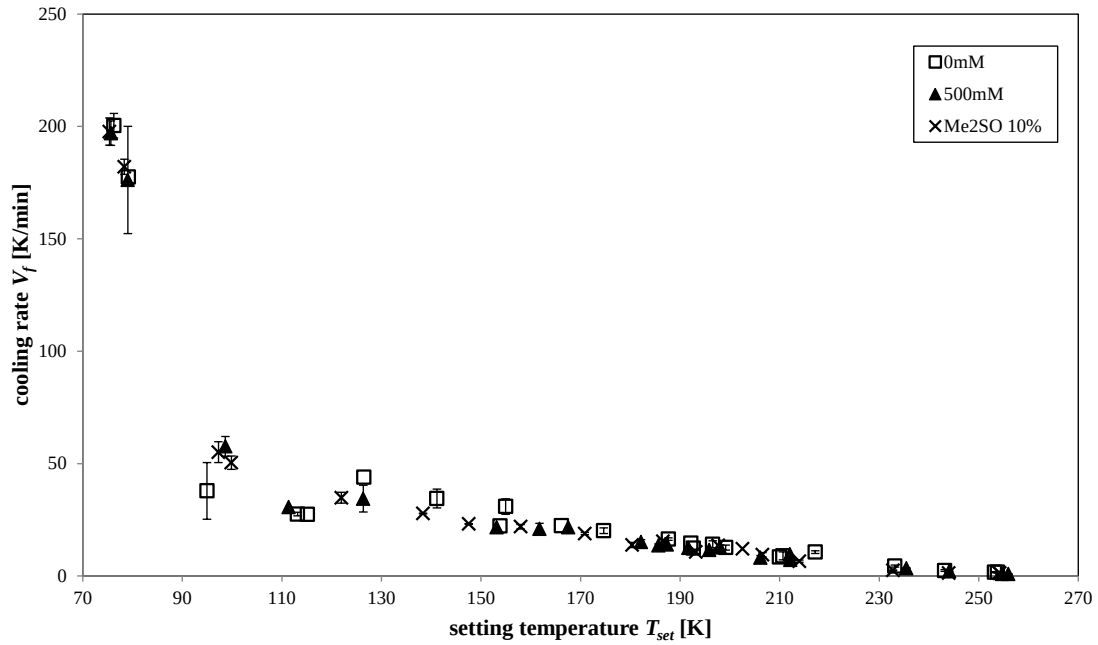


Fig. S4: Conversion diagram for T_{set} vs V_f of the medium (0 mM), of the medium including 500-mM trehalose, and of medium including 10 vol% Me₂SO. Error bars are $\pm$ SD.