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Modern methods for genome analysis (PřF:Bi7420)

Lecture 6 : smallRNA-seq and IP methods

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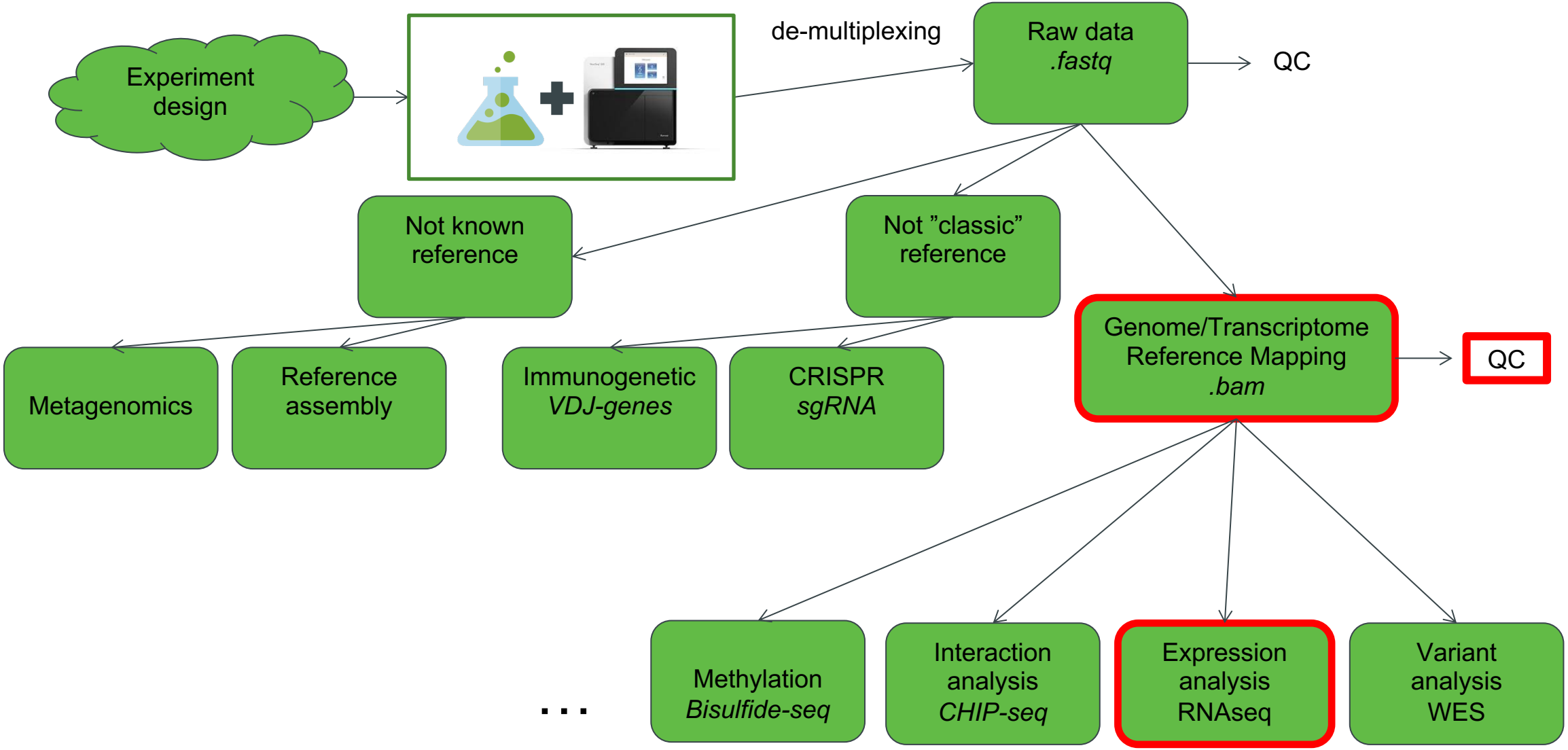
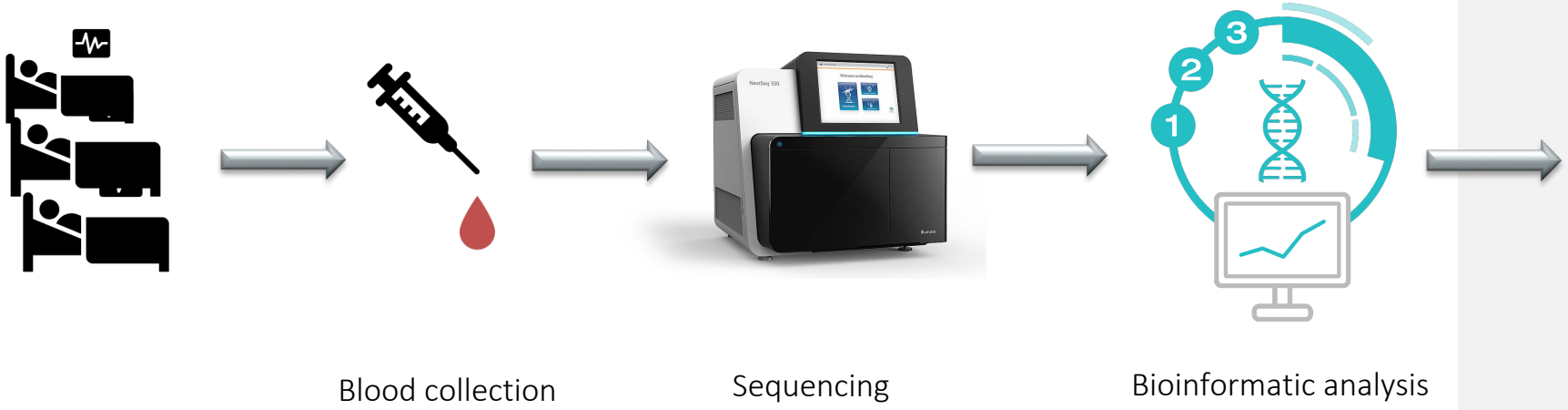
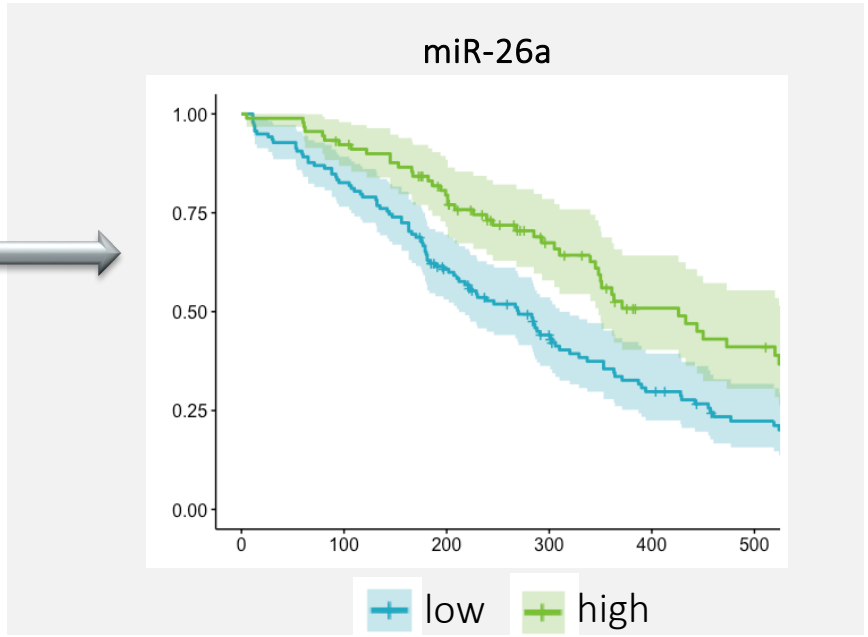
NGS data analysis



Small RNA-seq

- **Next-generation sequencing of short RNAs** allows for profiling of various short (non-coding) RNAs (microRNAs, piRNAs, tRNAs,…)
- Widely used method for **identification of disease biomarkers => cancer research**
- Special interest is in small RNAs that are part of circulatory system (biofluids) because these can serve as **non-invasive biomarkers**

