Transcriptional Interactome in Mouse Genome

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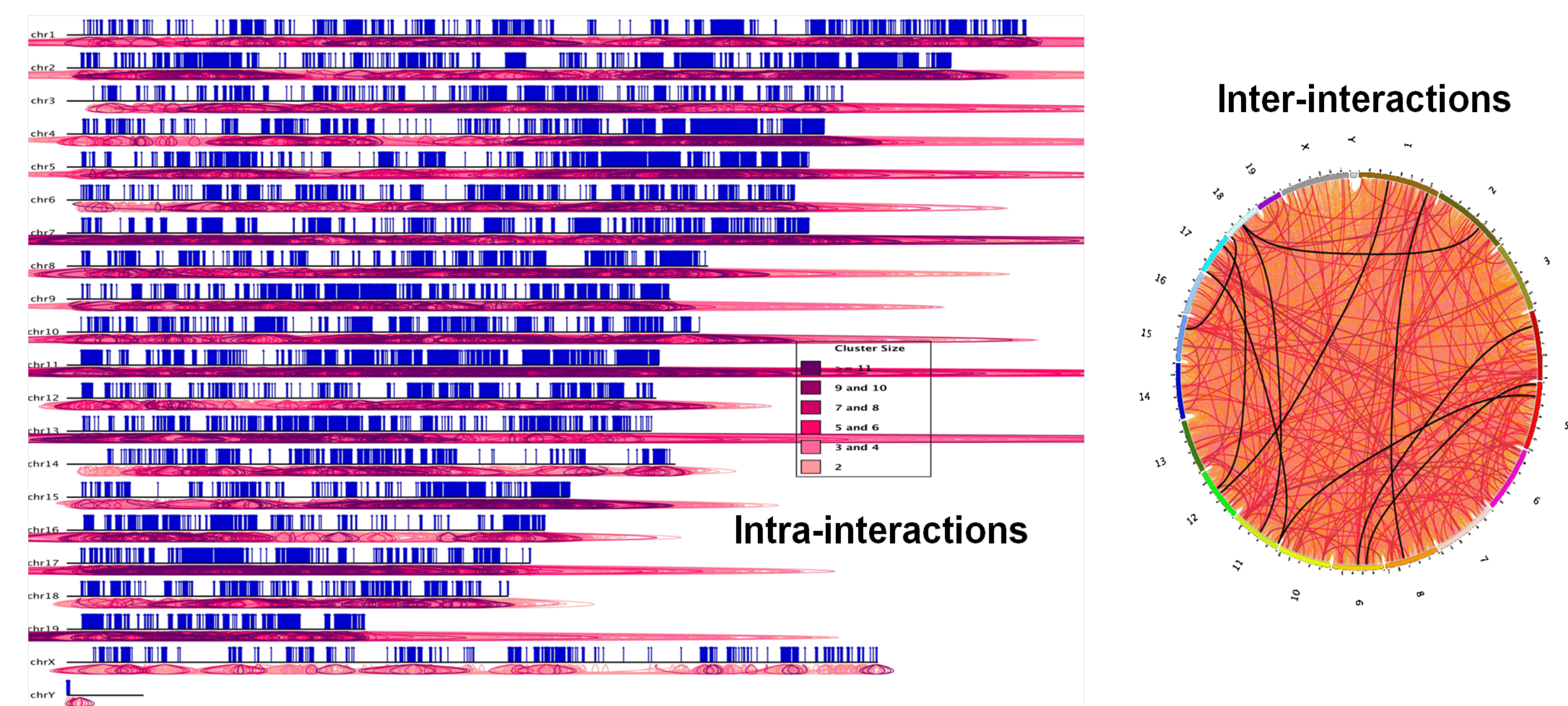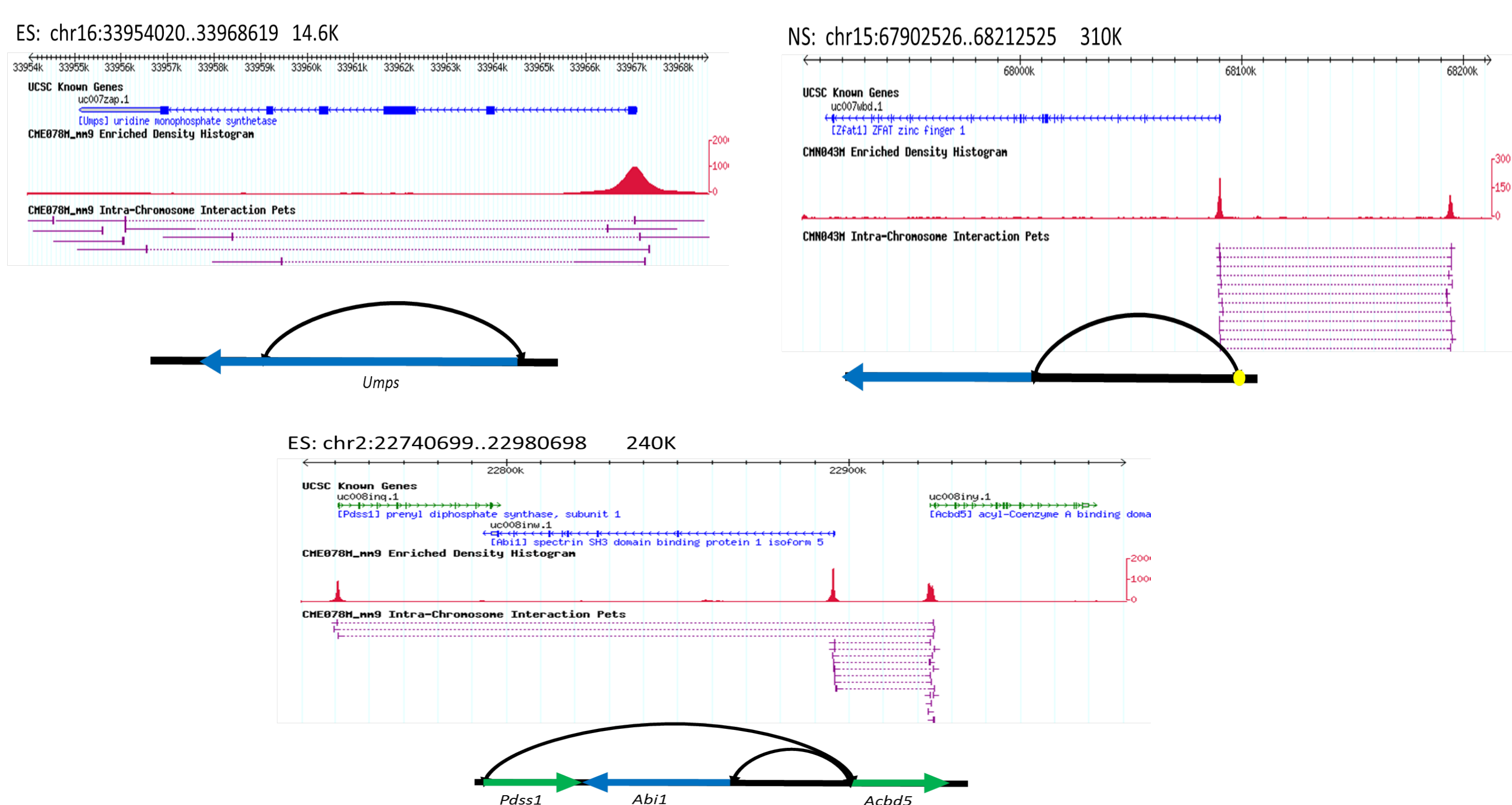
Abstract

