Annotation of *Seriola lalandi larval* reference transcriptome and differential gene expression between normal and skeletal deformed individuals

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Motivations

Phylum:	**Chordata**
Class :	**Actinopterygii**
Order :	**Perciformes**
Family :	**Carangidae**
Genus :	**Seriola**
Species:	**lalandi**

- ***Seriola lalandi*** **is widely distributed and recently being introduced to the aquaculture of Chile, North America, Japan**
- **No genomic resources available to date**
- **Incidence of skeletal deformity are high and it cause economic loss**

Objectives

- To obtain a *de novo* assembled trancriptome
- Annotation and reconstuction of pathways
- Identification of differentially expressed genes between normal and deformed individuals (bent in lower jaw) at 23 days post hatch
- Generation of hypothesis for jaw deformity in *S. lalandi* based on system biology knowledge

De novo assembly workflow

Illumina 2000^{TM}

- **Multiplexed**
- **102 X 2 cycles**

- **QC < 30**
- **Adapters**
- **Duplicates**

Trinity

- **All reads across samples**
- **Single transcriptome (CAP3)**

blast2go

- **BLASTx (< 1.0E-6)**
- **Nr-protein database of NCBI**

Differential expression and GO enrichment

CLC bio

- Length fraction >0.7 and similarity >0.9
- Reserved paired enformation

edgeR

- FDR < 0.01 and FC > abs. 2

GO enrichment

- Fisher’s exact test
- FDR (Benjamini-Hochberg) <0.05

REVIGO

reduce + visualize Gene Ontology

- Allowed similarity > 0.7
- Database = UniProt
- SimRel

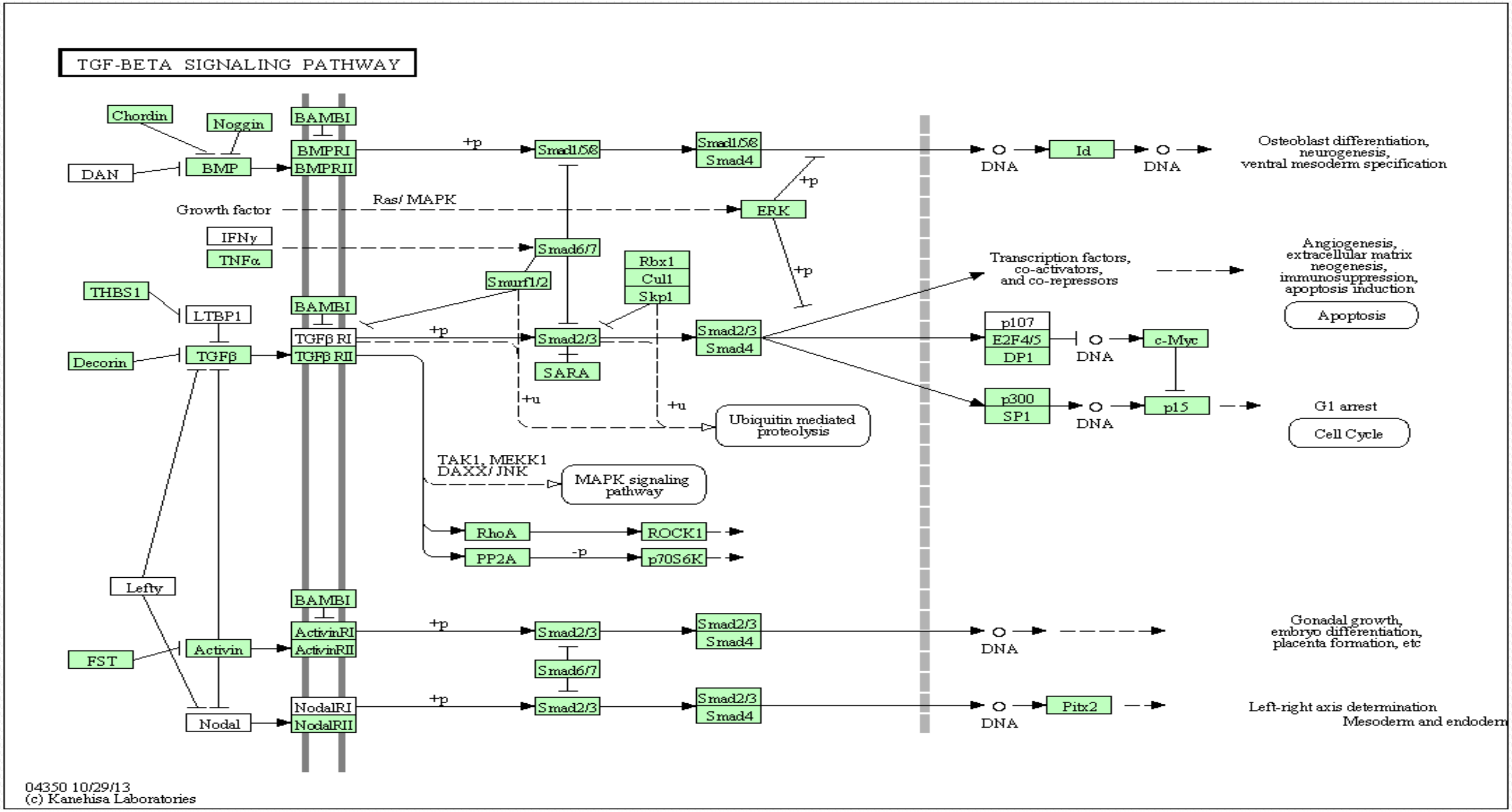
Statistics of *de novo* transcriptome

	Trinity assembly	Final transcriptome (after CAP3 clustering)
Total number of contigs (> 300 bases)	**95,495**	**40,066**
N90	**641 bases**	**788 bases**