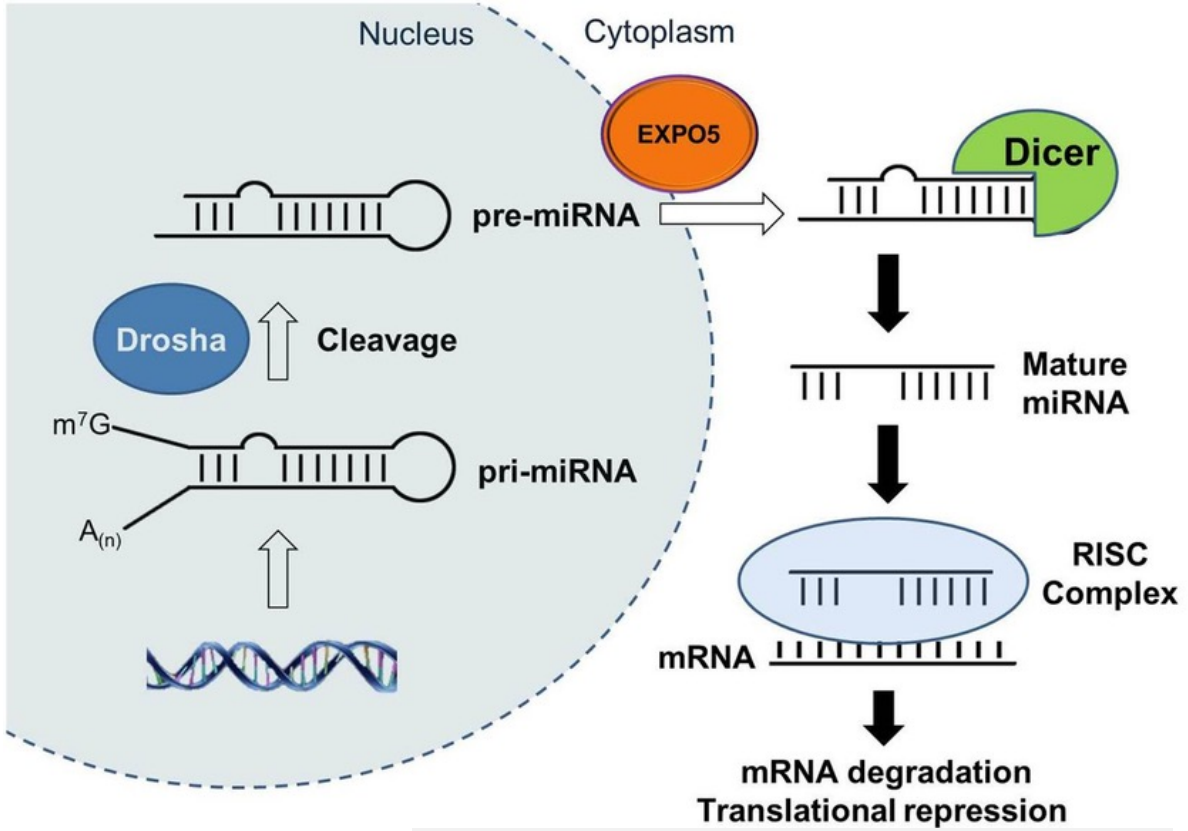




Small RNAs pool - microRNAs

- ~22nt long, regulate expression of other RNAs
- Mature miRNA binds to the 3’ UTR of coding RNA (mRNA) -> degradation
- ~2,000 mature miRNA known for human
- isomiRs = sequence variants of miRNA

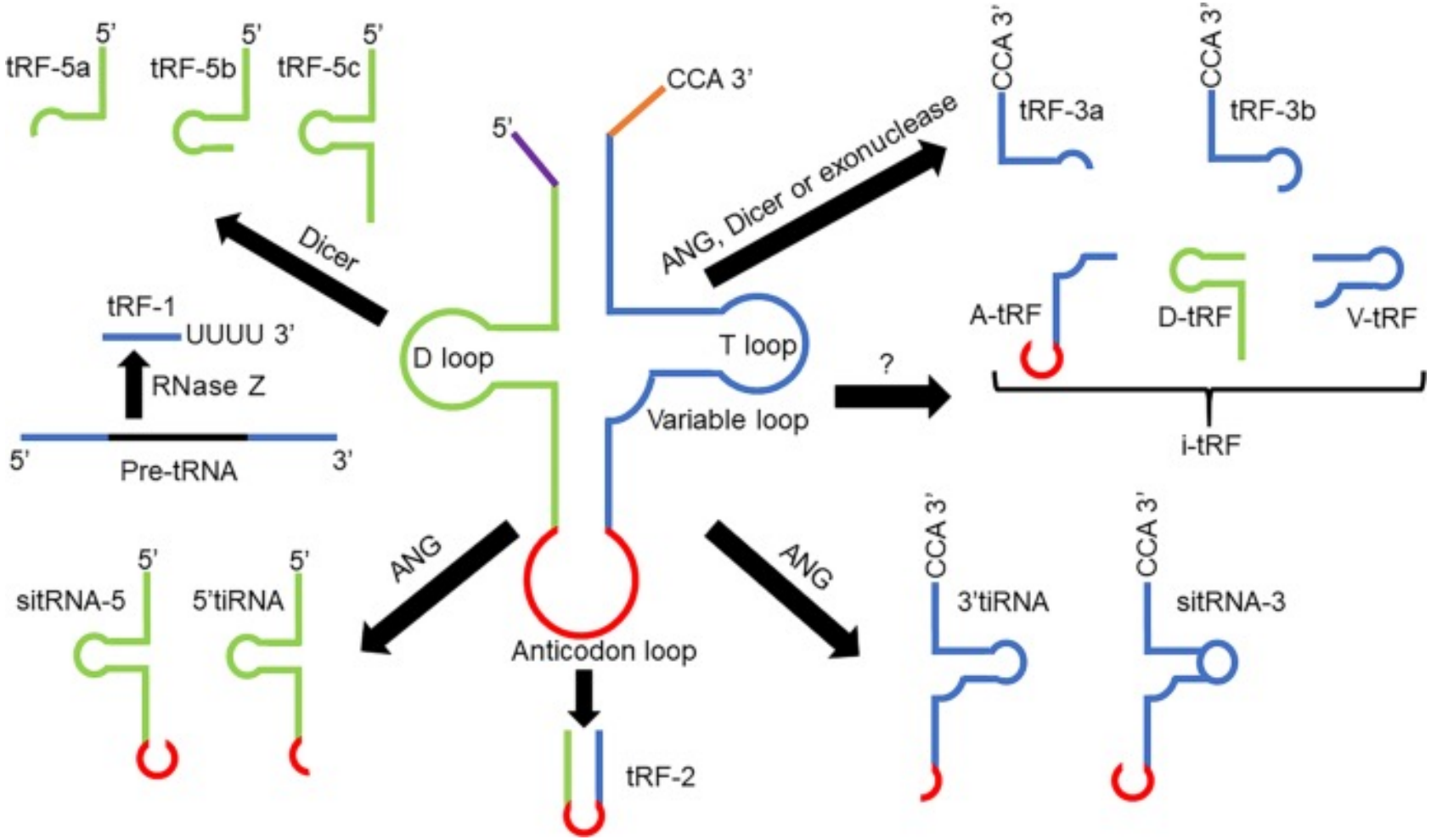
miRNA biogenesis



No protein

Small RNAs pool – tRNA fragments

- ~14-45nt long
- Participate in various biological processes -> research ongoing!



Small RNAs pool – other small RNAs

- **PIWI-interacting RNAs (piRNAs)** -> ~30nt long, most expressed in germinal cells where we know what they do; found to be expressed in the somatic cells as well but functions mainly unknown
- **Small nucleoler RNAs (snoRNAs)** -> chemical modification of other RNAs
- **Small nuclear RNAs (snRNAs)** -> pre-processing of coding RNAs in the nucleus
- Y RNA-derived small RNAs -> DNA replication?
- mRNA fragments -> random or not?

Module 1: **First QC**

- Quality control of raw sequencing data
- Scans FASTQ files for presence of adapters

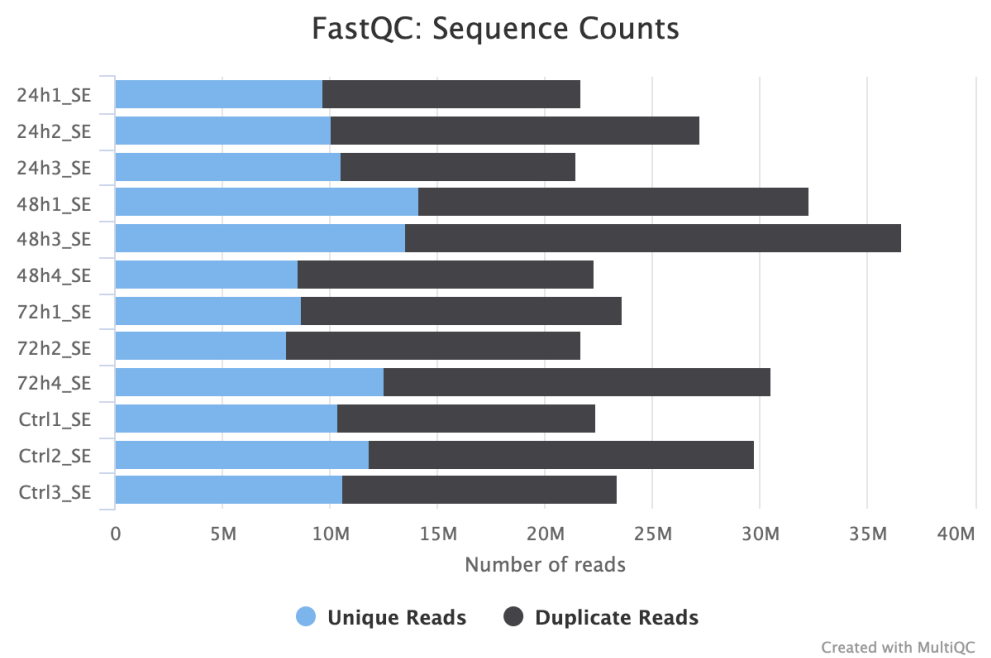
Results:

