Ablation of β-cells in Tg(*ins:NTR-mCherry*) transgenic zebrafish using metronidazole

Version: 1
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Summary:

To induce hyperglycemia in larval zebrafish, utilize a genetic β-cell ablation system involving the Tg(*ins:NTR-mCherry*) transgenic zebrafish strain (courtesy of Dr. Michael Parsons, Johns Hopkins University). This strain expresses the bacterial enzyme Nitroreductase (NTR), which is encoded by the *nfsB* gene, in β-cells of the pancreas starting around 24 hours post fertilization (hpf). Because NTR is genetically fused to the red fluorescent protein mCherry, β-cells expressing NTR can be visualized with fluorescence microscopy. Upon addition of the prodrug metronidazole (MTZ) to the embryo medium, NTR converts MTZ into a toxin, which selectively ablates NTR-expressing β-cells (Curado et al., Nature Protocols 3, 948-954 (2008). This can be monitored by the disappearance of the fluorescence under a confocal microscope.

Reagents and Materials:

Reagent/Material	Required concentration	Vendor	Order Number
Tg(*ins:NTR-mCherry*)		Dr. Michael Parsons, Johns Hopkins University	
Sea salt	0.3 g/ L	Instant Ocean (Pet store)	
1-phenyl-2-thiourea (PTU)	0.003 %	Sigma	P7629
Metronidazole	10 mM	Sigma	M3761
Dimethyl sulfoxide (DMSO)	0.5 %	Sigma	276855

Reagent Preparation:

Reagent 1: Embryo medium
Preparation: Add 0.03 % Instant Ocean salt (Pet store) into double distilled water.

Reagent 2: Embryo medium with 1-phenyl-2-thiourea (PTU) to prevent pigment formation.
Preparation: To embryo medium in a small beaker add a final of 0.003% PTU and dissolve overnight using a magnetic stir bar. Keep PTU solution in the dark.

Reagent 3: Metronidazole for β-cell ablation.
Preparation: First make a 2x MTZ stock (20 mM) by adding 0.17 g MTZ to a 50 ml Falcon tube. Next, add 49.5 ml of embryo medium containing 0.003 % PTU and 0.5 ml DMSO (makes 1 %). Dissolve MTZ by vortexing for ~ 5 minutes or until dissolved. Dilute the 2x MTZ stock 1:1 with embryo medium to make a 10 mM MTZ/ 0.5 % DMSO working solution. Store 50 % of 2x MTZ stock in the fridge overnight. Use the other half immediately. Discard the 2x MTZ stock latest after 2 days due to degradation of the MTZ.

Reagent 4: DMSO control solution.
Preparation: Make a 0.5 % DMSO solution by adding 0.25 ml DMSO to 50 ml of embryo medium.

Protocol:

Collect fertilized Tg(*ins:NTR-mCherry*) transgenic zebrafish embryos and incubate at 28ºC for 24 hours. Remove dead embryos and replace old embryo medium with fresh embryo medium containing 0.003 % PTU to prevent pigment formation. Remove chorions from embryos according to Protocol 1 and add the 1x MTZ working solution to the embryos. Incubate in the dark (due to light-dependent degradation of MTZ) at 28ºC for 24 hours. Assess ablation efficiency with confocal imaging. At 48 hpf, exchange media with fresh MTZ solution and incubate for another 24 hours. Repeat confocal analysis.

Potential Pitfalls:

1. Ablation efficiency is low: MTZ might be old or only incompletely dissolved in the embryo medium. MTZ might alternatively be degraded if embryos were incubated in light, as MTZ is light sensitive.
2. MTZ does not dissolve: DMSO and volume of initial solution is both crucial for dissolving the MTZ. High concentrations will not go into solution. Vortex for extended periods if it does not dissolve within 5 minutes.
3. MTZ treatment causes larvae to appear unhealthy or die: MTZ may be old or degraded due to excessive light exposure. We found that this affects the health of larval zebrafish. DMSO concentrations, if too high (above 1 - 2 %) may also cause larvae to appear unhealthy over time (bend axis). The same is true for larvae that are incubated for more than 2 days in MTZ. If ablation is not sufficient, try to incubate for 2 days and then let larvae recover for 1 or 2 days until a second MTZ treatment.