Transcriptome atlases of the craniofacial sutures

Harm van Bakel
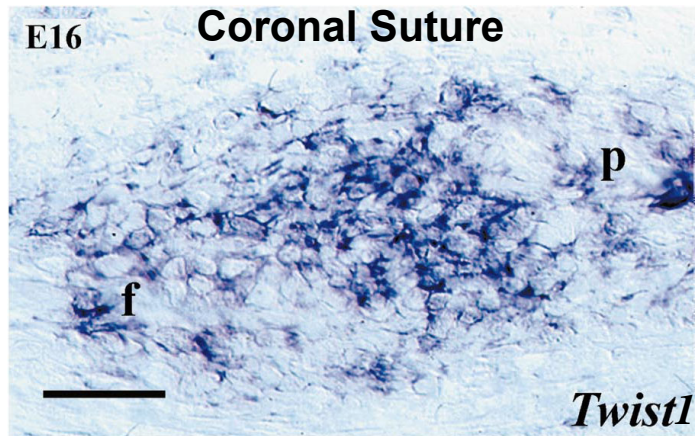
Icahn School of Medicine at Mount Sinai

Mount Sinai

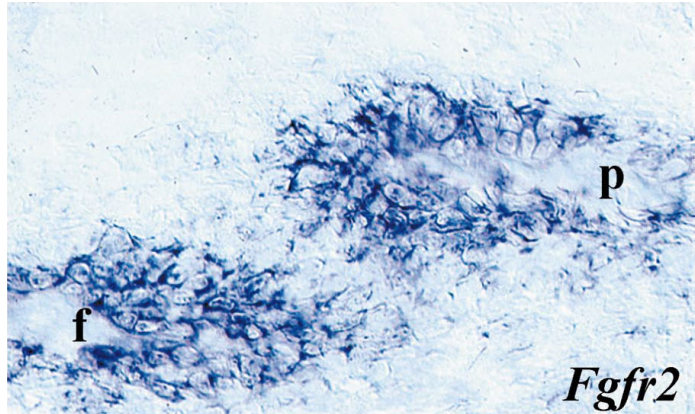# Differential gene expression & suture development

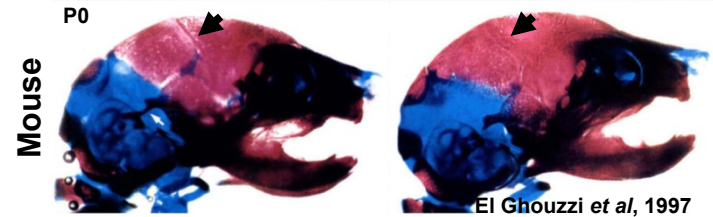Coronal Suture (E16) — *Twist1* and *Fgfr2* expression

Johnson *et al*, 2000
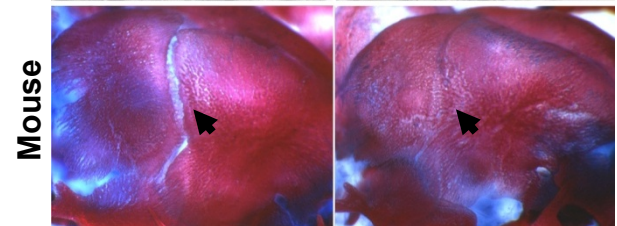
Twist1^+/- → Saethre-Chotzen Syndrome

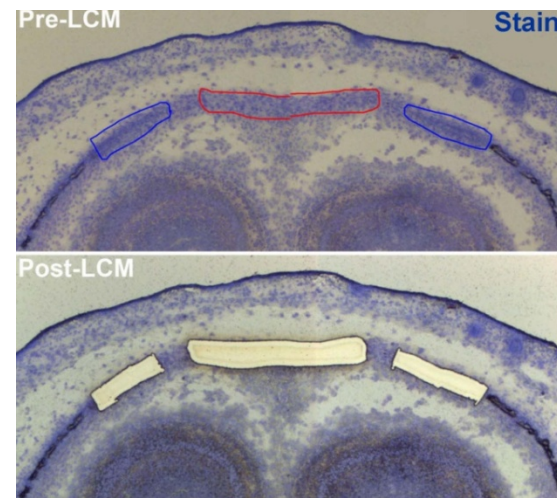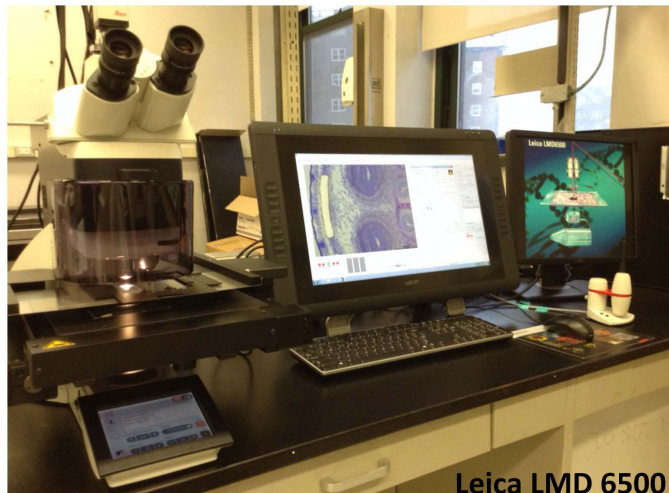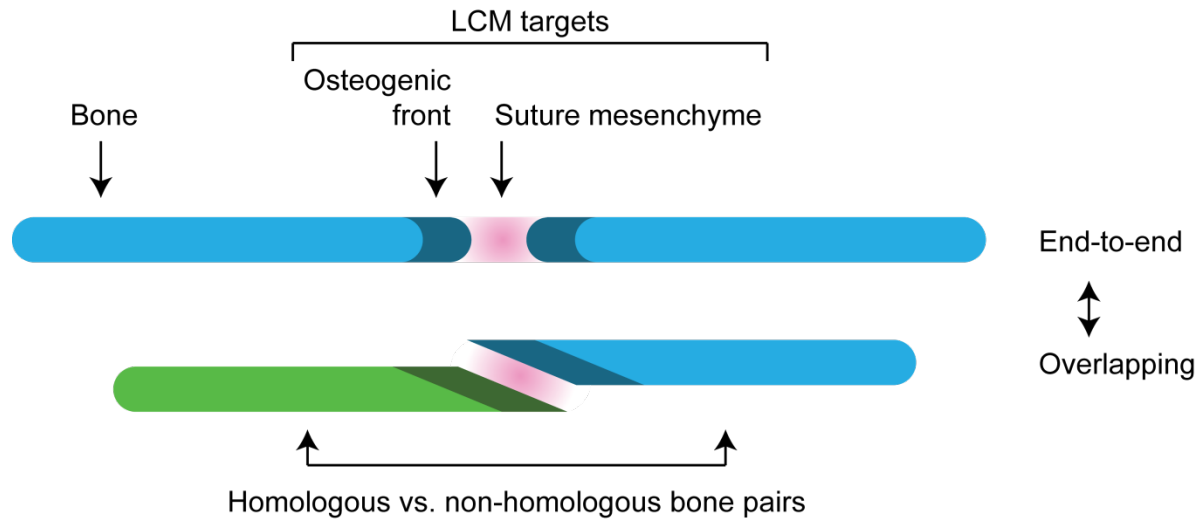*Fgfr2*^+/S252W → Apert syndrome
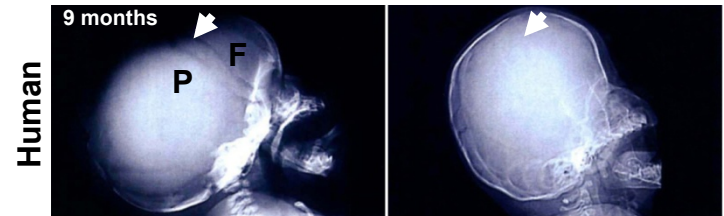
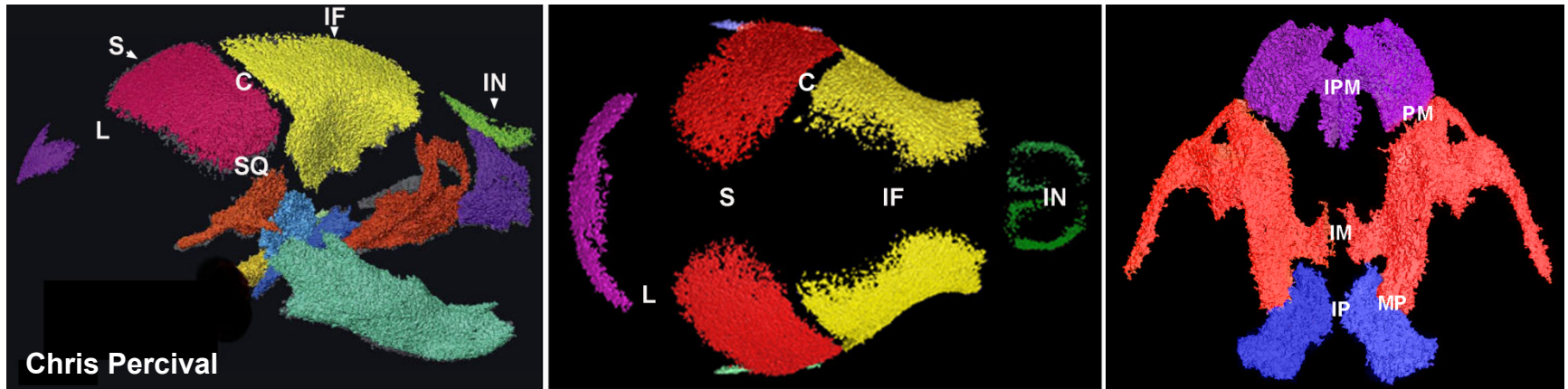El Ghouzzi *et al*, 1997
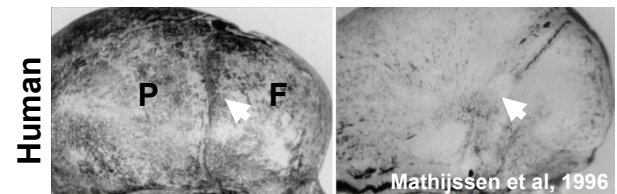
Mathijssen et al, 1996