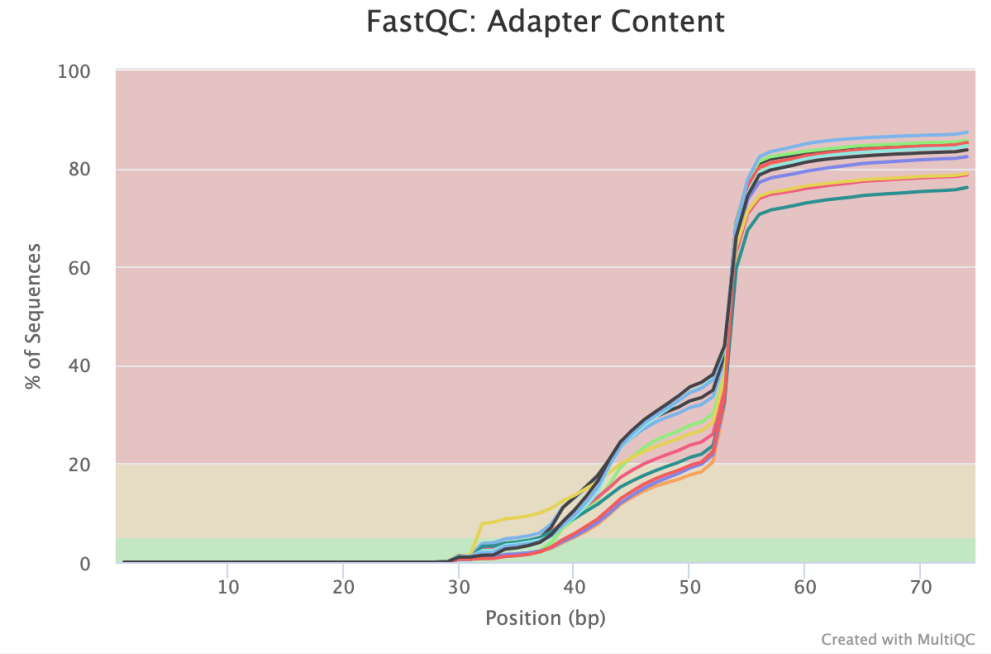
- List of detected adapters (exact sequences)

```
>24h1_SE
AAACGACGACGATCGCGATCCGC
>24h2_SE
AAACGACGACGATCGCGATCCGC
>24h3_SE
AAACGACGACGATCGCGATCCGC
```

→ Module 2

- Html/PDF report with plots and tables summarizing the quality of raw data





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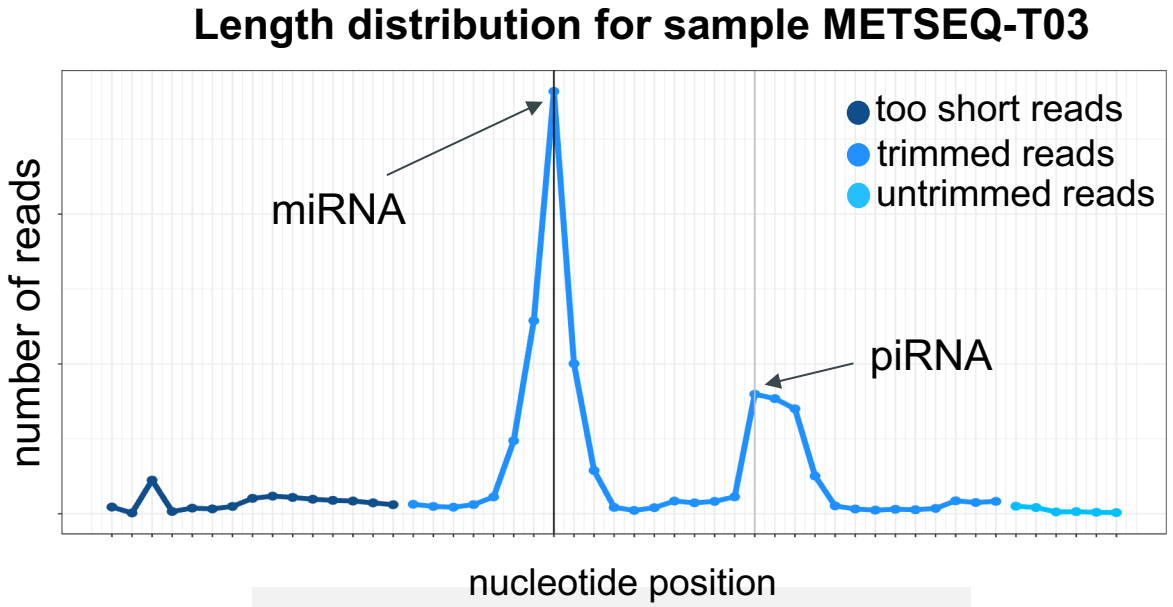
Module 2: **Pre-processing**

- Adapters trimming
- Trimming of low-quality bases, discarding of short reads
- Read collapsing based on UMIs (if present)

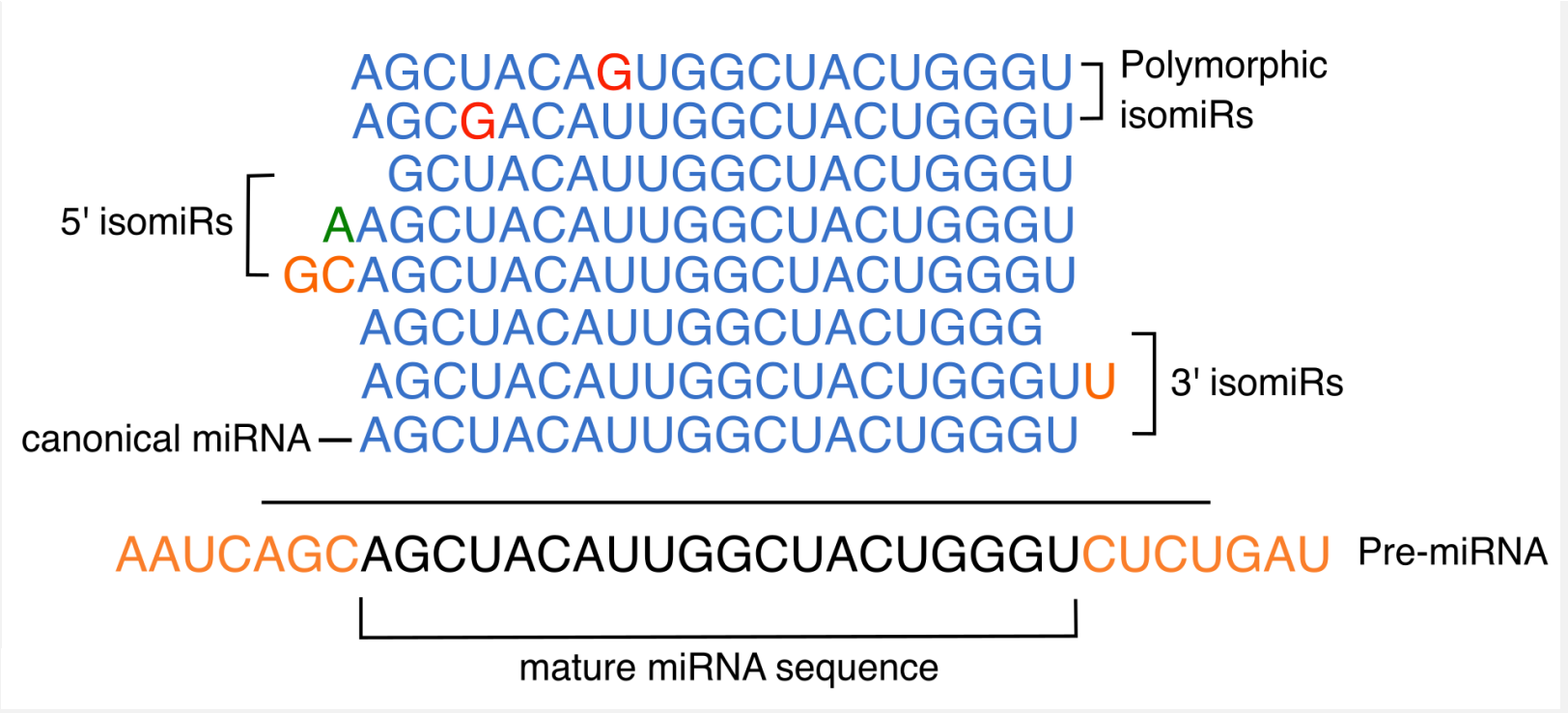
Results:

- Cleaned FASTQ files
- Html/PDF report with plots and table summarizing number of reads after each pre-processing step



