

# About OMICS Group

OMICS Group is an amalgamation of [Open Access publications](#) and worldwide international science conferences and events. Established in the year 2007 with the sole aim of making the information on Sciences and technology 'Open Access', OMICS Group publishes 500 online open access [scholarly journals](#) in all aspects of Science, Engineering, Management and Technology journals. OMICS Group has been instrumental in taking the knowledge on Science & technology to the doorsteps of ordinary men and women. Research Scholars, Students, Libraries, Educational Institutions, Research centers and the industry are main stakeholders that benefitted greatly from this knowledge dissemination. OMICS Group also organizes 500 [International conferences](#) annually across the globe, where knowledge transfer takes place through debates, round table discussions, poster presentations, workshops, symposia and exhibitions.

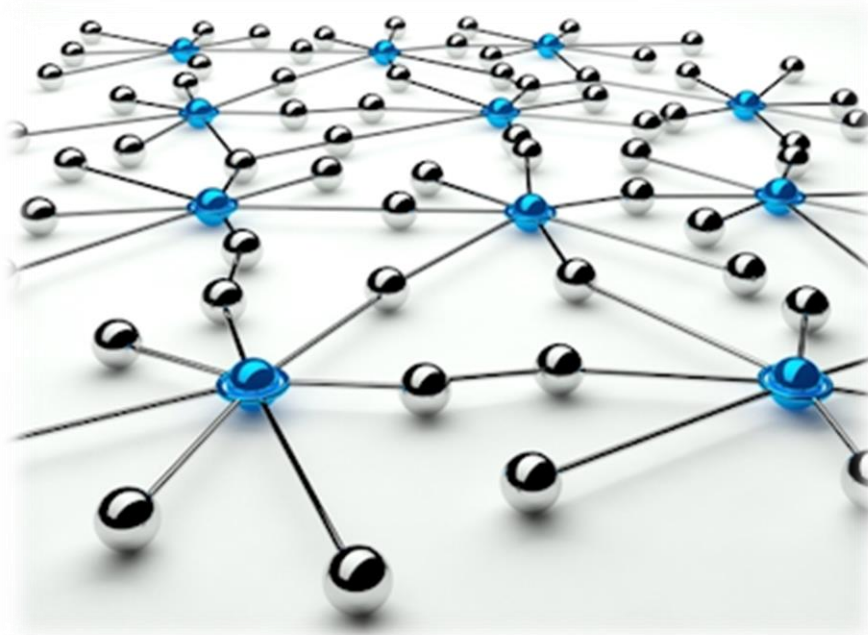
# About OMICS International Conferences

OMICS International is a pioneer and leading science event organizer, which publishes around 500 open access journals and conducts over 500 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

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# Transcriptional Interference Networks examined by single-molecule long-read sequencing



# The TIN hypothesis

HYPOTHESIS & THEORY ARTICLE

Front. Genet., 05 July 2012 | <http://dx.doi.org/10.3389/fgene.2012.00122>

**Transcriptional interference networks coordinate the expression of functionally related genes clustered in the same genomic loci**

Zsolt Boldogkői\*

Department of Medical Biology, Faculty of Medicine, University of Szeged, Szeged, Hungary

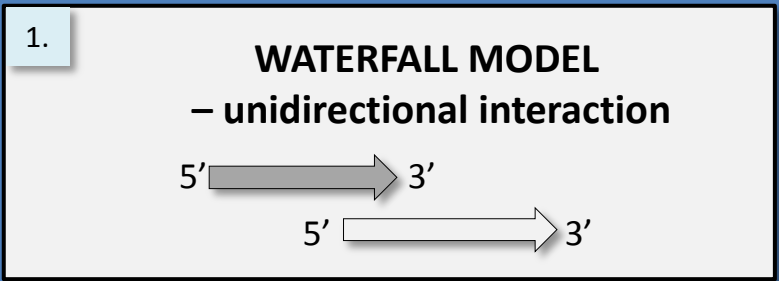
Adjacent genes interact with each other through **collision** of their transcriptional machineries at the transcriptionally overlapping regions.

These interactions form a **system-level self-regulatory network** and result in a strictly-controlled succession of the **ON/OFF states** of genes.

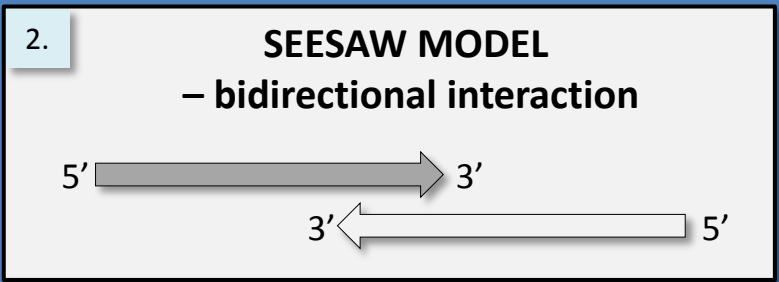
► A herpesvirus model for the investigation of TIN



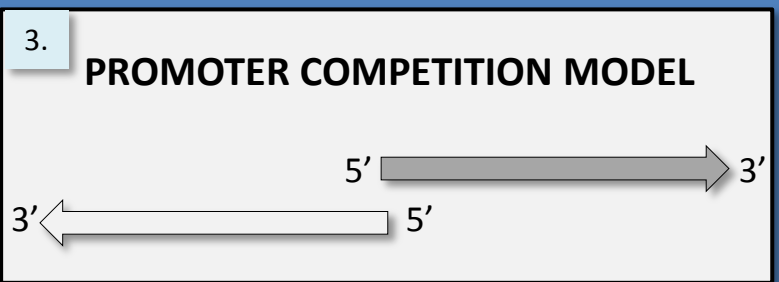
# Types of interactions



Dislocation of the transcription initiation complex



Collision of RNA pol molecules



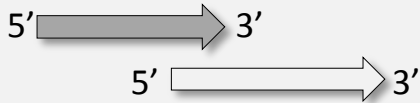
Competition between transcriptional machineries

Instead: joint coordination of genes

# Types of transcriptional interference

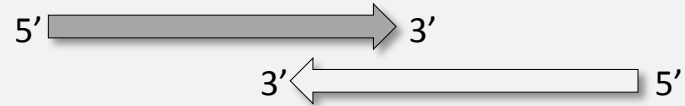
1.

## PARALLEL OVERLAP



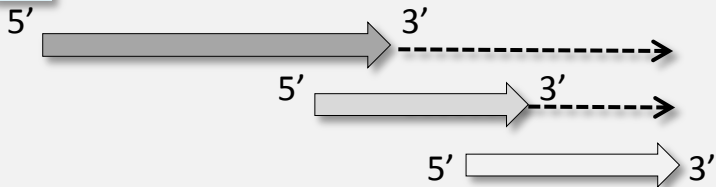
4.

## CONVERGENT ,HARD' OVERLAP



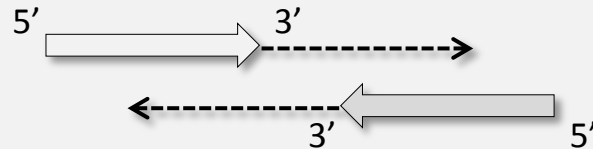
2.

## MULTIPLE PARALLEL OVERLAPS



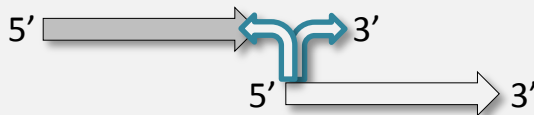
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## CONVERGENT ,SOFT' OVERLAP



3.

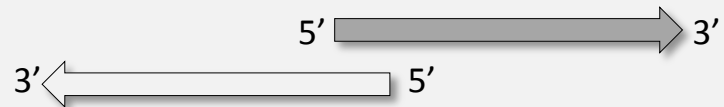
## BIDIRECTIONAL PROMOTER



6.

## DIVERGENT OVERLAP

(overlap of transcribed regions)



7.

## CONVERGENT OVERLAP

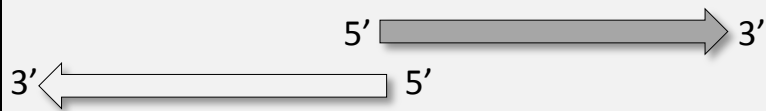
between coding – and non-coding genes



8.

## DIVERGENT OVERLAP

(overlap of promoters)



# Evidences for transcriptional interference between gene pairs

## VIRUSES:

Cullen BR, Lomedico PT, Ju G. [Transcriptional interference in avian retroviruses--implications for the promoter insertion model of leukaemogenesis.](#) **Nature.** 1984;307(5948):241-5.

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## BACTERIA:

Crampton N, Bonass WA, Kirkham J, Rivetti C, Thomson NH. [Collision events between RNA polymerases in convergent transcription studied by atomic force microscopy.](#) **Nucleic Acids Res.** 2006; 34(19):5416-25.

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## FUNGI:

Peterson JA, Myers AM. [Functional analysis of mRNA 3' end formation signals in the convergent and overlapping transcription units of the \*S. cerevisiae\* genes RHO1 and MRP2.](#) **Nucleic Acids Res.** 1993; 21(23):5500-8.

Prescott EM, Proudfoot NJ. [Transcriptional collision between convergent genes in budding yeast.](#) **Proc Natl Acad Sci USA.** 2002; 99(13):8796-801.

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## INSECTS:

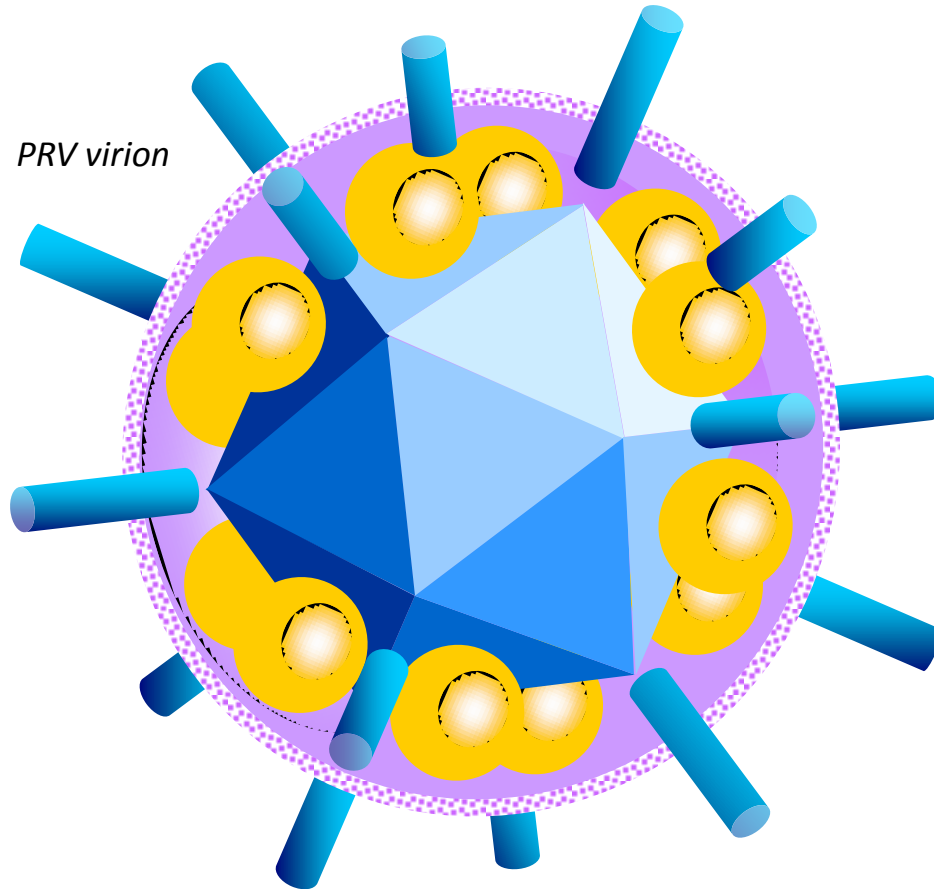
Petruk S, Sedkov Y, Riley KM, Hodgson J, Schweisguth F, Hirose S, Jaynes JB, Brock HW, Mazo A. [Transcription of bxd noncoding RNAs promoted by trithorax represses Ubx in cis by transcriptional interference.](#) **Cell.** 2006;127(6):1209-21.

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## MAMMALS:

Hu X, Eszterhas S, Pallazzi N, Bouhassira EE, Fields J, Tanabe O, Gerber SA, Bulger M, Engel JD, Groudine M, Fiering S. [Transcriptional interference among the murine beta-like globin genes.](#) **Blood.** 2007;109(5):2210-6.

# Pseudorabies virus (PRV)



Alpha-herpesvirus  
Natural host: pig  
Enveloped virus  
Large double-stranded DNA

**PRV is widely used as a**

- (1) model organism for the investigation of molecular pathogenesis of herpesviruses
- (2) trans-neuronal tract-tracing tool
- (3) gene delivery vector



## Strain Kaplan of Pseudorabies Virus Genome Sequenced by PacBio Single-Molecule Real-Time Sequencing Technology

Dóra Tombác<sup>a</sup>, Donald Sharon<sup>b</sup>, Péter Oláh<sup>a</sup>, Zsolt Csabai<sup>a</sup>, Michael Snyder<sup>b</sup>, Zsolt Boldogkői<sup>a</sup>

Author Affiliations

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<sup>b</sup>Department of Genetics, School of Medicine, Stanford University, Stanford, California, USA

# The PRV genome



**UL:** unique long region

**US:** unique short region

**IR:** internal repeat

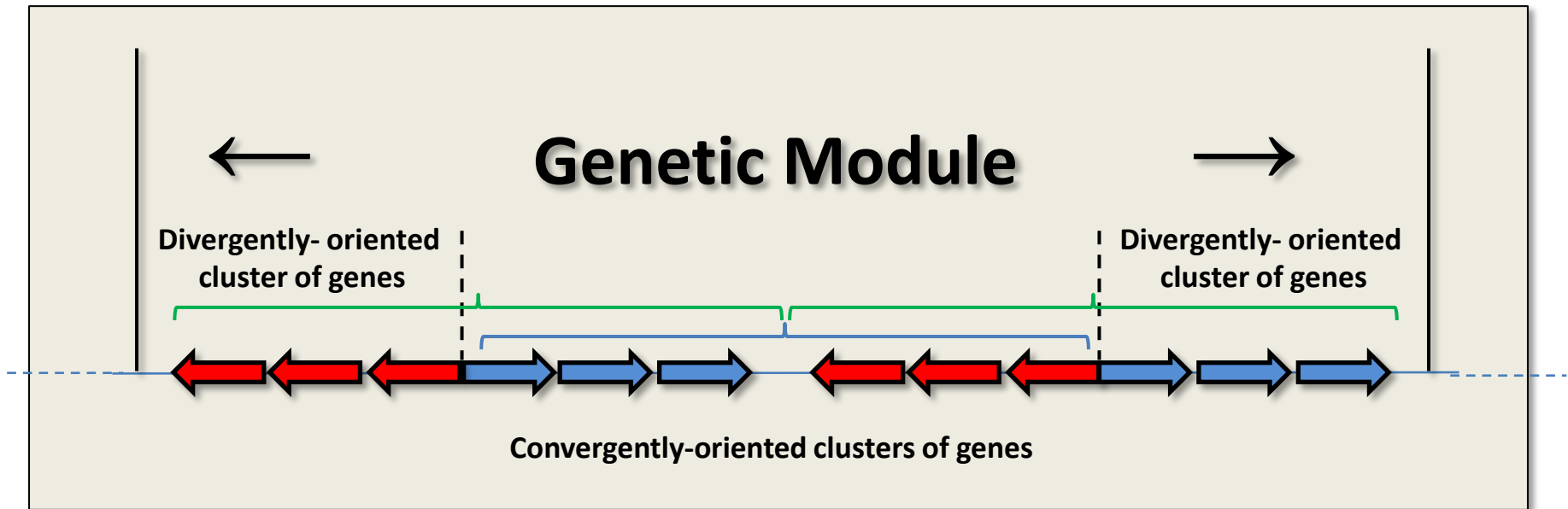
**TR:** terminal repeat

**PacBio RS II platform**



# Modular organization of PRV genome

The genome of herpesviruses exhibits a special architecture: it is composed of an array of module-like structures