Holmes *et al*, 2009

Comprehensive suture gene expression profiles are unknown

Laser Capture Microdissection of Suture Regions

Leica LMD 6500

Interfrontal Suture

Eleven craniofacial sutures for Atlases

Chris Percival

	Suture	Location	Lineage	Bone Pair Homology	Structure
*	Interfrontal (IF)	Cranial	Neural crest	Homologous	End-to-end
	Sagittal (S)	Cranial	Mixed	Homologous	End-to-end
*	Coronal (C)	Cranial	Mixed	Non-homologous	Overlapping
	Lambdoid (L)	Cranial	Mesoderm	Non-homologous	Overlapping
	Squamoparietal (SQ)	Cranial	Mixed	Non-homologous	Overlapping
*	Internasal (IN)	Facial	Neural crest	Homologous	End-to-end (broad)
*	Interpremaxillary (IPM)	Facial	Neural crest	Homologous	End-to-end (broad)
	Intermaxillary (IM)	Facial	Neural crest	Homologous	End-to-end
	Interpalatine (IP)	Facial	Neural crest	Homologous	End-to-end
	Premaxillary/maxillary (PM)	Facial	Neural crest	Non-homologous	End-to-end
	Maxillary/palatine (MP)	Facial	Neural crest	Non-homologous	Overlapping

Data generation for each suture
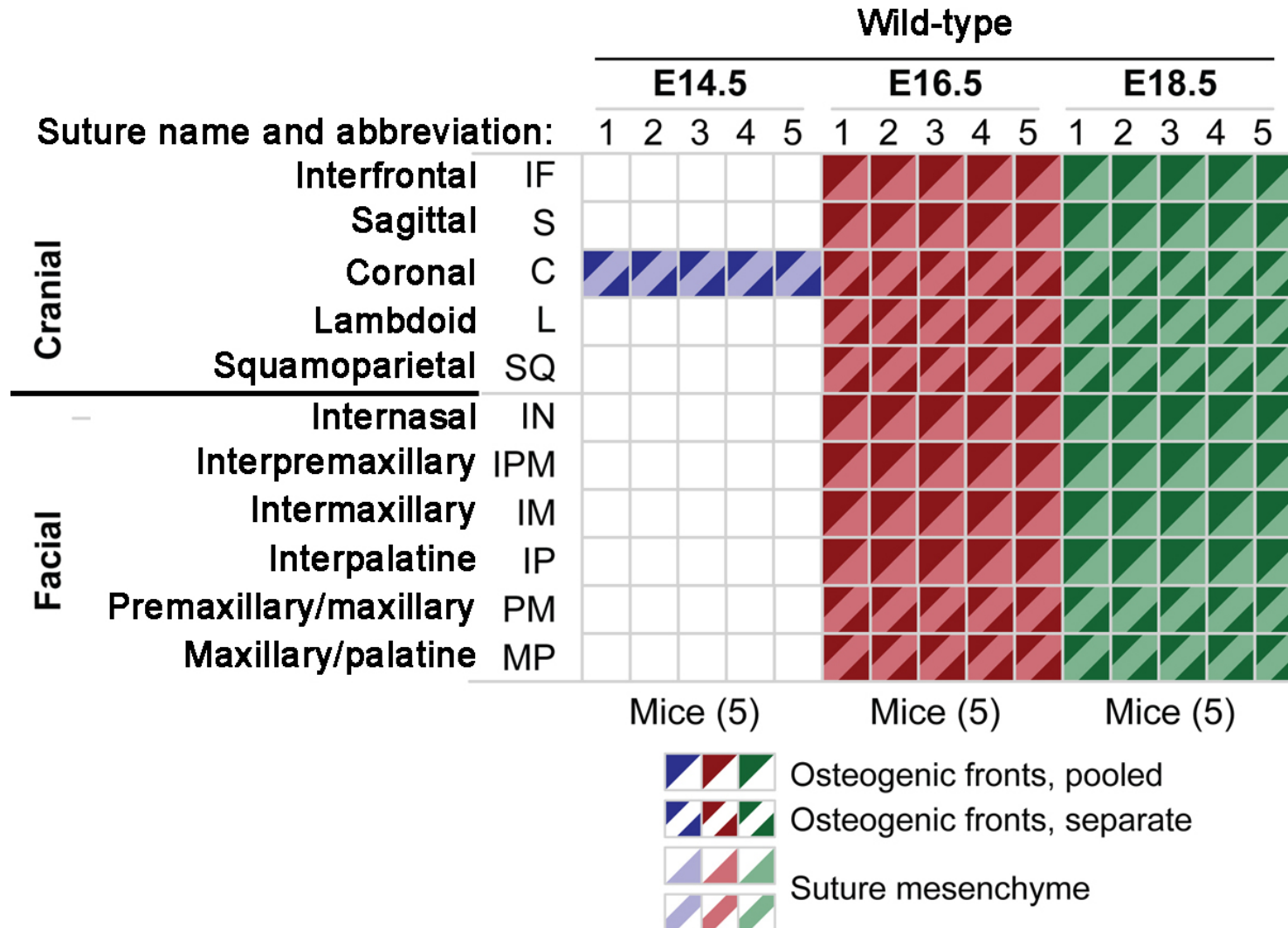
```
        ┌─────────────────┐
        │     Suture      │ ─────────→  → LCM
        └───┬─────────┬───┘
            ▼         ▼
 ┌──────────────┐ ┌──────────────────┐
 │  Mesenchyme  │ │ Osteogenic fronts │ ───→  → 5 replicates
 └──────┬───────┘ └─────────┬────────┘
        ▼                   ▼
        ┌─────────────────┐
        │  RNA extraction │ ─────────→  → 1-10 ng, RIN 7-8
        └────────┬────────┘
                 ▼
        ┌─────────────────┐
        │ SPI amplification │ ───────→  → rRNAs suppressed
        └────────┬────────┘
                 ▼
        ┌─────────────────┐
        │  Total RNA-Seq  │
        └────────┬────────┘
                 ▼
```
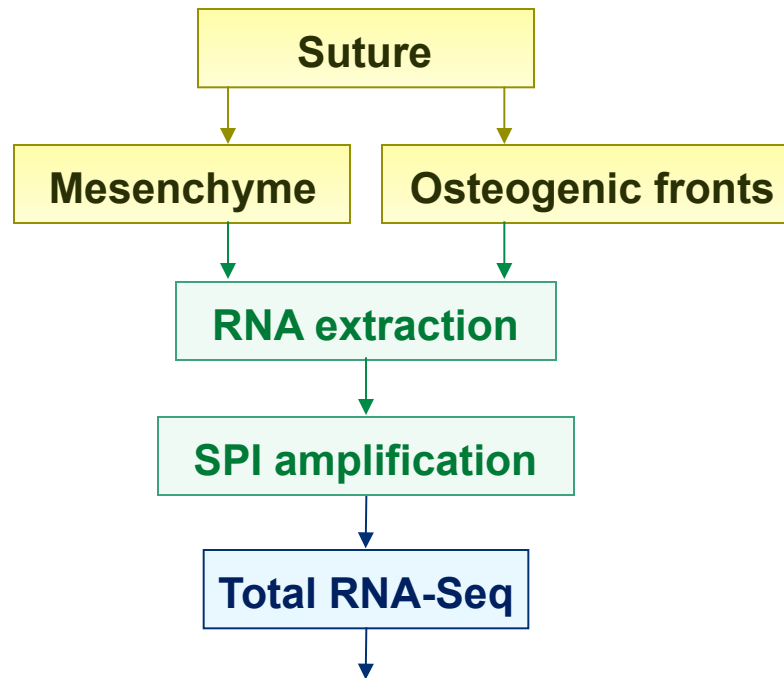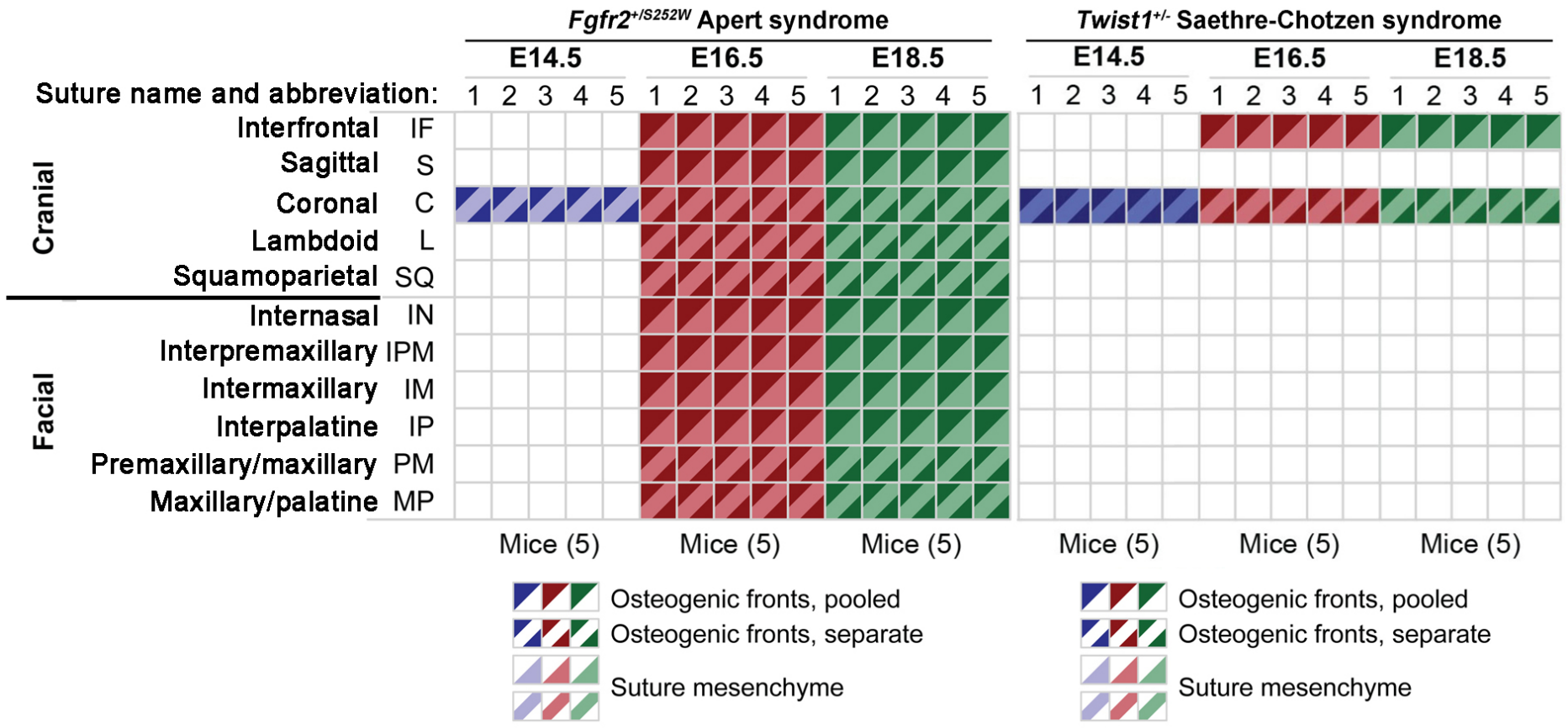
- Illumina HiSeq
- rRNA depleted
- Paired-end 100 nt reads
- Depth: ~40M read pairs