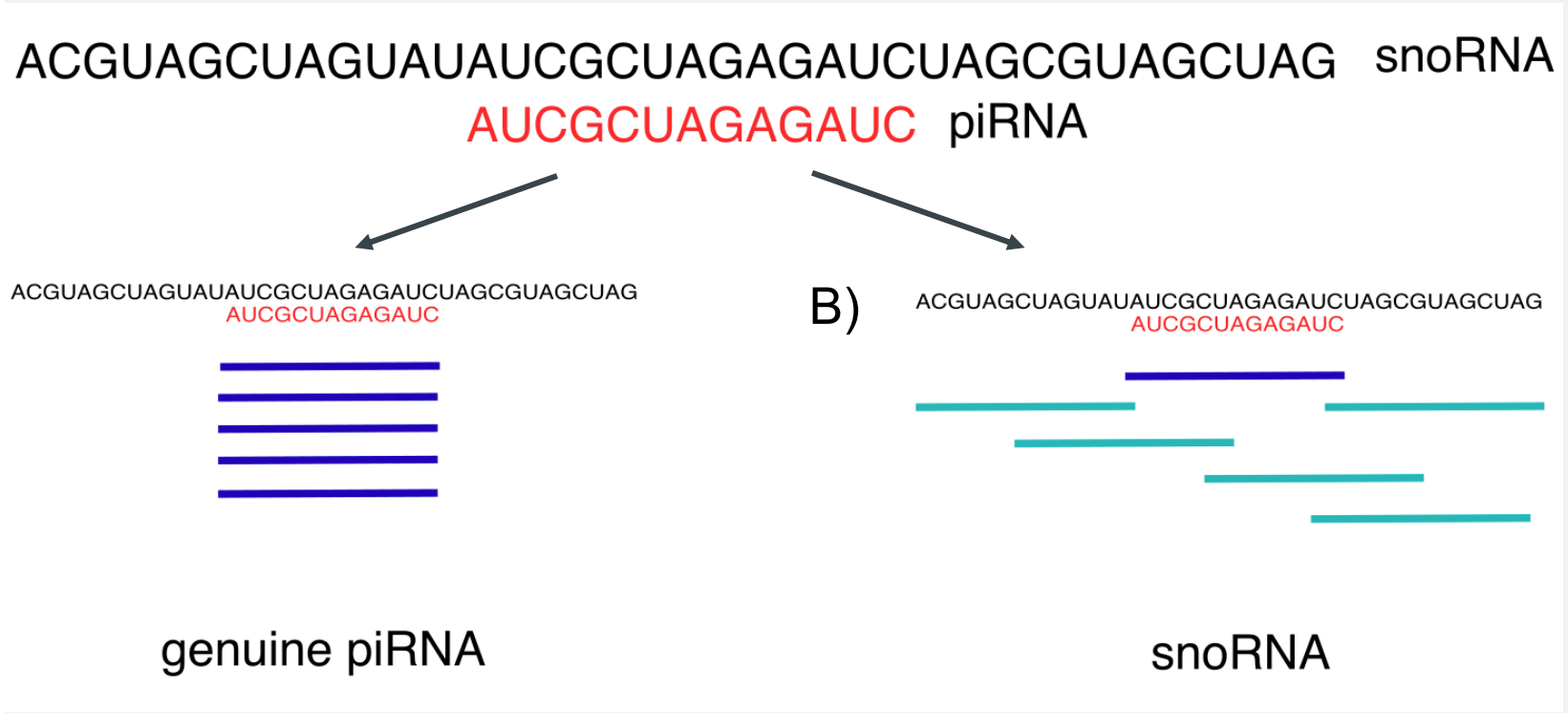
Module 3: **RNA quantification**

- Complicated due to complex nature of different short RNAs
- Requires individual approach for each class of short RNAs
  - **miRNA identification VS isomiRs identifications** (3'/5' additions, precursor ambiguity,…)



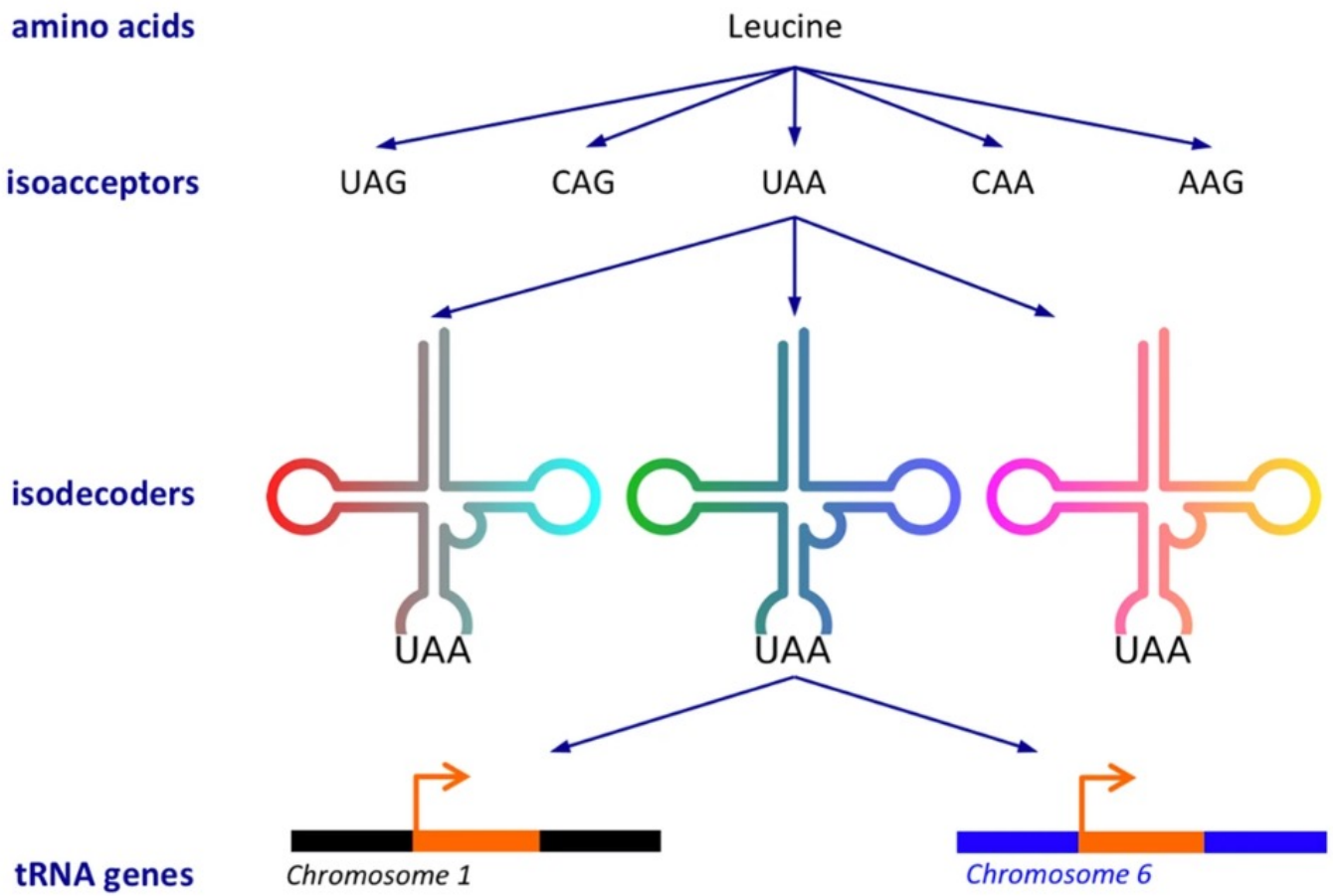
Module 3: **RNA quantification**

- Complicated due to complex nature of different short RNAs
- Requires individual approach for each class of short RNAs
  - **piRNAs nested within other coding/non-coding RNAs**

