RNA in situ conformation sequencing (RIC-seq) for RNA structure prediction

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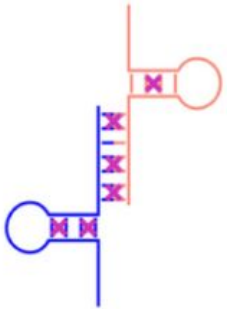
RNA proximity ligation assays

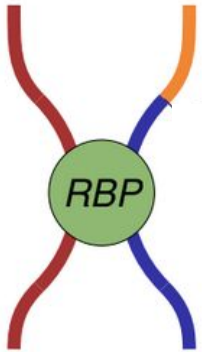
Direct crosslinking of RNA duplexes

- PARIS
- LIGR-seq
- SPLASH
- …

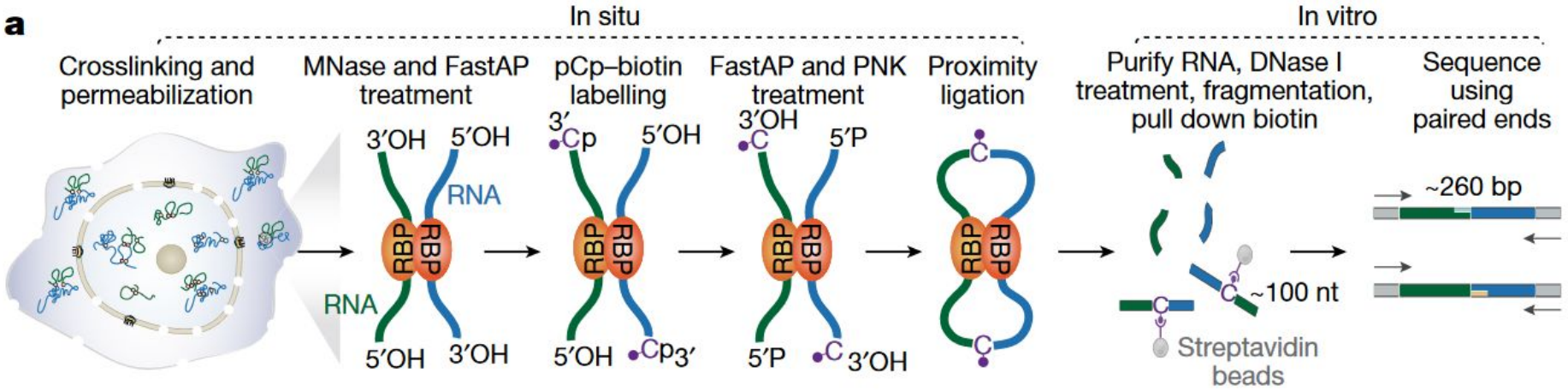


Protein-mediated crosslinking

- CLASH
- …
- **RNA in situ conformation sequencing (RIC-seq),** Cai et al., Nature, 432–437 (2020)