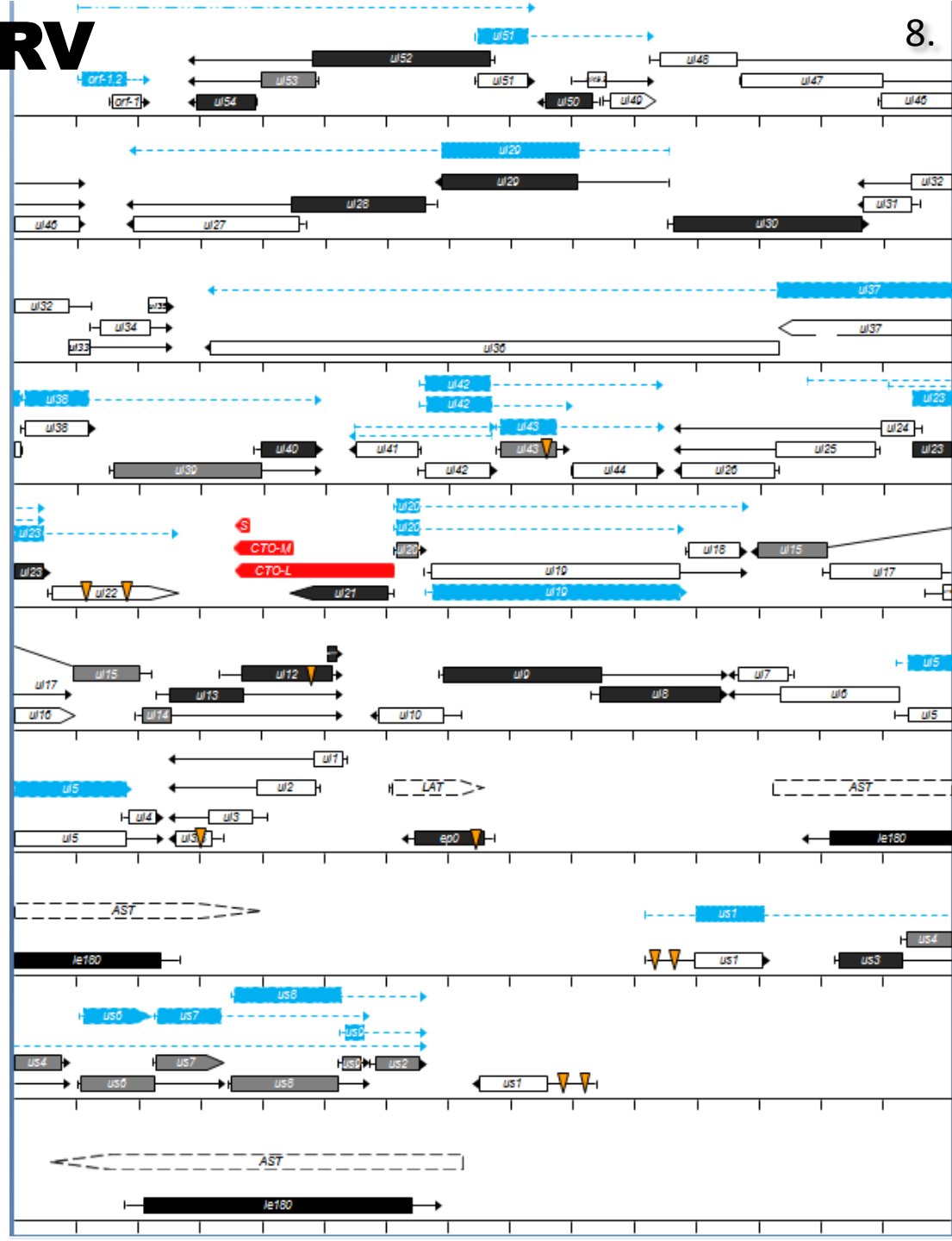


# Transcriptome of PRV

**Illumina HiSeq platform:**  
transcriptome with mixed transcriptional kinetics

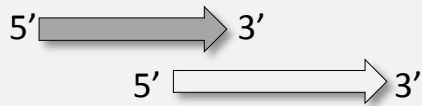


**PacBio RS II platform:**  
transcriptome at various phases of viral infection (1h, 2h, 4h, 6h, 8h, 12h)

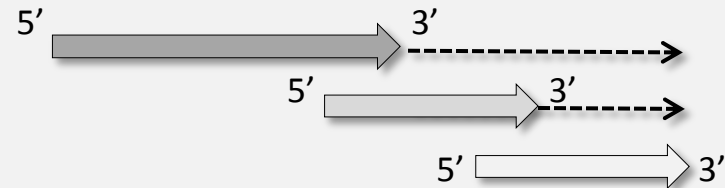


# Transcriptional overlap of tandem genes

## PARALEL OVERLAP



## MULTIPLE PARALLEL OVERLAPS



## Genetic Module

genes

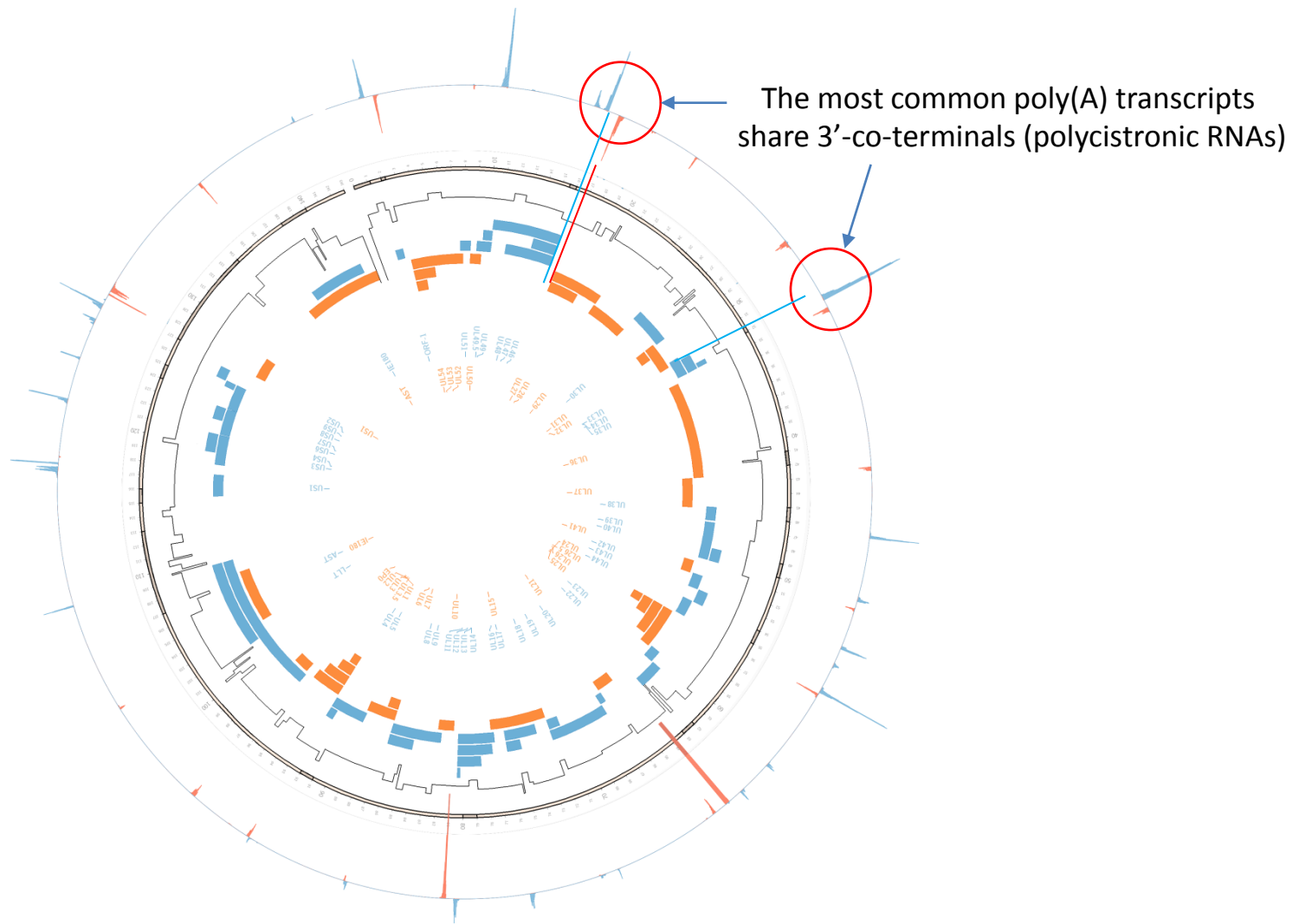
transcripts



### Two forms of parallel transcriptional overlaps:

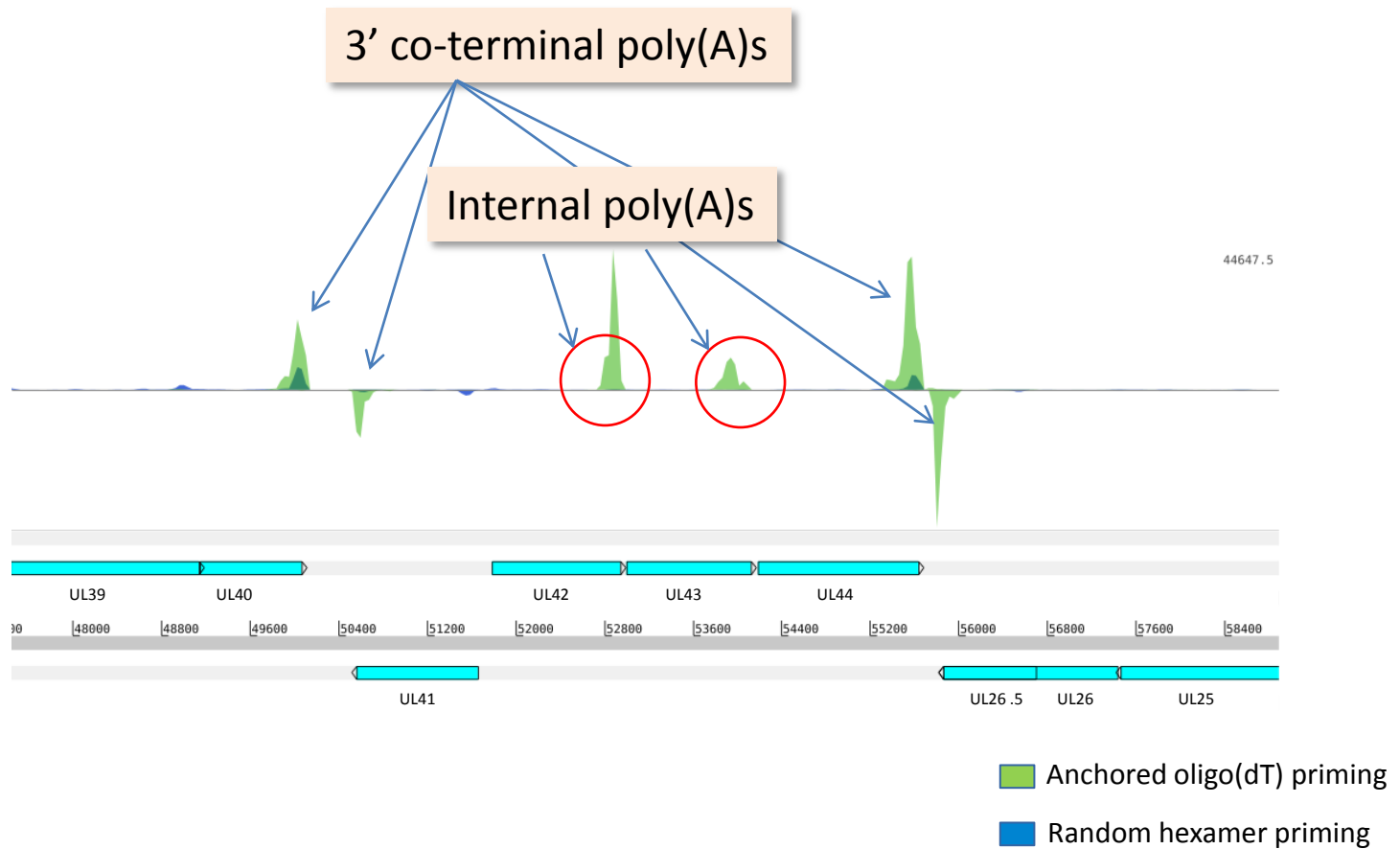
- (1) Most of the tandem genes overlaps at their 5' – 3' termini
- (2) Transcripts encoded by tandem genes share 3' co-terminals producing polycistronic transcripts

# Circos plot of the PRV transcripts

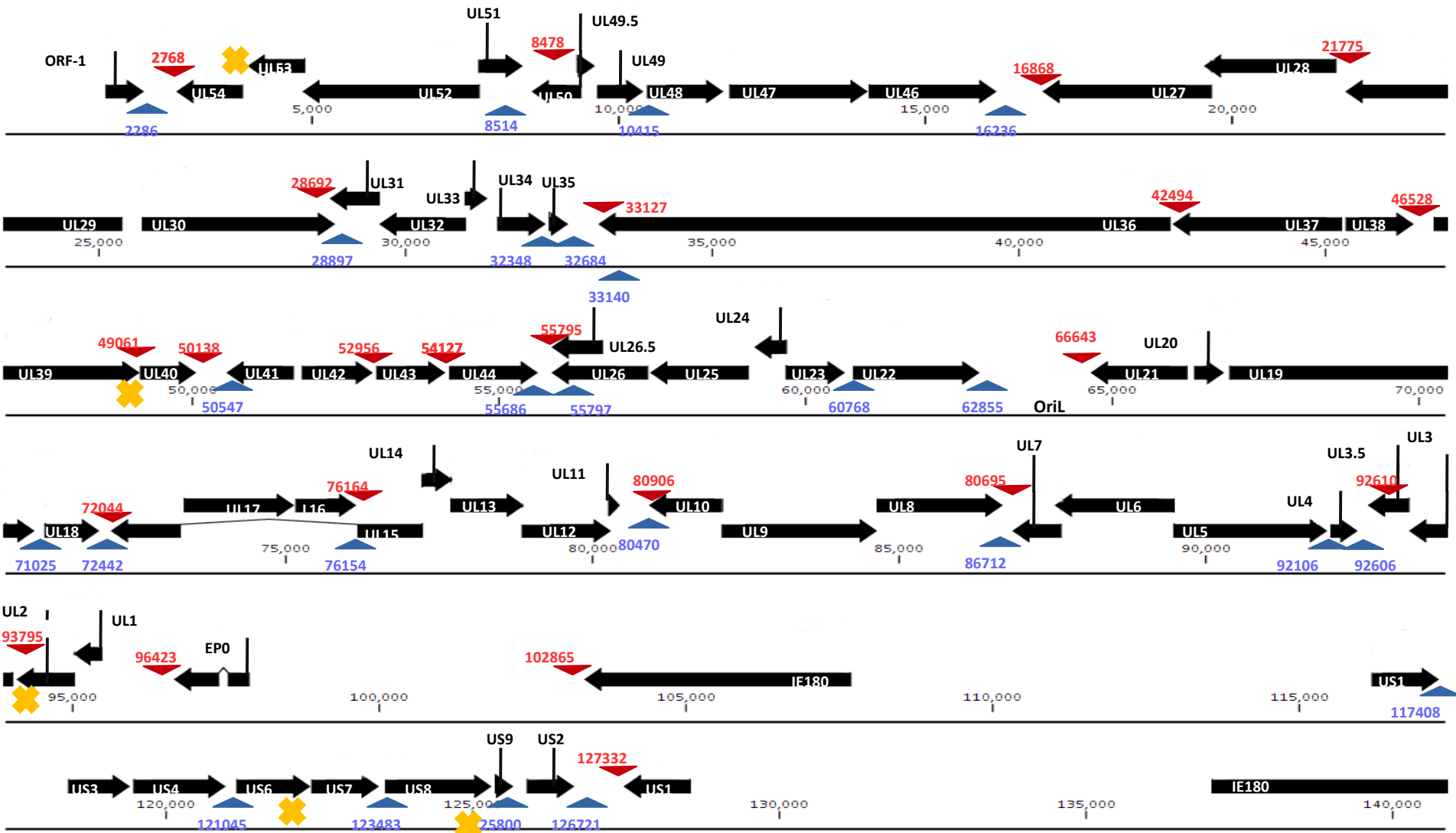


Illumina HiScanSQ platform – anchored oligo(dT) priming [poly(A)-seq]

# Transcription terminations of tandem genes



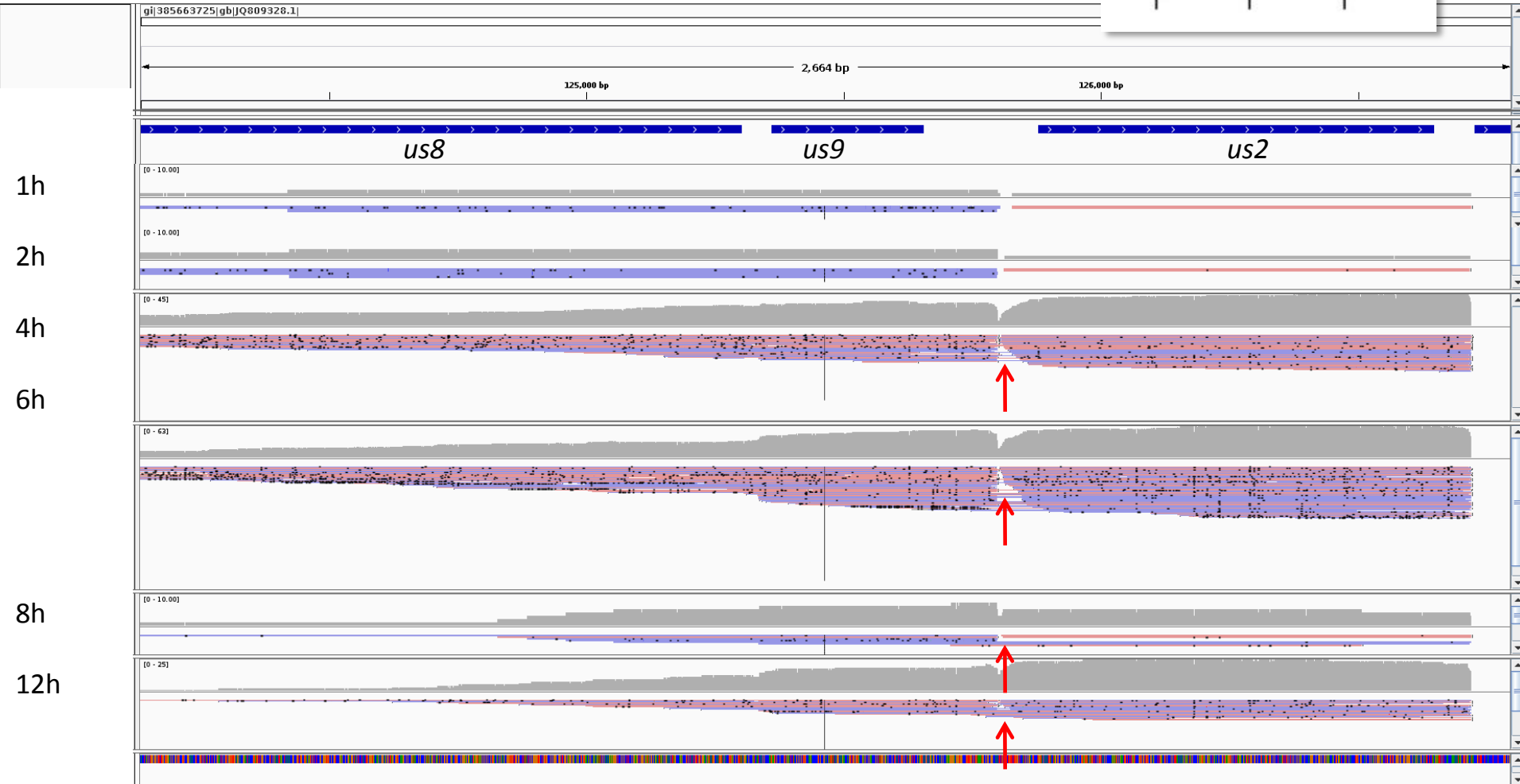
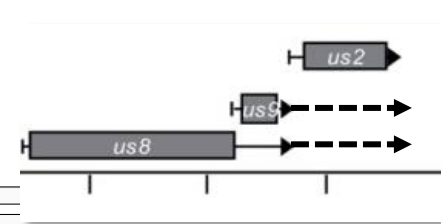
# Poly(A) signals of the upstream genes



- Coding region
- Major poly(A) signal, plus strand
- Major poly(A) signal, minus strand
- Weak poly(A) signal in tandem genes

# Dynamic transcriptome of PRV

9e.