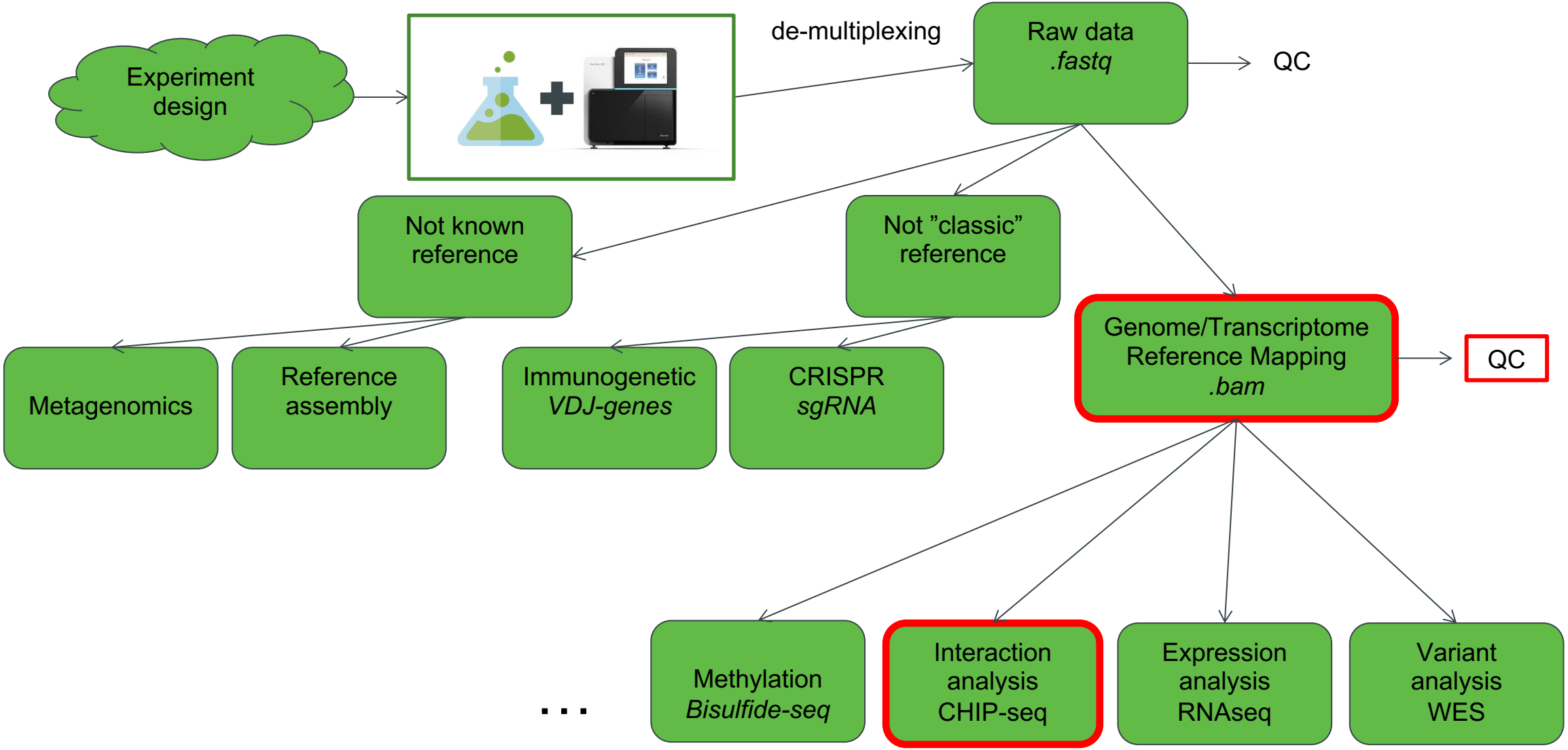


Module 3: **RNA quantification**

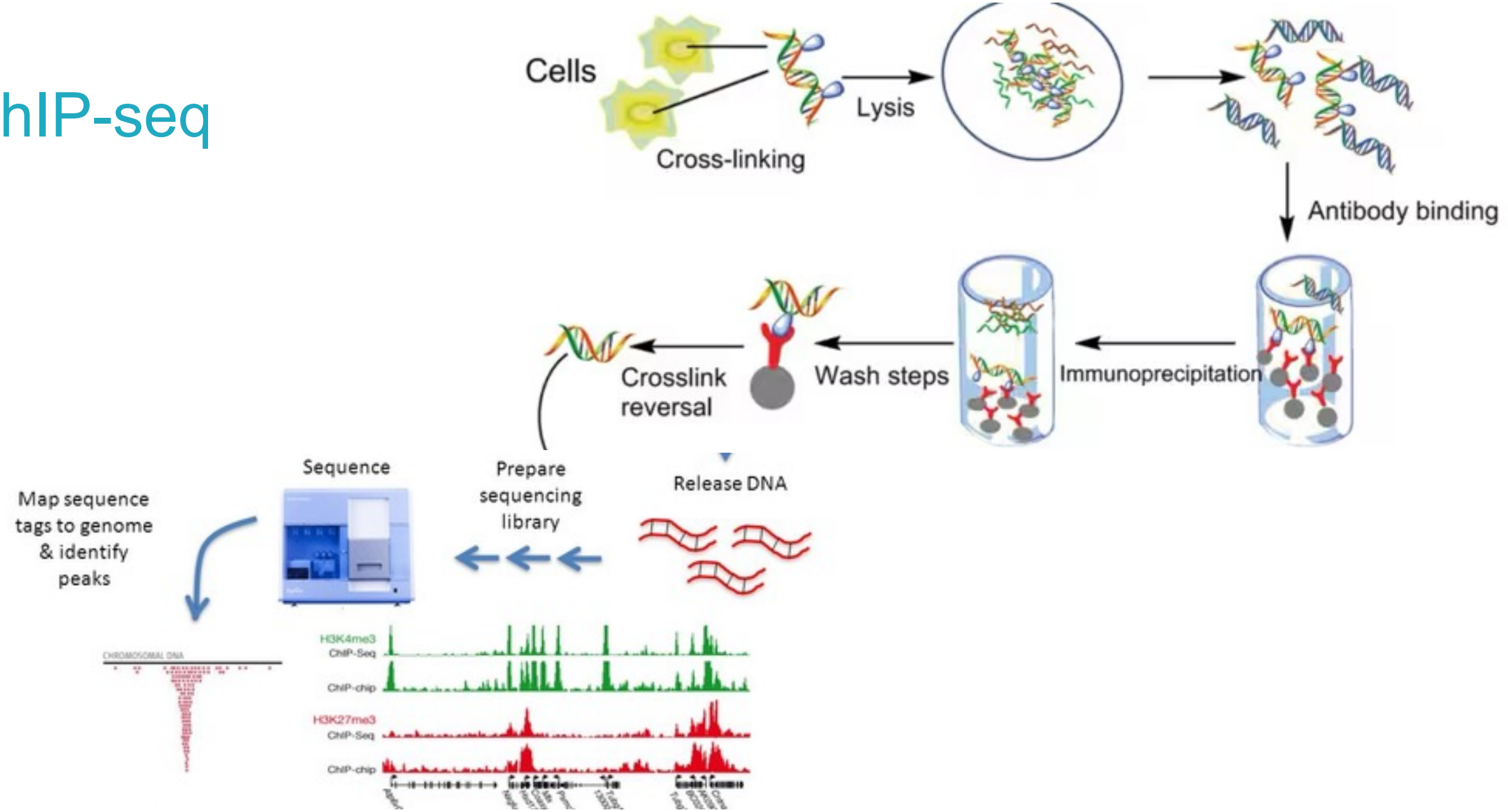
- Complicated due to complex nature of different short RNAs
- Requires individual approach for each class of short RNAs -> most problematic are tRFs and piRNAs



