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A mobile insulator system to detect and disrupt cis-regulatory landscapes in vertebrates.

SUPPLEMENTAL FIGURES

Supplemental Figure 1

Supplemental Figure 1 – Souther blot for 19 ED lines.

(A) Southern blot performed for 19 ED lines using genomic DNA extracted from a single F2 animal per line. Eleven of these animals presented single insertions. (B) A second Southern blot was performed for some lines that presented more than one insertion, using genomic DNA extracted from F4 animals. Three of these animals (ED52, ED112 and ED170) presented single insertions.
Supplemental Figure 2 – Comparison of tissue specific expression of ED lines and genes.

This analysis has been done by comparing the percentage of ED lines and genes that show expression in a given tissue. A paired t-test for these two groups did not show statistically significant differences (Ttest: p=0.36) indicating that in general the data from the ED transposon reflects the expression sites of genes during embryonic development. Analyzing each anatomical region per separate we observed that there are anatomical regions that show higher bias when comparing both groups, however only a minority show differences higher than four-fold (Liver 29.2; YSL 6.4; Neural crest 6.4; Dorsal root ganglia 5.7; Posterior lateral line 5.6; Neuromasts 4.9). It should be noted that the limited number of ED lines expressed in those tissues reduces the statistical significance of these differences. In addition, these differences could be also due to the technical differences for detection of gene expression in both groups (fluorescence vs in situ hybridization). Finally, genes with known expression patterns might not be completely randomly distributed; for instances genes associated to exhaustively studied tissues might be over-represented (e.g. CNS; almost 180.000 entries in Pubmed), while genes associated to tissues not as well known might be under-represented (e.g. notochord; almost 3.000 entries in Pubmed). Data on gene tissue specific expression was extracted from zfin.org (Bradford et al. 2011).
Supplemental Figure 3 – Cre mediated excision of the ED's Insulator/RFP cassette.

The loxP flanked cassette (yellow triangles) that includes the Insulator and the RFP enhancer trap (A) can be excised by the activity of Cre recombinase (B). In this case, the downstream enhancer (a; red box) is now able to interact with the GFP minimal promoter (B), resulting in a shift of RFP expression (A; lower embryo) to GFP expression in the eye (B; lower embryo). In addition, the upstream enhancer (A; green box) can now interact with the promoter of the nearby gene (B), rescuing the loss of expression in the hindbrain that results from the insulator activity (A and B; higher embryos).
Supplemental Figure 4 - Eight ED lines injected with Cre recombinase.

Each line is documented for GFP (first column and third column) and RFP (second column and fourth column) expression, in a Cre injected (second, fourth, sixth and eighth rows; Cre) and not injected (first, third, fifth and seventh rows; No Cre) background. The line ED60 shows a shift of RFP to GFP expression in the forebrain (arrow), when comparing the control (No Cre) with the Cre injected background (Cre). The line ED77 shows a shift of RFP (No Cre) to GFP (Cre) expression in the notochord (arrow). In ED162 a shift of RFP (No Cre) to GFP (Cre) expression is observed in the posterior region of yolk syncytial layer (arrow). The ED185B line shows a shift of RFP (No Cre) to GFP (Cre) expression in the notochord (arrow). ED186 line shows a shift of RFP (No Cre) to GFP (Cre) expression in the central nervous system (arrow) and eye (asterisk). ED219 line shows a shift of RFP (No Cre) to GFP (Cre) expression in the notochord (arrow). In ED270 a shift of RFP (No Cre) to GFP (Cre) expression is observed in the posterior retina (arrow). ED272 line shows a shift of RFP (No Cre) to GFP (Cre) expression in the spinal cord (arrow).
**Supplemental Figure 5** - ED insertions on the zebrafish genome. (A) Distribution of ED mapped insertions (red triangles) on the zebrafish genome. (B) Distribution of insertions between genes (intergenic, blue; 54%), in introns (orange; 44%) and in exons (yellow; 2%).
Supplemental Figure 6

ED5

Genomic Landscape: chr6:50395584-50496447
Size of Landscape: 100863bp
Associated Gene: mych
ED20 And ED21

Genomic Landscape: chr15:37061399-37803435
Size of Landscape: 742036 bp
Associated Gene: robo1
ED25

