



The Twinkle Factory

match_{dark}

a.k.a. HBIR-3M

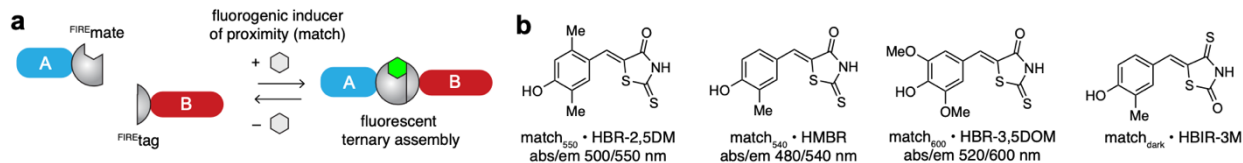
Reference matchDark-50X

Quantity 63 µg

Store at 2-8 °C

match_{dark} is a molecular glue that can be used to selectively induce the dimerization of proteins fused to cognate ^{FIRE}mate and ^{FIRE}tag. One vial includes 250 nmol of **match_{dark}**, enabling to prepare 50 mL of a 5 µM dimerizing solution.

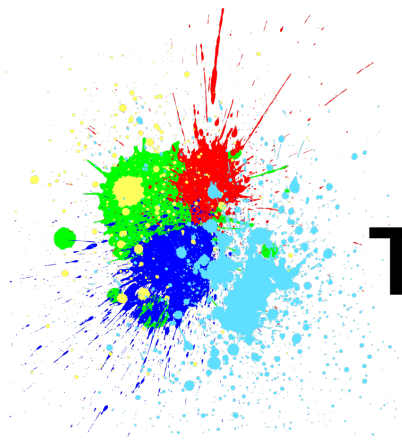
The Twinkle Factory technology CATCHFIRE (Chemically Assisted Tethering of Chimera by Fluorogenic Induced Recognition) is a self-reporting chemically induced dimerization technology that enables one not only to artificially control the proximity of two proteins of interest in cells, but also to see their interactions. The two proteins are genetically fused to two small protein domains (^{FIRE}mate et ^{FIRE}tag) which are capable of interacting together in a reversible fashion in the presence of small fluorogenic molecular glues. When the two domains interact, the fluorescence of the assembly increases 100-fold, enabling to see the newly induced interaction by fluorescence microscopy. Unlike fluorogenic ligands, **match_{dark}** is a non-fluorogenic ligand which induces fast dimerization while remaining dark, hence freeing observation channels.



The use of CATCHFIRE implies cloning and expressing proteins fused to ^{FIRE}mate and ^{FIRE}tag, and treating cells with **match_{dark}**. The protocol is described below. Note that proteins of interest can be expressed with ^{FIRE}mate and ^{FIRE}tag as either N- or C-terminal fusions.

The Twinkle Factory provides a range of fluorogenic dimerizers of various emission wavelengths, **match₅₄₀**, **match₅₅₀**, & **match₆₀₀**, and a non-fluorogenic dimerizer, **match_{dark}**. All of them are compatible with ^{FIRE}mate and ^{FIRE}tag.

Cells expressing proteins fused to ^{FIRE}mate and ^{FIRE}tag are not supplied by The Twinkle Factory. Plasmids containing ^{FIRE}mate and ^{FIRE}tag genes are available at Addgene www.addgene.org/Arnaud_Gautier/.



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Protocol of labeling in living cells

Dissolve one vial of **match_{dark}** in 50 μL of DMSO to yield a 5 mM stock solution. Mix by vortexing for few seconds until all the **match_{dark}** is dissolved. Note that different stock concentrations can be made depending on your requirements. **match_{dark}** is soluble in DMSO up to at least 50 mM.

Dilute the stock solution 1:1000 in medium or buffer to yield a 5 μM dimerizing solution. Mix thoroughly by vortexing. For best performance, add **match_{dark}** to serum-free medium or buffer, and do not keep/store the dimerizing solution. Note that different concentrations can be made depending on your requirements. Optimal concentrations range from 1 to 10 μM .

Remove the cell culture medium, wash with D-PBS, and replace the buffer with the dimerizing solution to induce protein dimerization. Image with appropriate settings.

To reverse the dimerization, remove the dimerizing solution, wash with D-PBS, and replace with culture medium.

Storage

Dry **match_{dark}** should be stored at 2-8 $^{\circ}\text{C}$ in the dark. Once dissolved in DMSO, the solution should be aliquoted to avoid repeated freeze/thaw cycles and stored at -20°C in the dark. With proper storage, **match_{dark}** should be stable at least three years dry or 6 months dissolved in DMSO.

Purity and Characterization

Purity of **match_{dark}** was determined to be $> 99\%$ by nuclear magnetic resonance (NMR) and elementary analysis.

References

A fluorogenic chemically induced dimerization technology for controlling, imaging and sensing protein proximity. *Nature Methods* **20**, 1553–1562 (2023). doi.org/10.1038/s41592-023-01988-8

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- EP 3,164,411; JP 2017-527,261; US 10,138,278 (Fluorogen activating and shifting tag (FAST))
- EP 3,719,007; US 2022-0,169,682 (Split photoactive yellow protein complementation system and uses thereof)
- EP Appl. 22,306,308.2 (Proximity inducing system and uses thereof)

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