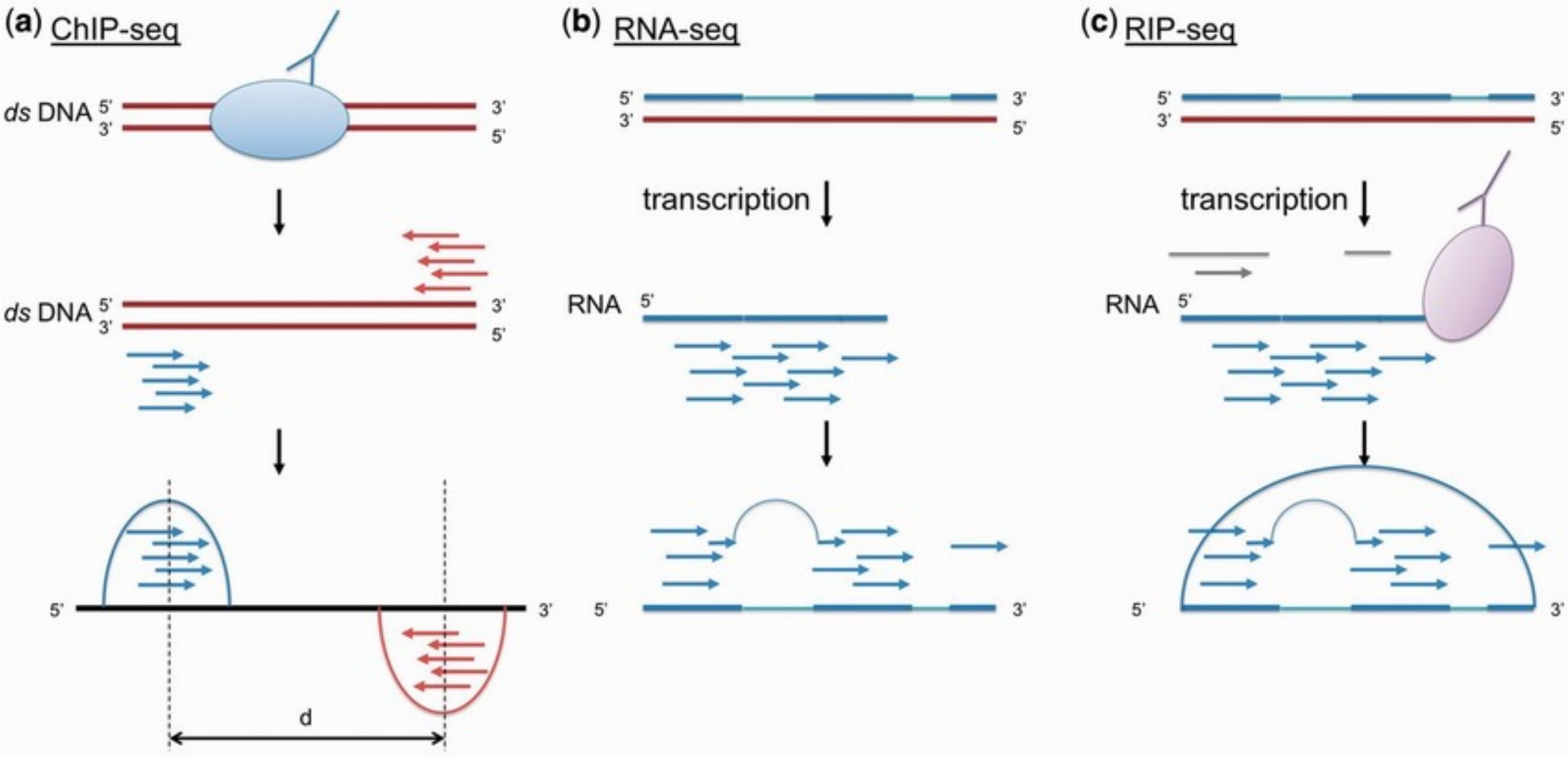
NGS data analysis



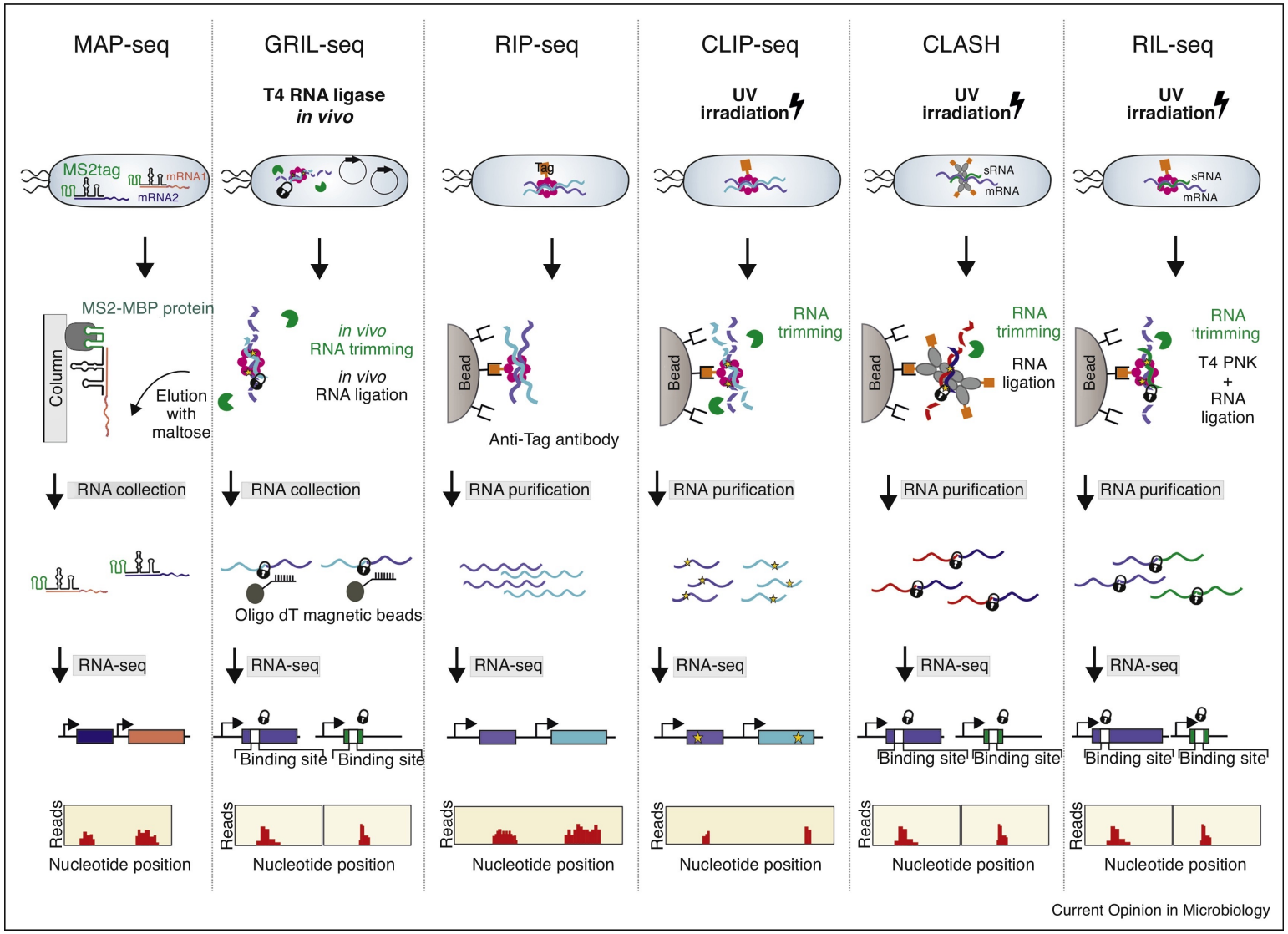
ChIP-seq


Cells
Cross-linking
Lysis
Antibody binding
Immunoprecipitation
Wash steps
Crosslink reversal
Release DNA
Prepare sequencing library
Sequence
Map sequence tags to genome & identify peaks
CHROMOSOMAL DNA
H3K4me3
ChIP-Seq
ChIP-chip
H3K27me3
ChIP-Seq
ChIP-chip

IP methods


(a) ChIP-seq
ds DNA 5' 3'
3' 5'
ds DNA 5' 3'
3' 5'
5'
3'
d
(b) RNA-seq
5' 3'
3' 5'
transcription
RNA 5'
5'
3'
(c) RIP-seq
5' 3'
3' 5'
transcription
RNA 5'
5'
3'

RNA IP methods



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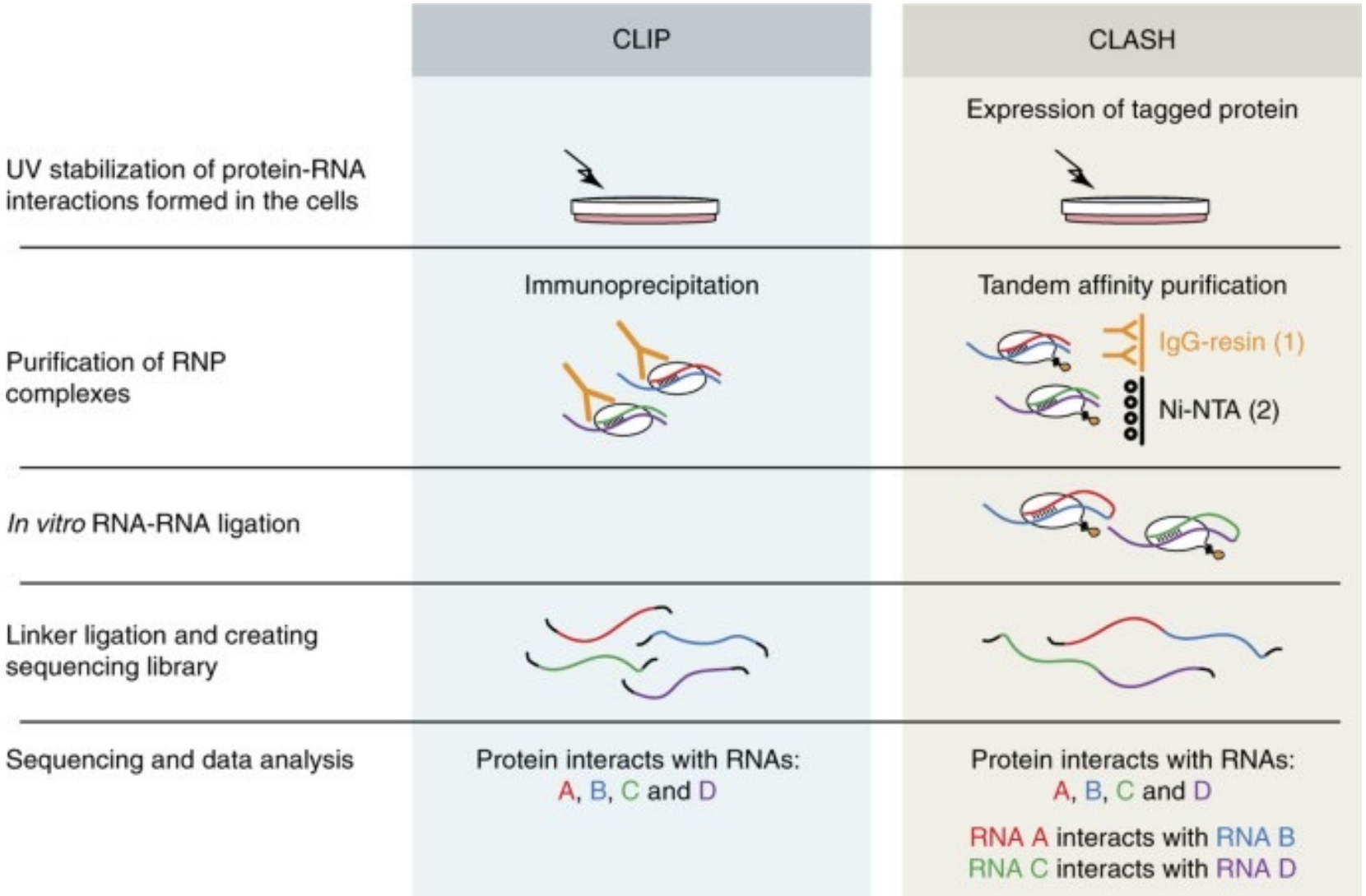
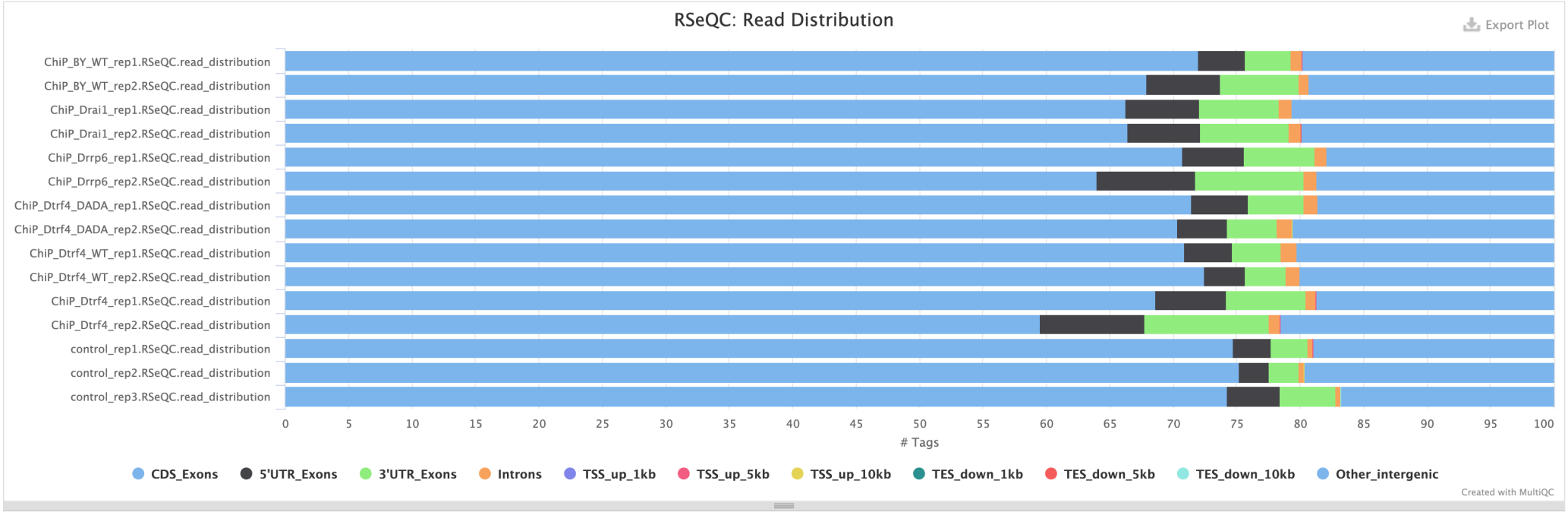
RNA IP methods



