



Universiteit  
Leiden  
The Netherlands

## Fish genomes : a powerful tool to uncover new functional elements in vertebrates

Stupka, E.

### Citation

Stupka, E. (2011, May 11). *Fish genomes : a powerful tool to uncover new functional elements in vertebrates*. Retrieved from <https://hdl.handle.net/1887/17640>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/17640>

**Note:** To cite this publication please use the final published version (if applicable).

# Fish genomes: a powerful tool to uncover new functional elements in vertebrates

**Elia Stupka**

This work was carried out with support from the European Commission Framework VI grant TRANSCODE (LSHG-CT-2004-511990 ) as well support from A-STAR Singapore and Temasek Life Sciences Laboratory, Singapore

# Fish genomes: a powerful tool to uncover new functional elements in vertebrates

PROEFSCHRIFT

ter verkrijging van de graad van Doctor aan de Universiteit Leiden,  
op gezag van Rector Magnificus prof.mr. P.F. van der Heijden,  
volgens besluit van het College voor Promoties  
te verdedigen op woensdag 11 Mei 2011  
klokke 16.15 uur

door  
Elia Stupka

door

Geboren te Quartu Sant'Elena, Italy in 1977

## PROMOTIE COMISSIE

### *Promotor*

Prof. Dr. J.N. Kok

### *Co-promotor*

Dr. Ir. F.J. Verbeek

### *Overige Leden*

Prof. Dr. H.P. Spaink

Prof. Dr. J. Den Hertog

Dr. P. Sordino (Stazione Zoologica Anton Dohrn, Naples, Italy)

To the two shining stars in my life, Ann and Anais

To my guiding light, my grandmother Giuliana

To my grandfather Aurelio and his free spirit

<b>Chapter 1: Introduction .....</b>	<b>8</b>
<b>Introduction .....</b>	<b>8</b>
Fish as model organisms .....	8
Fish genomes .....	9
Comparative Genomics .....	10
Transcriptomics .....	12
<b>Organization of the thesis.....</b>	<b>12</b>
<b>Bibliography.....</b>	<b>14</b>
<b>Chapter 2: Whole-Genome Shotgun Assembly and Analysis of the Genome of Fugu rubripes .....</b>	<b>16</b>
<b>Abstract.....</b>	<b>17</b>
<b>Introduction .....</b>	<b>18</b>
<b>Methods .....</b>	<b>19</b>
Sequencing Methods .....	19
Assembly .....	22
Repeats and assembly .....	25
Annotation methods.....	29
<b>Results.....</b>	<b>36</b>
Whole-Genome Shotgun Sequencing and Assembly of the Fugu rubripes Genome .....	36
Preliminary Annotation and Analysis of the Fugu Genome .....	40
Introns in Fugu .....	54
Structuring of the Fugu Genome over Evolutionary Time.....	58
Comparison of Fugu and Human Predicted Proteomes.....	68
<b>Conclusions.....</b>	<b>78</b>
<b>References and Notes.....</b>	<b>81</b>
<b>Chapter 3: Shuffling of cis-regulatory elements is a pervasive feature of the vertebrate lineage .....</b>	<b>90</b>
<b>Abstract.....</b>	<b>91</b>
<b>Introduction .....</b>	<b>92</b>
<b>Results .....</b>	<b>96</b>
Identification of mammalian regionally conserved elements.....	96
Shuffling of conserved elements is a widespread phenomenon.....	99
Shuffled conserved regions cast a wider net of nongenic conservation across the genome .....	103
The proximal promoter region is a shuffling 'oasis' .....	105
Shuffled conserved regions are able to predict vertebrate enhancers .....	109
Shuffled conserved regions act as enhancers <i>in vivo</i> .....	110
<b>Discussion .....</b>	<b>115</b>
Widespread shuffling of cis-regulatory elements in vertebrates .....	115
Conservation versus function .....	117
Toward improved detection of cis-regulatory elements.....	124
In <i>vivo</i> transient assays .....	126
Mechanisms for genome-wide shuffling.....	127
<b>Conclusion.....</b>	<b>129</b>
<b>Materials and methods .....</b>	<b>129</b>
Selection of genes and sequences.....	129
Identification of mammalian regionally conserved elements.....	130
Identification of shuffled conserved regions .....	131
Gene Ontology analysis .....	131
Mapping of conserved elements .....	132