Distant regulatory elements have been shown to influence gene function *via* long-range chromatin interactions. In this study, we depict the dynamic transcriptional interactomes mediated by RNA polymerase II (RNAPII) with the newly developed Chromatin Interaction Analysis by Pair End diTag (ChIA-PET) to define the chromatin organizations pertinent to transcription regulation and epigenetic control in pluripotent embryonic stem cells (mES), neural stem cells (mNS) and neurosphere cells (mNP). With over 150 million ChIA-PET sequencing, thousands of *cis-* and *trans-* chromatin interactions tethered by RNAPII are defined. We plan to integrate the transcription interactome maps with global transcription expression changes, promoter activities, gene activities and major epigenomic features to define spatial distribution and temporal regulation of transcription networks.

I. Project goal

Transcription Interactomes in Mouse Stem Cells

- Identify distal regulatory elements
- Relationships with target genes
- 3-D gene regulation networks
- Dynamic transcriptional interactomes
- Potential unknown mechanism(s)

2. approaches used

CHIA-PET Procedure

Cross-link cells
Sonicate chromatin
ChIP

Add linker

Circularize DNA
• self-ligation
• Inter-ligation

Extract PETs
•Mmel digestion

Amplify, Purify and Sequence PETs

Map PETs to genome

Chimera ratio

Linker ligation: Split the chromatin to 2 aliquot. Add different linkers to each aliquot.

Proximity ligation: Mix the chromatin, elute them off the ChIP beads, and conduct dilute ligation.

Result

I. CHIA-PET sequencing and mapping summary

Library ID		ES_CME078	NS_CMN043	NP_CMN041
Total PETs		160.9M	152.6M	166.7M
Unique mapped PETs		94.6M (58.8%)	72.1M (47.3%)	79.8M (47.9%)
Merged Unique PETs (±2bp)		66.9M (41.6%)	38.7M (25.4%)	8.1M (4.9%)
Self-ligation PETs	PETs	26.9M (40.3%)	5.6M (14.3)	721K (8.9%)
	Binding Sites	24,201	14,867	11,819
Intra-chromsomal	PETs	3.0M (4.5%)	2.1M (5.5%)	765K (9.4%)
	Interactions (2+)	72,427	9,671	6,581
Inter-chromsomal	PETs	36.M (54.6%)	30.1M (79.7%)	6.5M (79.8%)
	Interactions (2+)	278,656	59,152	1,700