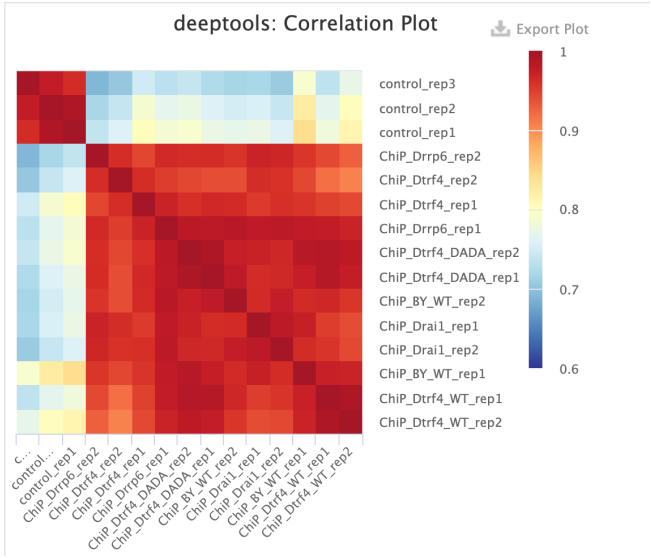
Primary analysis + QC

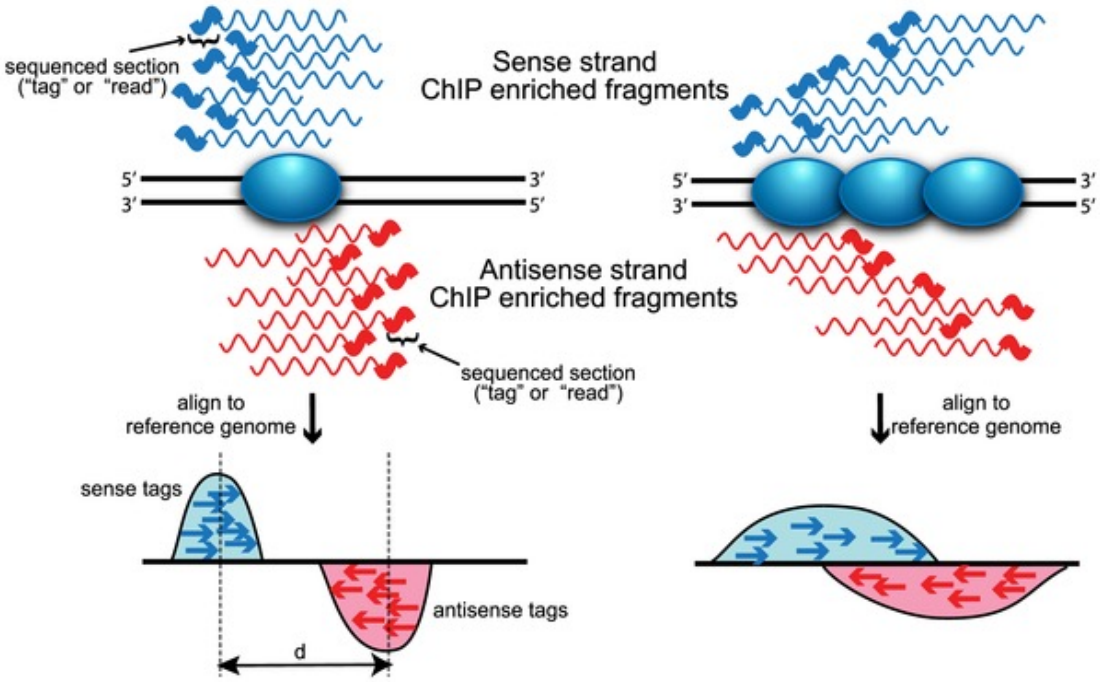
- Alignment – standard DNA (RNA for CLIP)
- RNA-seq like QC
  - Check sequencing quality
  - RSeQC – Read Dstribution



Primary analysis + QC

- IP experiment quality control
  - Sample correlation
    - Replicates control treatment
  - Strand cross-correlation
    - Shift of strand mapping
    - Shift should correlate with expected fragment size





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Primary analysis + QC

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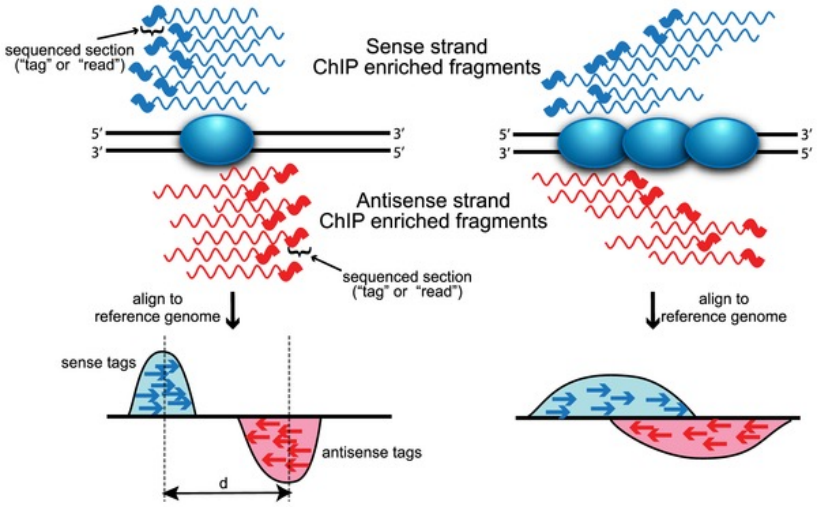
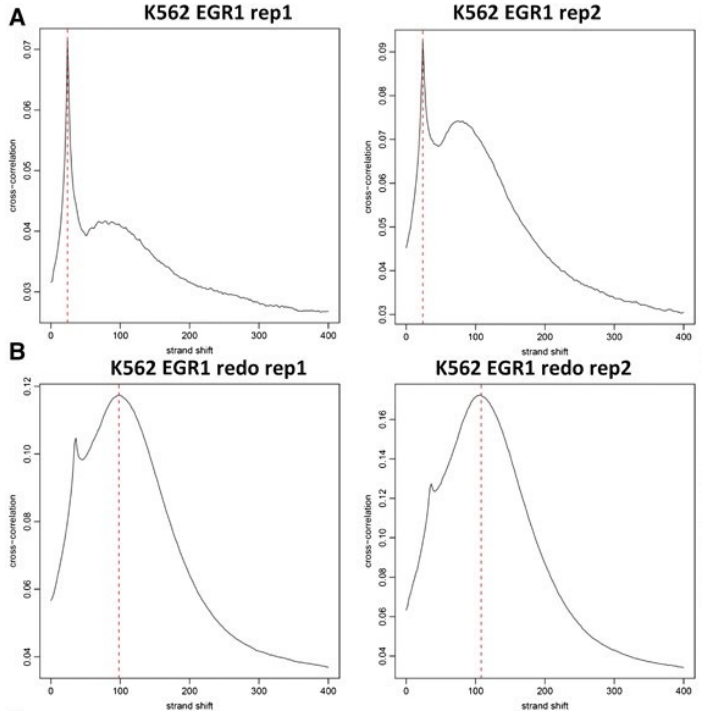
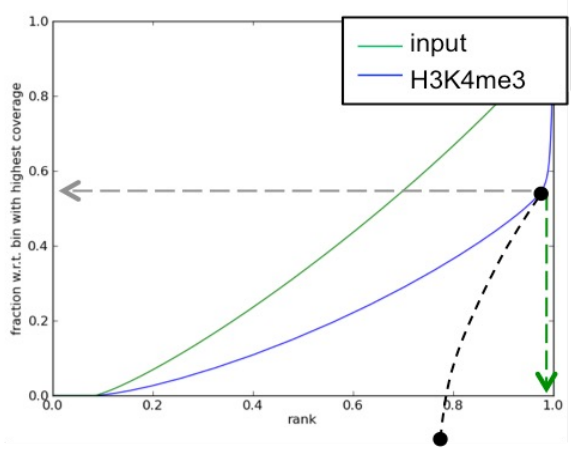




