

Description of Additional Supplementary Files

File Name: Supplementary Data 1

Description: Raw fluorescence intensities time courses and the extracted mRNA levels for the natural enhancers (*Hb_ant* and *Kr_CD2*).

File Name: Supplementary Data 2

Description: Extracted fluorescence intensities obtained with the MS2-MCP system for the *Hb_ant* and the *Bcd3* enhancers.

File Name: Supplementary Data 3

Description: Raw fluorescence intensities time courses and the extracted mRNA levels for the natural enhancers (*Bcd3*-proximal, *Bcd 3*, and *Zld-Bcd3*).

File Name: Supplementary Movie 1

Description: Spatiotemporal activity of the *hb_ant* enhancer imaged by confocal microscopy (maximum intensity projection) and using the sensitive mNeonGreen reporter system.

File Name: Supplementary Movie 2

Description: Spatiotemporal activity of the *hb_ant* enhancer imaged by confocal microscopy (maximum intensity projection) and using the MS2-MCP reporter system. The typical MS2-MCP fluorescent spots can be observed in the anterior half of the embryo.

File Name: Supplementary Movie 3

Description: Spatiotemporal activity of the *Bcd3* enhancer imaged by confocal microscopy (maximum intensity projection) and using the sensitive mNeonGreen reporter system. we detect a weak gradient of reporter expression that slowly decreases.

File Name: Supplementary Movie 4

Description: Spatiotemporal activity of the *Bcd3* enhancer imaged by confocal microscopy (maximum intensity projection) and using the MS2-MCP reporter system. A limited number of very weak fluorescent dots are almost indistinguishable from the background noise.

File Name: Supplementary Movie 5

Description: Spatiotemporal activity of the *Kr_CD2* enhancer imaged by confocal microscopy (maximum intensity projection) and using the sensitive mNeonGreen reporter system.