2. Evaluation noise level via chimera library

Non-chimeric library Chimeric library

CME078_ES Pol II

CMN043_NS Pol II

CMN041_NP Pol II

3. Overview of the mES Interactome across Genome

Inter-interactions

Intra-interactions

4. Interactions identified by CHIA-PET in mES

ES: chr16:33954020..33968619 14.6K

NS: chr15:67902526..68212525 310K

ES: chr2:22740699..22980698 240K

5. Specific Interactions

a. Common Interactions identified

chr9:79606065-79642534 36.5K
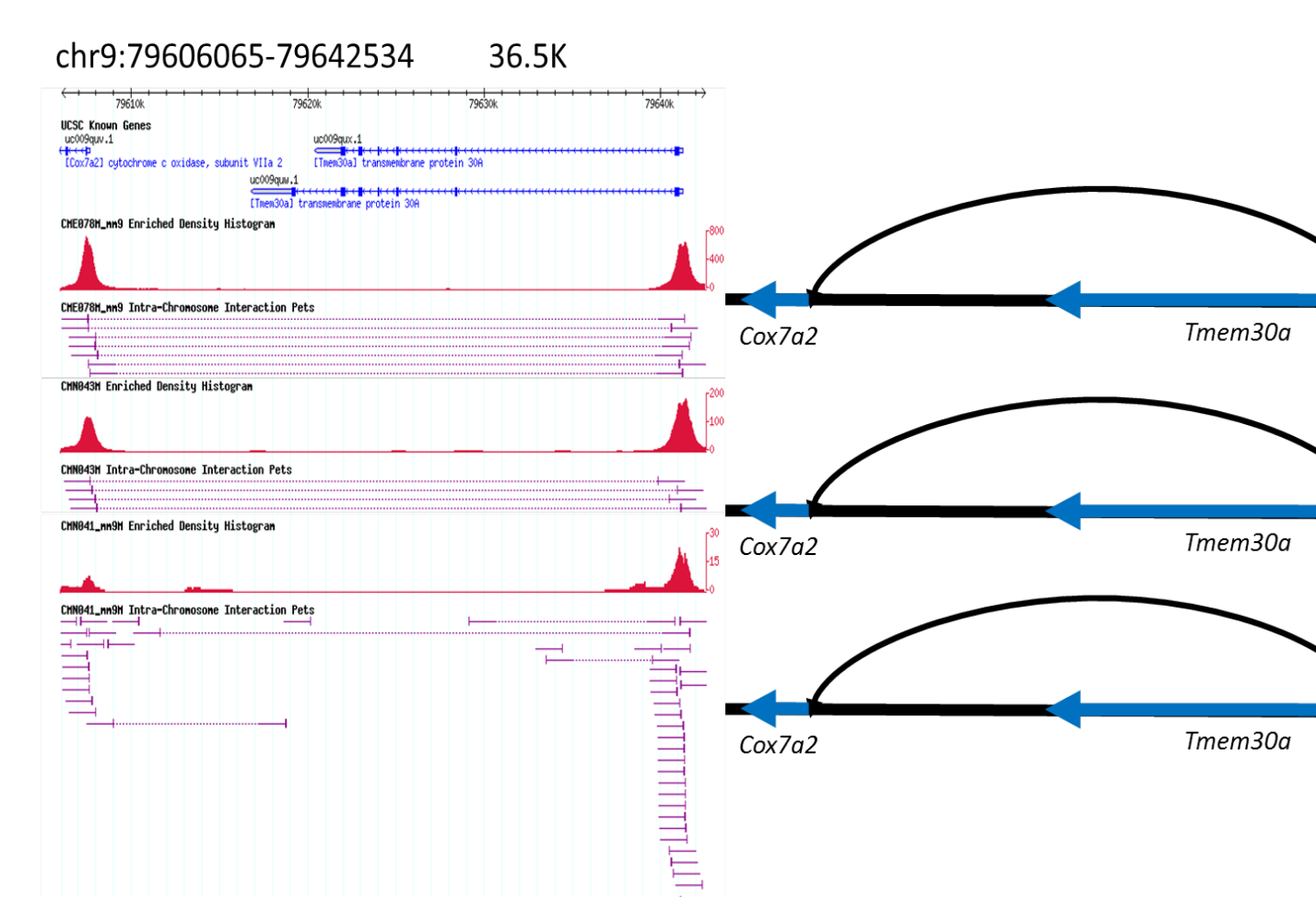
b. Tissue specific interactions

Oct4 chr17:35620353..35650352 30K
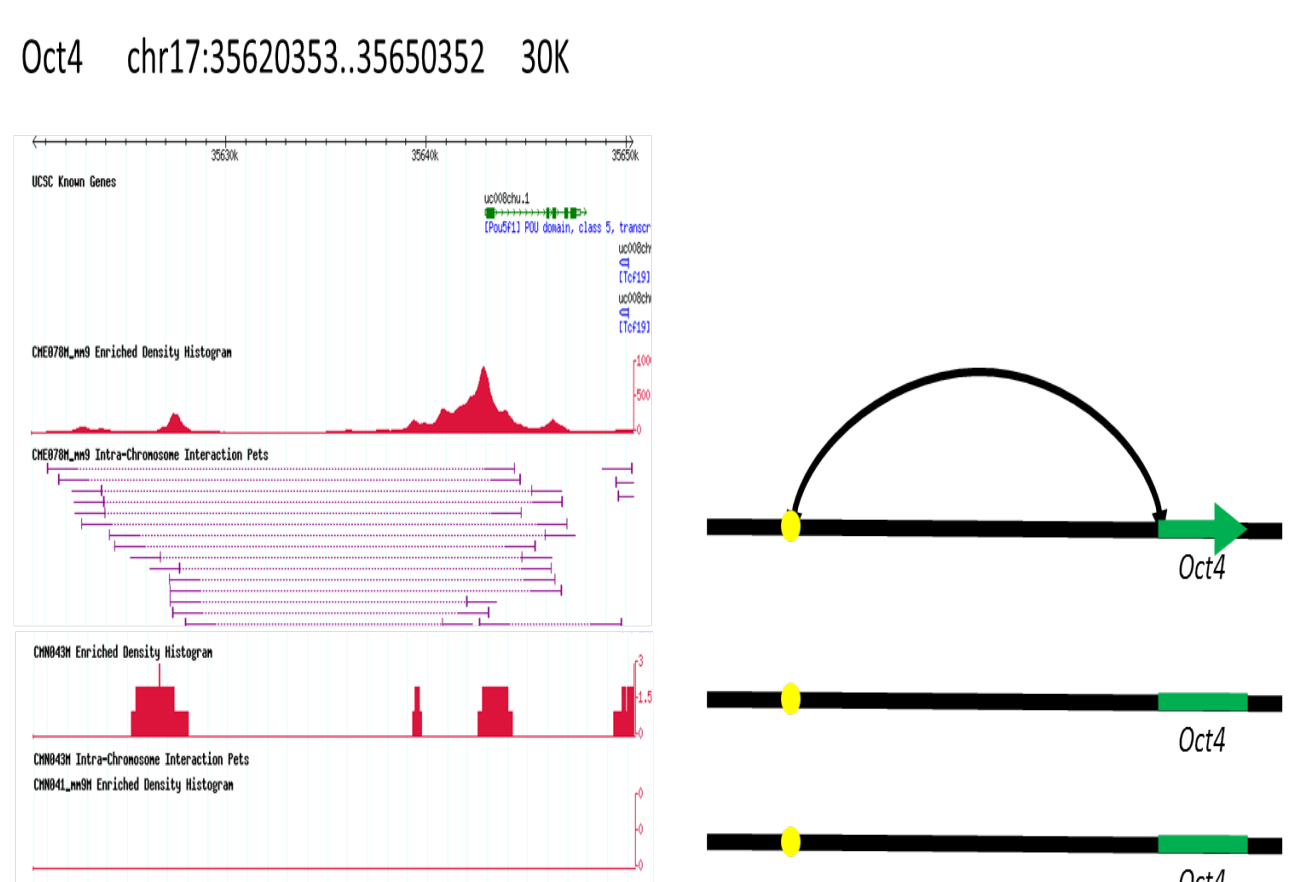
6. Dynamic interactome across three cell lines