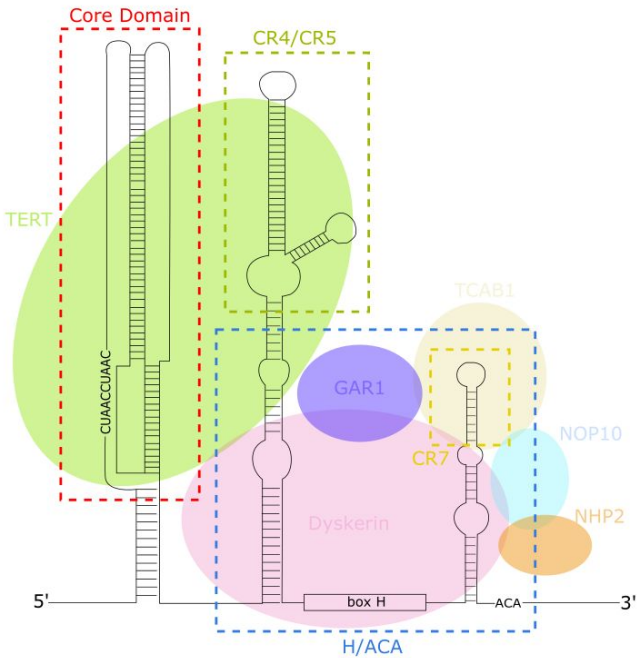


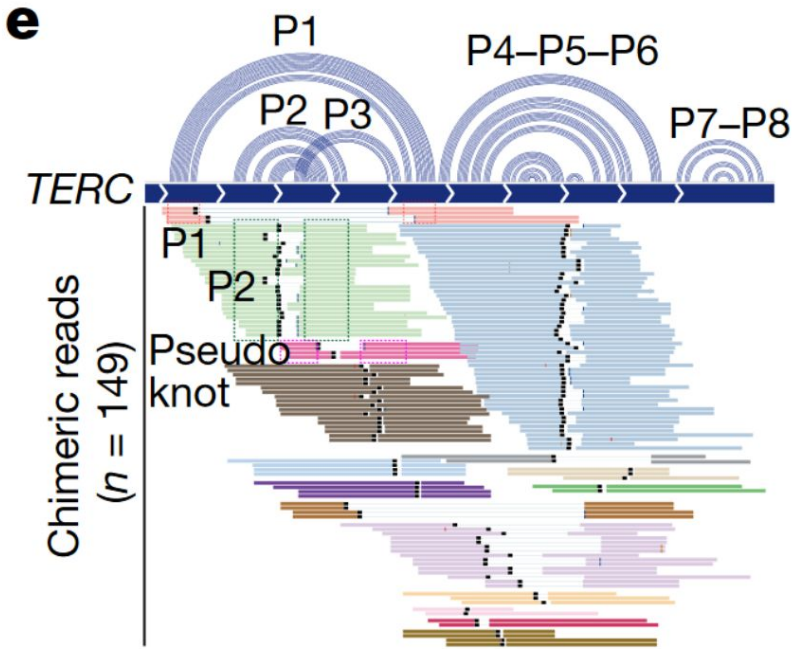
RIC-seq: protein-mediated RNA proximity ligation



Cai et al., Nature, 432–437 (2020)

RIC-seq: telomerase RNA component structure





Cai et al., Nature, 432–437 (2020)

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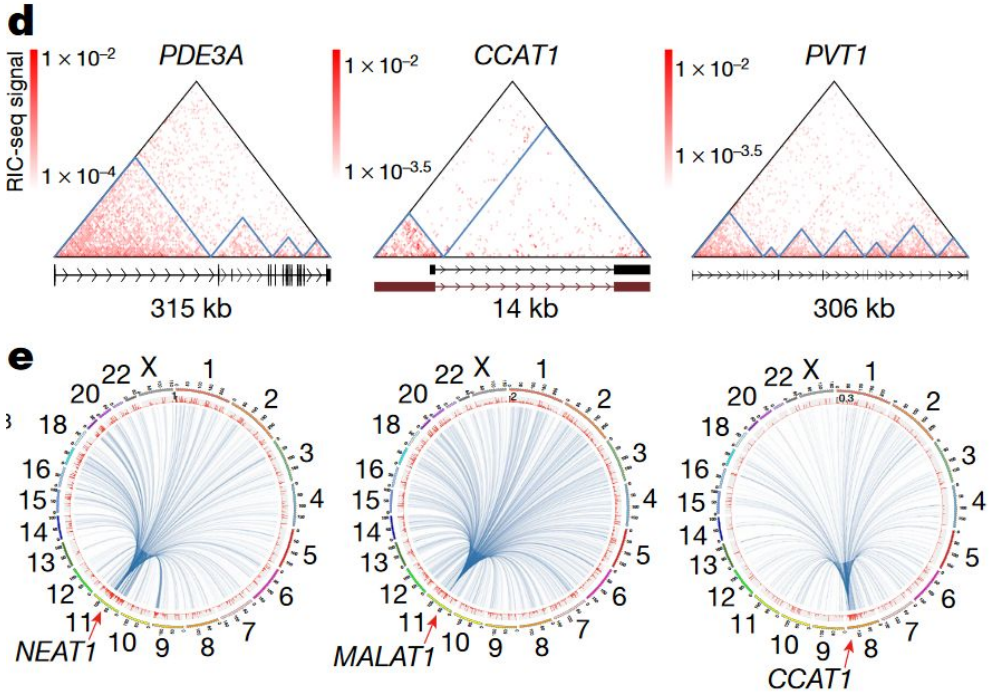
RIC-seq: local structure and lncRNA targets



Cai et al., Nature, 432–437 (2020)