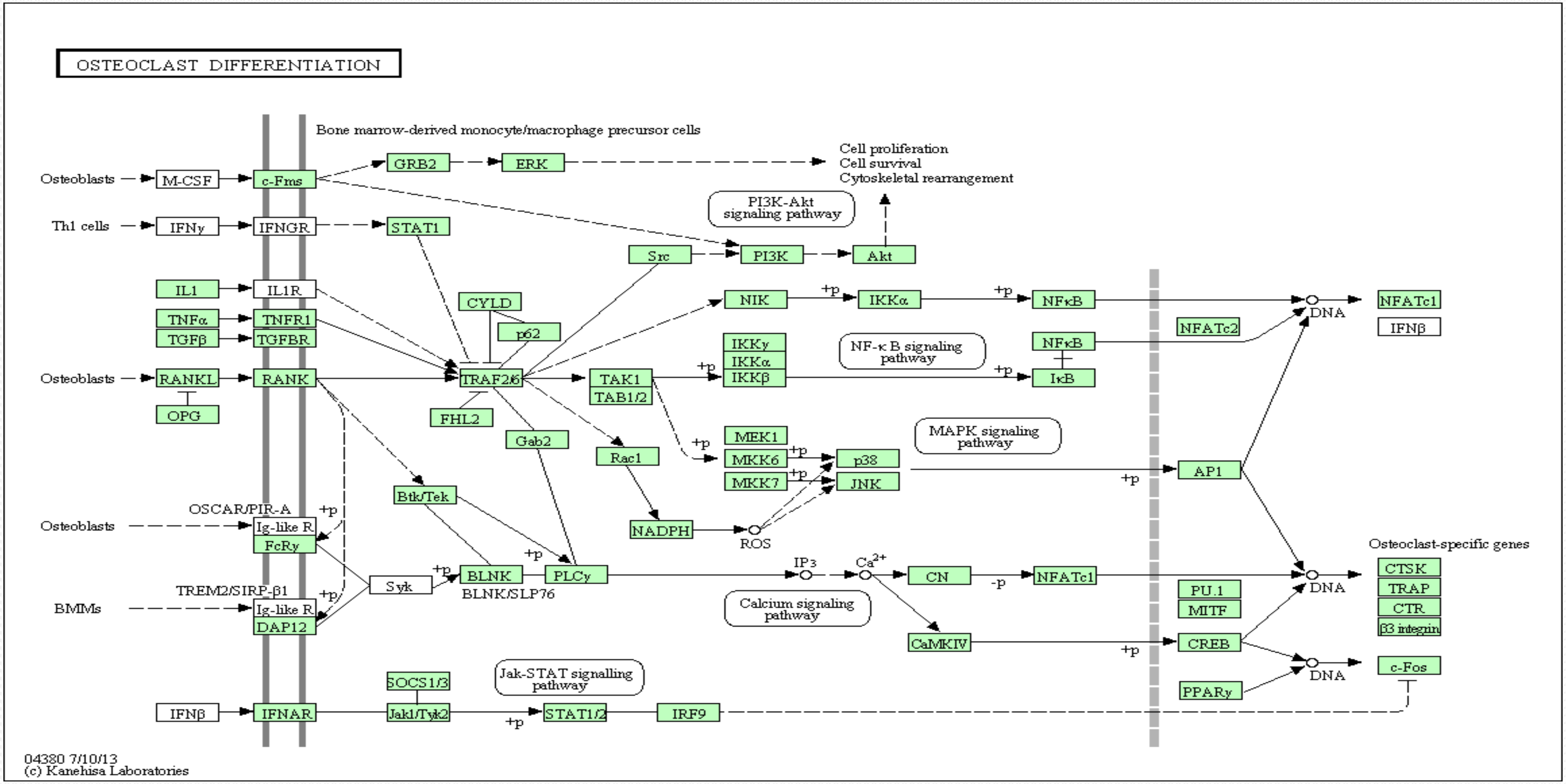
Blast, Mapping and Annotation results

Type	Number of genes	Possible reasons
Without Blast Hits	12,733	• **Lack of closely related species' sequences, UTRs, LnRNA** • **Assembly artifacts**
Annotated Sequences from BLASTx	7,150	
Annotated Sequences from InterProScan	8,694	
Final Annotations	**15,744 (39%)**	
Total Contigs	40,066	