## Transcriptional cascade:

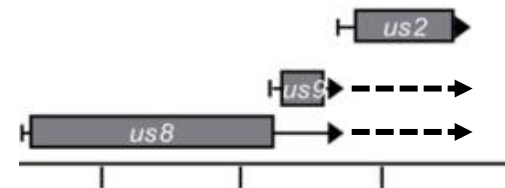
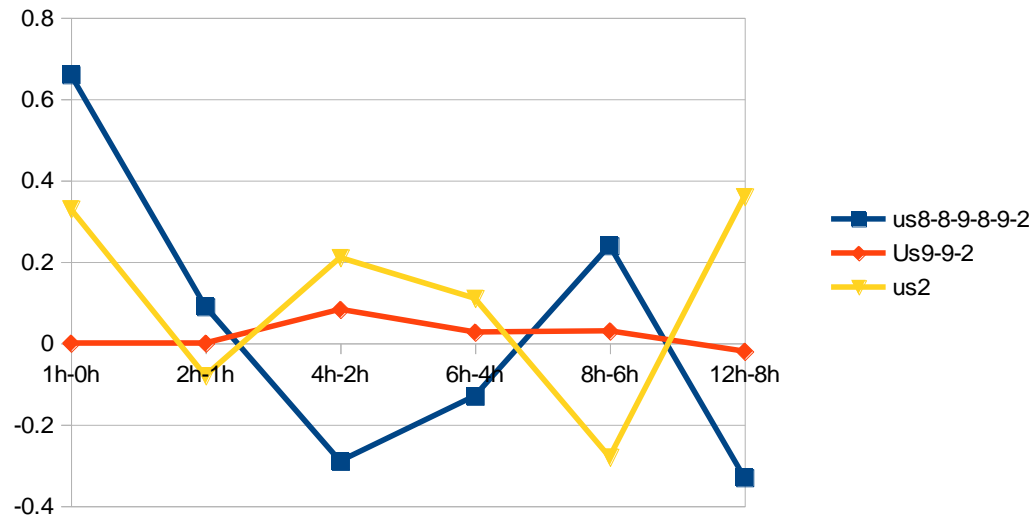
1. us8-us9 (only the us8 is translated)
2. us9 + us2 (both are translated)
3. us9-us2 (only the us9 is translated)
4. us8-us9-us2 (only the us8 is translated)

Pacific Biosciences RSII platform –  
combined anchored oligo(dT) and random hexamer priming



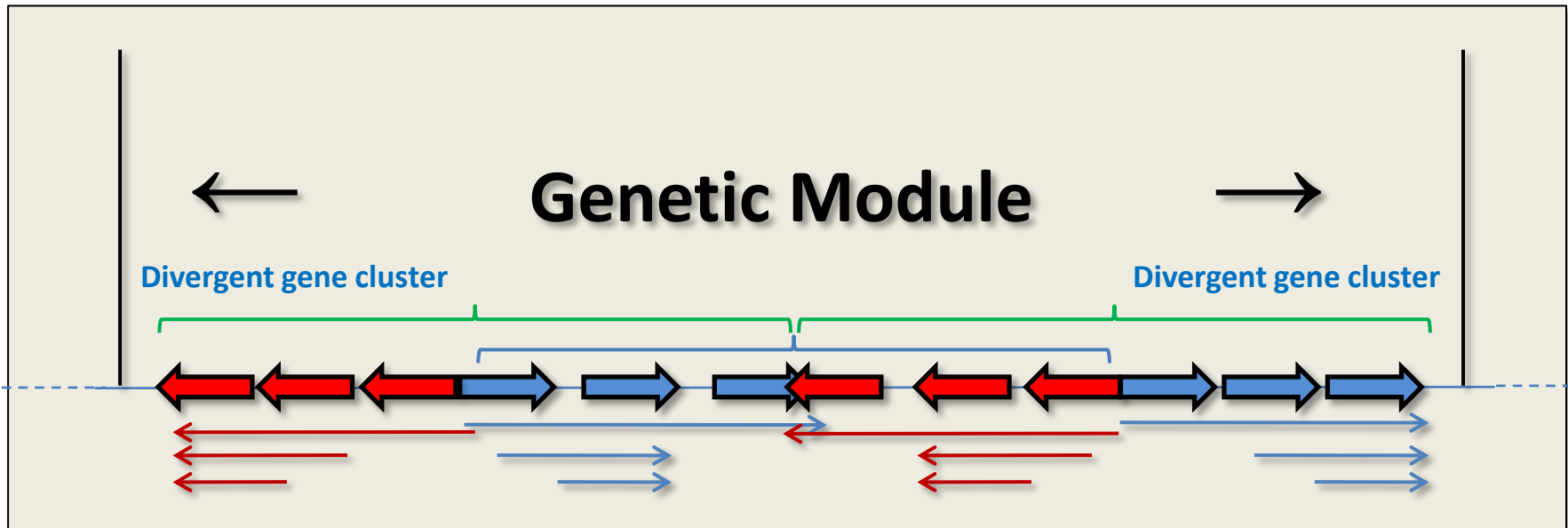
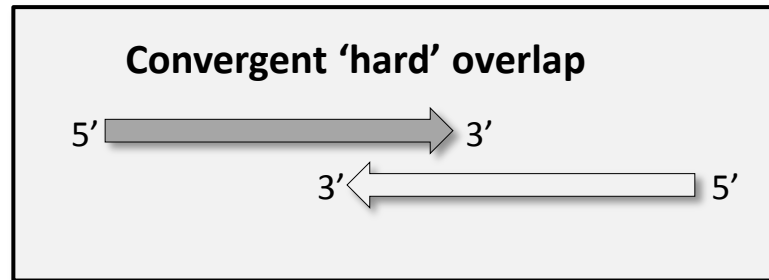
# Changes of expression kinetics of the translated genes across the viral life cycle

$$R_{\Delta} = R_t - R_{(t-1)}$$



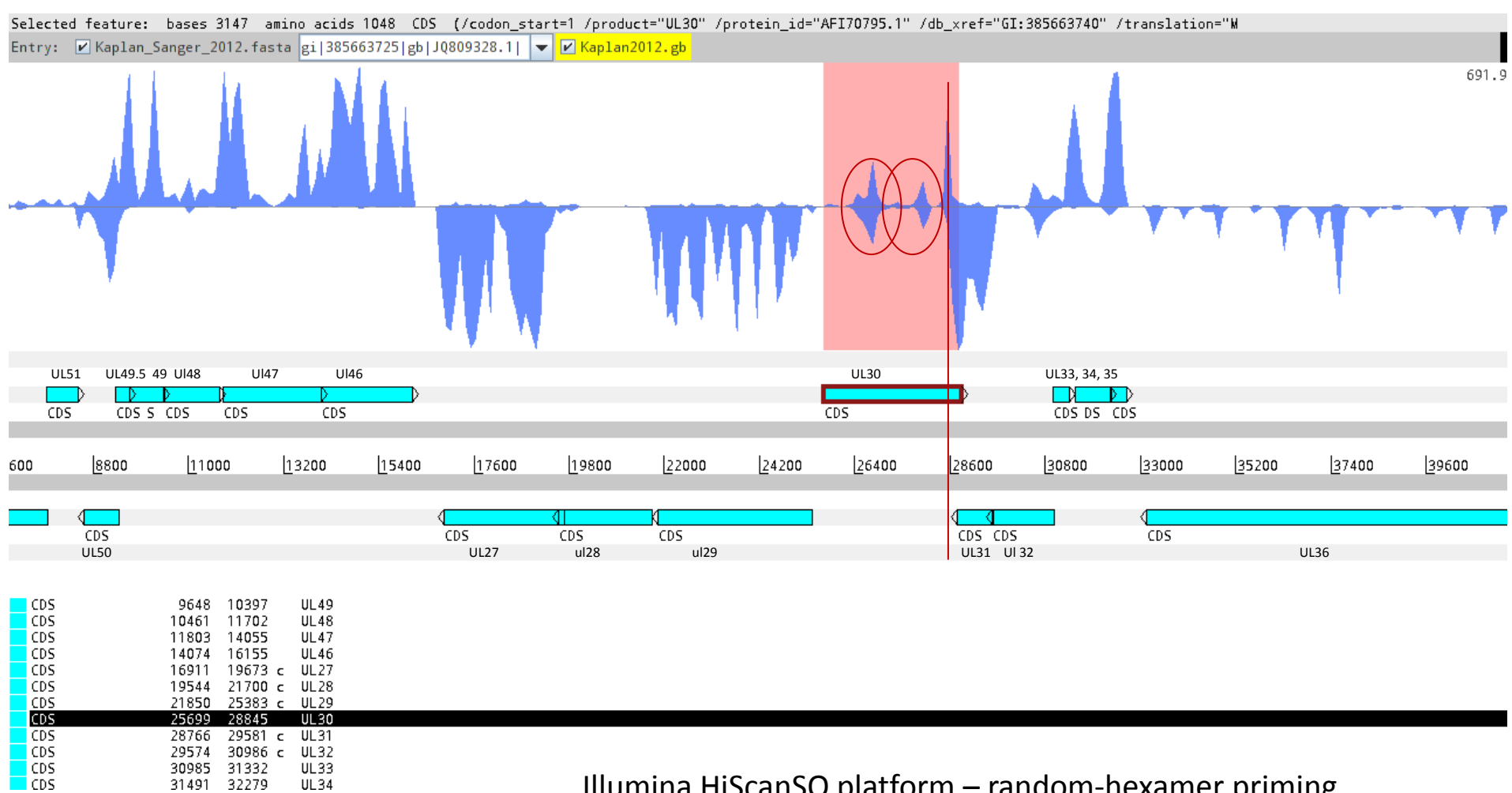
# Convergent transcriptional overlaps

## - 1. 'hard' overlaps



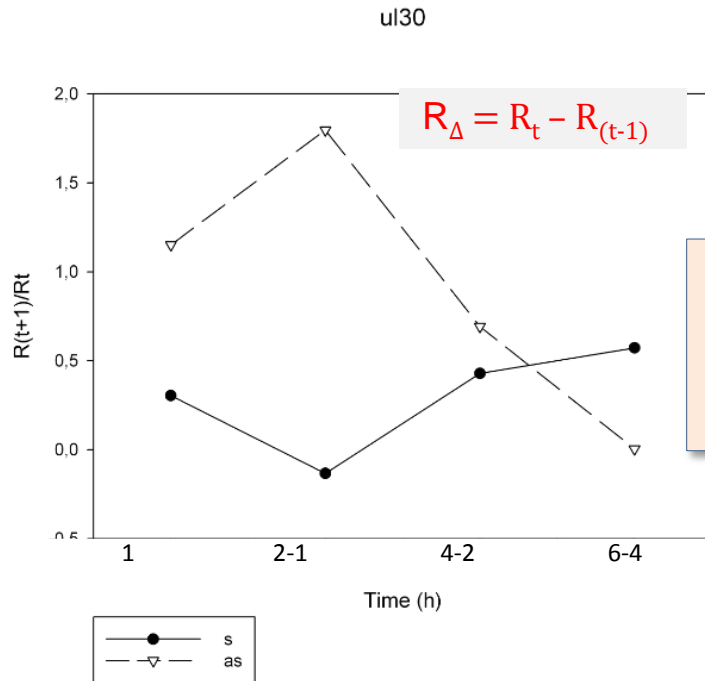
# Convergent transcriptional overlaps

## - 1. 'hard' overlaps: ul30-31 gene pairs

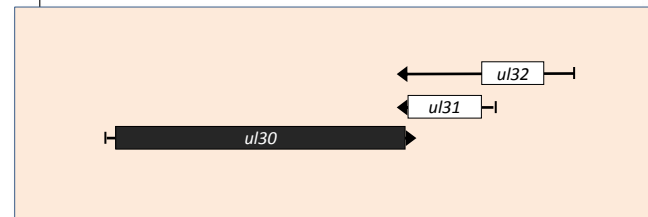


# ul30 vs. antisense ul30

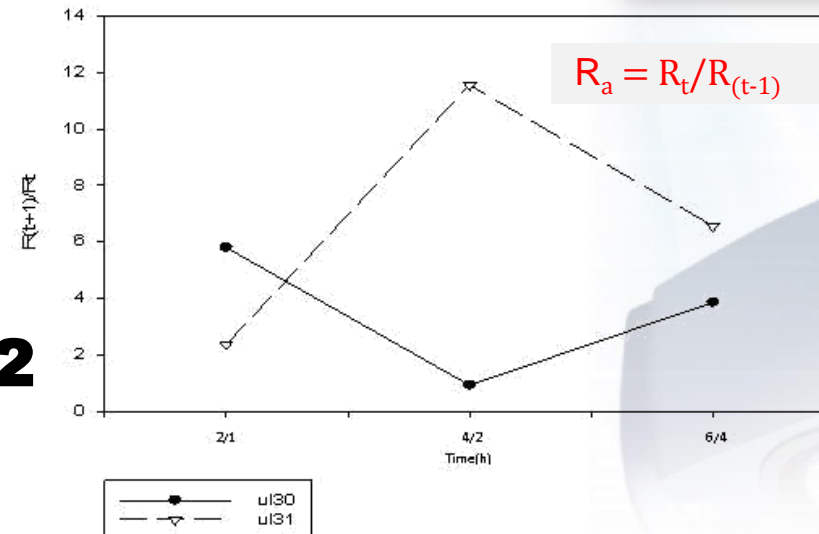
$$R: \text{relative expression ratio} = \frac{(E_{\text{sample@max}})^{Ct_{\text{sample@max}}}}{(E_{\text{sample}})^{Ct_{\text{sample}}}} : \frac{(E_{\text{refmax}})^{Ct_{\text{refmax}}}}{(E_{\text{ref}})^{Ct_{\text{ref}}}}$$