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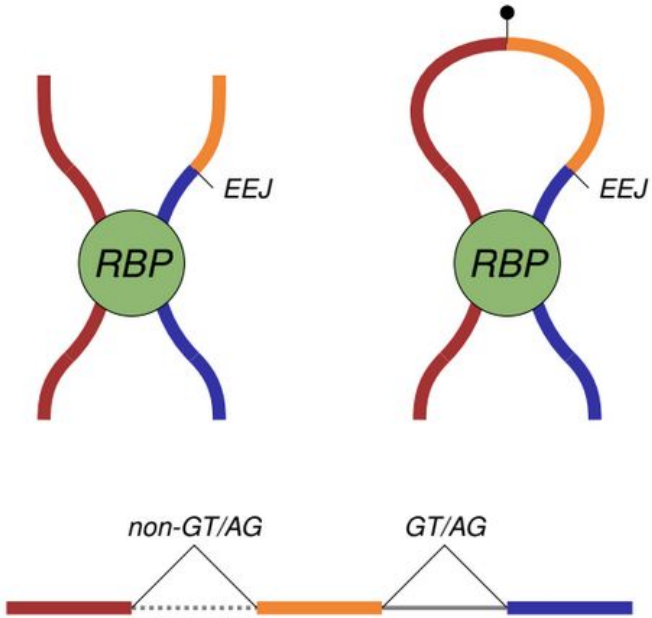
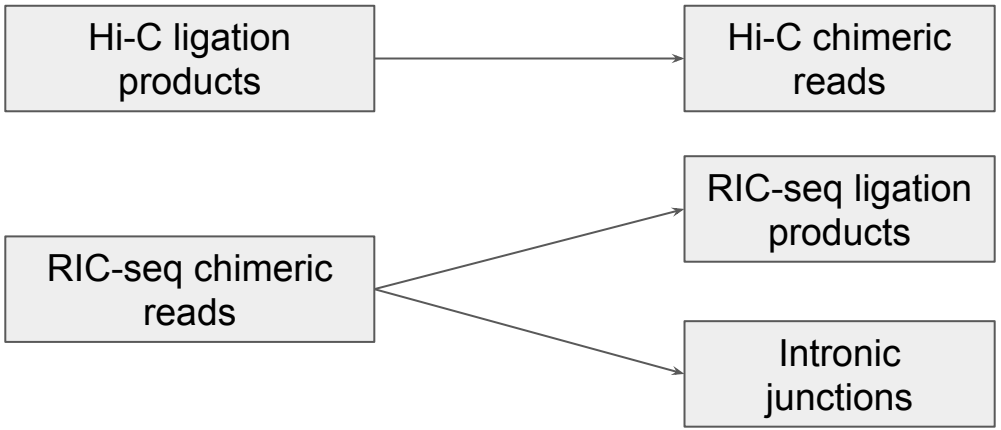
RIC-seq data analysis: problem statement





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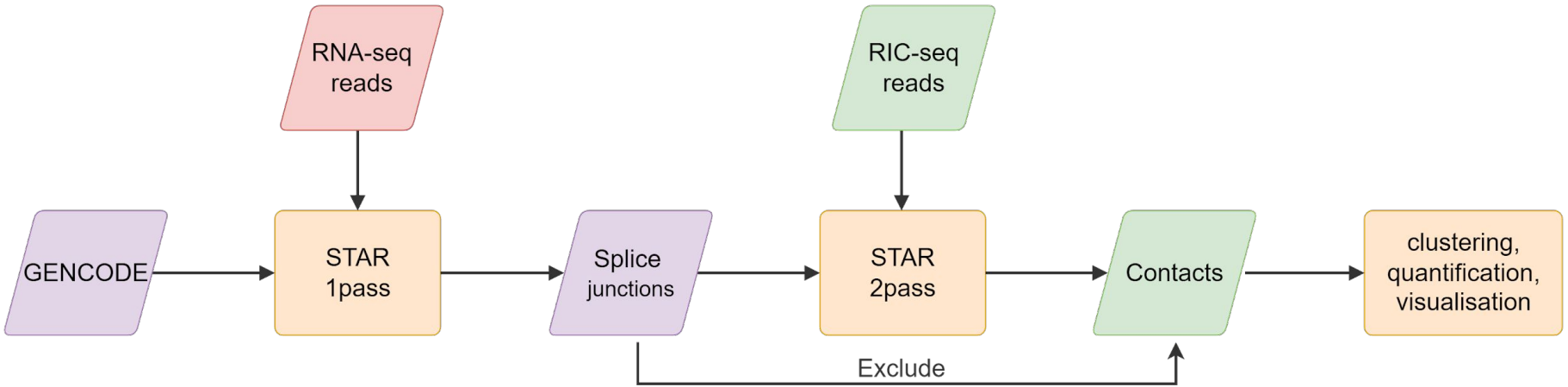
RNAcontacts pipeline