mES vs mNS

1,516 — 9,440 — 11,786

mES vs mNP

1,317 — 9,421 — 12,004

- Common interactions
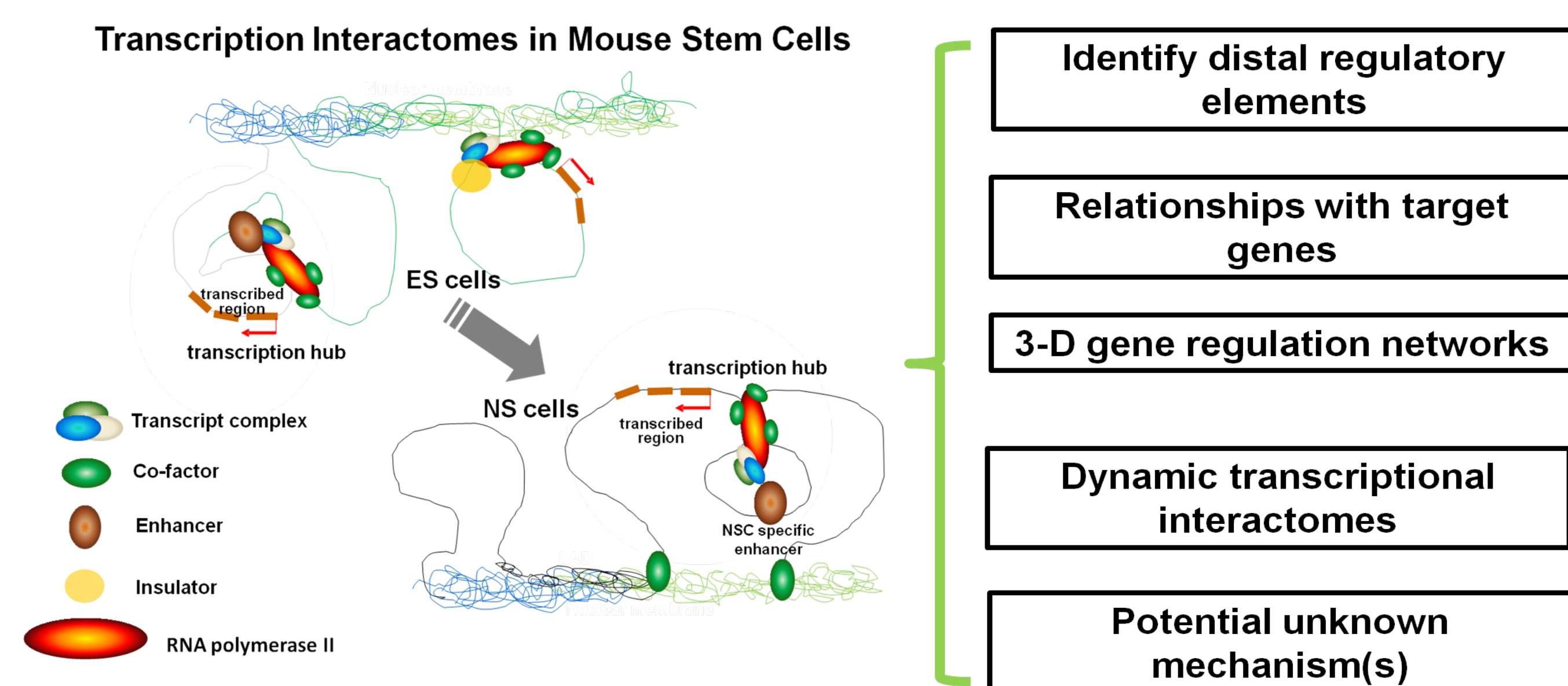- Usage specific interactions
- Specific interactions
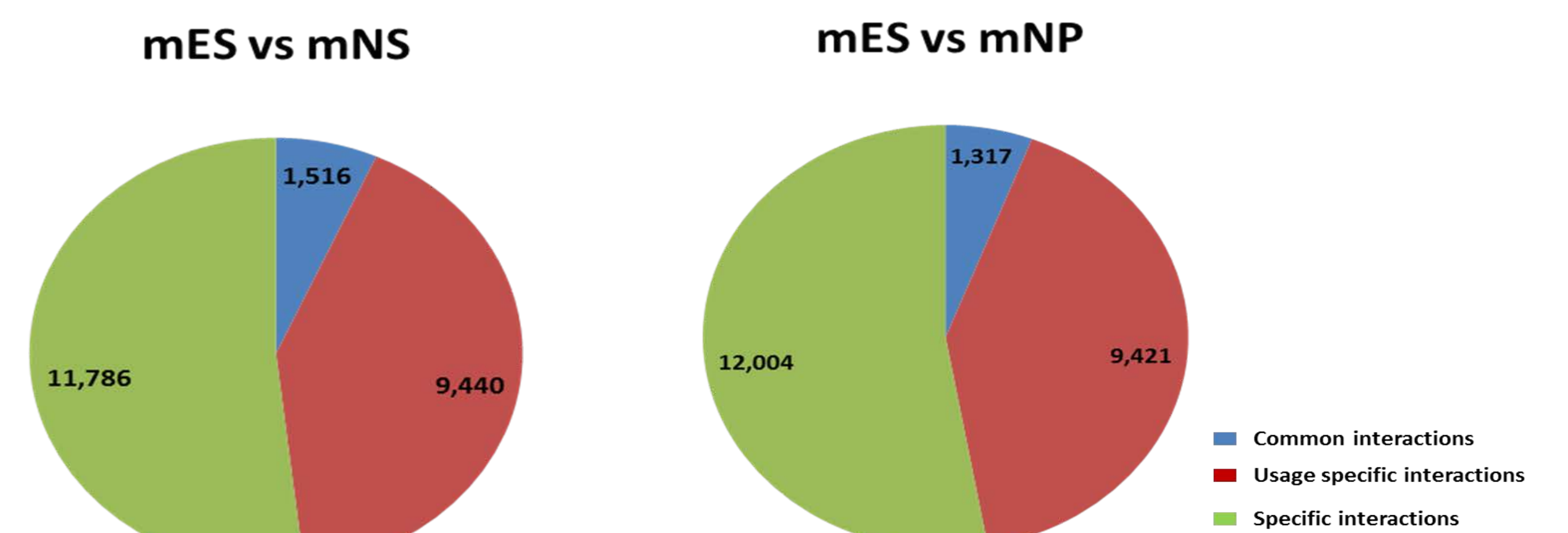
Ongoing works