low-titer infection: 0.1 pfu/cell  
**Convergently-oriented genes**



Real-time RT-PCR

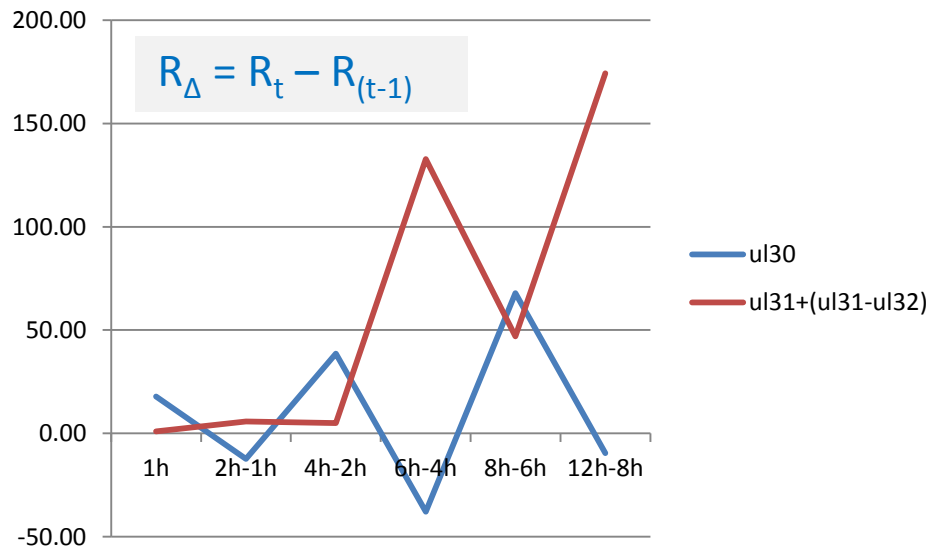


**ul30 vs. ul30 + ul31-32**

# ul30 vs. ul31 + ul31-32

high-titer infection: 10 pfu/cell

## Convergently-oriented genes

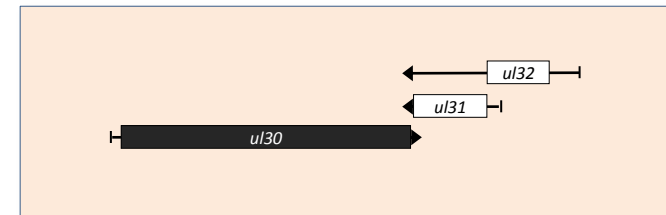


$$r = \frac{\sum_{i=1}^n (X_i - \bar{X})(Y_i - \bar{Y})}{(n-1)S_X S_Y}$$

Pearson's correlation coefficient: - **0.51**

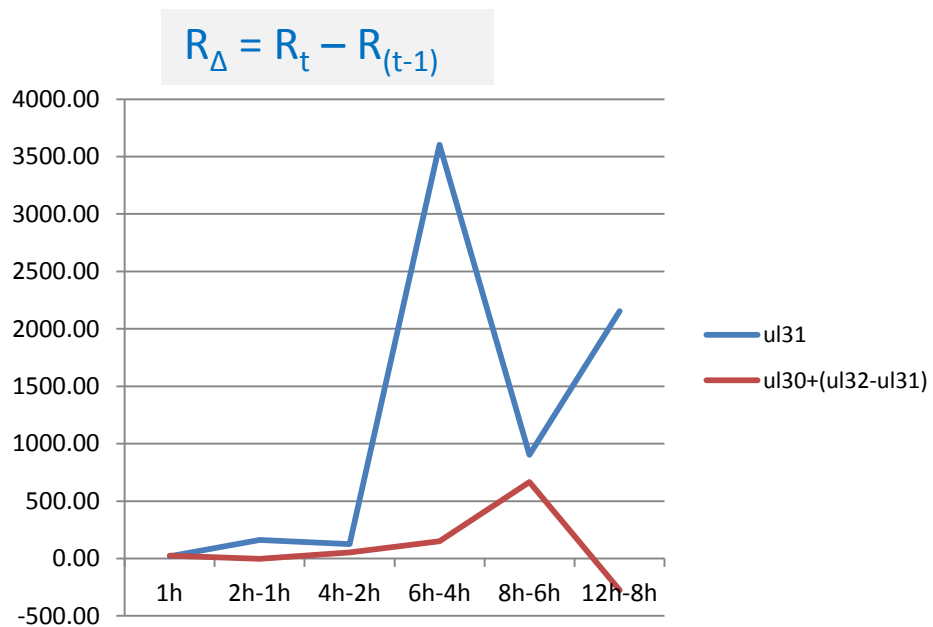


PacBio RS II

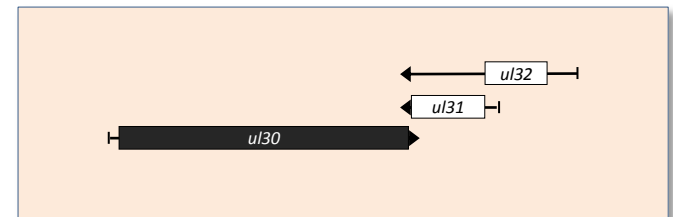


# ul31 vs. ul30 + ul31-32

## Tandem & convergently-oriented genes

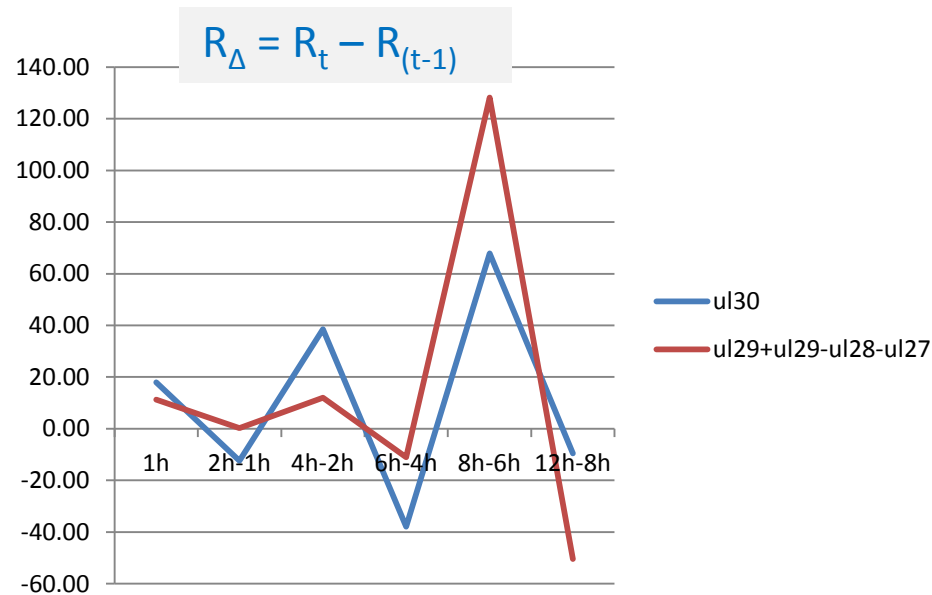


PacBio RS II



# ul30 vs. ul29 + ul29-28-27

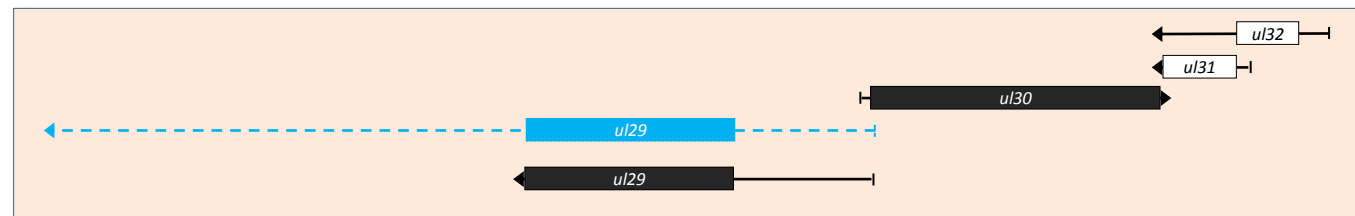
## Divergently-oriented genes



**Not promoter competition but joint control!**

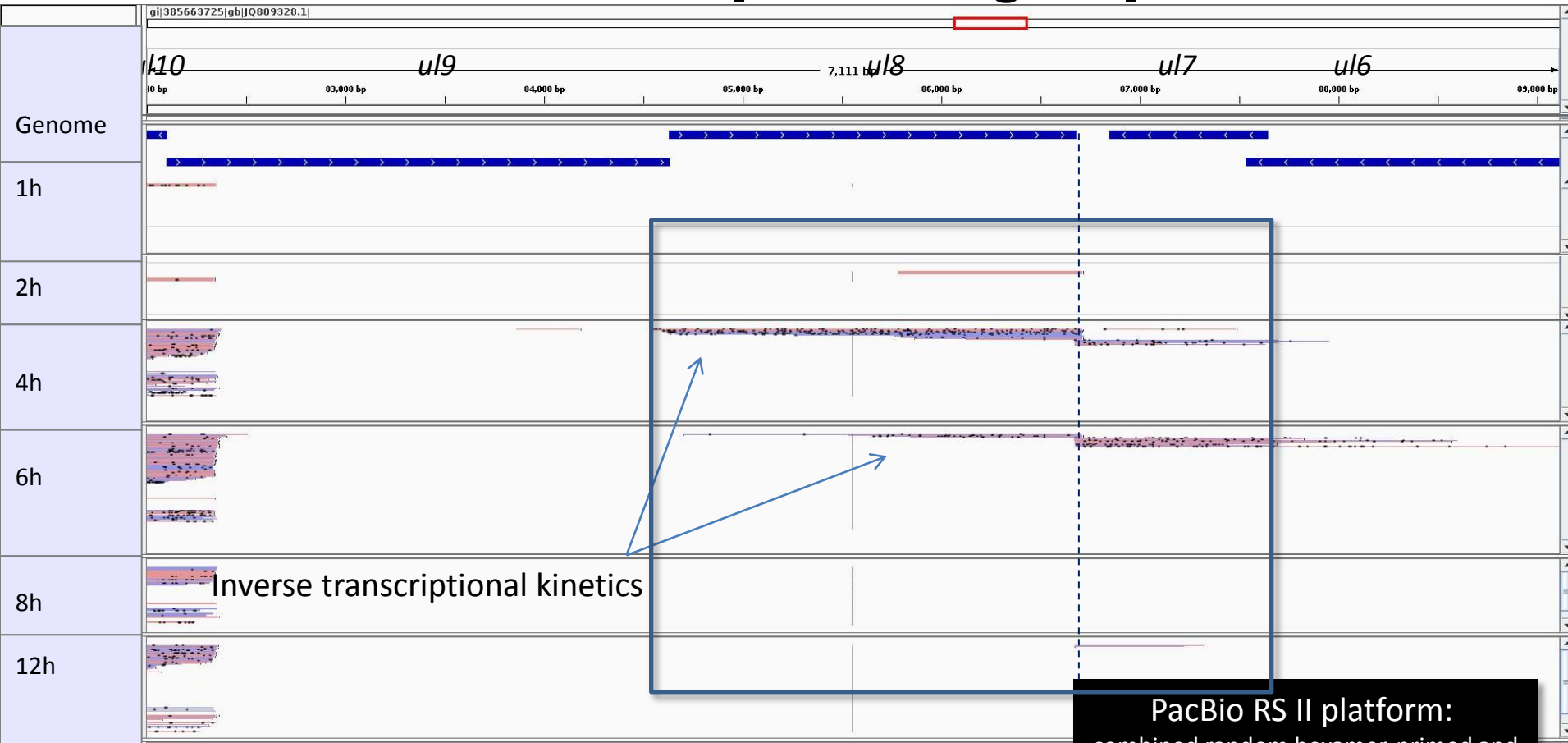


**PacBio RS II**



# Convergent transcriptional overlaps

## - 1. 'hard' overlaps: *ul8-ul7* gene pairs



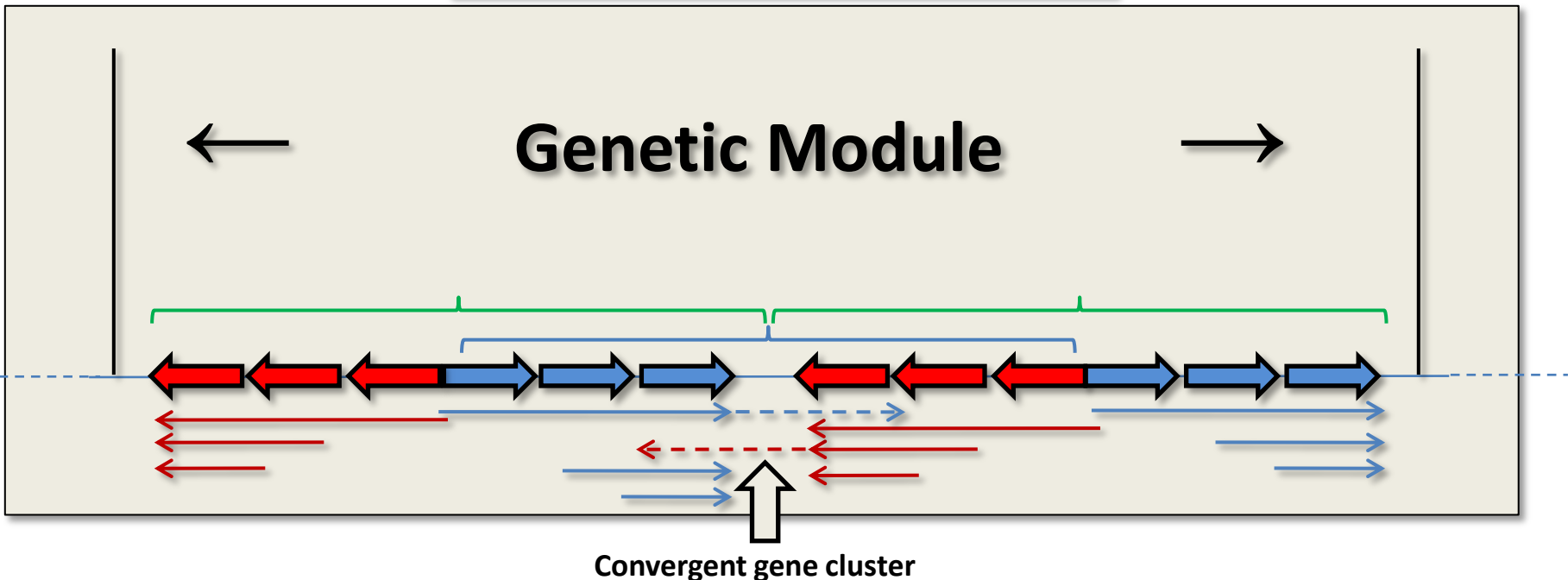
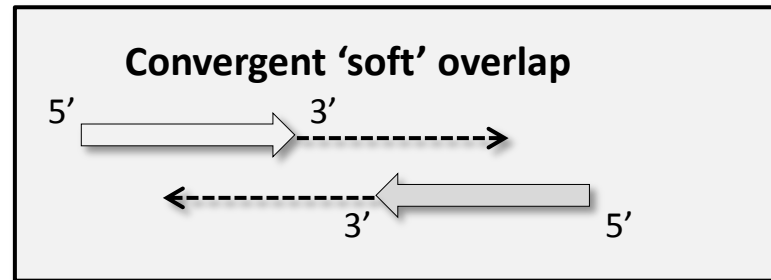
PacBio RS II platform:  
combined random hexamer-primed and  
anchored oligo(dT) primed [poly(A)-seq]





# Convergent transcriptional overlaps

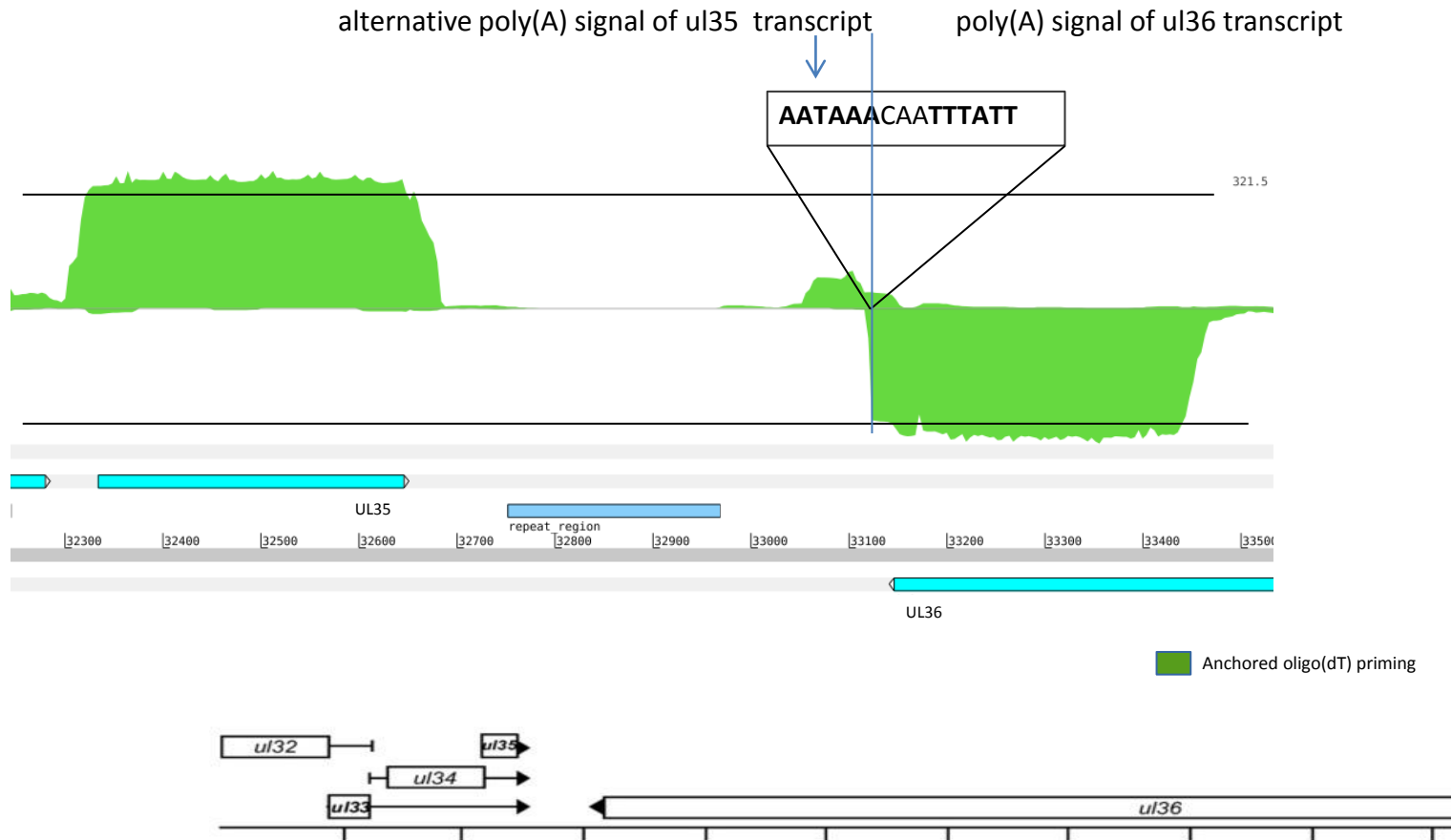
## - 2. 'soft' overlaps with alternative poly(A) signals



- RNS pol-II : inefficient recognition of major transcription termination signals
- Collision of RNA pol molecules
- Generation of antisense transcripts

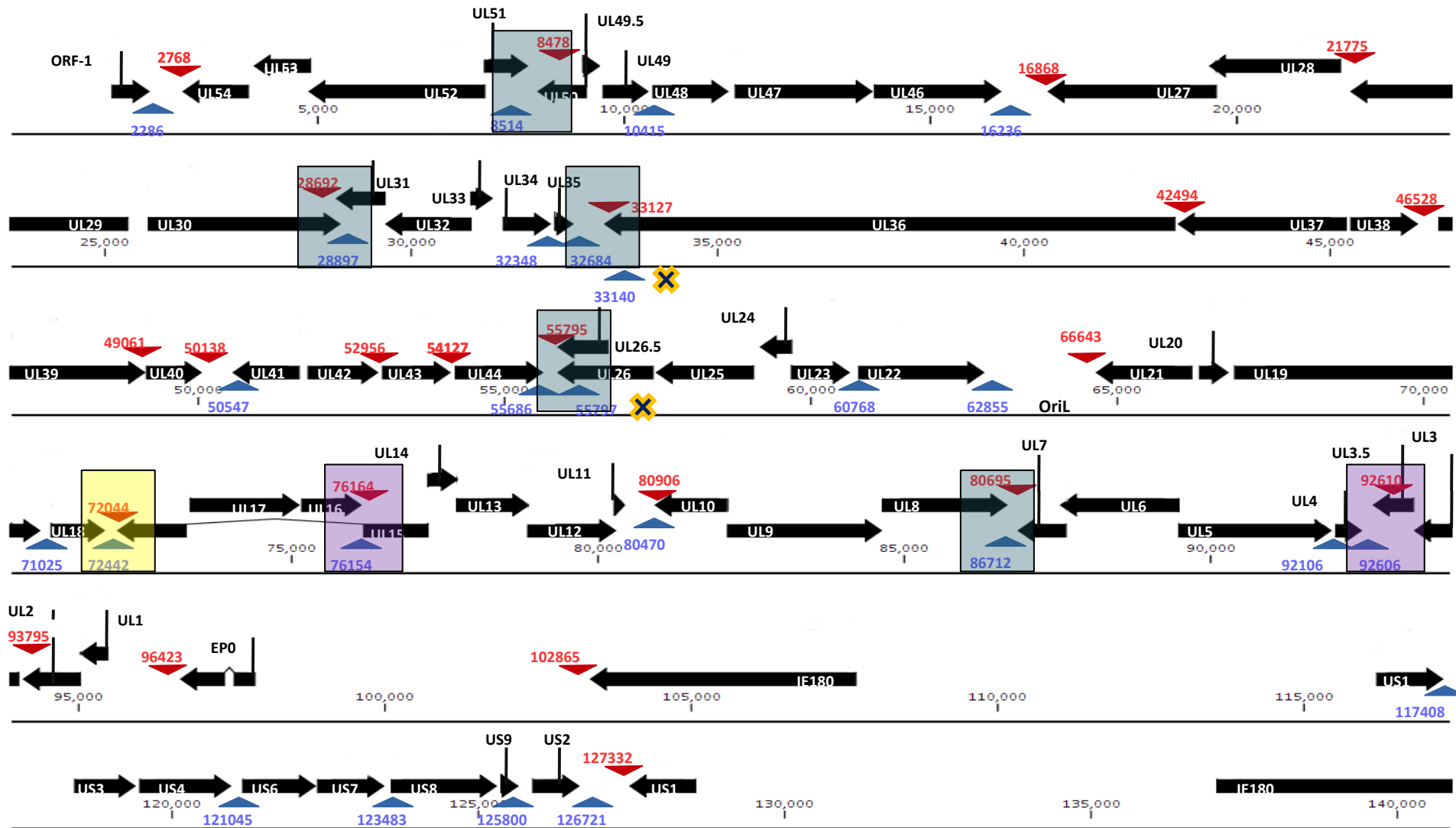
# Convergent transcriptional overlaps

## - 2. 'soft' overlaps with alternative poly(A) signals



Illumina HiScanSQ platform – anchored oligo(dT) priming [poly(A)-seq]





# Whole-genome convergent overlaps



Hard overlaps: ul7-8, ul30-31, ul50-51

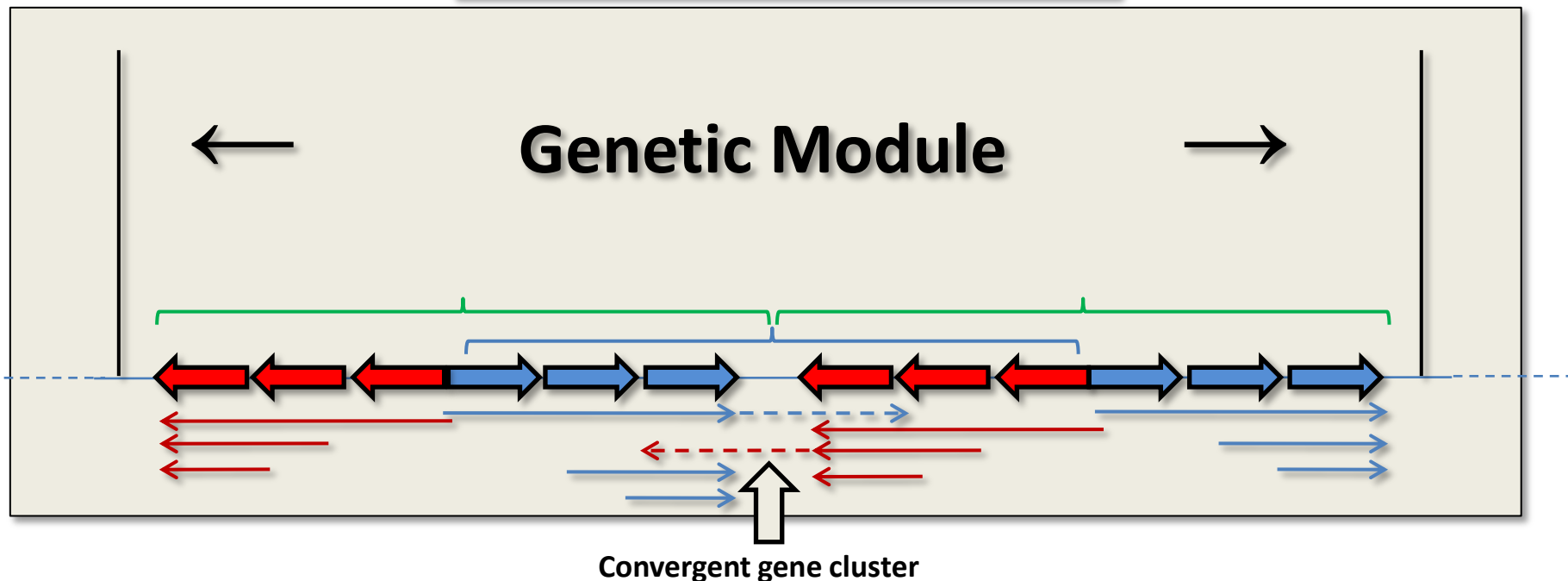
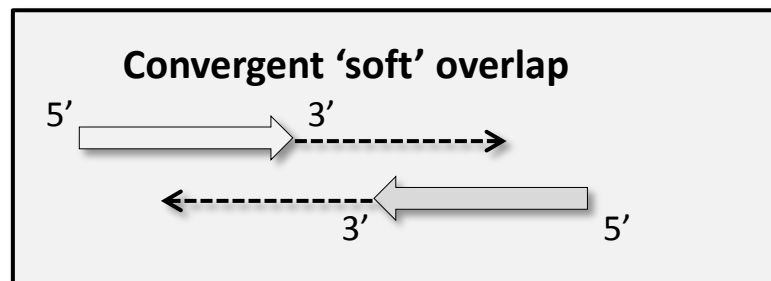
Soft overlaps: ul35-36, ul44-26.5

Very close locations: ul15-18, ul15-16, ul3-3.5

-  Coding region
-  Major poly(A) signal, plus strand
-  Major poly(A) signal, minus strand
-  Weak poly(A) signal in convergent genes

