

TB-HOST GENES workflow

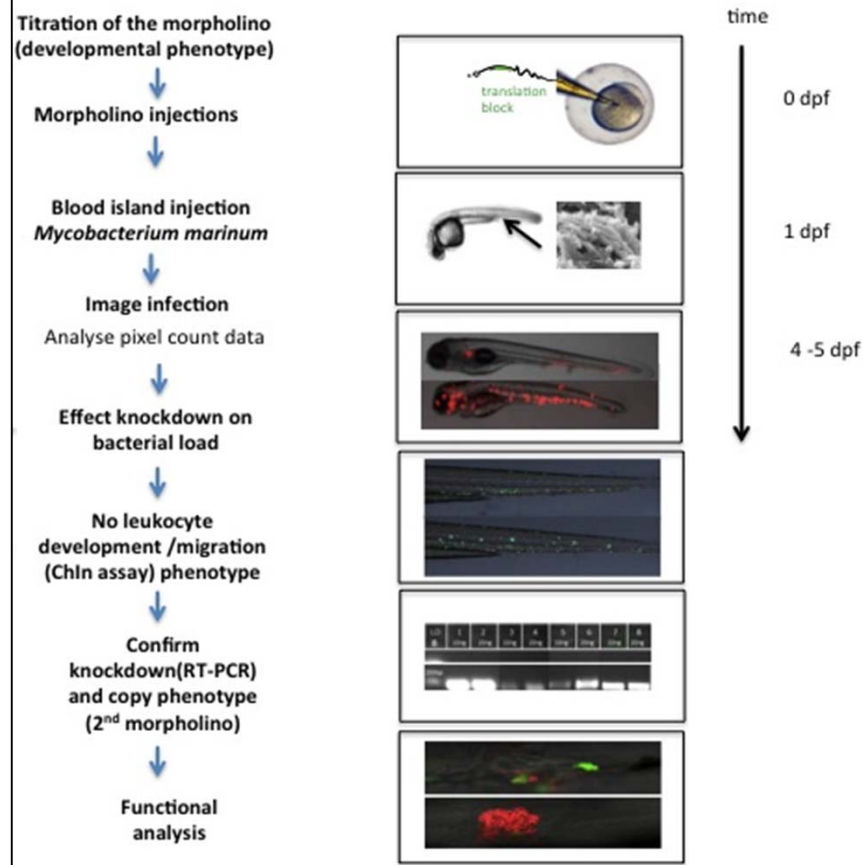


Figure 1. Workflow of the TB host genes project.

Host genes for morpholino knockdown screening were selected from prior data linking their expression profiles to innate immunity and mycobacterial infection. Gene-specific antisense morpholino oligonucleotides were injected at the 1-cell stage, followed by infection with fluorescently labeled mycobacteria at 1 day post fertilization (dpf). Fluorescent pixel quantification software was used to compare bacterial burden and granuloma formation between knockdown and control groups at 4-5 dpf. The specificity of the morpholino effect was checked by histochemical staining of leukocytes, chemically-induced leukocyte migration assays (Chin assay), RT-PCR, and phenocopy effect of a second morpholino against the same target gene. The initial screen resulted in more than 10 positive hits and follow-up studies provided further evidence for functions of two macrophage-specific genes in early stages of mycobacterial infection.