RNAcontacts: HeLa alignment statistics

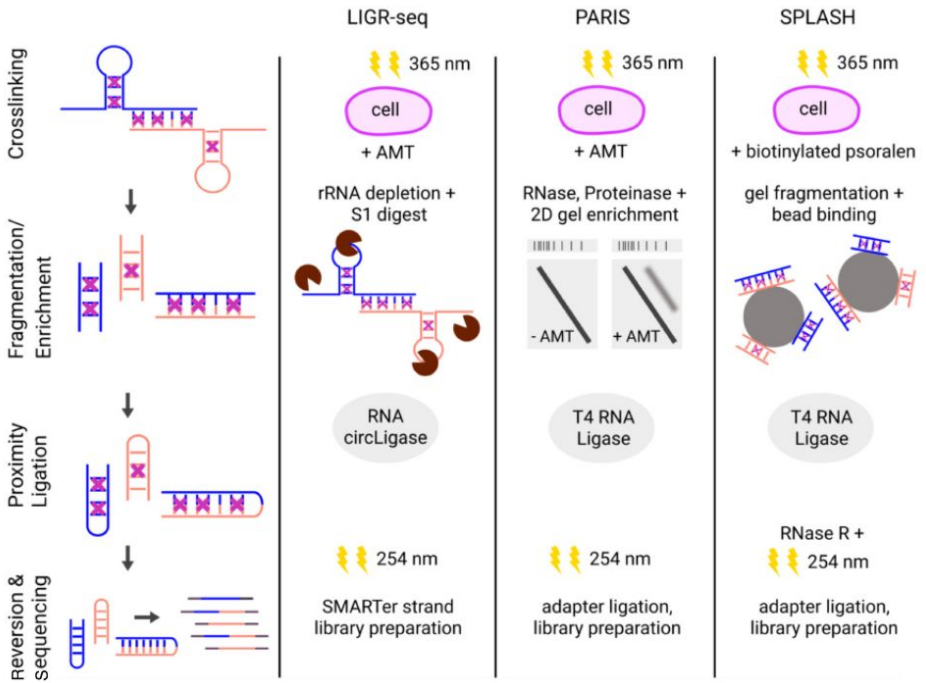
Replicate	Mate	Total	Mapped, %	Mapped unique, %
1	1	56329082	95.10%	71.59%
1	2	56329082	93.62%	70.53%
2	1	55853750	94.96%	73.39%
2	2	55853750	93.43%	72.37%

RIC-seq: high proportion of chimeric reads

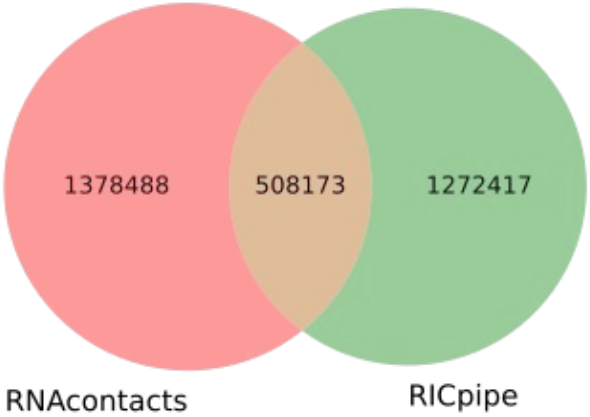
	LIGR-seq	PARIS	SPLASH
Total number of sequencing reads	171,239,817	99,698,824	189,340,955
Chimeric reads	6,614,251 (~3.9%)	2,077,743 (~2%)	1,038,801 (~0.5%)
RNA–RNA interactions	1,029	232,031[a]	4,026

Schönberger, et al. 7: F1000 Faculty Rev-1824 (2018)

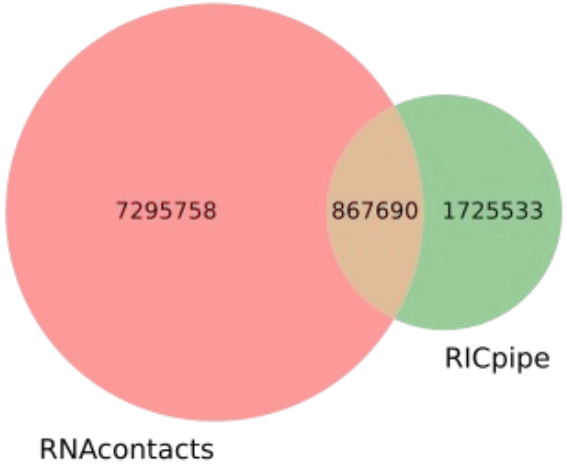
RNAcontacts extracts 18.5% of chimeric reads from RIC-seq