# Convergent transcriptional overlaps

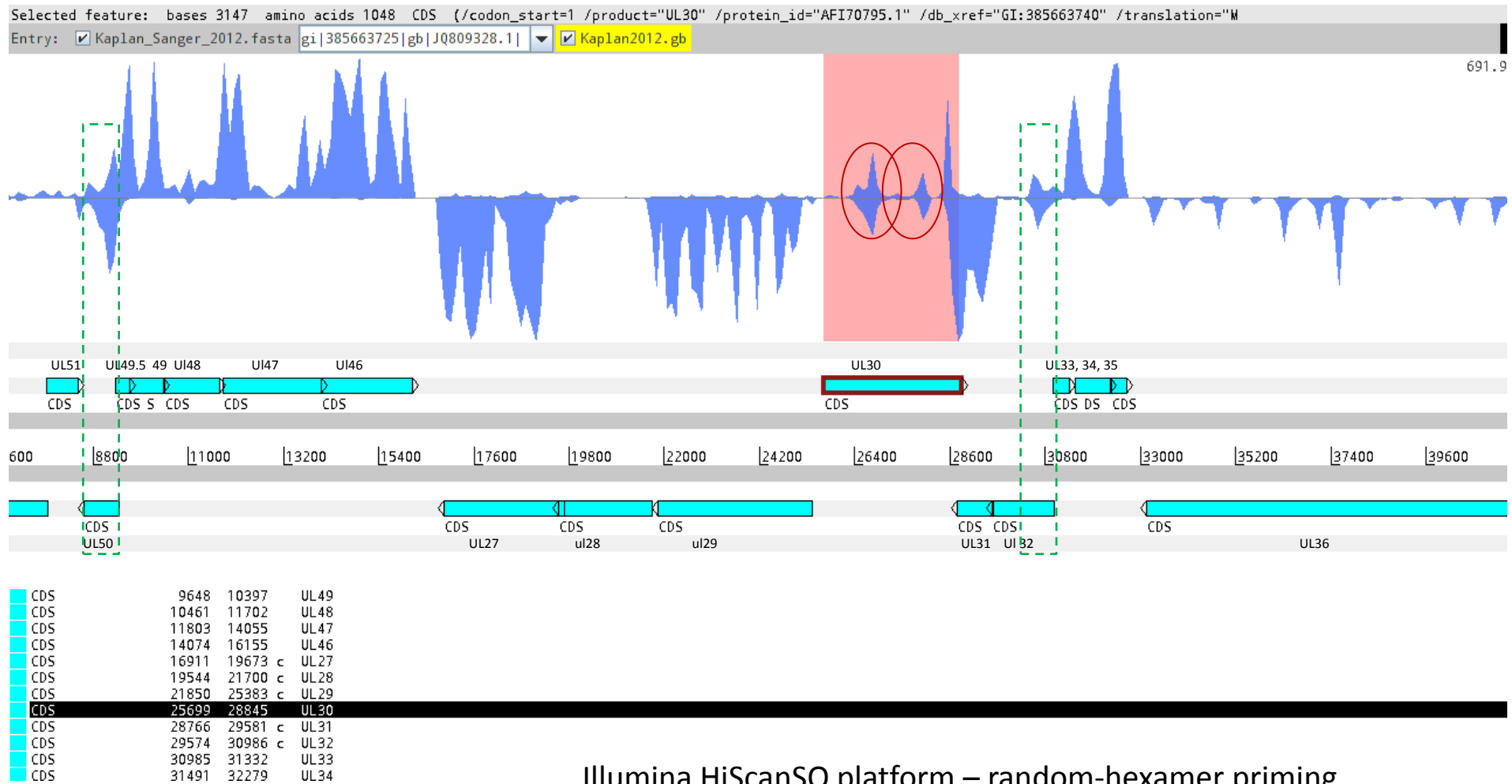
## - 3. 'soft' overlaps, no poly(A) signals



- RNA pol II : inefficient recognition of main transcription termination signals
- Collision of transcriptional machineries
- Generation of antisense transcripts

# Convergent transcriptional overlaps

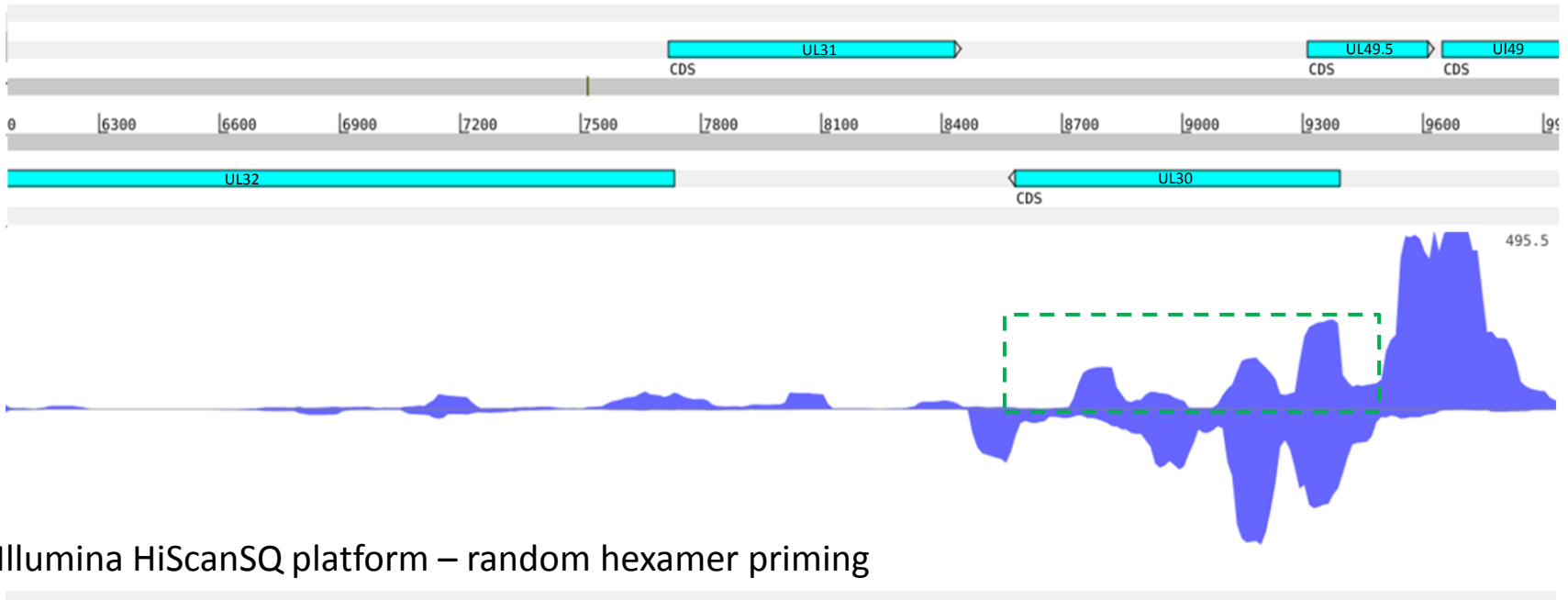
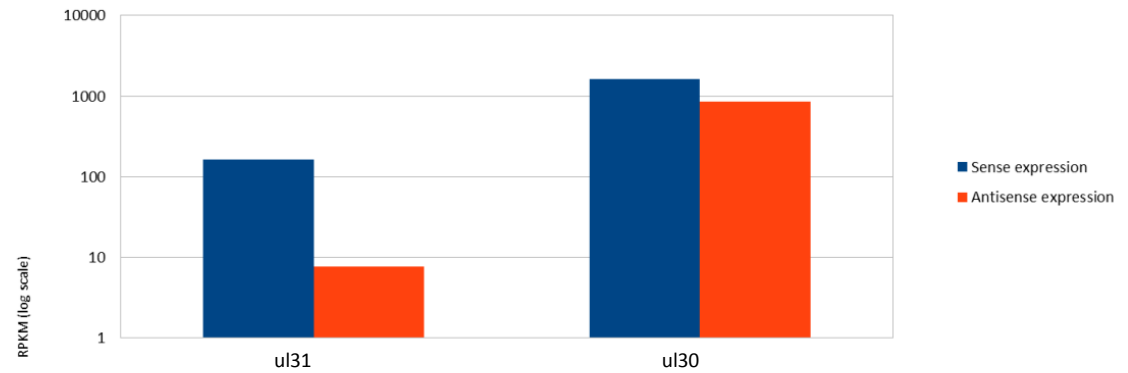
## - 3. 'soft' overlaps, no poly(A) signals



Illumina HiScanSQ platform – random-hexamer priming

# Convergent transcriptional overlaps

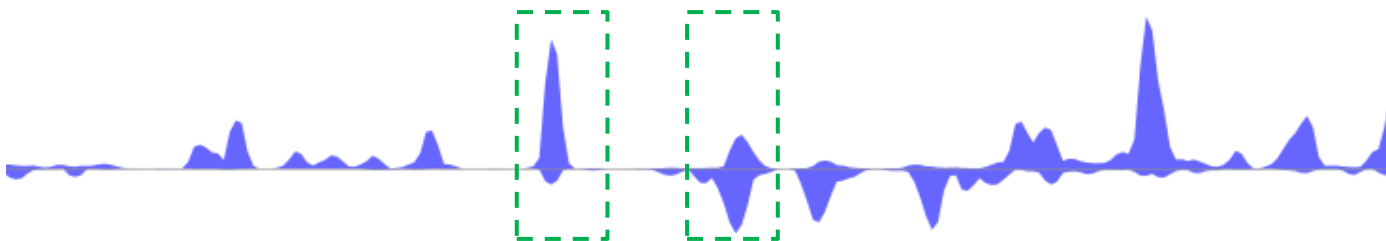
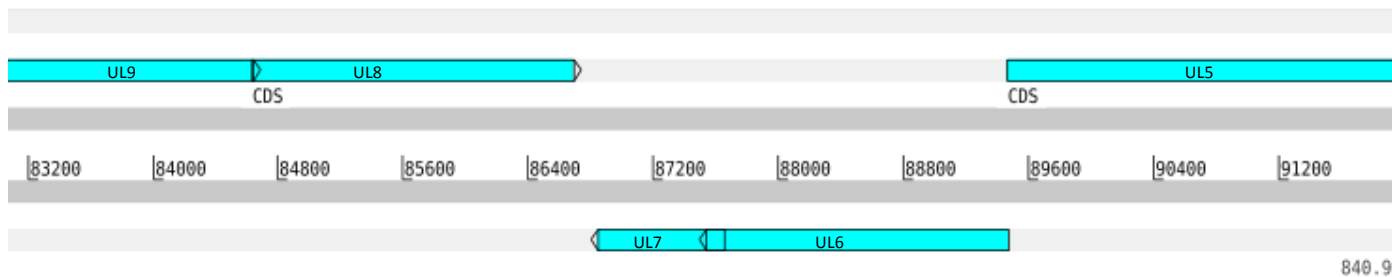
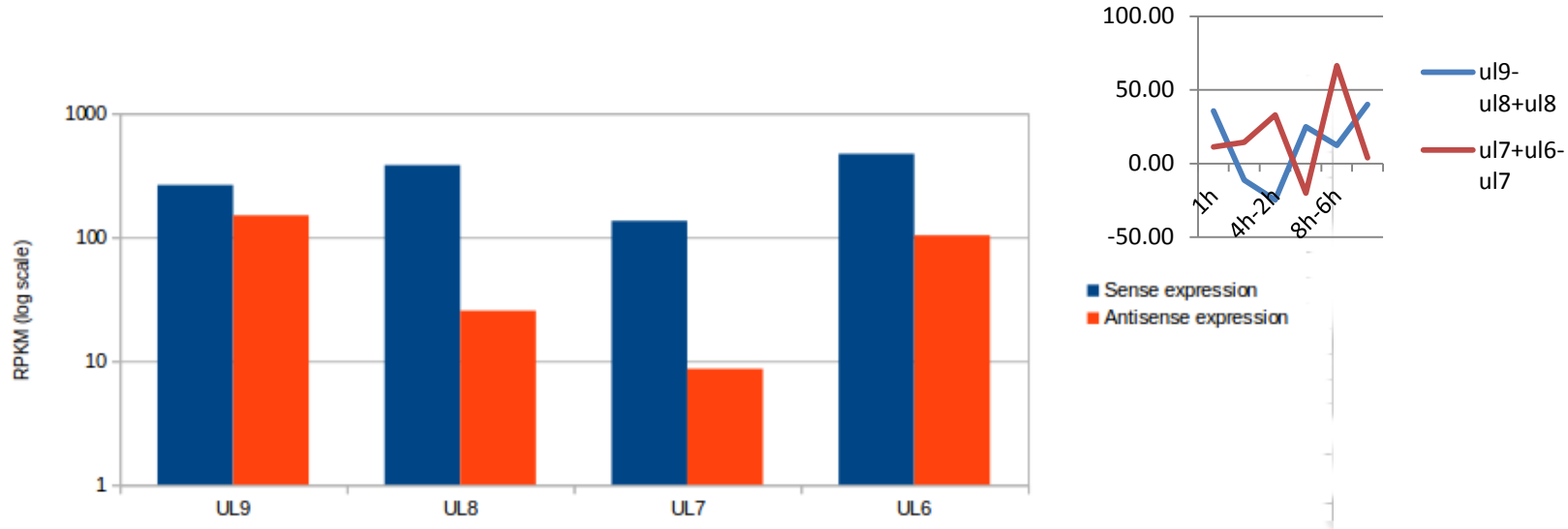
## - 3. 'soft' overlaps, no poly(A) signals



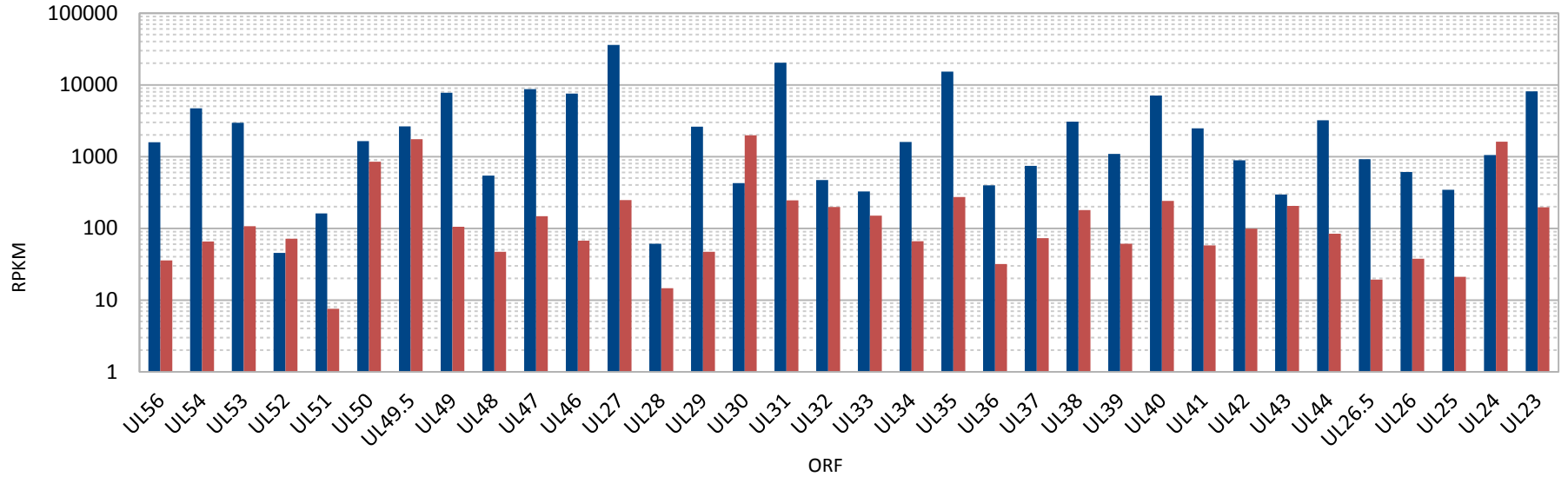
Illumina HiScanSQ platform – random hexamer priming

# Convergent transcriptional overlaps

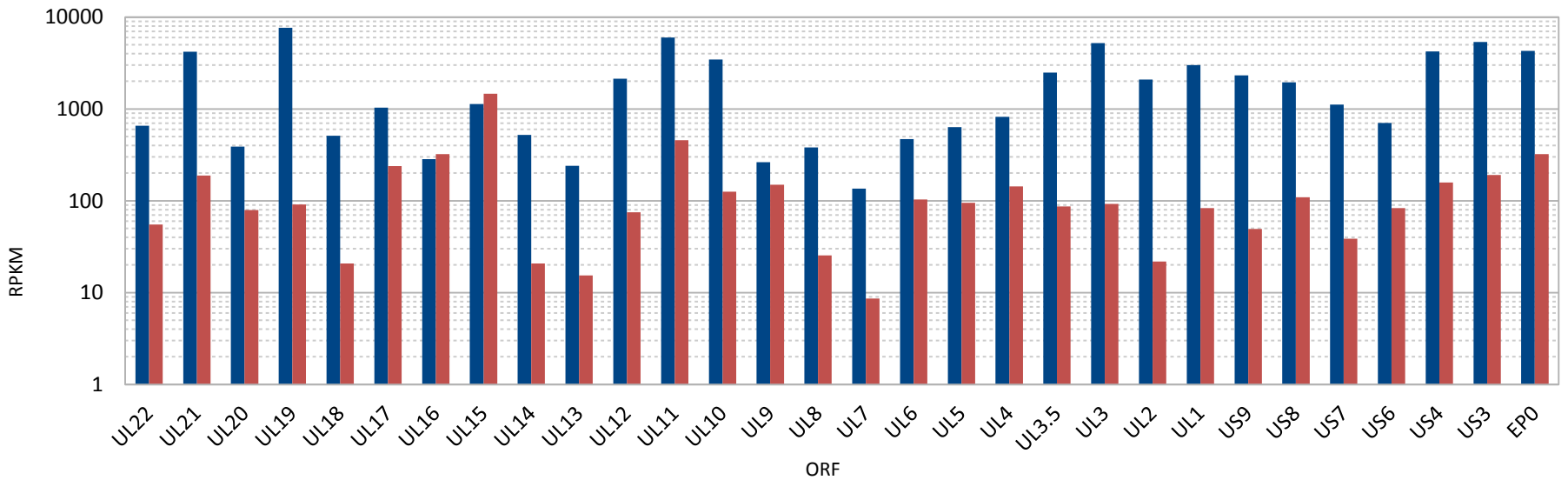
## - 3. 'soft' overlaps, no poly(A) signals



# Overall antisense RNA expression

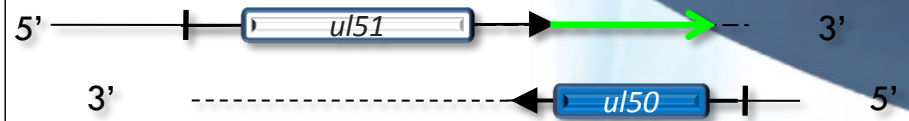
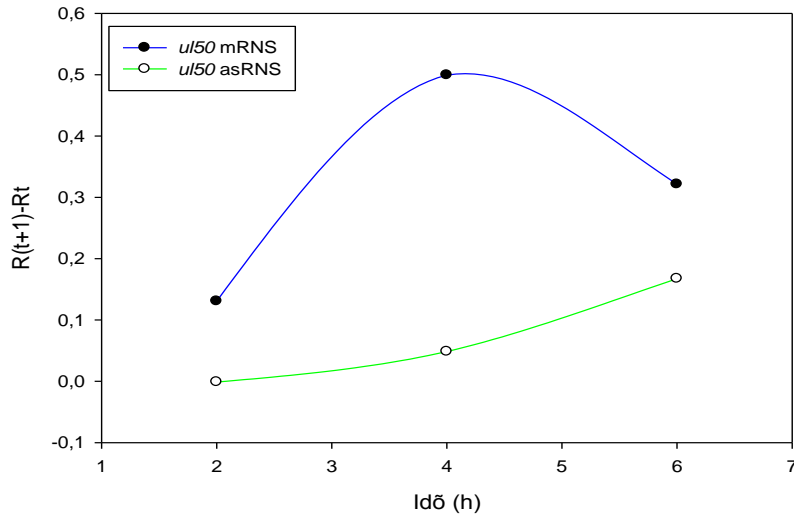