Genomic Landscape:
chr2:32977192-33081797

Size of Landscape
104605 bp

Associated Gene:
klf4b
ED26

Genomic Landscape: chr13:9813235-10220458
Size of Landscape: 407223 bp
Associated Gene: six3a
ED27

Genomic Landscape: chr21:33320279-33735792
Size of Landscape: 415513 bp
Associated Gene: dacha
ED31

Genomic Landscape:
chr11:45555315-45655324

Size of Landscape:
100009 bp

Associated Gene:
irf2bp2b
ED33

Genomic Landscape:
chr15:14528990-14866507
Size of Landscape:
337517bp
Associated Gene:
traf4a
ED49

Genomic Landscape:
chr3:56507796-56734626
Size of Landscape
226830 bp
Associated Gene:
axin2
ED59

Genomic Landscape: chr17:25732569-26579075

Size of Landscape: 846506 bp

Associated Gene: calm1a
Genomic Landscape: chr18:24522878-24725643
Size of Landscape: 202765 bp
Associated Gene: 
rgma
Genomic Landscape: chr2:25121146-25788771
Size of Landscape: 667625 bp
Associated Gene: pld1a
Genomic Landscape: chr4:15114897-15318480
Size of Landscape: 203583 bp
Associated Gene: *atp2b1a*
ED87

Genomic Landscape: chr17:20476343-20948904
Size of Landscape: 472561 bp
Associated Gene: vsx1

![Image of genomic landscape and molecular markers]
ED97

Genomic Landscape: chr2:31732031-31995185
Size of Landscape: 263154 bp
Associated Gene: mycb
ED98

Genomic Landscape: chr22:31317812-31652128
Size of Landscape: 334316 bp
Associated Gene: mir216a-1
ED101

Genomic Landscape: chr17:18573131-18719447
Size of Landscape: 146316 bp
Associated Gene: zgc:165635
ED103

Genomic Landscape: chr22:16511237-16535821
Size of Landscape: 24584 bp
Associated Gene: lrrc39
Genomic Landscape: chr14:9669475-9703440
Size of Landscape: 33965 bp
Associated Gene: atoh8
ED114

Genomic Landscape:
chr6:36319151-36341285

Size of Landscape
22134 bp

Associated Gene:
her6
Genomic Landscape:
chr2:27080519-27252823
172304 bp
Associated Gene:
cdh7
ED155

Genomic Landscape: chr15:32970160-33170167
Size of Landscape: 200007 bp
Associated Gene: postnb
ED162

Genomic Landscape:
chr19:31785663-31936390

Size of Landscape:
150727 bp

Associated Gene:
fam49a
ED165

Genomic Landscape: chr24:23499725-24278704
Size of Landscape: 778979 bp
Associated Gene: zfhx4

Scale 200 bp

Ensembl Gene Predictions - Ensembl 71
- ENSDART00000120238
- ENSDART00000143862
- ENSDART00000135946 (zfhx4)
- ENSDART00000112236 (zfhx4)
- ENSDART00000120189
ED170

| Genomic Landscape: | Size of Landscape | Associated Gene: |
|-------------------|-------------------|-----------------|
| chr24:7703271-7783173 | 79902 bp          | ptrfb           |
ED180

Genomic Landscape:
chr7:31620580-31708059
87479 bp

Associated Gene:
aldh1a2
Genomic Landscape: chr16:26144614-26263494
Size of Landscape: 118880 bp
Associated Gene: apoa4 and apoeb
ED186

Genomic Landscape:
chr23:17942332-17964259

Size of Landscape
21927 bp

Associated Gene:
mir124-5
ED191

Genomic Landscape: chr15:26276119-26730220
Size of Landscape: 454101 bp
Associated Gene: tbx2b
ED196

Genomic Landscape:
chr1:35599004-35664148

Size of Landscape:
65144 bp

Associated Gene:
smad1
ED198

Genomic Landscape: chr15:32570115-32993341
Size of Landscape: 423226 bp
Associated Gene: mab21l1
Genomic Landscape: chr18:20040522-20265304
Size of Landscape: 224782 bp
Associated Gene: ddb2
Supplemental Figure 6 - ED mapped lines associated to a gene.