Planned wild-type mouse suture profiles

285 WT profiles

Planned *Fgfr2*$^{+/S252W}$ and *Twist1*$^{+/-}$ suture profiles


350 mutant profiles → 635 total

RNA-Seq Data Analysis & QC pipeline

▶ <u>Raw reads</u> → assessment of base-level qualities

▶ Adapter and quality filtering

▶ Map filtered reads to mouse genome (mm10)
  – <u>Mapped read bam files</u> and genome browser tracks

▶ Quality control of mapped reads
  – Global stats: % mapped reads, % unique reads, duplicated reads, intronic and exonic read mapping rates, library diversity, etc.

▶ Generate <u>gene, transcript and exon-level read counts</u> per sample

Data is made available through <u>https://facebase.org/</u>
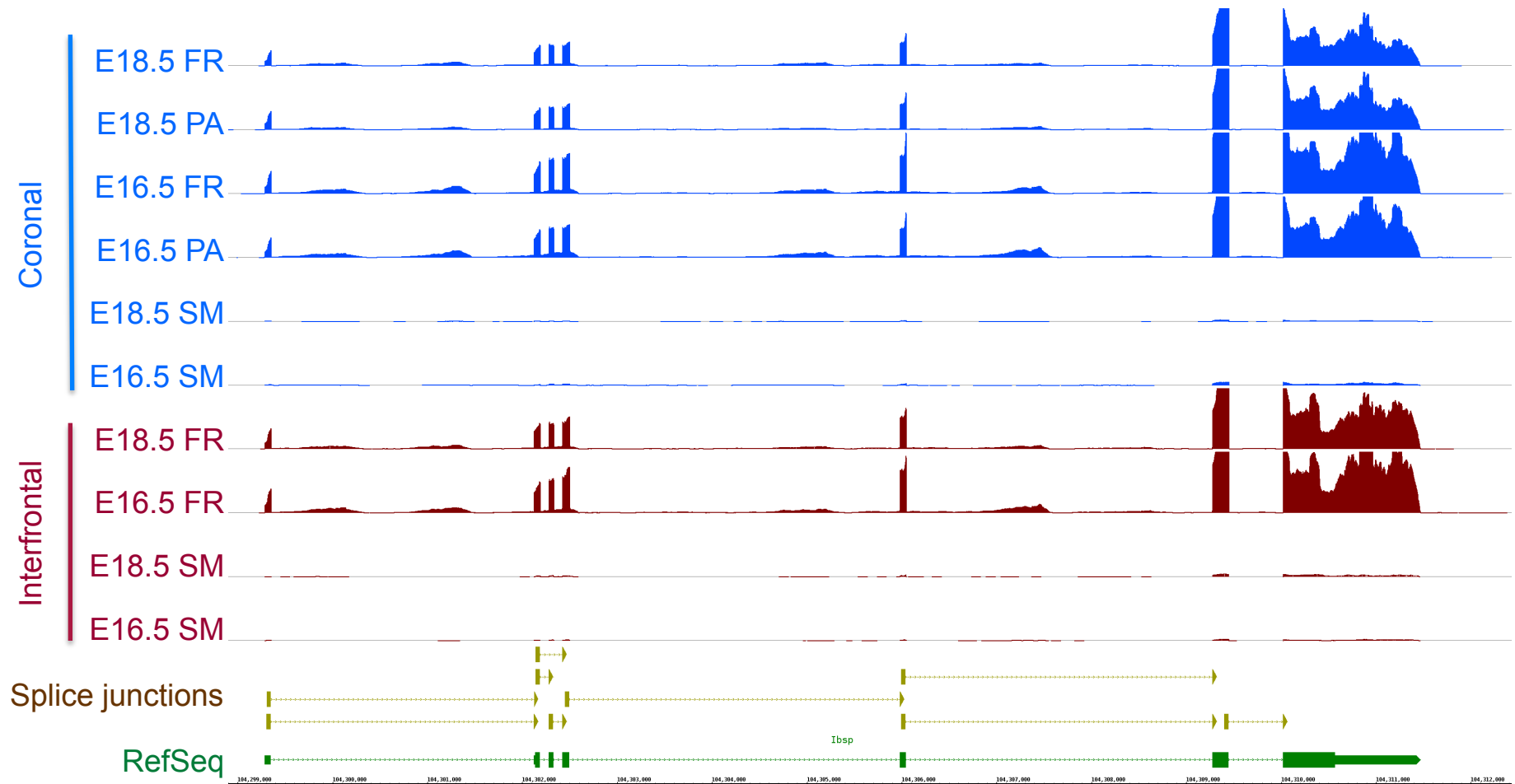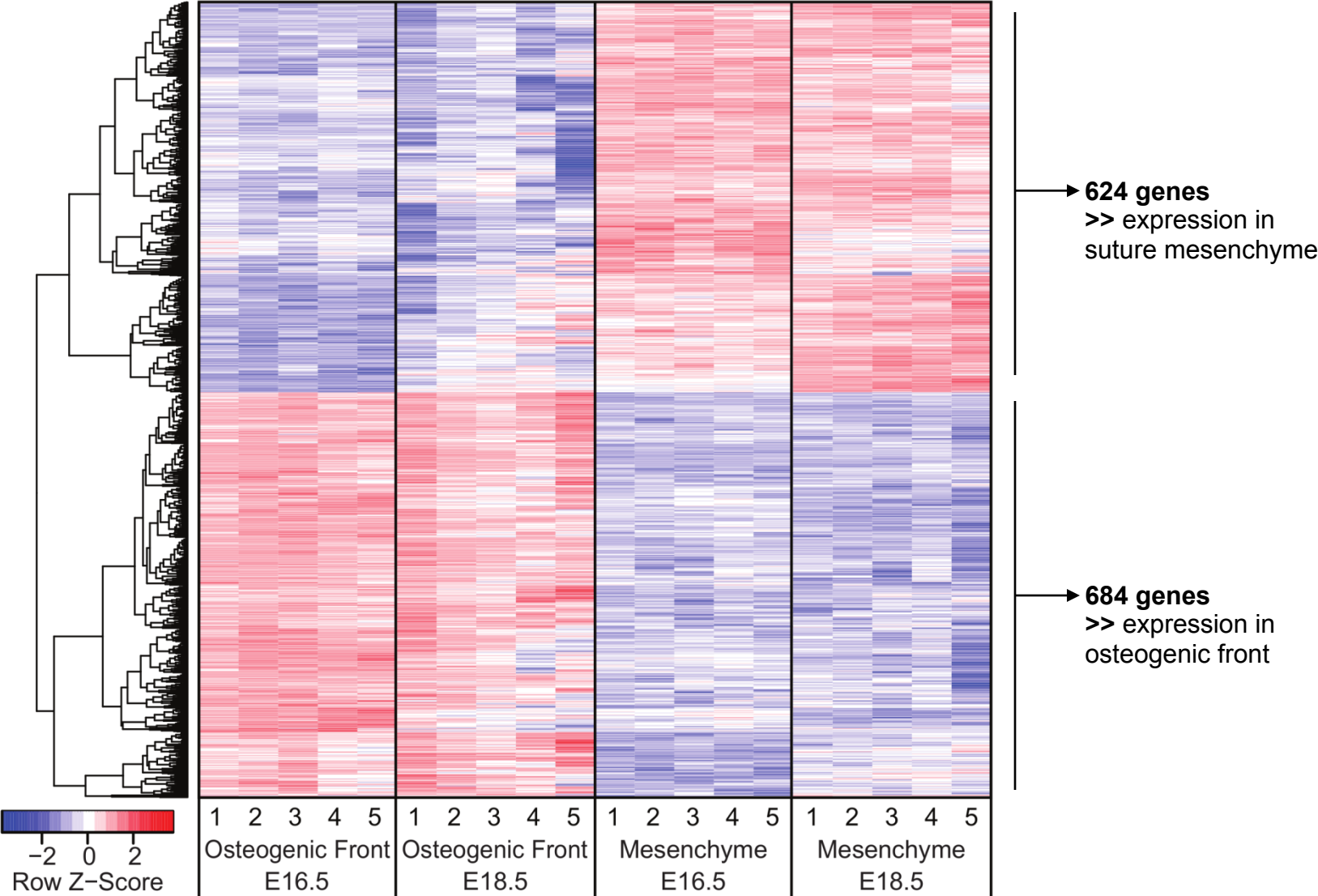
Total RNA-Seq – Mapping statistics
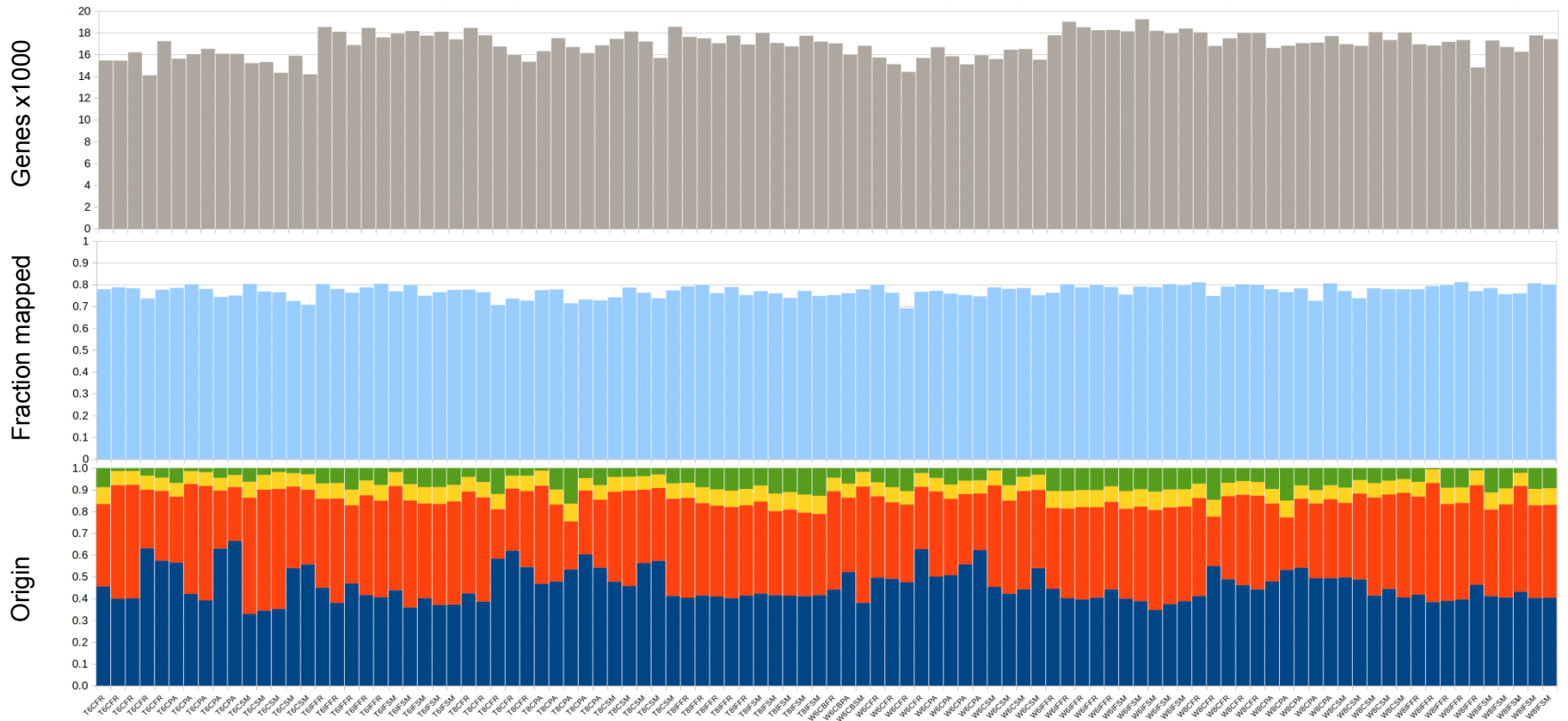
Mapping statistics

▶ Mapping rate ~80%