## Illumina HiScanSQ platform

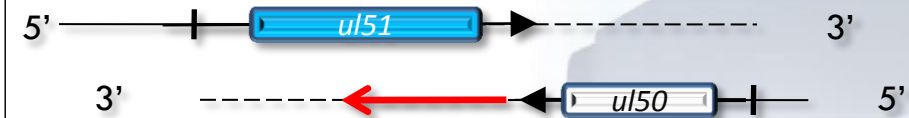
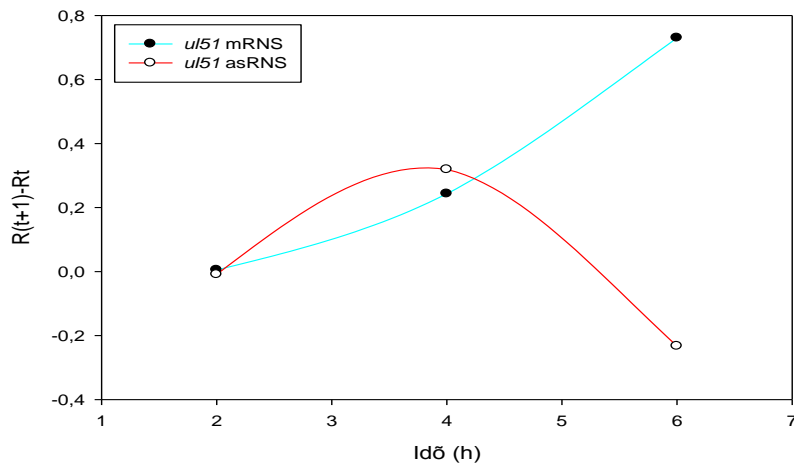




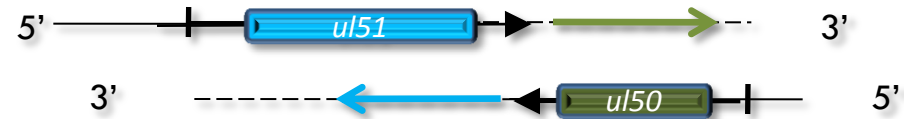
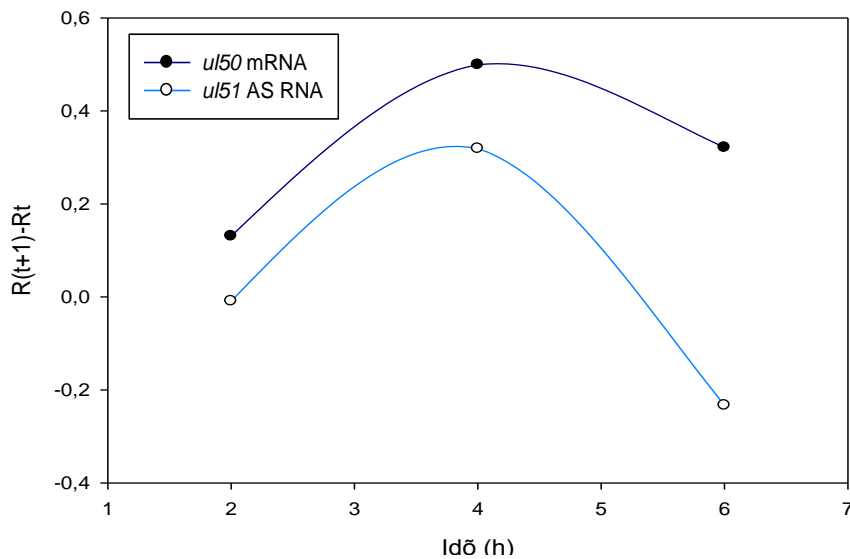
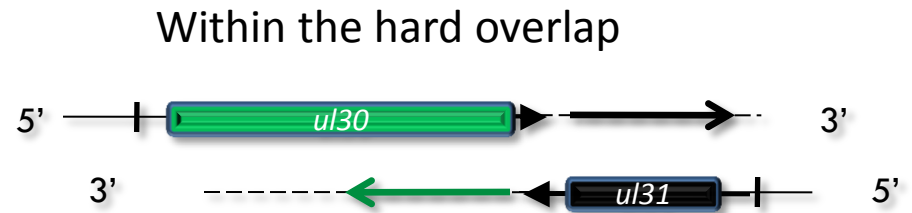
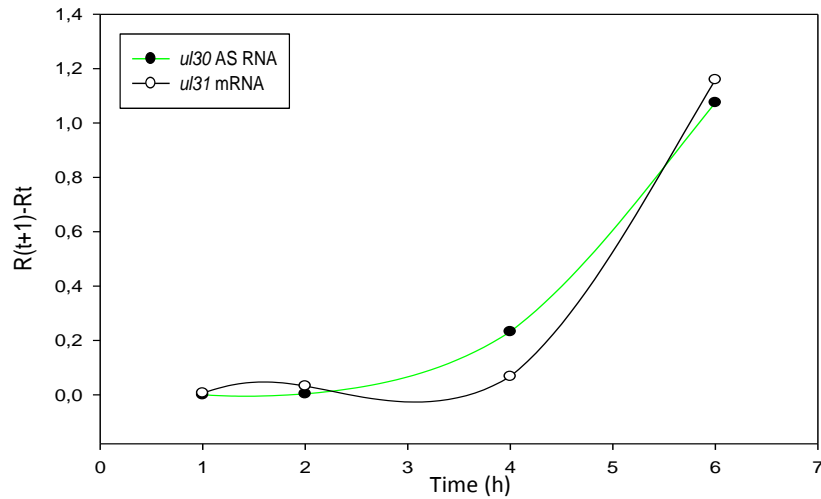
# Inverse expression of mRNAs and their antisense RNA partners



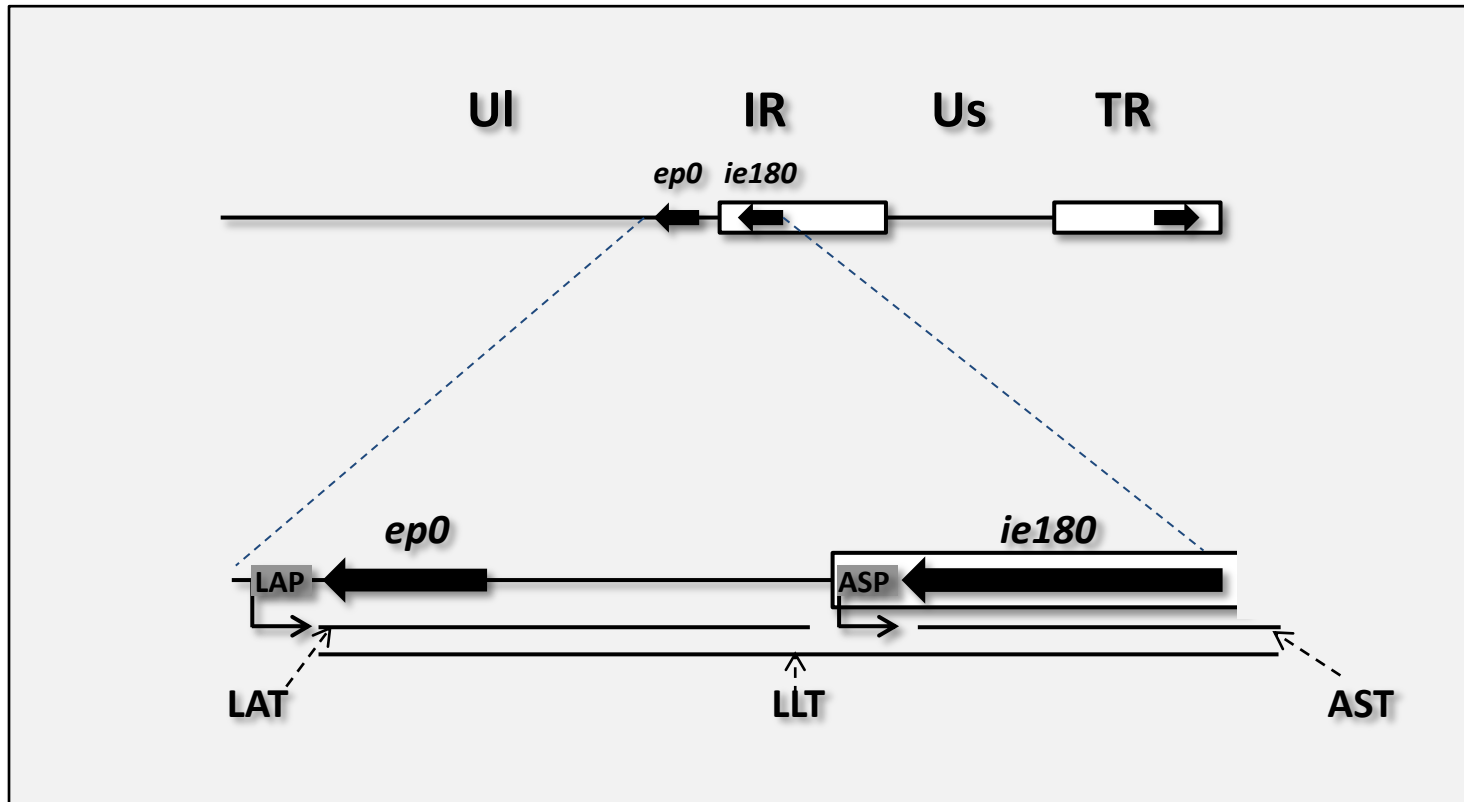
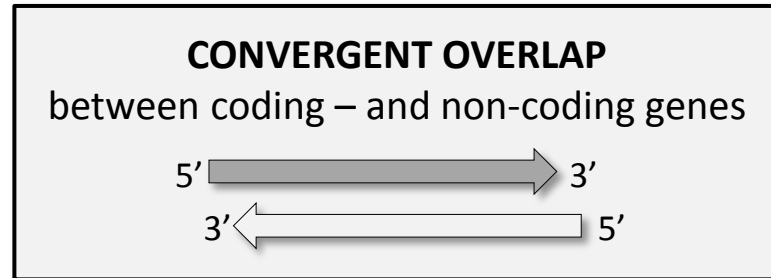
Real-time RT-PCR



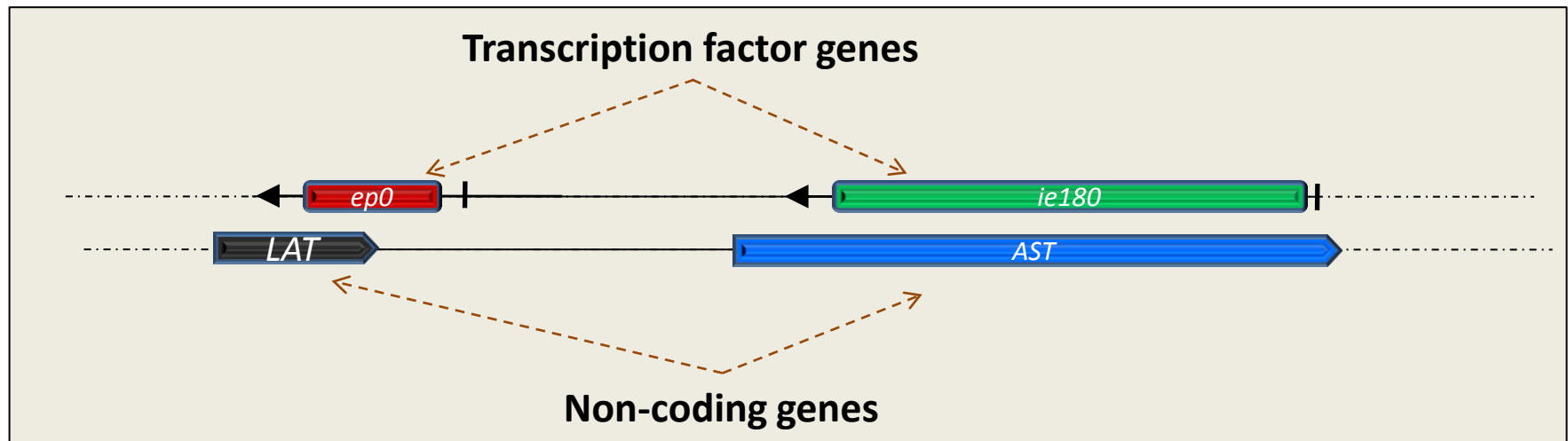
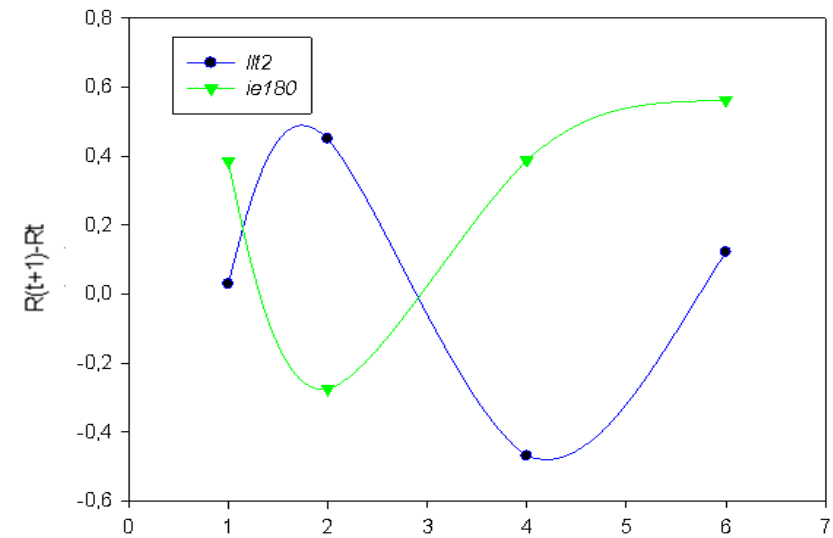
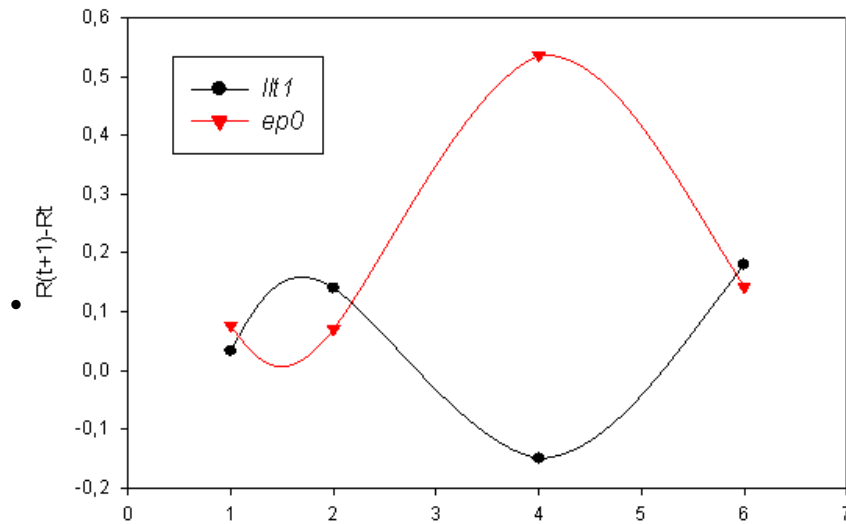
# Positive correlation between mRNA and antisense RNA expressions



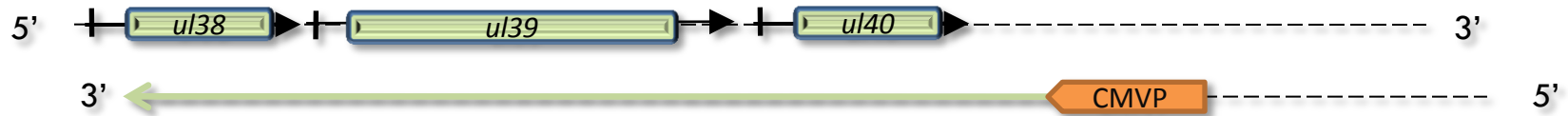
# Long non-coding RNAs of PRV



# Inverse expression patterns between mRNA and antisense RNA expressions



# An experimental system for the modification of convergent transcriptional read-through

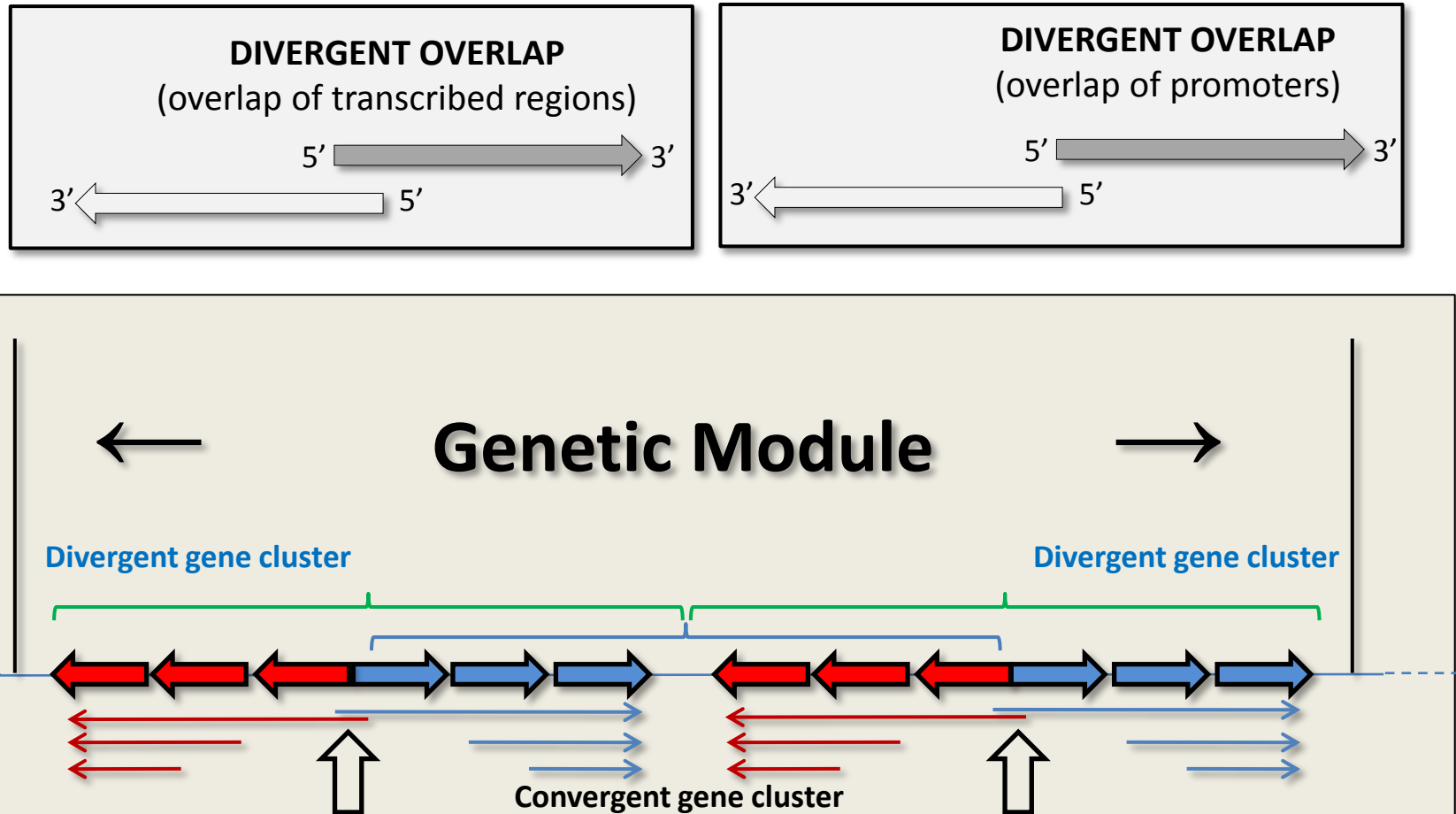


Gene	Time	$R_{AS}/R_S$ (wt)	$R_{AS}/R_S$ (mut)	$R_{AS}/R_S$ (mut/wt)
<i>ul38</i>	1h	0,093	0,181	<b>1.951</b>
	2h	0,032	2,025	<b>63.257</b>
	4h	0,212	0,291	<b>1.375</b>
	6h	0,323	1,514	<b>4.694</b>
	8h	0,146	0,624	<b>4.258</b>
	12h	0,240	0,303	<b>1.263</b>