RNAcontacts: comparison with RICpipe

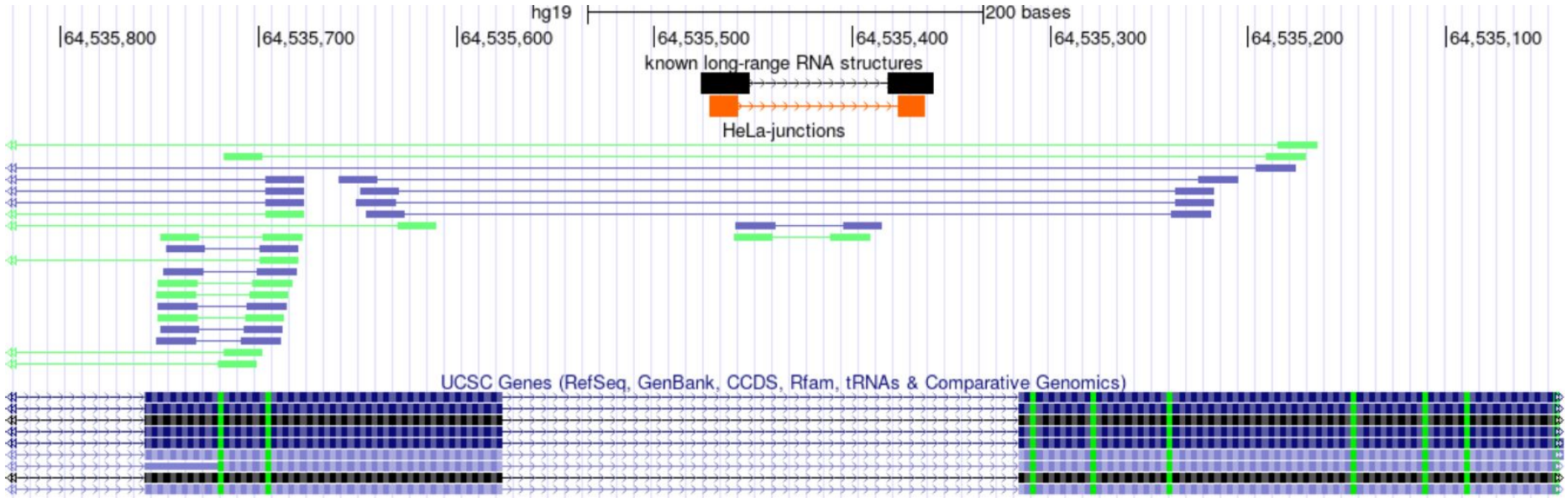


junctions

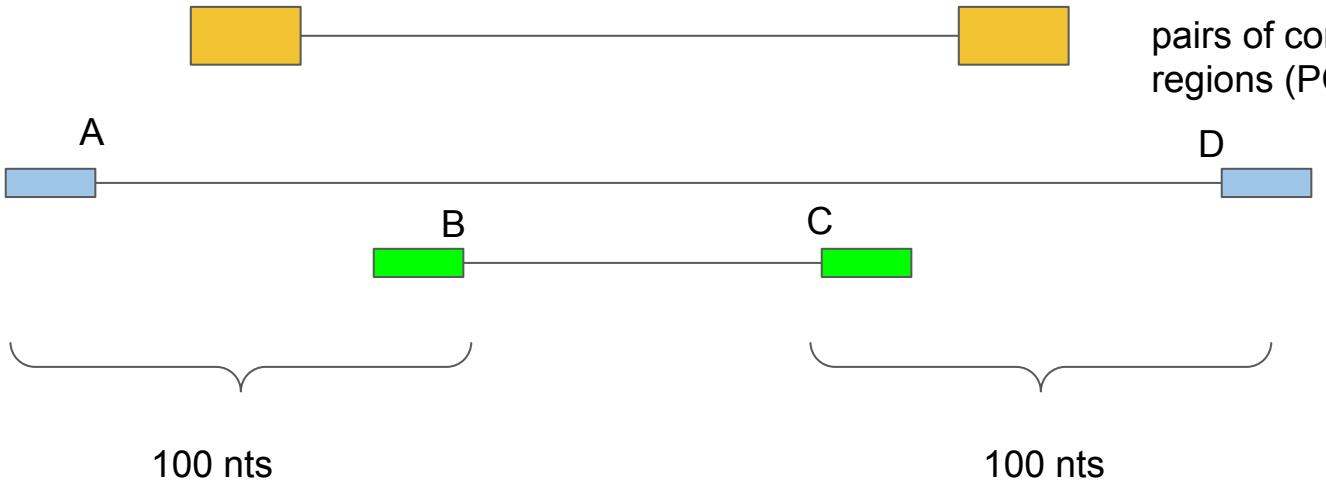


reads

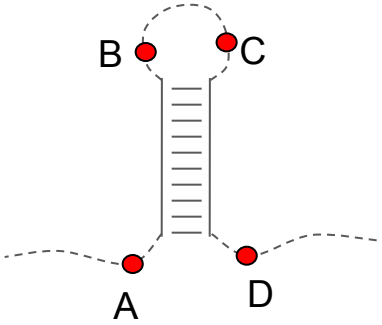
RIC-seq: SF1 structure



Conserved RNA structure with **bilateral** RIC-seq contacts



pairs of conserved complementary regions (PCCRs)