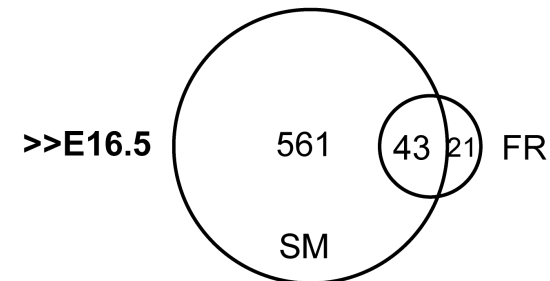▶ rRNA contamination < 10%

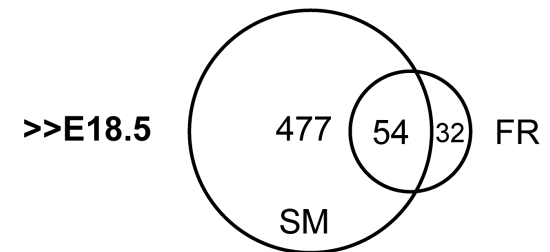# Genome browser example – *Ibsp* expression profiles

Expression differences in Interfrontal suture regions

Interfrontal suture gene ontology analysis

Description	# Genes	P-value
* skeletal system development	27	5.93E-07
* biomineral formation	11	1.55E-06
* bone development	17	2.03E-06
* ossification	16	2.62E-06
sterol metabolic process	12	2.13E-04
* regulation of ossification	10	4.33E-04
cholesterol metabolic process	11	5.00E-04
sterol biosynthetic process	8	5.27E-04
cholesterol biosynthetic process	7	1.26E-03
regulation of epithelial cell proliferation	10	1.15E-03
regulation of cell proliferation	29	1.62E-03

Developmental time differences – E16.5 vs. E18.5

Suture mesenchyme (SM)

>> E18.5
531 genes

>> E16.5
604 genes

E16.5   E18.5

Osteogenic front (FR)

