

# Cracking the genome's second code: Enhancer detection by combined phylogenetic footprinting and transgenic fish and frog embryos

Allende, Miguel L.

Manzanares, Miguel

Tena, Juan J.

Feijóo, Carmen G.

Gómez-Skarmeta, José Luis

Genes involved in vertebrate development are unusually enriched for highly conserved non-coding sequence elements. These regions are readily detected *in silico*, by genome-wide sequence comparisons between different vertebrates, from mammals to fish (phylogenetic footprinting). It follows that sequence conservation must be the result of positive selection for an essential physiological role. An obvious possibility is that these conserved sequences possess regulatory or structural functions important for gene expression and, thus, an *in vivo* assay becomes necessary. We have developed a rapid testing system using zebrafish and *Xenopus laevis* embryos that allows us to assign transcriptional regulatory functions to conserved non-coding sequence elements. The sequences are cloned into a vector containing a minimal promoter and the GFP reporter, and are assayed for their putative cis-regulatory activity in zebrafish or *Xenopus* transgenic experiments. Vectors used include plasmid DNA and the T