Transforming growth factor beta pathway



Osteoclast differentiation pathways



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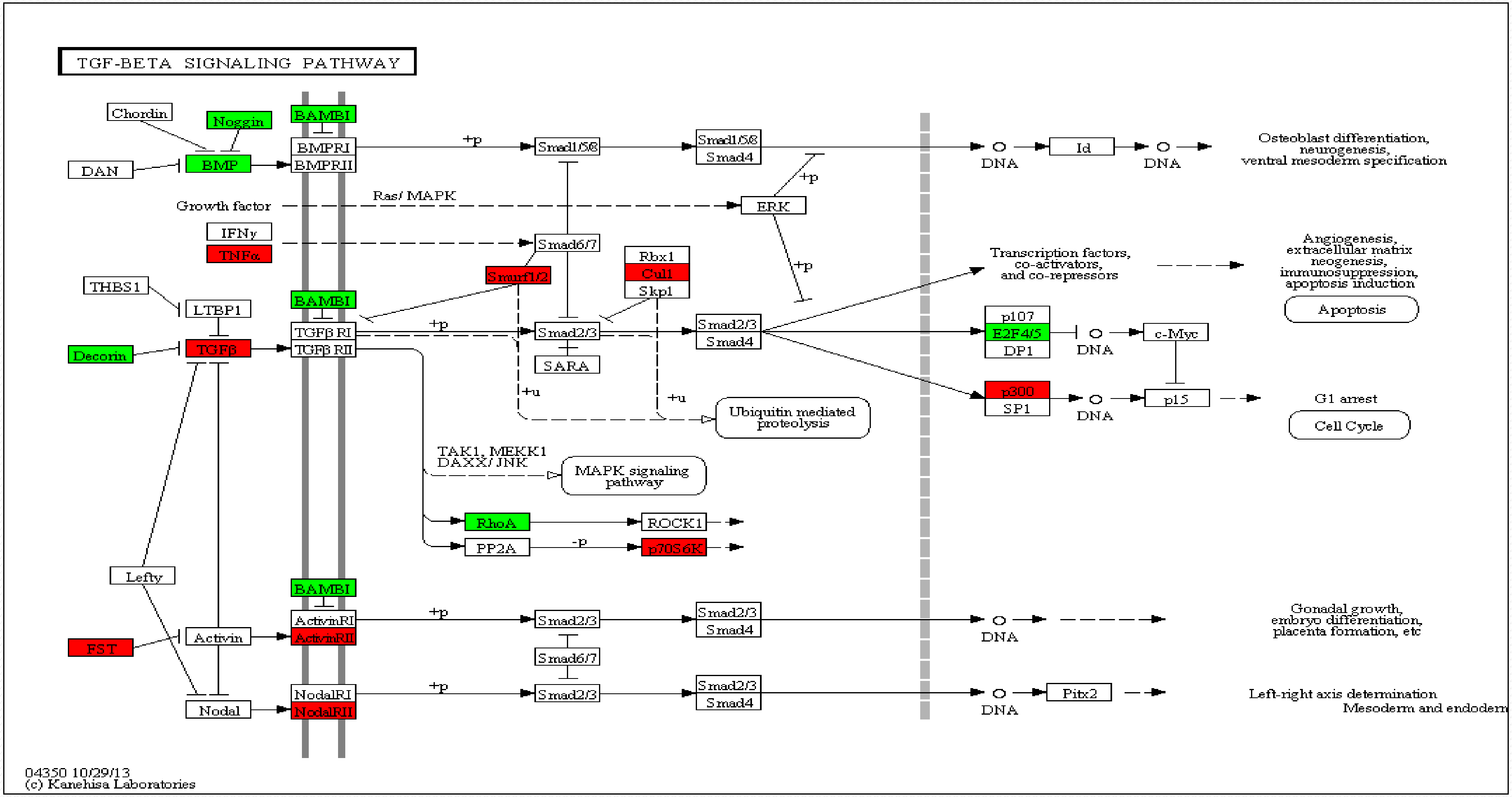
Results of DGE