>> E18.5
86 genes

>> E16.5
64 genes

E16.5   E18.5

>>E18.5   477   54 32   FR
SM

>>E16.5   561   43 21   FR
SM
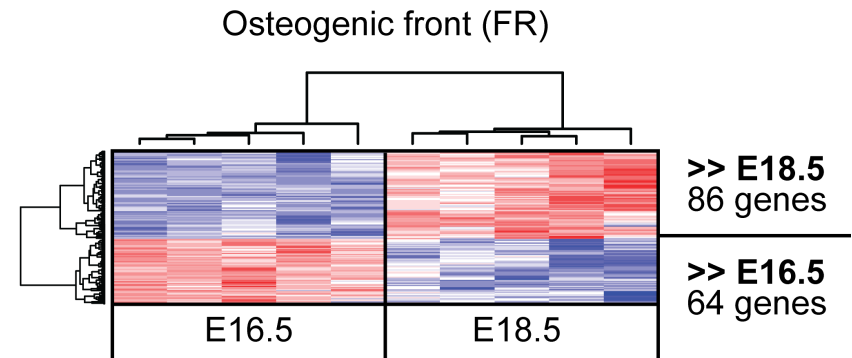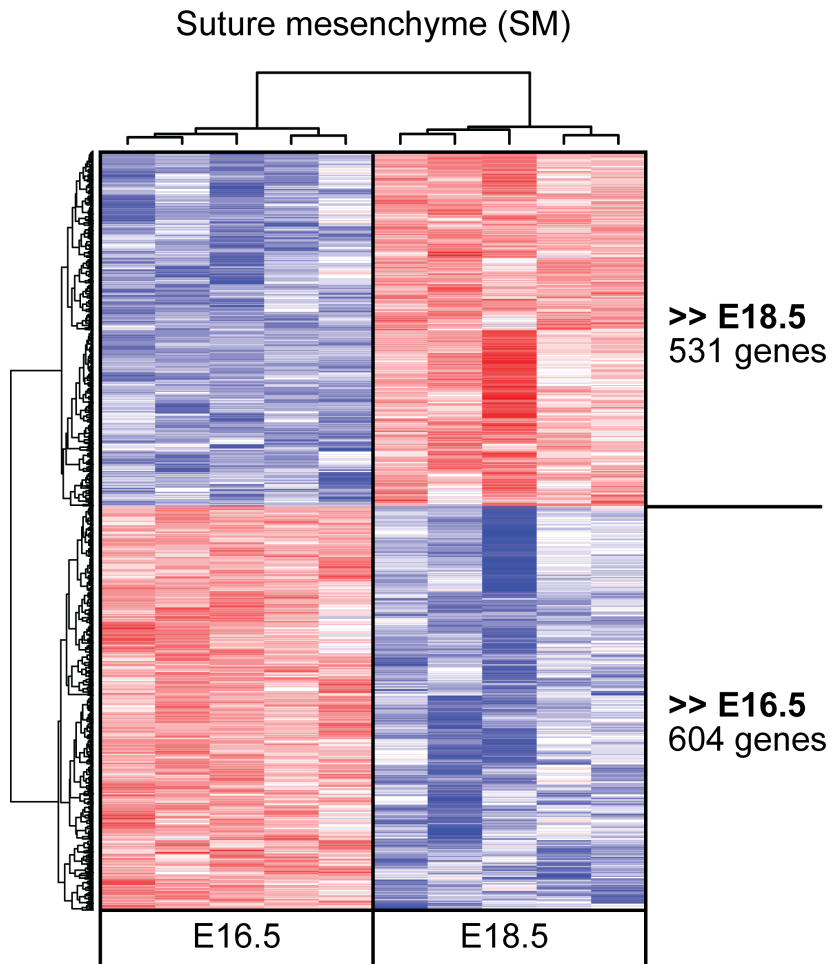
Combined analysis: Interfrontal & Coronal Sutures
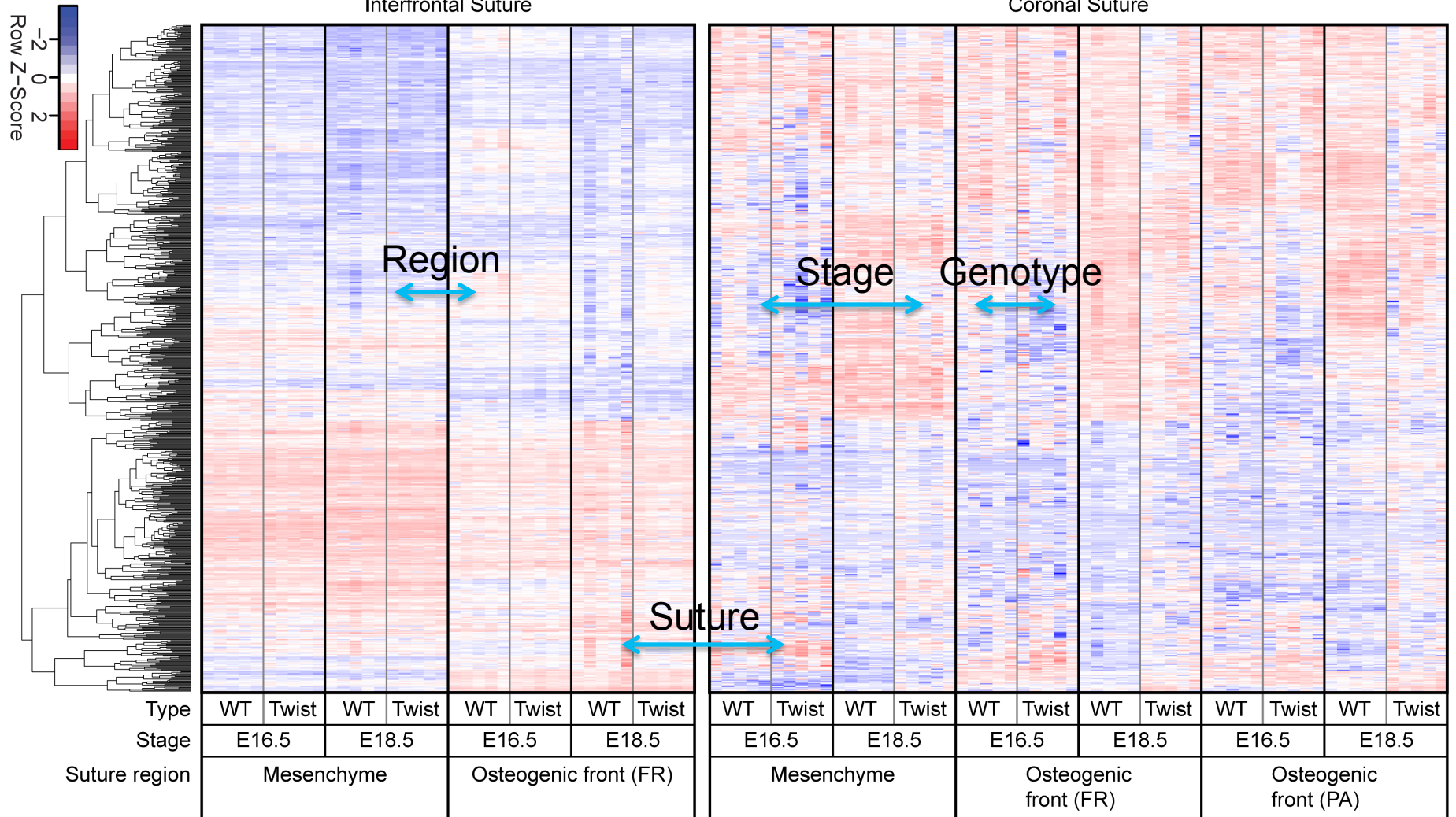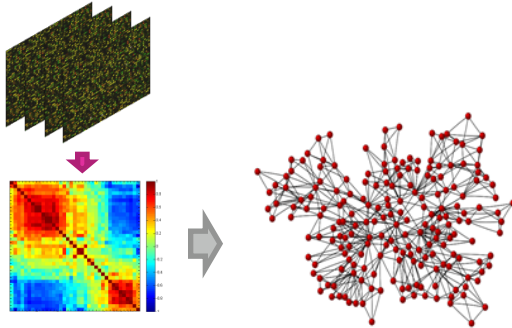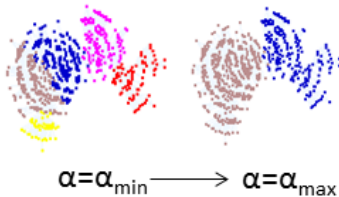
Multiscale embedded co-expression network analysis

Network construction

- ❖ Screening: pairs with FDR < 0.05
- ❖ Parallelization of network construction
- → **Reduce false-positives, improve time complexity.**

Multi-scale cluster detection

$\alpha = \alpha_{min} \longrightarrow \alpha = \alpha_{max}$

- ❖ Unsupervised hierarchical divisive approach to identify multiscale organization
- → **Identification of modular structures.**