3616 PCCRs supported by RIC-seq from both sides

120 additionally contain an exon inside

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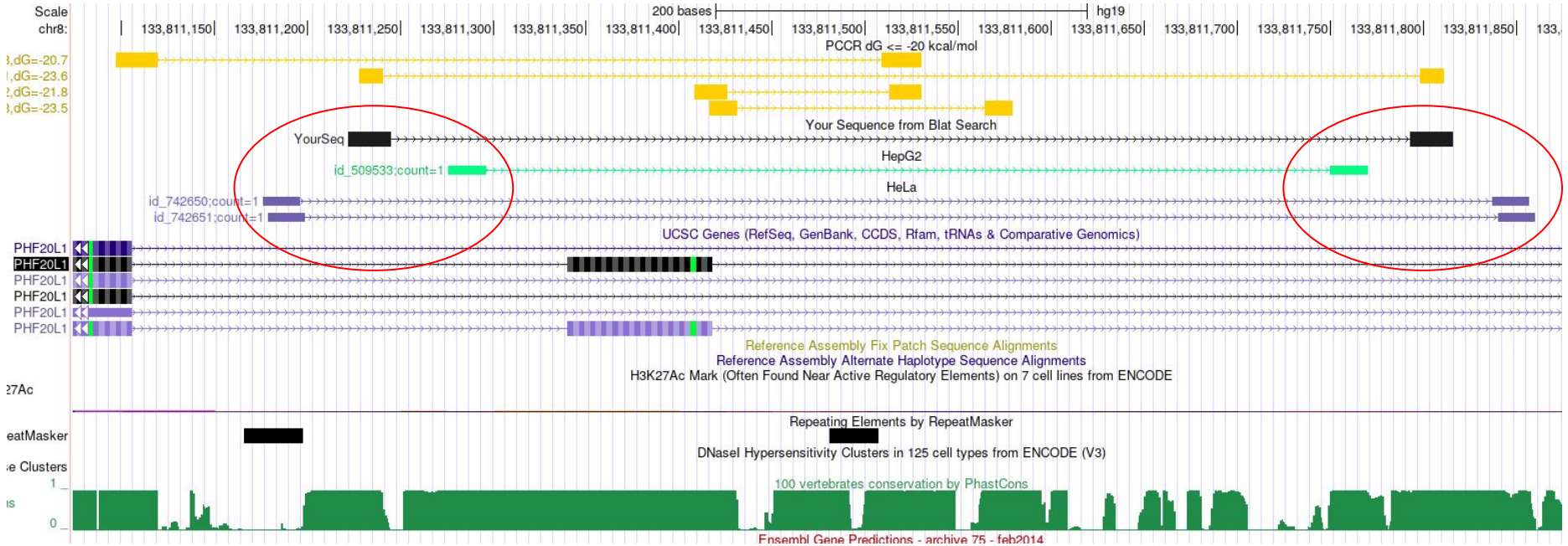
PHF20L1



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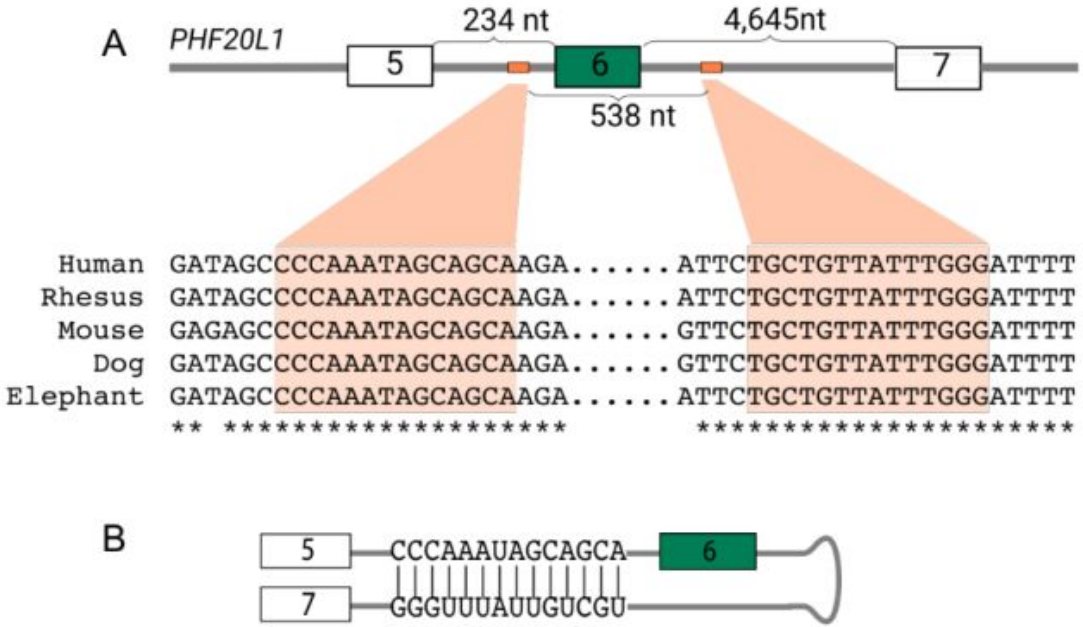
PHF20L1 (PHD Finger Protein 20 Like 1)



