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ABSTRACT FULL TEXT

Genetic Analysis of Digestive Physiology Using Fluorescent Phospholipid Reporters

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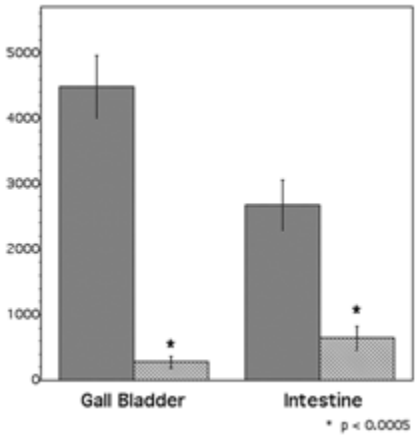
Supplementary Material

BODIPY FR-PC synthesis. Bis-BODIPY-FL-C_{11}-PC (Molecular Probes Inc., Eugene, OR) was deacylated with *N. naja* PLA_2 at the *sn*-2 position as described (*1*). The lysophospholipid (lyso-BODIPY-FL-C_{11}-PC) was acylated with BODIPY(558/568)-dodecanoic acid (Molecular Probes) in the presence of dicyclohexylcarbodiimide and 4-(dimethylamino)pyridine as described (*2*) and the product which was purified by column chromatography on silica gel (eluted with $CHCl_3$-CH_3OH-H_2O, 65/35/2). Further purification was achieved by preparative TLC on silica gel using $CHCl_3$-CH_3OH-HOAc-H_2O (50/30/8/4). The silica gel containing a single spot of pure lipid was eluted with methanol by vortexing and centrifugation. The visible spectrum in ethanol showed peaks at 558 nm and 503 nm due to BODIPY(558/568) and BODIPY-FL(503/512) respectively. The peak at 558 nm was used with an extinction coefficient of 91,000 M^{-1} cm^{-1} (Molecular Probes Inc.: www.probes.com/servlets/datatable?id=28830) to determine concentration.

To view these movies, download a QuickTime viewer.

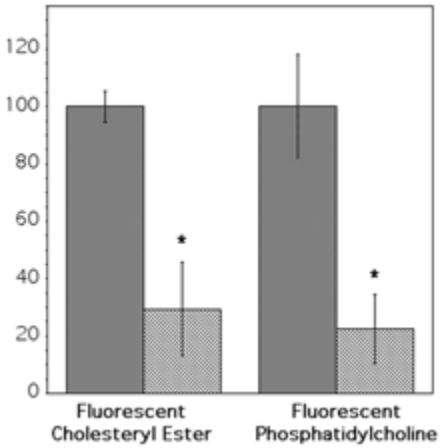
- Movie 1
  Web movies 1 and 2. Movies following PED6 labeling. To study intestine and gall bladder function, the intensity of fluorescence was observed in digestive organs and recorded in time-lapse movies of gall bladder contraction and intestinal motility. After PED6 ingestion, secretion of fluorescent bile from the gall bladder was observed, as was excretion of fluorescent bile. Time-lapse movies also illustrate how PED6 labeling facilitates the visualization of intestinal morphology and motility in zebrafish.
- Movie 2

Supplemental Figure 1. Comparison of fluorescent emissions from WT and *fat free* larval digestive organs; data represent mean ± SEM, $n = 7$ *fat free*: 14 WT, $^*P < 0.0005$.



Medium version | Full size version

Supplemental Figure 2. Quantification of TLC analysis. Data represent mean ± SEM, *n* = 4, **P* < 0.05, WT represented by solid bars.



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References

1. S. Ali, R. Bittman, *Chem. Phys. Lipids***50**, 11 (1989).

2. S. F. Martin *et al.*, *J. Org. Chem.* **59**, 4805 (1994).