Downstream analyses
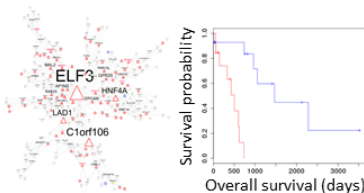
- ❖ Identification of:
- - Significant modules,
- - Significant hubs,
- - Identify suture development modules
- - Association with suture phenotypes
- → **potential drivers/mechanisms of disease etiology**

Global network: Interfrontal Suture
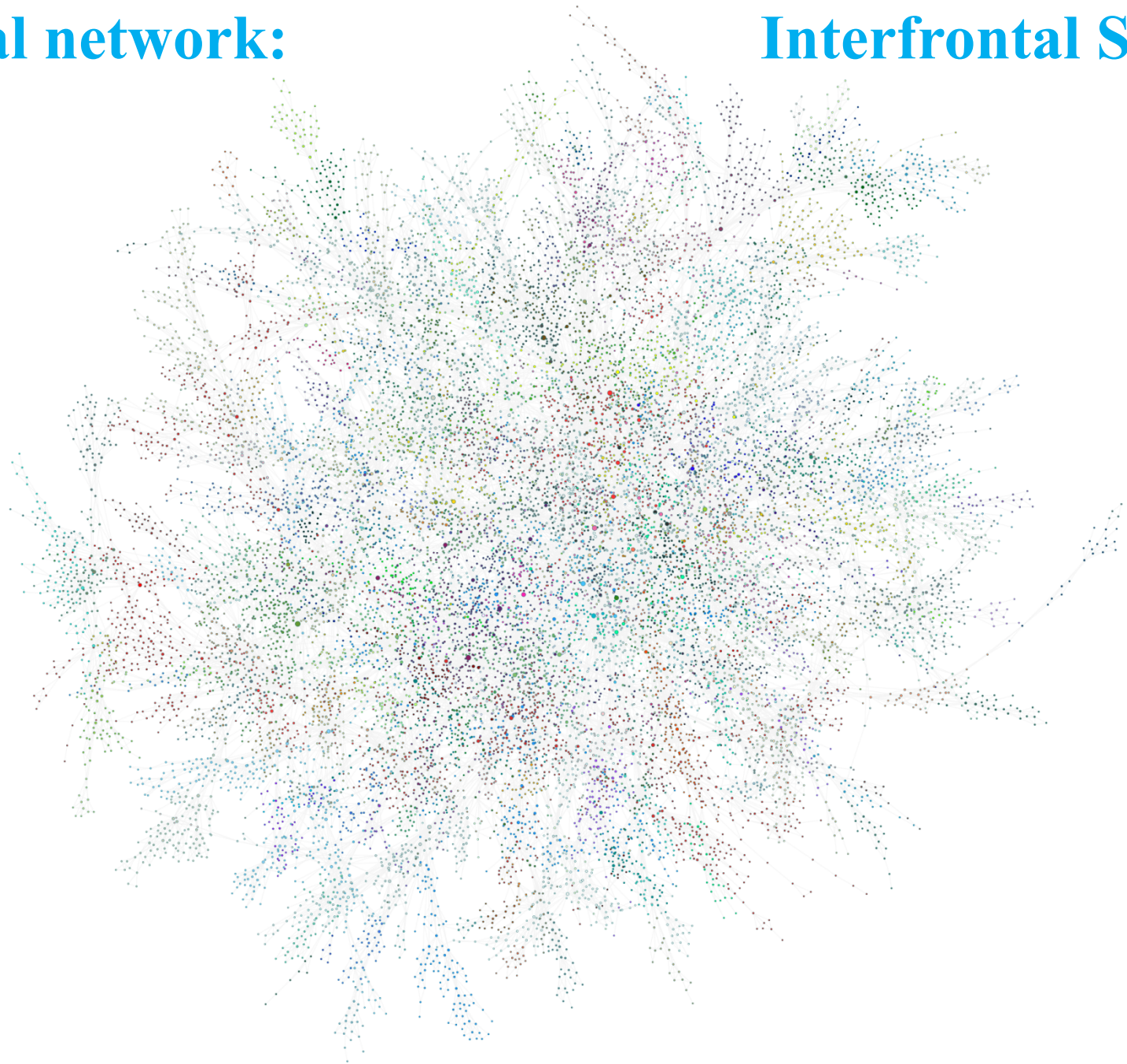
Global network: Interfrontal Suture

Interfrontal Suture – Osteogenic front vs. Mesenchyme

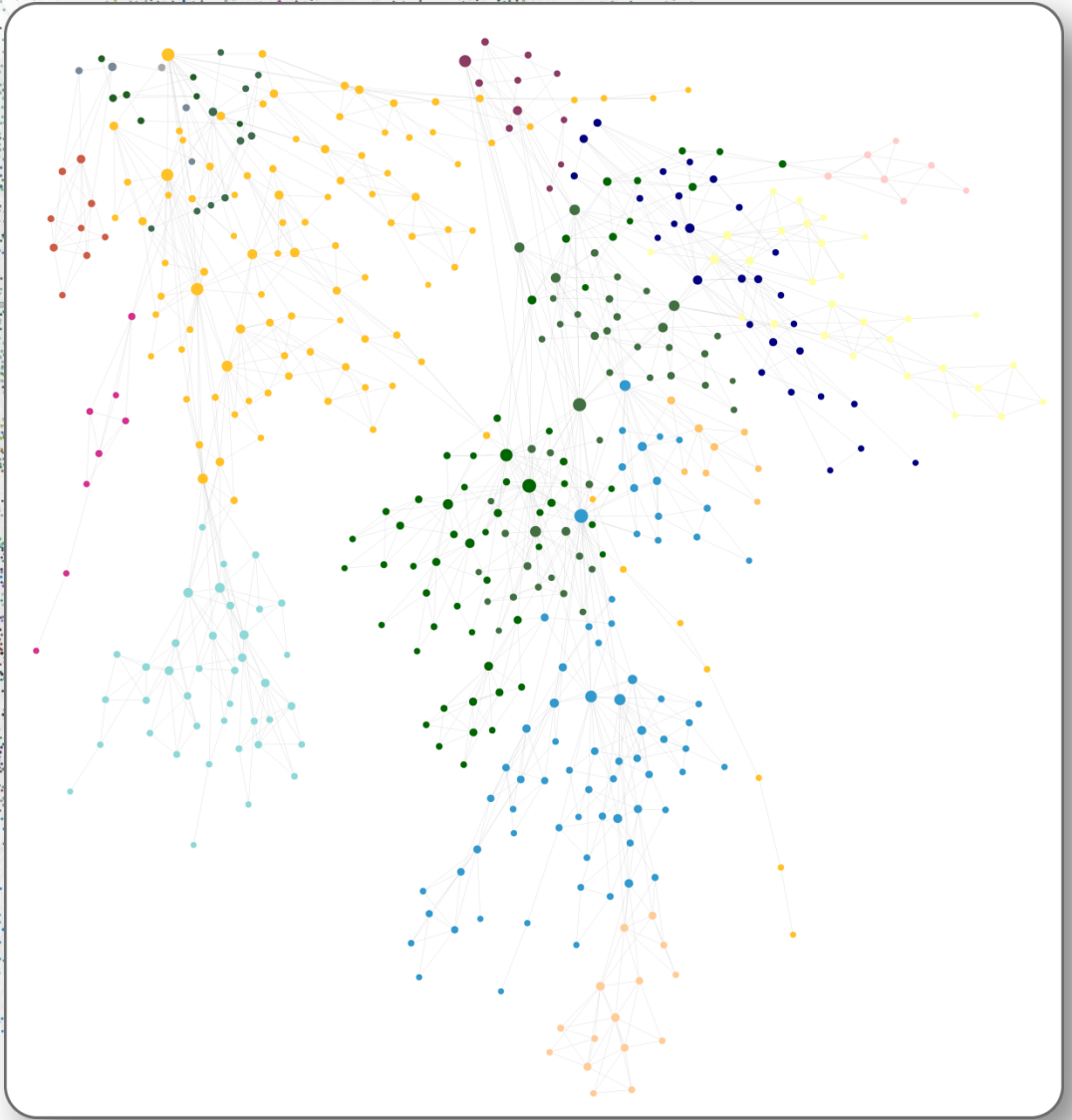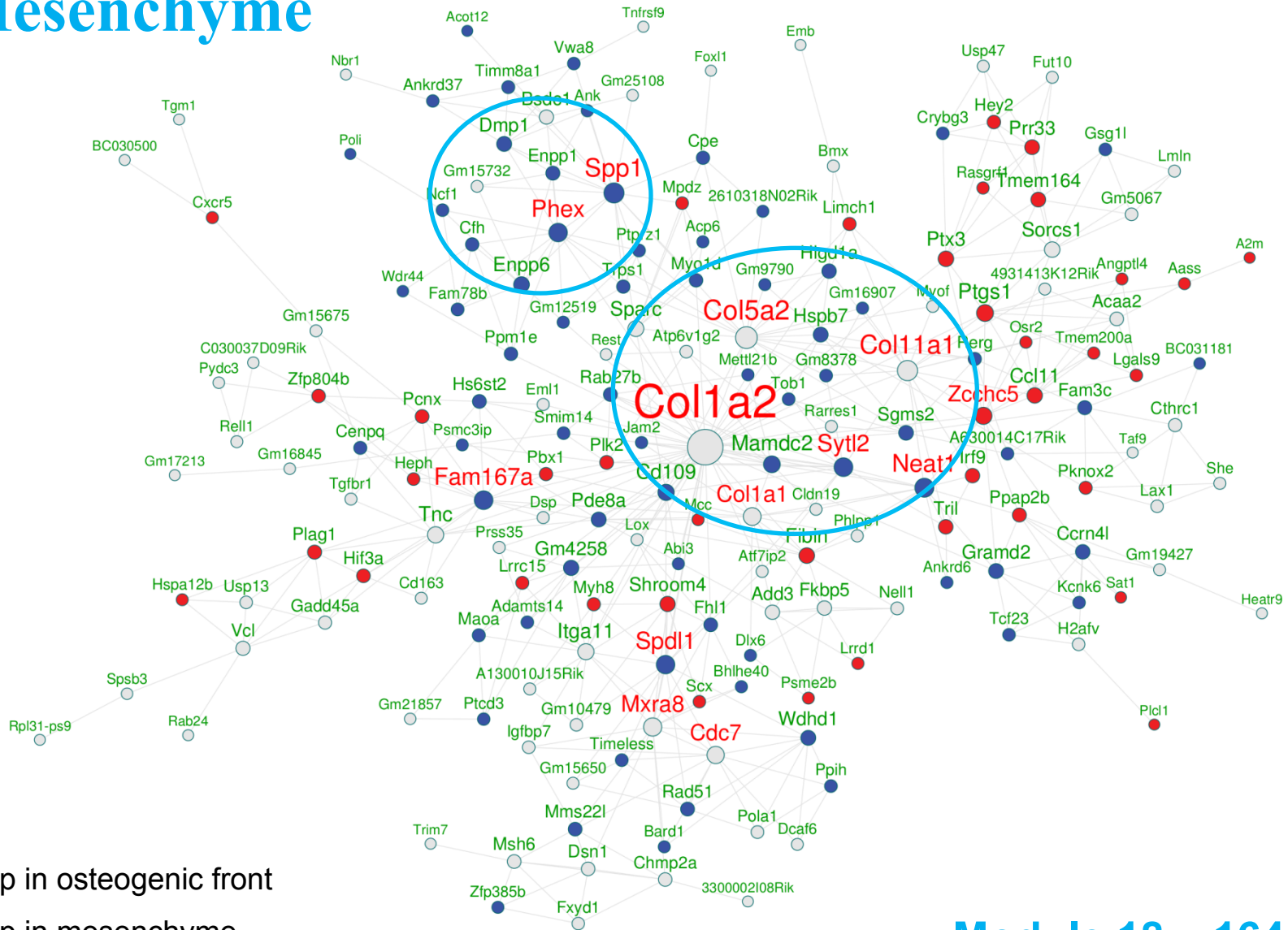Up in osteogenic front
Up in mesenchyme