Gene	Time	$R_{AS}/R_S$ (wt)	$R_{AS}/R_S$ (mut)	$R_{AS}/R_S$ (mut/wt)
<i>ul39</i>	1h	0,044	0,072	<b>1.638</b>
	2h	0,062	0,048	<b>0.781</b>
	4h	0,137	0,654	<b>4.783</b>
	6h	0,141	0,972	<b>6.896</b>
	8h	0,197	0,870	<b>4.420</b>
	12h	0,153	0,676	<b>4.425</b>

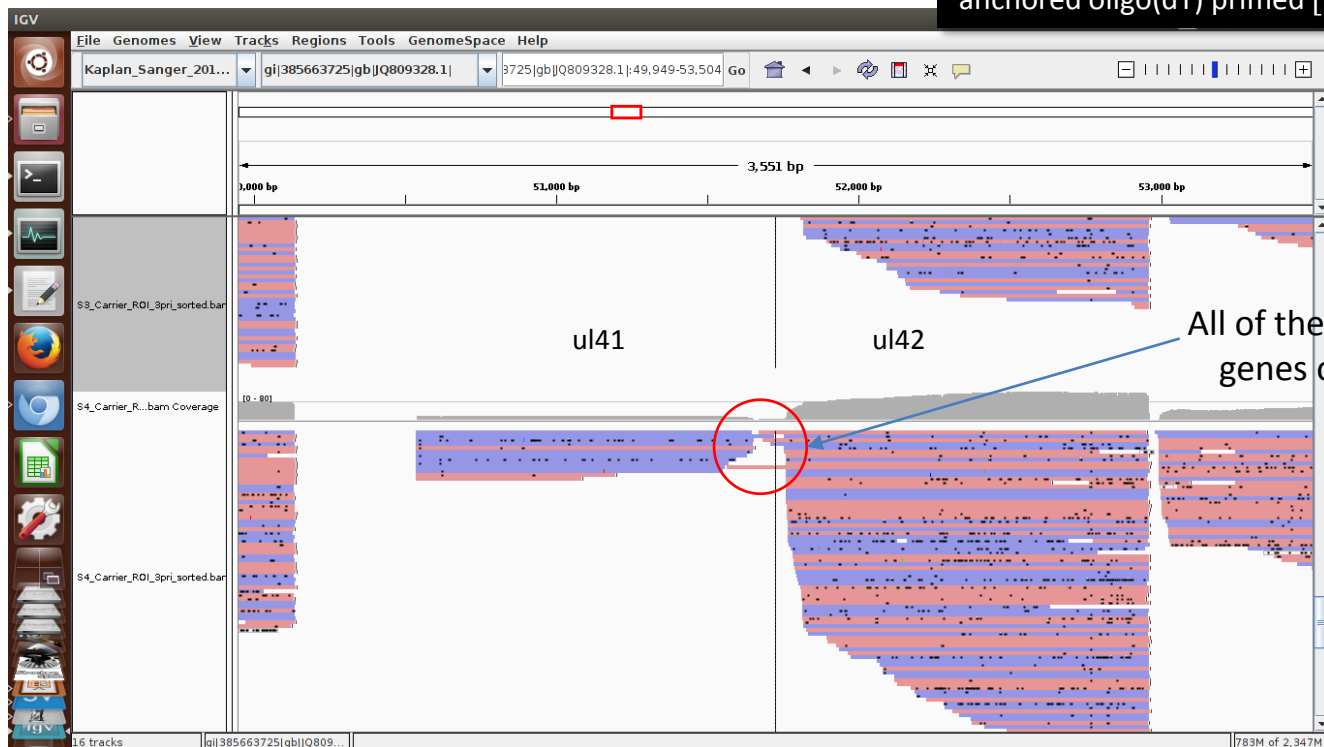
Gene	Time	$R_{AS}/R_S$ (wt)	$R_{AS}/R_S$ (mut)	$R_{AS}/R_S$ (mut/wt)
<i>ul40</i>	1h	0,023	0,036	<b>1.554</b>
	2h	0,015	0,021	<b>1.366</b>
	4h	0,085	0,407	<b>4.761</b>
	6h	0,152	0,854	<b>5.604</b>
	8h	0,180	0,746	<b>4.136</b>
	12h	0,155	0,358	<b>2.311</b>

# Transcriptional overlap in divergently-oriented genes

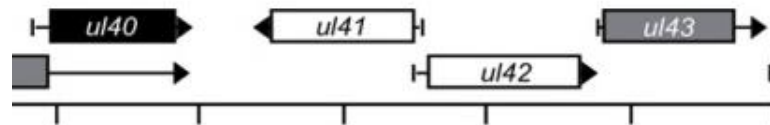


# Transcriptional overlap of divergently-oriented genes

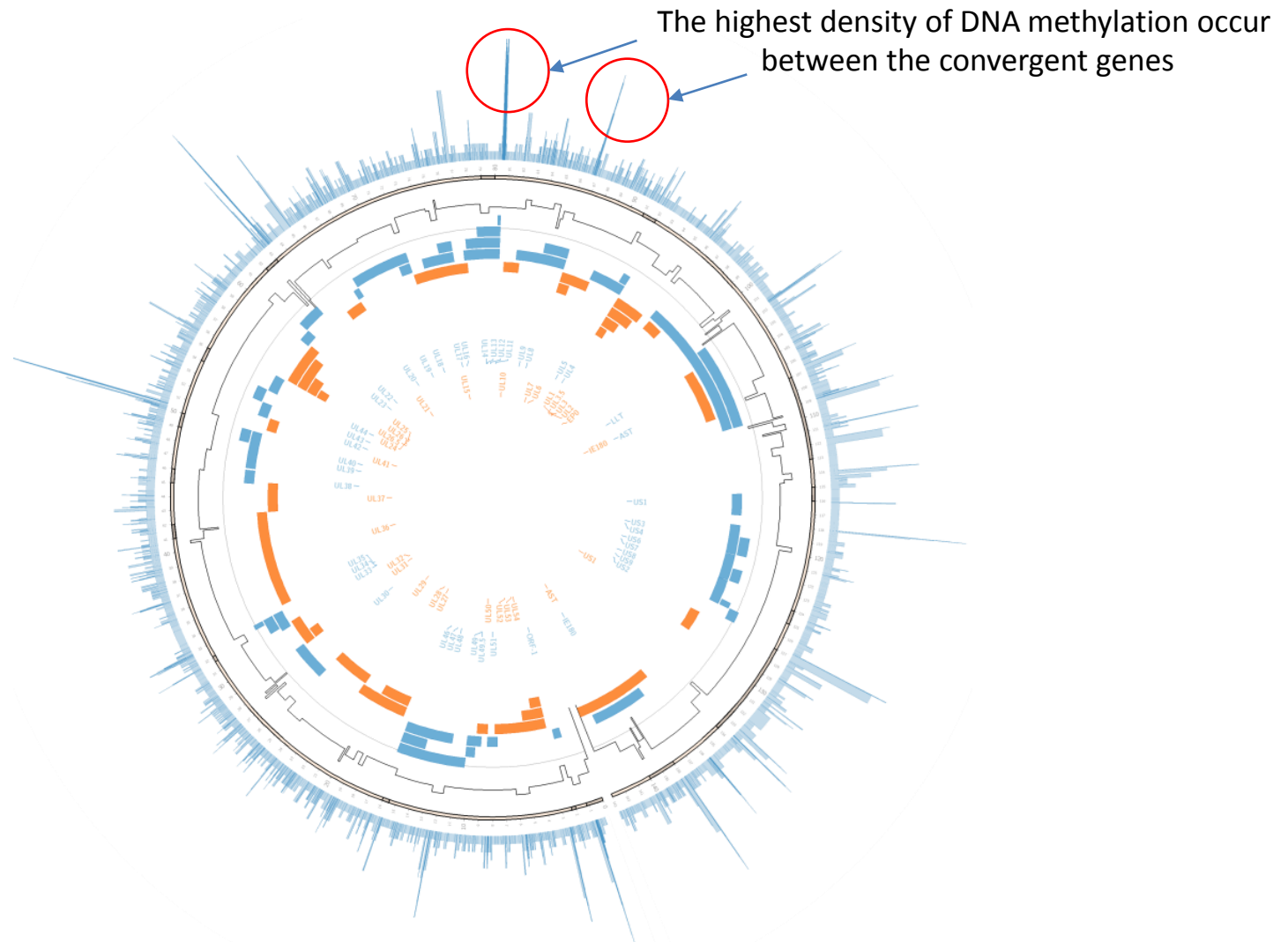
PacBio RS II platform:  
combined random hexamer-primed and  
anchored oligo(dT) primed [poly(A)-seq]



All of the divergent  
genes overlaps



# Methylation of genome of PRV virion



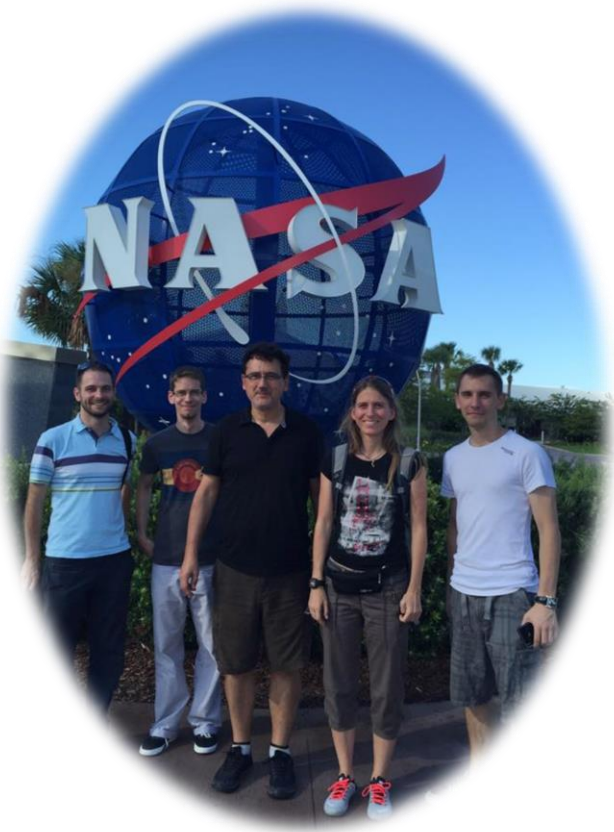


# The putative functions of TINs

- (1) **Automatism** in the alteration of transcriptional activity of gene clusters
- (2) **Synchronization** and negative synchronization of gene activities ➤ well-defined temporal patterns of gene expressions
- (3) Reduction of **transcriptional noise**

TINs are assumed to be especially important in biological processes following a **definite time course** of gene expressions, such as embryonic development, tissue regeneration, response to external stimuli, metabolism, viral replication, etc.

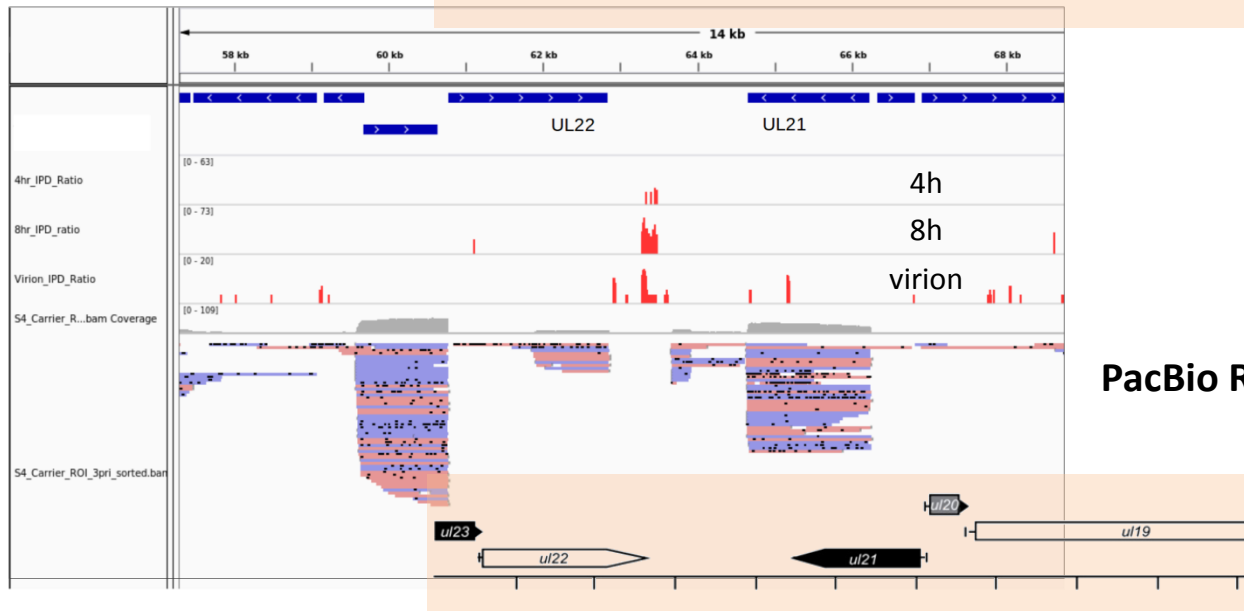
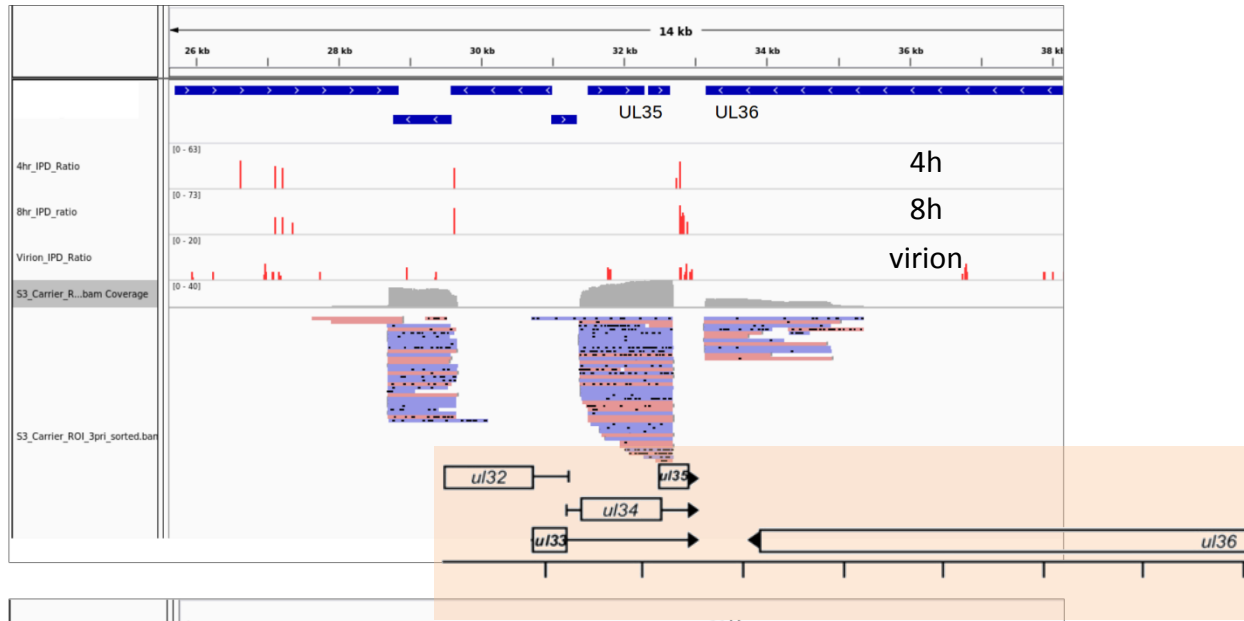
## Acknowledgements:



**Mike Snyder**

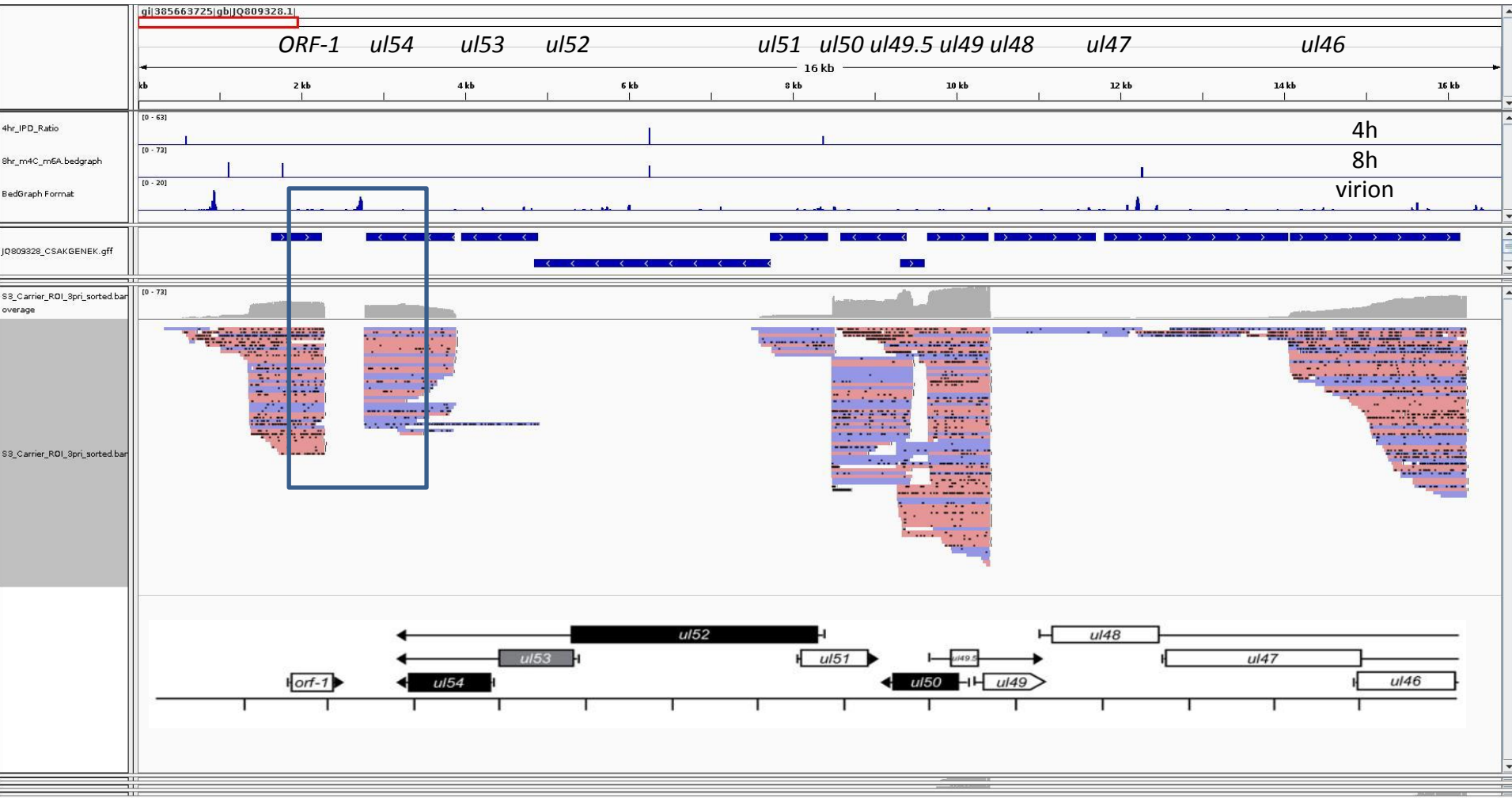
Director, Stanford Center for Genomics and Personalized Genetics