Predicted to be involved in histone acetylation and regulation of transcription by RNAPII. Tudor and PHD domains interact with H3K4me1, H4K20me1, H3K27me2 and DNMT1

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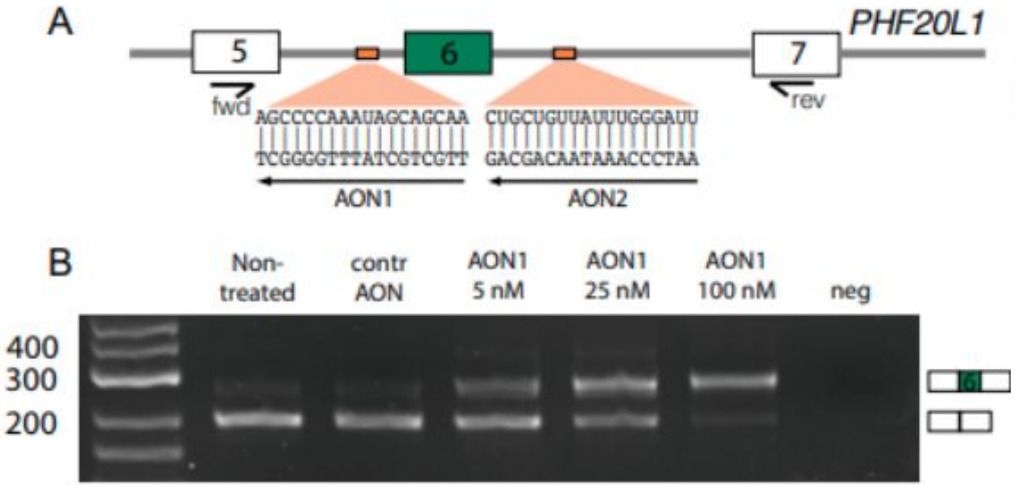
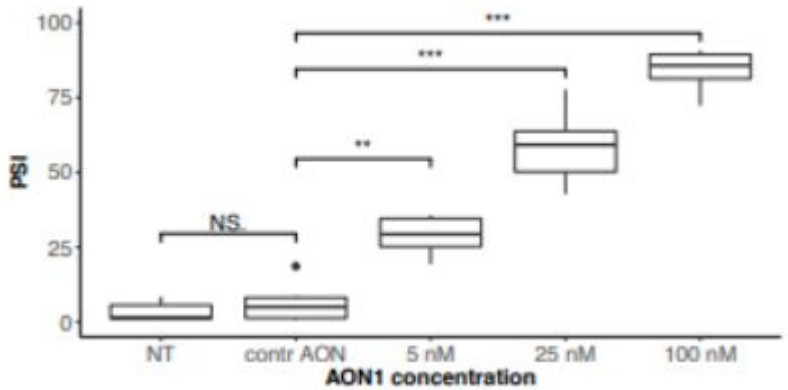
Blocking RNA structure leads to inclusion of exon 6





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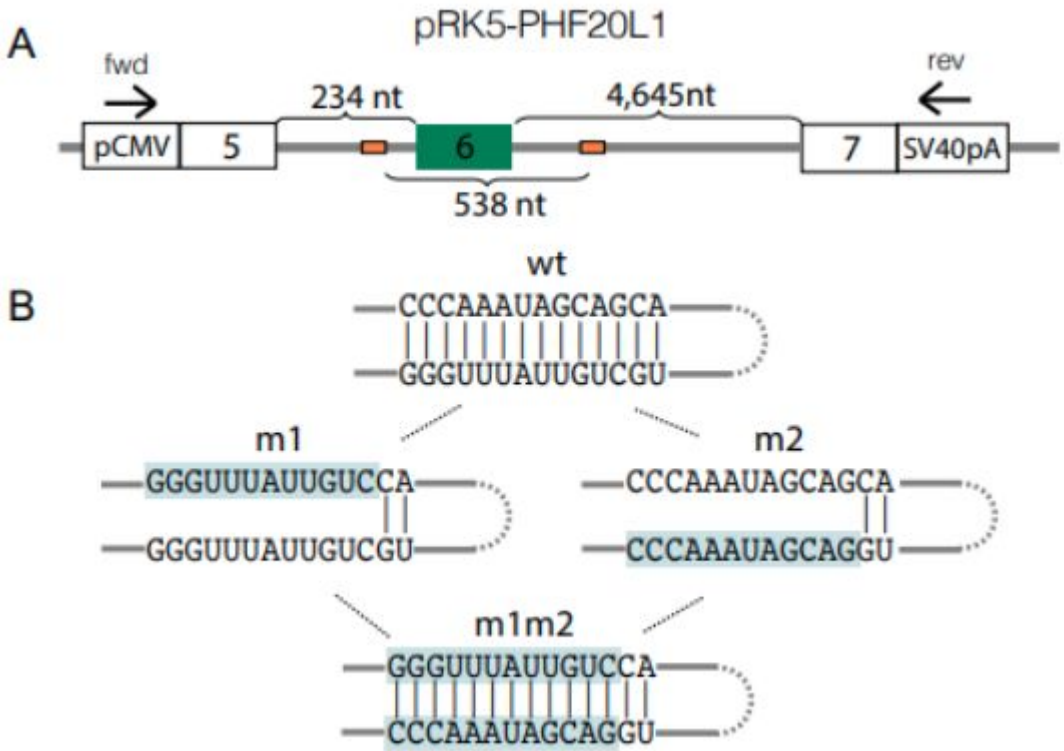
Mutagenesis PHF20L1 in a minigene