Module 18 – 164 genes

Top 10 enriched biological processes (Module 18)

Name	Term gene	Overlap	FE	Pvalue	Padjust
* collagen fibril organization	37	8	18.9	7.28E-09	0.0009
* ossification	316	16	4.4	7.01E-07	0.0453
* biomineral tissue development	104	9	7.6	2.96E-06	0.1274
* regulation of ossification	161	9	4.9	9.85E-05	1
response to yeast	2	2	87.3	0.00013	1
* inorganic diphosphate transport	2	2	87.3	0.00013	1
* extracellular matrix organization	169	9	4.6	0.000143	1
extracellular structure organization	170	9	4.6	0.000149	1
organ development	2104	42	1.7	0.000165	1
protein trimerization	33	4	10.6	0.000524	1

Coronal Suture – Osteogenic front vs. Mesenchyme

Module 168 – 253 genes

Up in osteogenic front

Up in mesenchyme

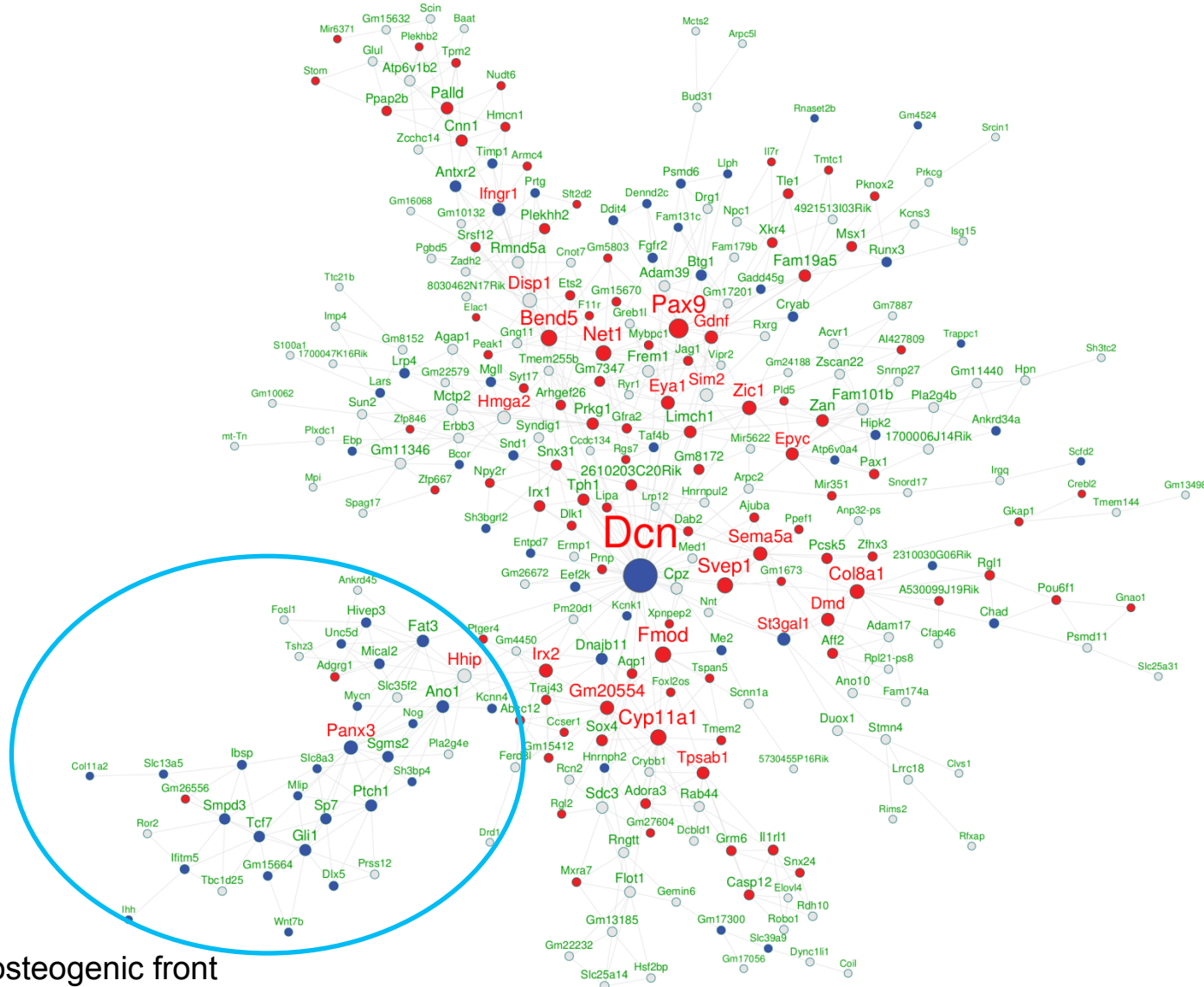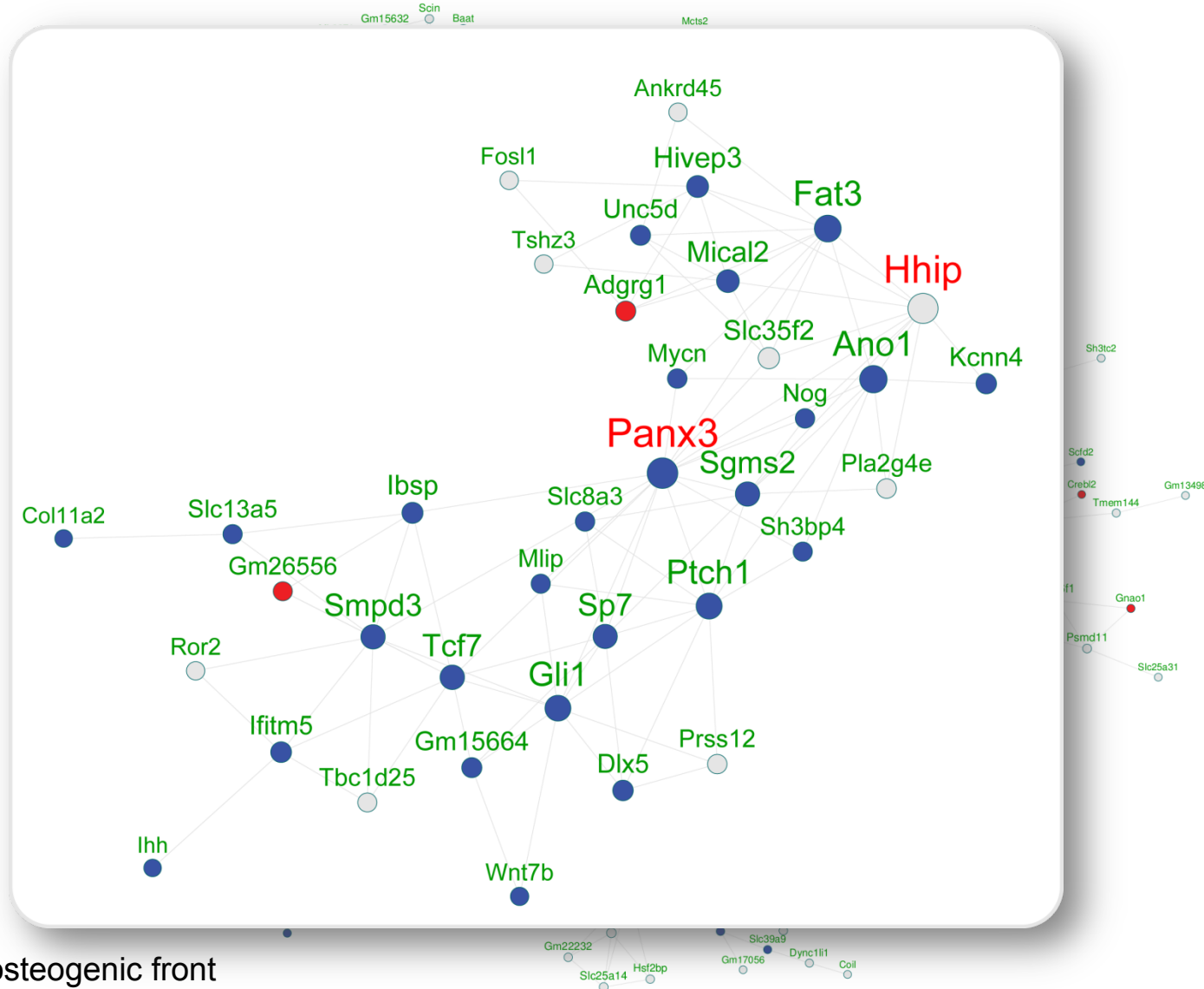# Coronal Suture – Osteogenic front vs. Mesenchyme