# Dynamics of DNA methylation throughout the viral replication



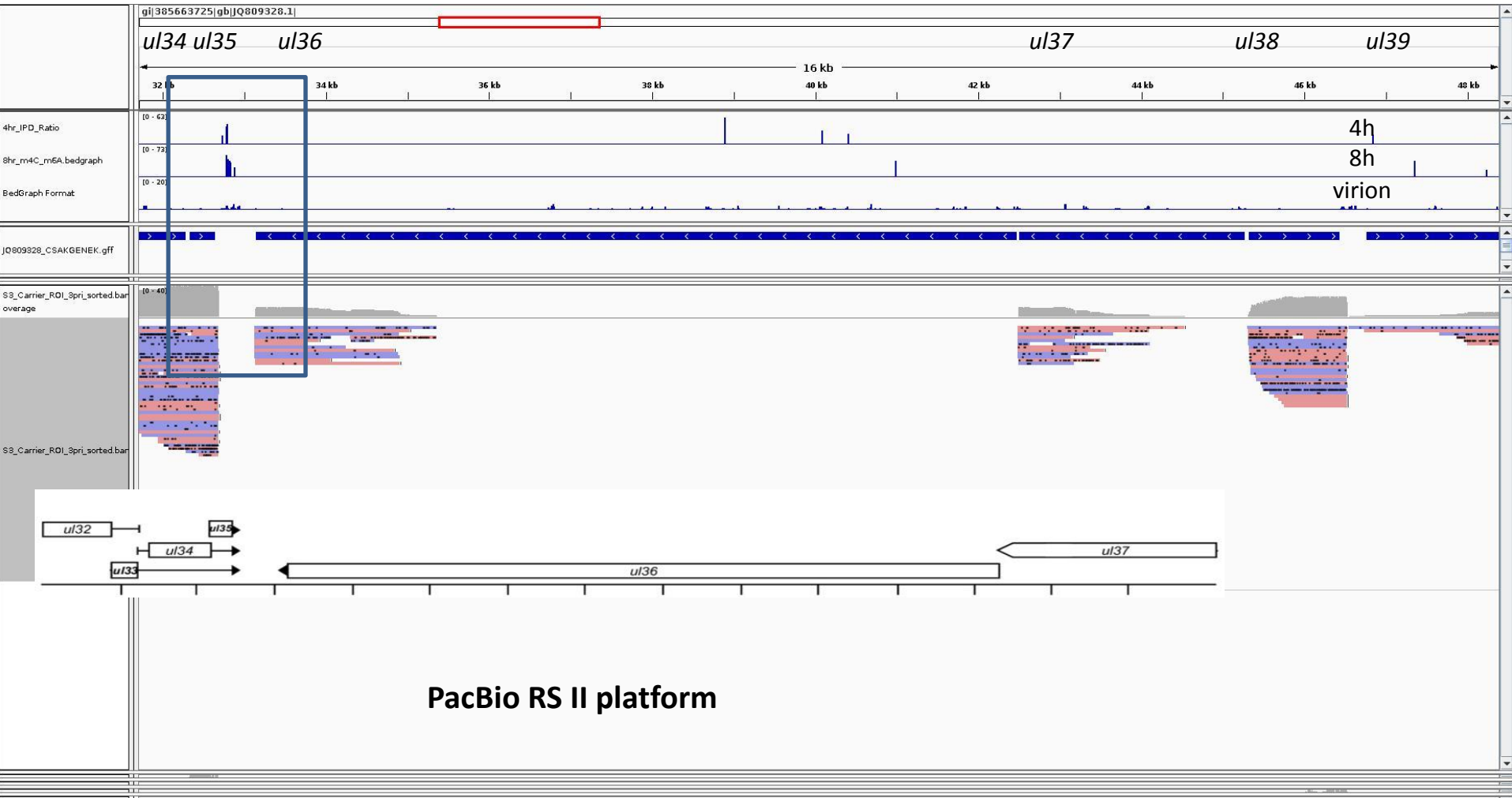
PacBio RS II platform

# Dynamics of DNA methylation throughout the viral replication

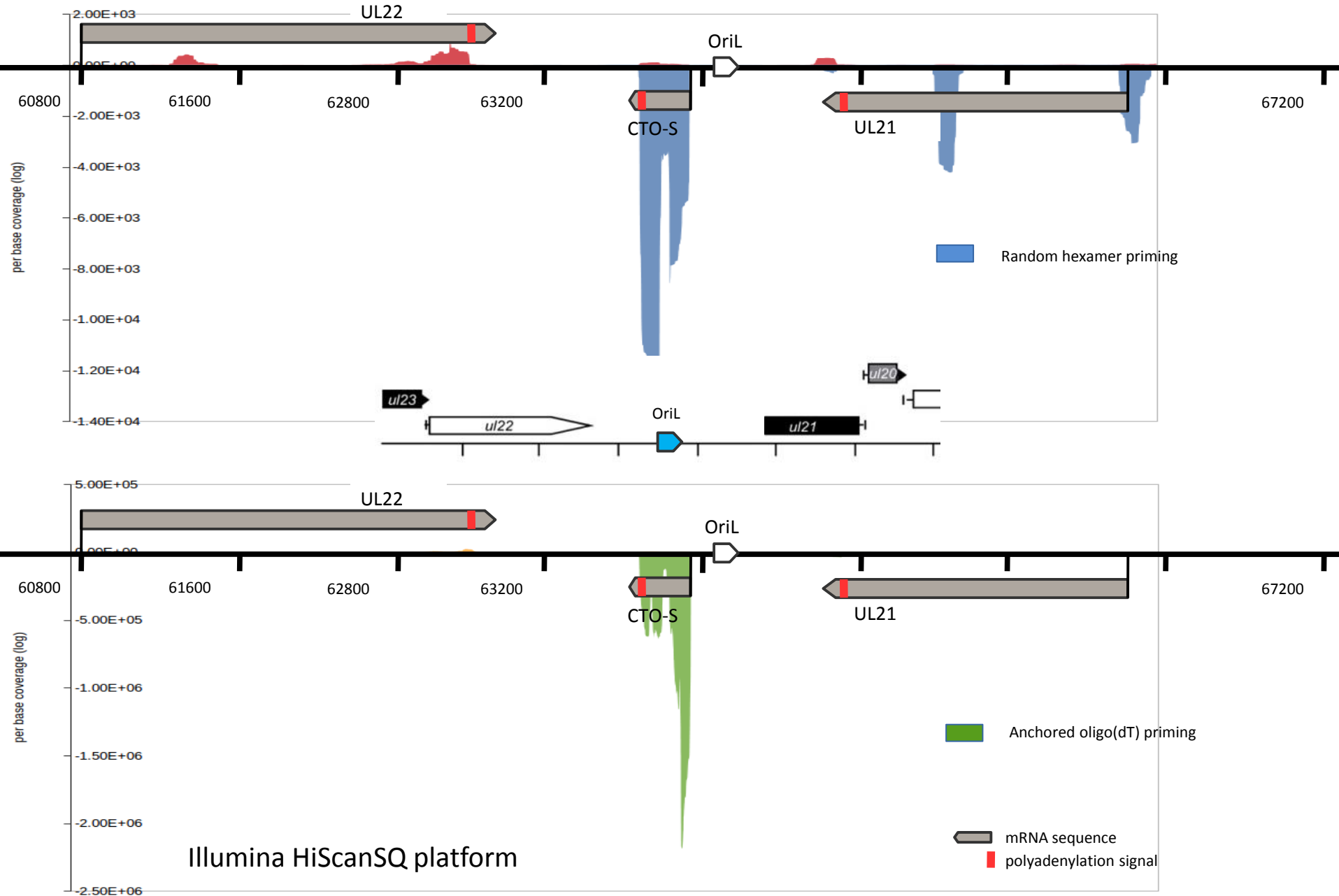


PacBio RS II platform

# Dynamics of DNA methylation throughout the viral replication

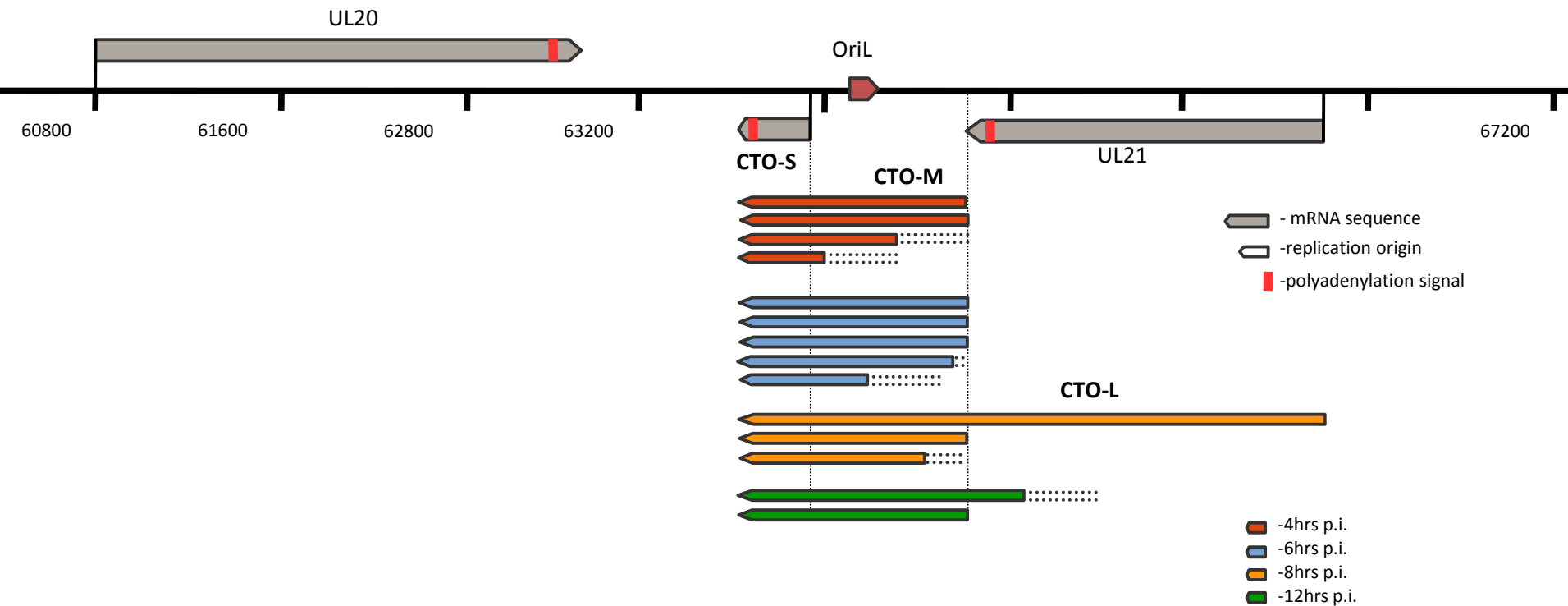


# CTO - a novel Inc-RNA



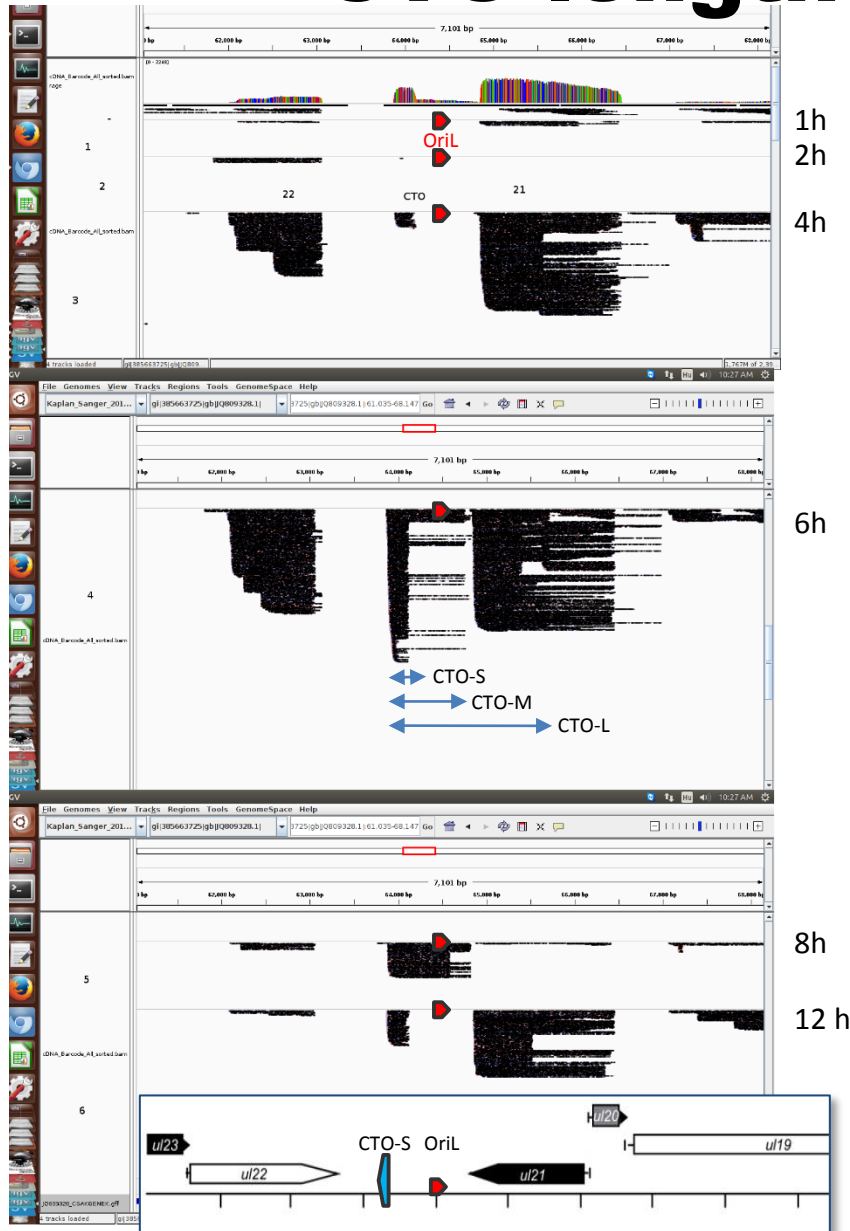
# Transcriptional kinetics of CTO length variants

**Clash between the transcription and DNA replication machineries?**



**PacBio RS II platform** – combined random hexamer-primed and anchored oligo(dT) primed [poly(A)-seq]

# Transcriptional kinetics of CTO length variants



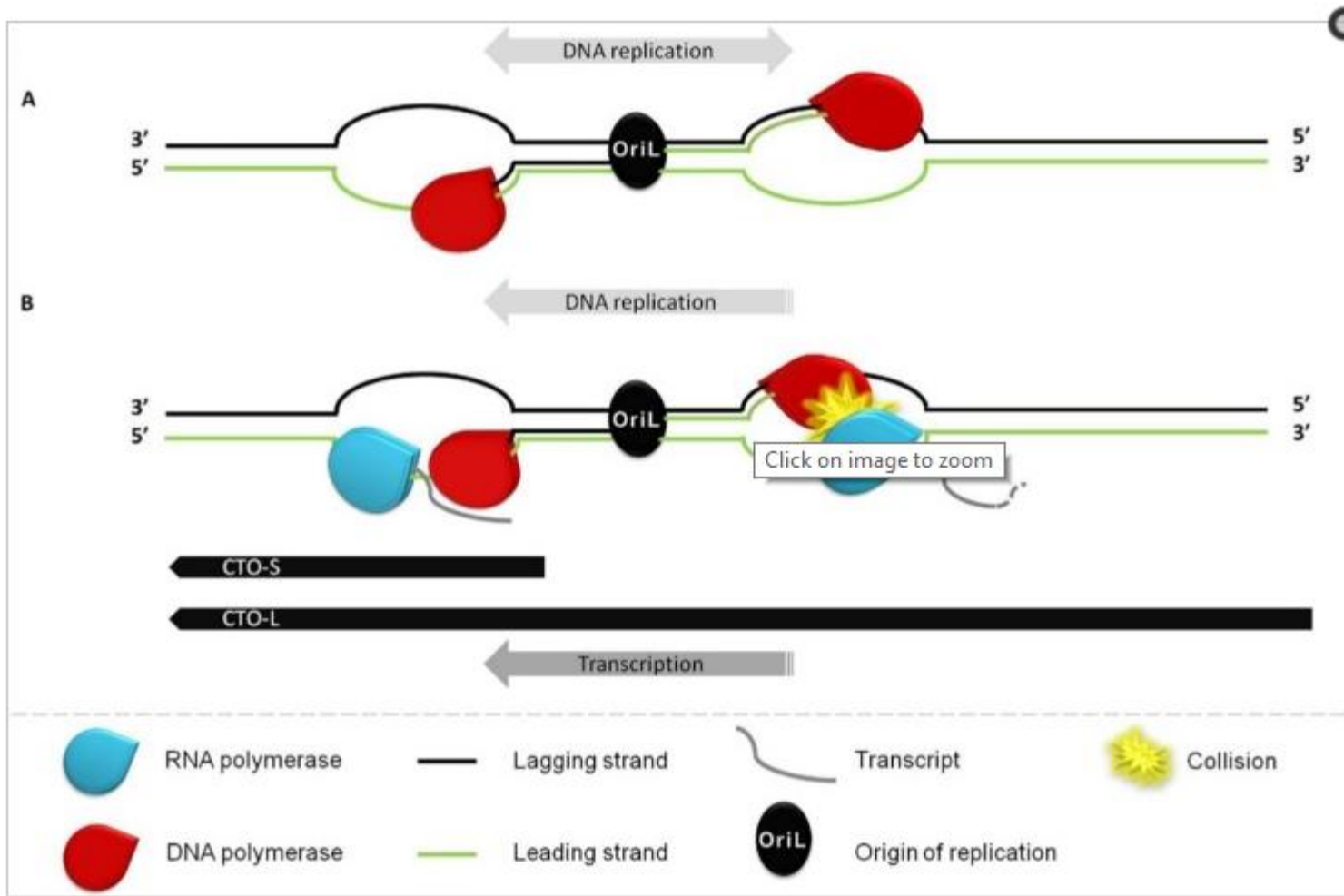
Confrontation of transcription and DNA replication machinery?

PacBio RS II platform:  
combined random hexamer-primed and anchored oligo(dT) primed [poly(A)-seq]

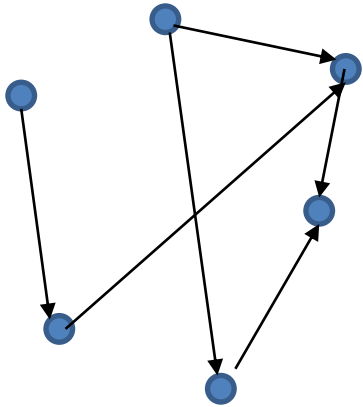


# Confrontation between transcription and replication machineries?

17b.



# Graph-based modeling of TIN



TIN Model Demo

**Genes**

Gene1
Gene2
Gene3
Gene4
Gene5
Gene6

**Properties**

TI = 70
BI = 30
R = 0.6

**Interactions**

```
Gene4==>CH
Gene1==>DTB
```

**Phases**

Phase0
Phase1
Phase2

**Phase properties**

Gene1 TI=90
Gene2 TI=70
Gene3 TI=100
Gene4 TI=65
Gene5 TI=20
Gene6 TI=60

Custom model... Open

Prev. phase Next phase Phase: 0 model props

- Java SDK 1.7
- NetBeans 7.4 IDE
- jgrapht – 0.9
- Platform-independent
- XML based storage of data

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