Deformed	
UP	1,047 ⬆
Down	1,262 ⬇

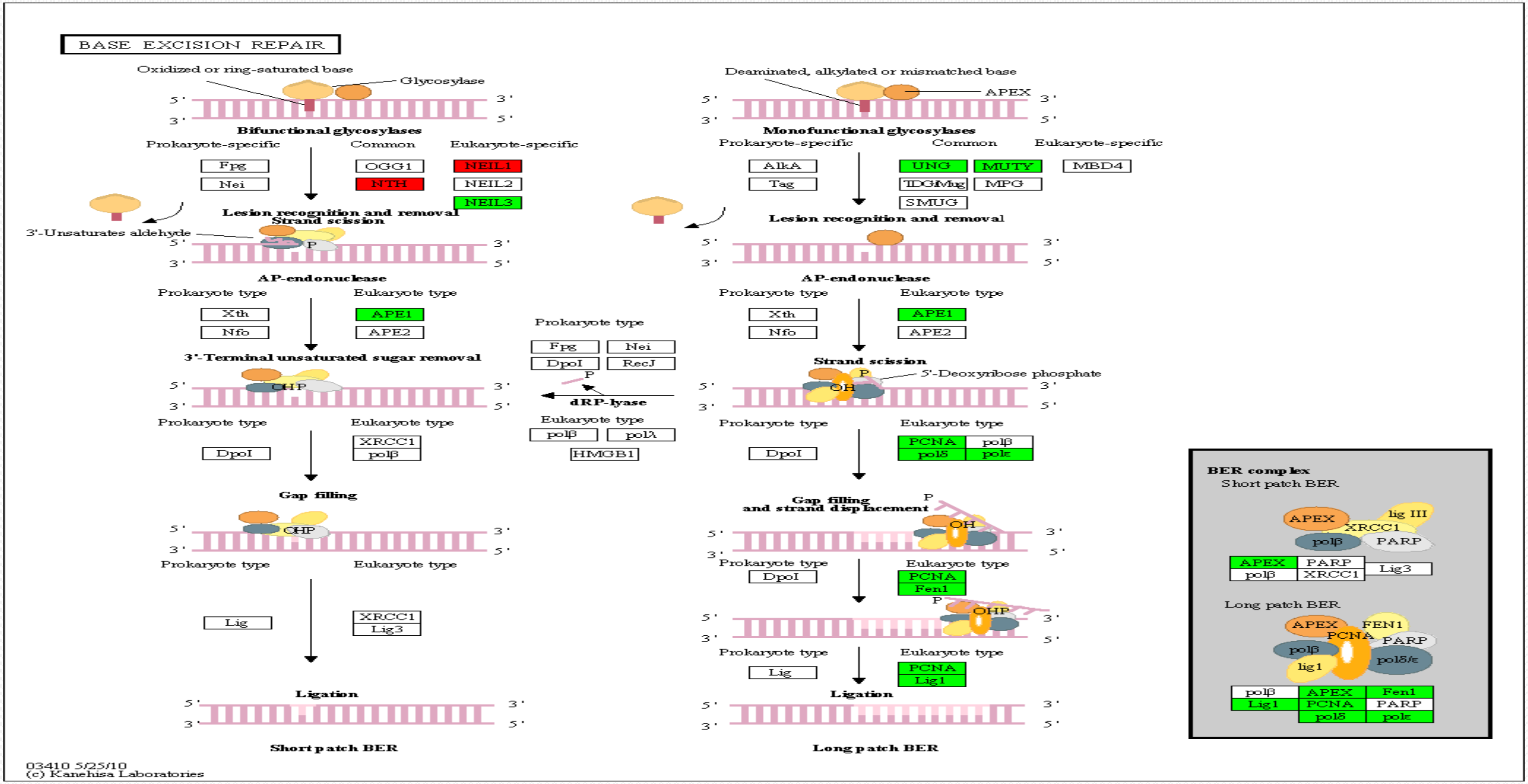
GO enrichment and Semantic similarity clustering

Term_ID	Description	Domain
GO:0006260	DNA replication	BP
GO:0007067	mitotic nuclear division	BP
GO:0007059	chromosome segregation	BP
GO:0051301	cell division	BP
GO:0006323	DNA packaging	BP
GO:0006333	chromatin assembly or disassembly	BP
GO:0000228	nuclear chromosome	CC
GO:0005578	proteinaceous extracellular matrix	CC
GO:0000786	nucleosome	CC