Up in osteogenic front

Up in mesenchyme

Module 168 – 253 genes

Top 10 enriched biological processes (Module 168)

Name	Term genes	Overlap	FE	Pvalue	Padjust
pattern specification process	304	24	4.4	1.16E-09	8.88E-05
organ morphogenesis	680	37	3.0	1.38E-09	8.88E-05
organ development	2079	73	2.0	4.42E-09	0.00019
* skeletal system development	365	25	3.8	9.51E-09	0.000307
* ossification	314	22	3.9	5.15E-08	0.001329
tissue development	1251	49	2.2	1.23E-07	0.002638
system development	2905	87	1.7	1.79E-07	0.003296
appendage morphogenesis	125	13	5.8	3.71E-07	0.005324
limb morphogenesis	125	13	5.8	3.71E-07	0.005324
* skeletal system morphogenesis	176	15	4.8	6.35E-07	0.008199

Interfrontal Suture – Development network (E16.5 vs E18.5)
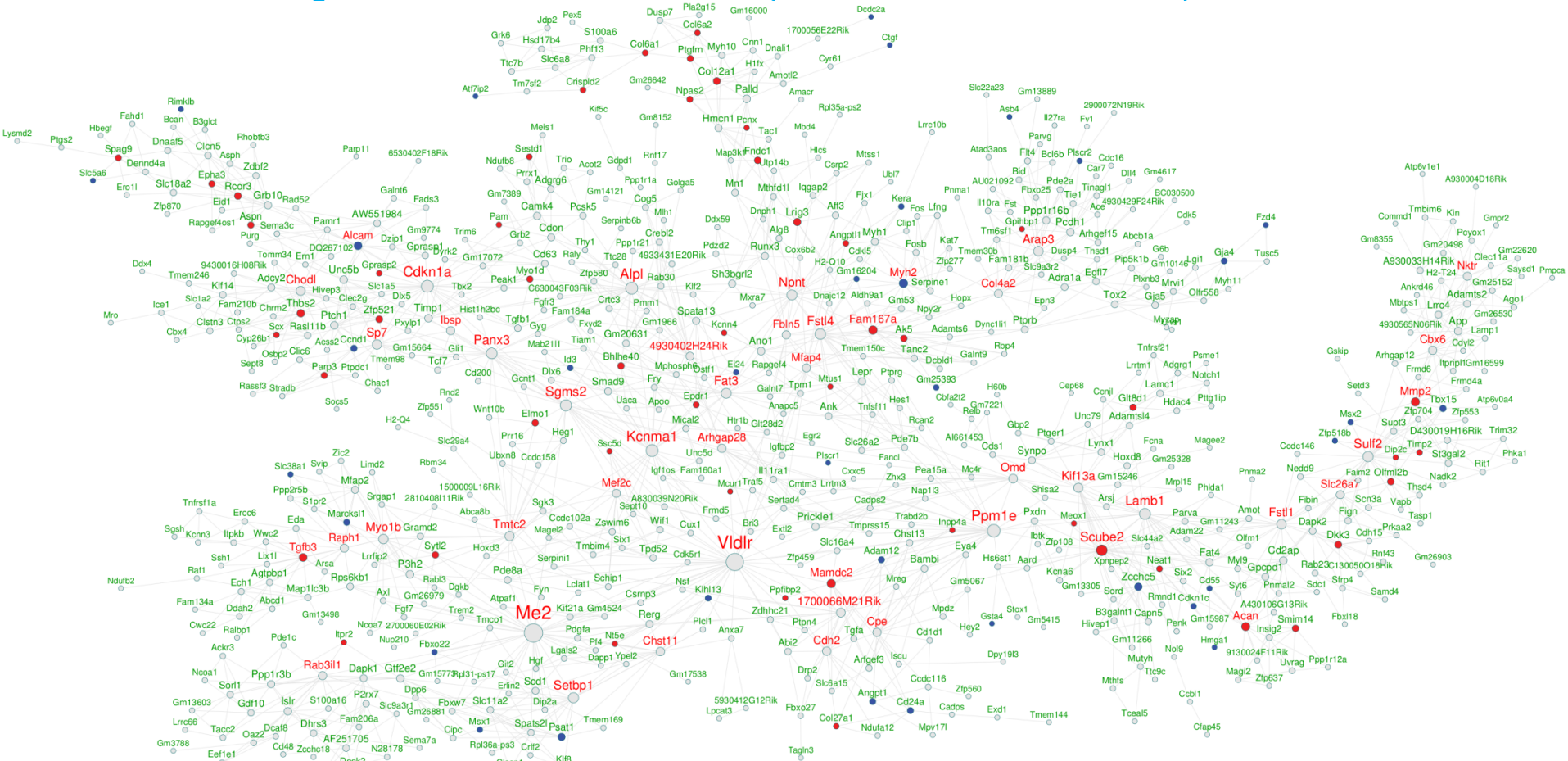
Up at E16.5

Up at E18.5

Module 6 – 651 genes

Top 10 enriched biological processes (Module 6)

Name	Term genes	Overlap	FE	Pvalue	Padjust
single-multicellular organism process	4130	265	1.4	3.14E-11	2.62E-06
anatomical structure morphogenesis	1861	144	1.7	4.21E-11	2.62E-06
multicellular organismal process	4234	269	1.4	6.07E-11	2.62E-06
multicellular organismal development	3323	220	1.5	2.88E-10	9.30E-06
system development	2930	199	1.5	3.81E-10	9.84E-06
locomotion	1061	92	1.9	1.13E-09	2.44E-05
regulation of multicellular organismal process	1835	137	1.6	1.65E-09	3.05E-05
single-organism developmental process	3904	246	1.4	1.94E-09	3.13E-05
organ morphogenesis	686	67	2.1	2.52E-09	3.62E-05
tissue development	1262	103	1.8	2.83E-09	3.65E-05

Conclusions

▶ Analysis of datasets for Interfrontal and Coronal sutures at E16.5 and E18.5 for wild-type and *Twist1*$^{+/-}$ mutant mice

▶ High-quality LCM RNA-Seq captures known and novel aspects of suture biology

▶ Embedded co-expression network analysis has enabled us to construct regulatory networks and identify candidate regulatory 'hubs'

  – Additional suture datasets will allow further refinement of network modules

Acknowledgements

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