1. Validation our dataset via various experiments, such as 3C, FISH

2. integrating with other genomic datasets, CHIP-Seq, RNA-Seq, RIP-Seq, methylome

3. Dynamic interactome analysis across the mouse genome
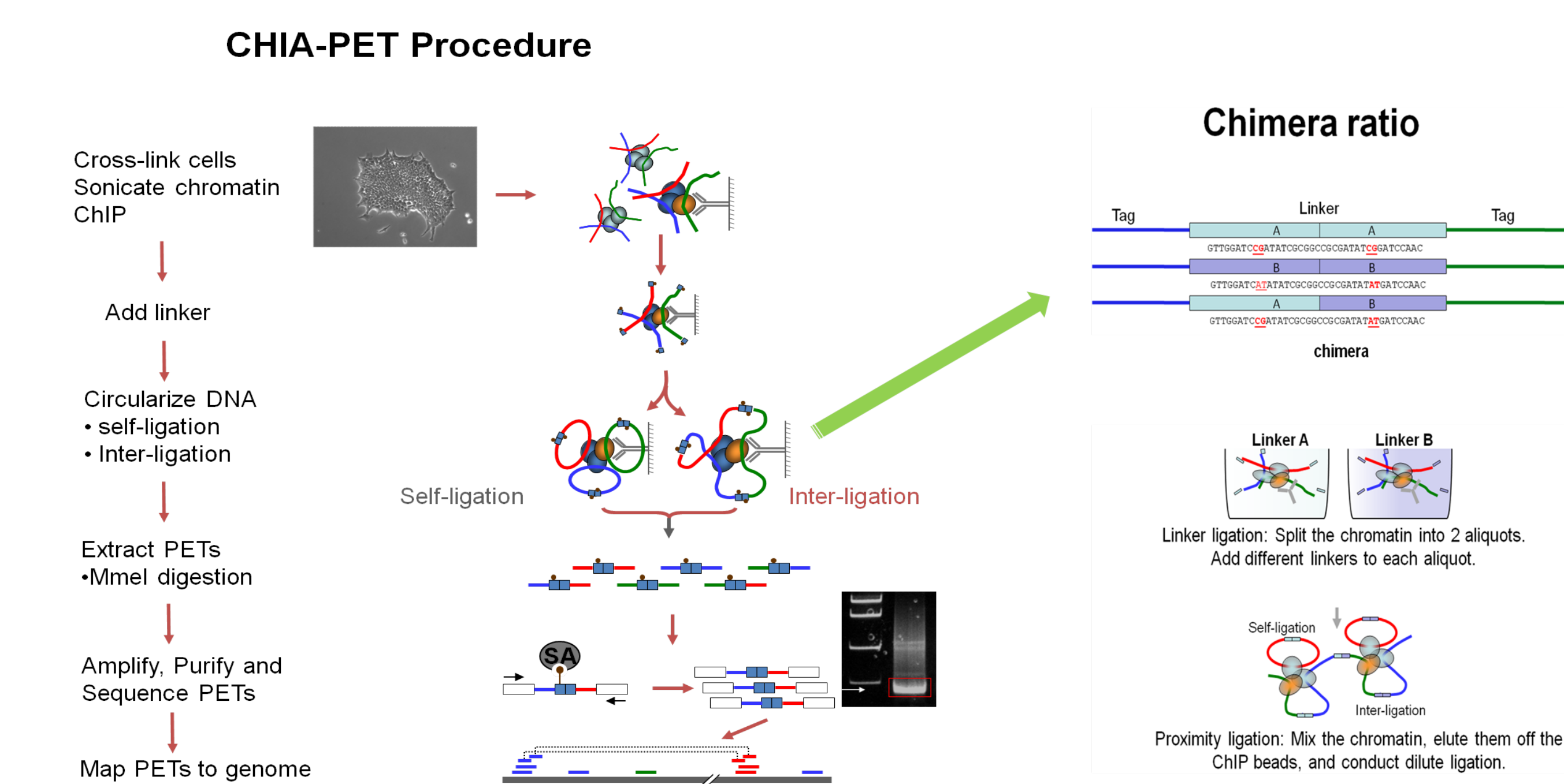
4. Modeling the 3-D gene regulation networks

References

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