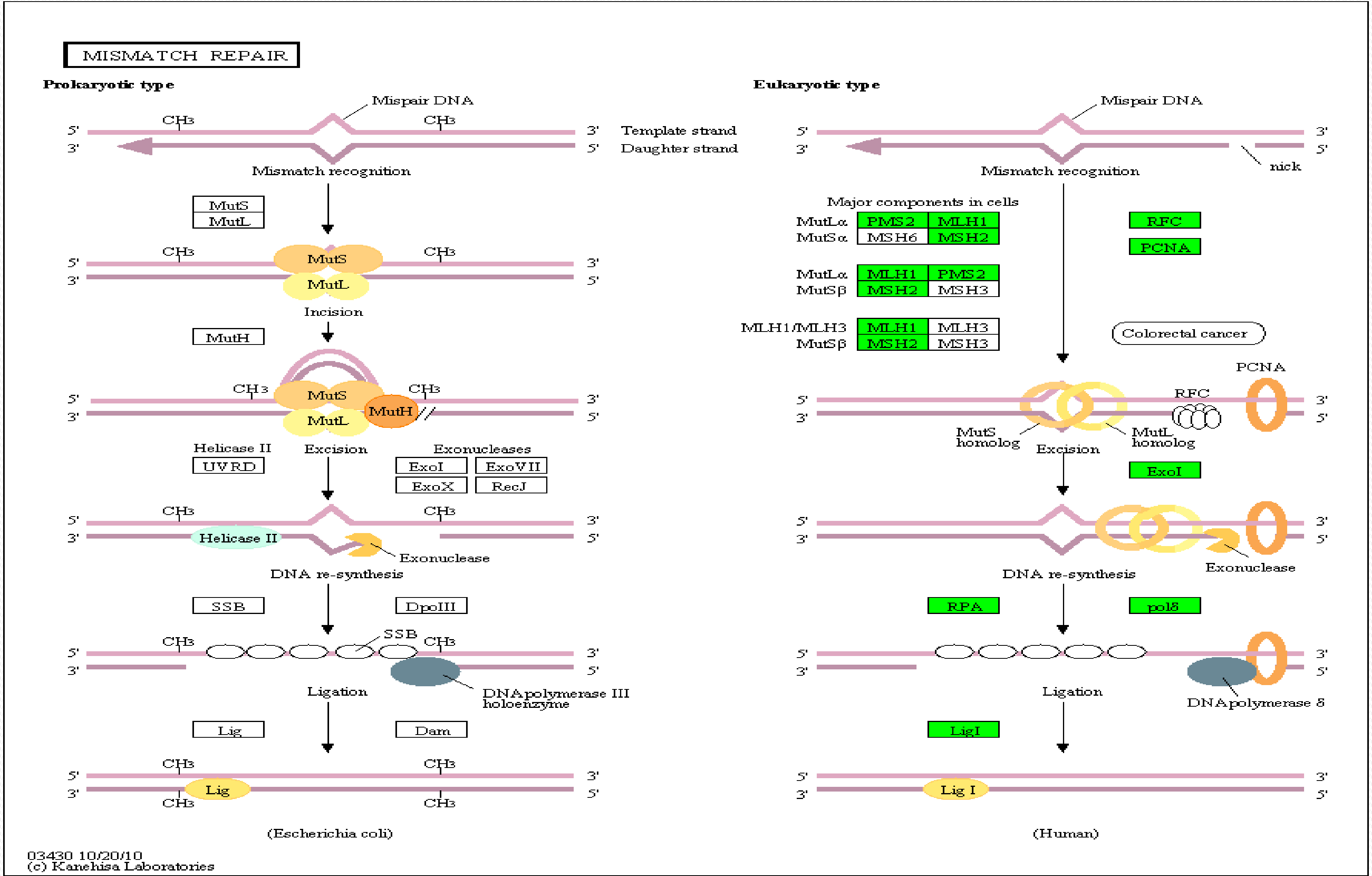
Transforming growth factor beta pathway



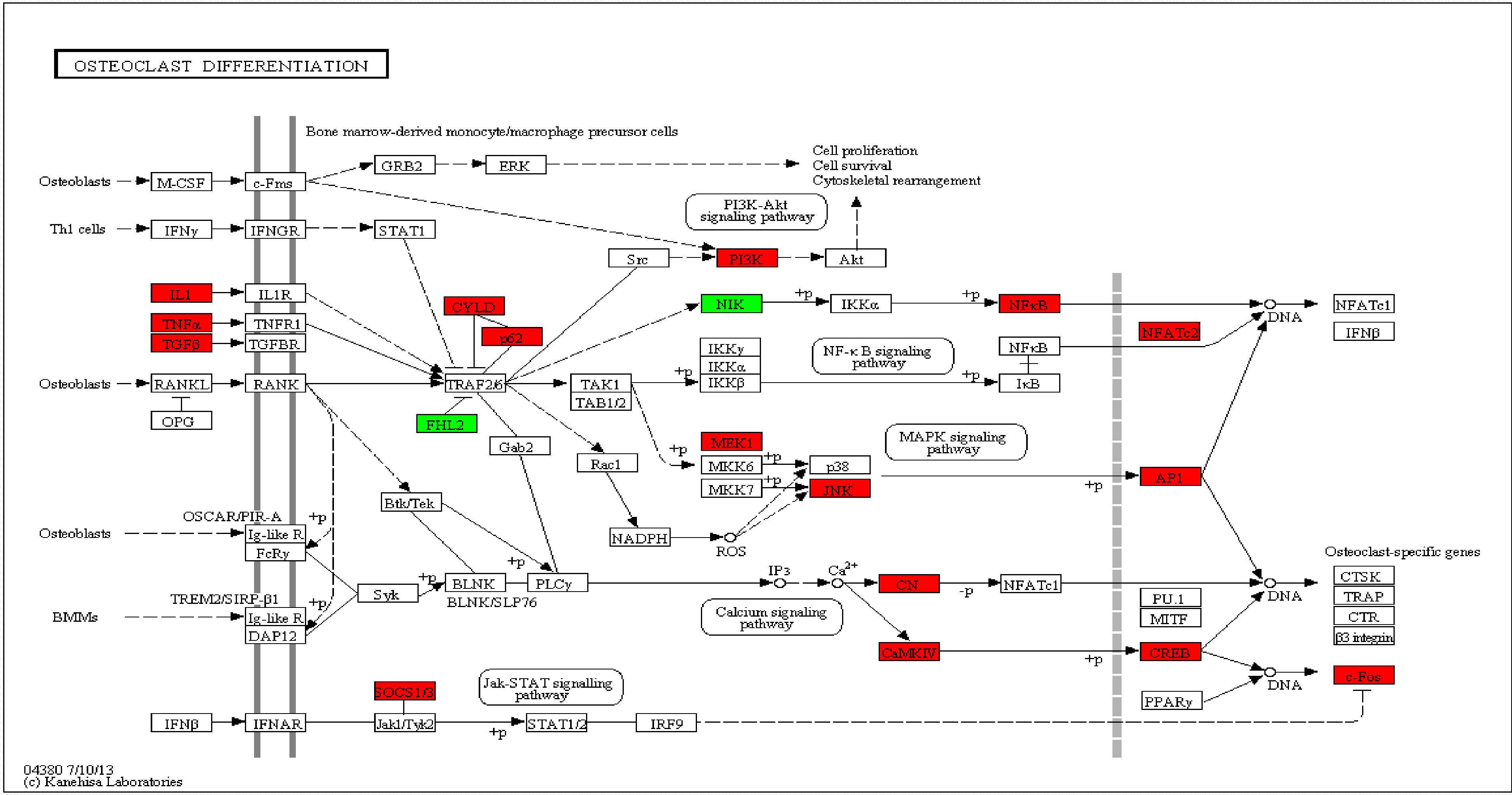
Base excision repair



Mismatch Repair



Osteoclast differentiation



Individual candidate genes

- Specific genes such as BMP-2, BMP-5, COL1A1 were found to be down-regulated in deformed individuals.

Conclusions

- Fish bones contain high 24-90% lipid and highly vulnerable to lipid peroxidation.

- It is an autocatalytic process initiated by free radicals produced during aerobic metabolisim

- Oxidized lipids could inhibit osteoblast differentiation and induce osteoclast differentiation contributing imbalance in the bone remodelling equilibrium causing bone loss.

- Therefore, nutritional profile is an important factor in jaw development, and suggest to keep good balance of oxident/antioxident compound in the diet of developing embryos

Future work

- Identification of SNPs in DEGs
- Tissue specific expression study of candidate genes along the diffrent developmental stages

Acknowledgment

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