For each line, a diagram representing the genomic landscape of the ED insertion is available. The first track of this diagram represents the ED insertion point and its orientation (GFP RFP or RFP GFP) in the respective genomic landscape. The second track plots genes present in this genomic landscape (RefSeq genes). Third to eighth tracks represent epigenetic marks obtained by chip-seq (Bogdanovic et al. 2012). Each mark is plotted at two different developmental times, 24hpf (higher track) and 80% epiboly (lower track). Third and fourth tracks plot the H3K27 acetylation mark, the fifth and sixth tracks plot H3K4 mono methylation mark and seventh and eighth tracks plot H3K4 tri methylation mark. Genomic coordinates of the respective landscape are displayed under “Genomic Landscape:” (Zv9/danRer7 zebrafish genome assembly). The size of the landscape is displayed under “Size of Landscape”. The gene associated to the ED line is named under “Associated Gene:” and its expression pattern is shown in the lower panels in the image containing the corresponding gene name. Images of the GFP and RFP expression patterns for each ED line are also included in the lower panels. In some cases a probe against krox20 was used as an internal control for the in situ hybridization and its expression pattern is noted with asterisks. apoeb, atp2b1a, calm1a, ddb2, irf2bp2b, postnb, robo1, traf4a, vsx1, and zfhx4 in situ hybridization images were obtained from The Zebrafish Model Organism Database (Bradford et al. 2011). The expression patterns of atoh8, mir124-5, mir216a-1 and pld1a are described by Yao and colleges (Yao et al. 2010), Shkumatava and colleges (Shkumatava et al. 2009) and Zeng and colleges (Zeng et al. 2009).
Supplemental Figure 7 – Enhancer assay for the 0.9 kb fragment downstream of ED186 insertion.

Three different F0 embryos (rows) injected with a reporter vector (Bessa et al. 2009) to test the enhancer activity of the 0.9 kb fragment downstream of the ED186 insertion. In this assay GFP expression (green) reports enhancer activity, which is not detected at 24 (first column) or 48hpf (second column). RFP expression (Red) is used as an internal control of transgenesis (third column).
Supplemental Figure 8

**Supplemental Figure 8** - Detection of ptrfb expression by fluorescent *in situ* hybridization in a wild type and ED170 homozygous mutant background, with and without Cre recombinase.

Wild type embryos present a strong expression of *ptrfb* (green) in the notochord (left image). In ED170 homozygous mutant background *ptrfb* expression levels are strongly reduced (middle image). Cre recombinase injection in ED170 homozygous mutant embryos induces mosaics of cells expressing similar to wild type *ptrfb* levels (arrows; right image). Embryos were stained with the nuclear marker DAPI (blue) and images were acquired with a confocal microscope. Cre injected embryos displaying a high degree of mosaicism were selected to show differences of transcript levels in rescued cells versus non rescued cells within the same sample.
Supplemental Figure 9 – Selection of putative enhancers of ptrfb.

Based on H3K4me1 (green) or H3K27Ac (blue) marks at dome, 80%, epiboly and 24hpf (shown here) developmental stages, 4 candidate enhancer sequences where selected, 1 to 4 (black boxes). The H3K4me3 promoter associated mark is also displayed (orange).
Supplemental Figure 10 - Phenotypes associated to the *klf4b*/ED25 mutant and to a strong specific knockdown of *ptrfb* using a morpholino.

(A) *beta E3 globin* is expressed in blood cells of wild type embryos at 24hpf (left). This expression is reduced in the *klf4b*/ED25 mutant (right). (B) The knockdown of *ptrfb* by injecting 10 ng of a morpholino that targets specifically *ptrfb* generates a strong phenotype characterized by a dramatically bent tail.
Supplemental Figure 11 – Diagram of the different versions of ED vectors used in the ED screen.

Four different vectors are represented, IMP16, IMP17, IMPCherry and IIC. Each vector is composed by two Tol2 transposon terminal inverted repeats (orange boxes; Tol), two minimal promoters (blue boxes; MP), one insulator (purple, Insulator), two loxP sequences (yellow triangles) and two reporter genes, eGFP (green boxes; GFP) and RFP (red boxes). Different versions of RFP were used, DsRed2 fused to a myc tag (myc_DsRed2), mCherry fused to a myc tag (myc_mCherry) and mCherry alone. The vast majority of the ED lines, 223 in total, were generated using the IIC vector.

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