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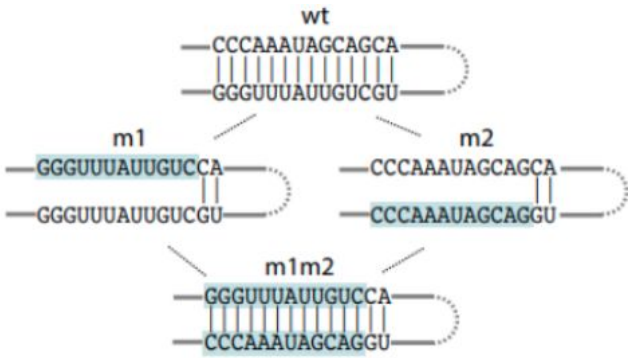
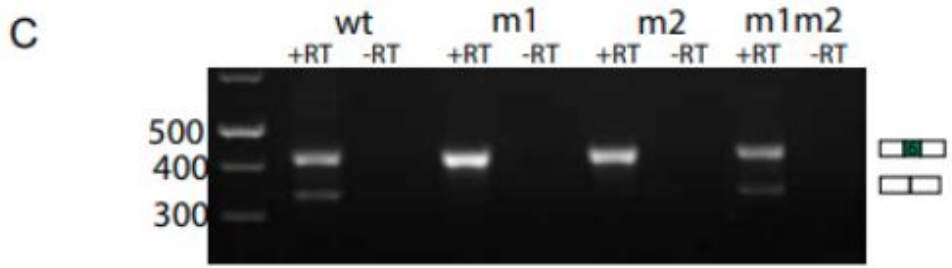
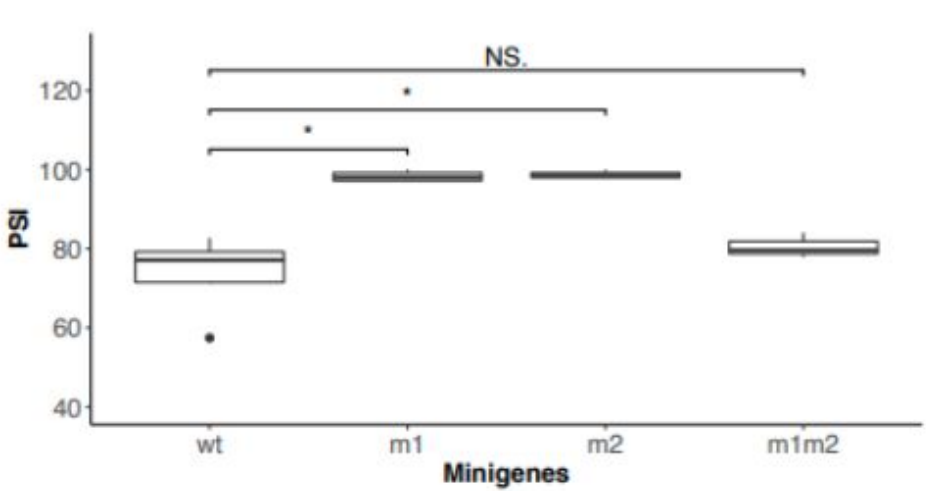
Exon 6 inclusion is regulated by RNA structure







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Conclusions

RNAcontacts, a pipeline for detecting contacts in RNA proximity ligation data (https://github.com/smargasyuk/RNAcontacts)

RIC-seq-derived RNA contacts in known structures are sparse

Nevertheless, they can be used to discover novel functional long-range RNA structures

Acknowledgements

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