BLAST versus CHAOS comparison .....	132
Overlap analysis .....	133
Identification of control fragments .....	133
Zebrafish embryo injections .....	133
Analysis of transgene expression .....	134
<b>Acknowledgements .....</b>	<b>136</b>
<b>References .....</b>	<b>136</b>
<b>Chapter 4: The TATA-binding protein regulates maternal mRNA degradation and differential zygotic transcription in zebrafish .....</b>	<b>146</b>
<b>Abstract.....</b>	<b>147</b>
<b>Introduction .....</b>	<b>148</b>
<b>Results.....</b>	<b>150</b>
TBP regulates specifically a subset of mRNAs in the dome-stage embryo.....	150
Most TBP activated genes are dynamically regulated during zebrafish ontogeny	154
TBP dependence of transcription from isolated zebrafish promoters .....	156
TBP is required for degradation of a large number of maternal mRNAs.....	158
Identification of TBP-dependent maternal transcripts.....	160
TBP regulates a zygotic transcription-dependent mRNA degradation process .....	162
Degradation of maternal mRNA by the miR-430 microRNA is specifically affected in TBP morphants.....	164
Redundant and specific function of TBP in the activation of subsets of genes at MBT .....	167
<b>Discussion .....</b>	<b>168</b>
Redundant and specific function of TBP in the activation of subsets of genes at MBT .....	169
TBP limits certain gene expression activities in the zebrafish embryo .....	172
The mRNA degradation machinery active during maternal to zygotic transition requires TBP function.....	173
<b>Materials and methods .....</b>	<b>175</b>
Embryo injection experiments .....	175
Whole-mount <i>in situ</i> hybridisation and immunostaining .....	177
RT-PCR analysis of maternal mRNA degradation.....	177
Gene identification and statistical analysis of EST microarray data .....	177
Annotation of ESTs of the TBP microarray, in relation to the stage-dependence array and to the zebrafish genome .....	178
Degradation pattern of maternal transcripts.....	179
Identification of miR-430 targets among the genes of the TBP microarray .....	179
<b>Acknowledgements .....</b>	<b>180</b>
<b>References .....</b>	<b>180</b>
<b>Chapter 5: Assembly of the carp genome .....</b>	<b>184</b>
<b>Abstract.....</b>	<b>185</b>
<b>Introduction .....</b>	<b>186</b>
<b>Results.....</b>	<b>187</b>
Initial Dataset: pseudo-tetraploid material.....	187
Preliminary Genome Assembly .....	188
Haploid material assembly .....	189
<b>Varying the K parameter in SOAPdenovo .....</b>	<b>190</b>
<b>Varying the L parameter in SOAPdenovo.....</b>	<b>193</b>
<b>Testing read trimming strategies .....</b>	<b>195</b>
<b>Testing combination of assembly softwares.....</b>	<b>198</b>
<b>Adding BAC end reads.....</b>	<b>198</b>
<b>Assembly Statistics .....</b>	<b>199</b>

<b>Largest scaffolds .....</b>	<b>201</b>
<b>Quality Assessment.....</b>	<b>203</b>
Coverage of existing BAC clones .....	203
Coverage of all carp Genbank sequences .....	204
<b>Gap Filling .....</b>	<b>207</b>
Mitochondrial genome .....	208
RNA-Seq Analysis .....	209
<b>Methods .....</b>	<b>212</b>
Genome Assembly .....	212
QC Analysis.....	214
Graphical Reporting .....	215
<b>Discussion .....</b>	<b>215</b>
Initial pseudo-tetraploid ABYSS based assembly.....	215
Evaluation of ABYSS .....	216
Haploid DNA CLC Bio and SOAP de novo based assembly .....	216
CLC Bio Contig Assembly .....	217
The K parameter .....	218
Other SOAPdenovo parameters .....	218
BAC end reads .....	219
Assembly Assessment and QC.....	220
<b>References .....</b>	<b>222</b>
<b>Chapter 6: Discussion .....</b>	<b>224</b>
Impact of next-generation sequencing on genome research .....	224
Searching for regulatory elements.....	225
Transcriptomics.....	227
Genome Assembly .....	228
<b>References .....</b>	<b>230</b>