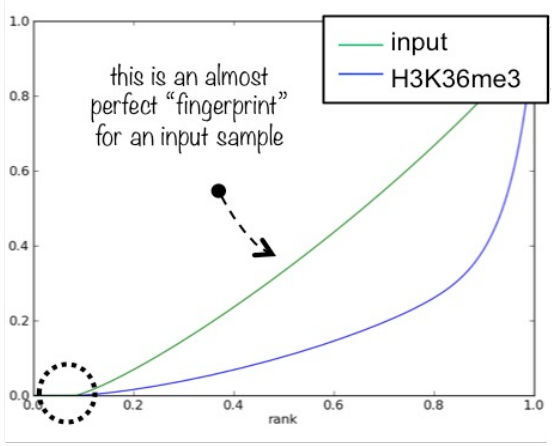
Primary analysis + QC

- **Fingerprint profile**
  - **profile of cumulative read coverages**
  - how evenly are the reads distributed over the genome

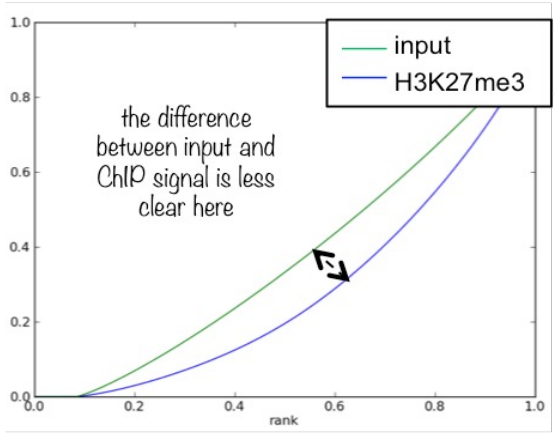


when counting the reads contained in 97% of all genomic bins, only ca. 55% of the maximum number of reads are reached, i.e. 3% of the genome contain a very large fraction of reads!

this indicates very localized, very strong enrichments!
(as every biologist hopes for in a ChIP for H3K4me3)



pay attention to where the curves start to rise – this already gives you an assessment of how much of the genome you have not sequenced at all (i.e. bins containing zero reads – for this example, ca. 10% of the entire genome do not have any read)



H3K27me3 is a mark that yields broad domains instead of narrow peaks

it is more difficult to distinguish input and ChIP, it does not mean, however, that this particular ChIP experiment failed

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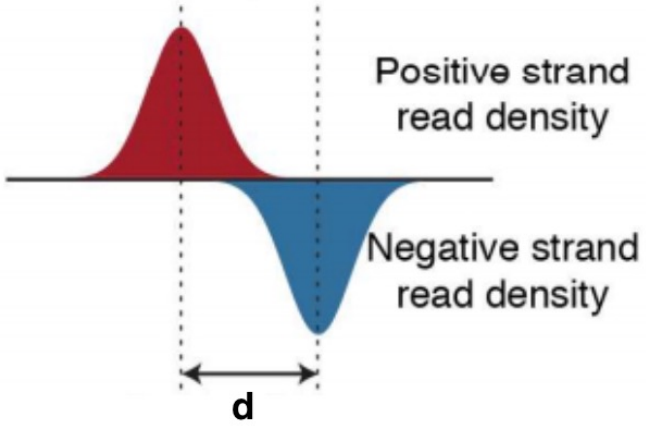
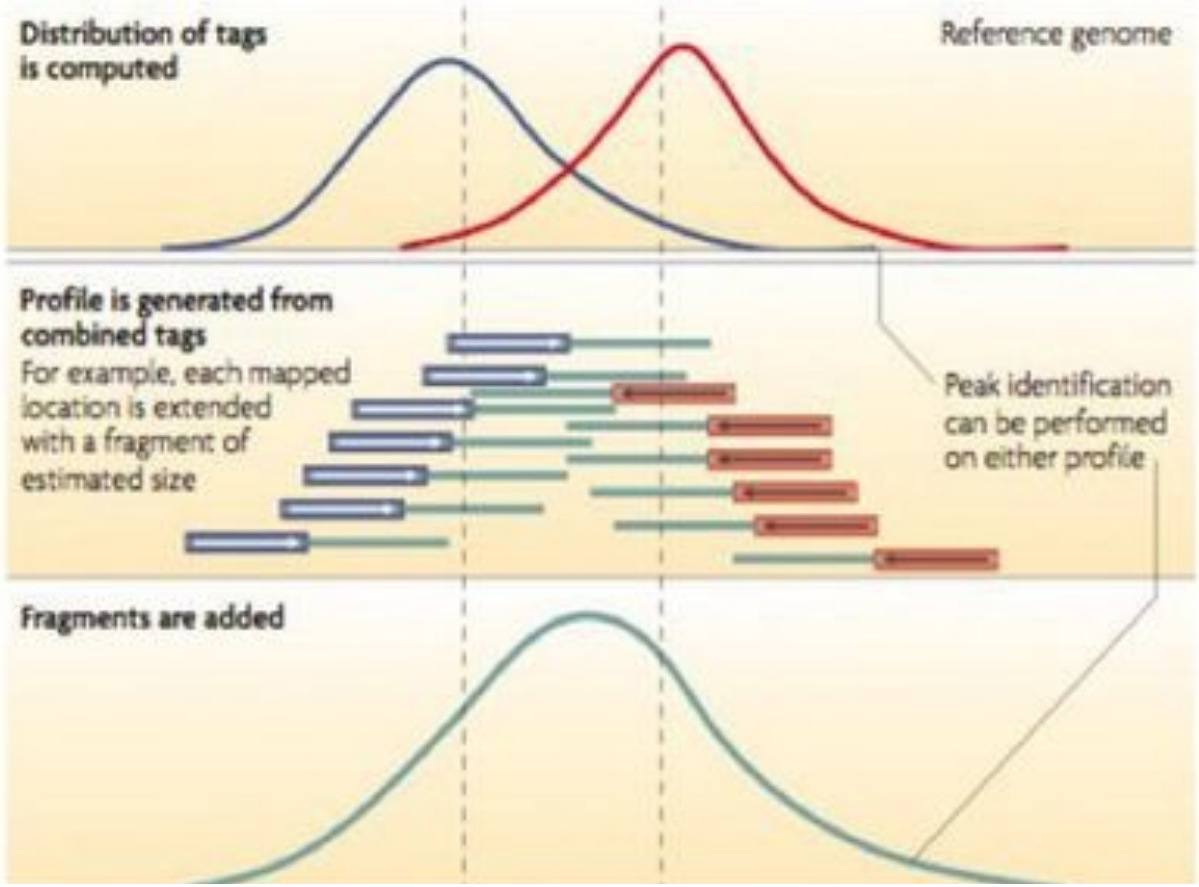
Peak calling

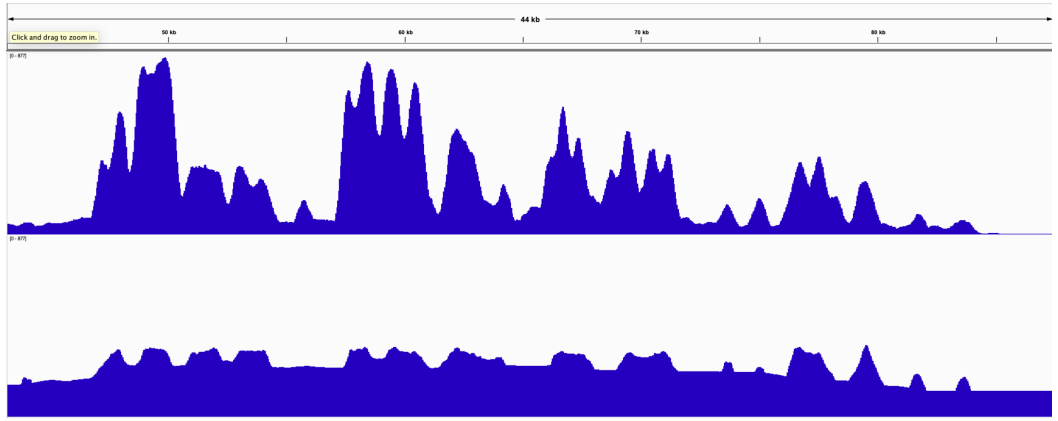
- Read extension





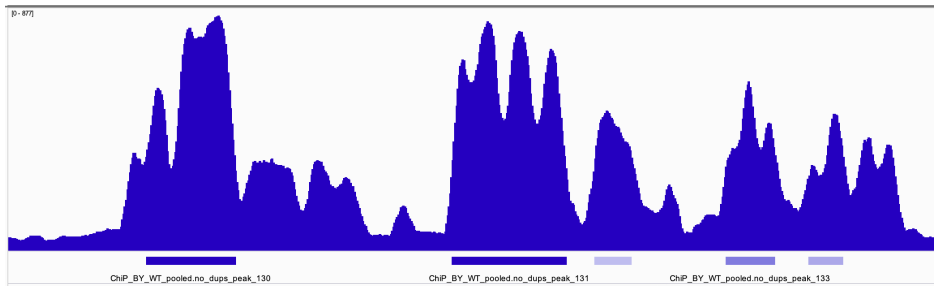
Peak calling

- Statistical assessment of peaks against background
- Background
  - Control sample – recommended
  - Model background from overall coverage of the sample

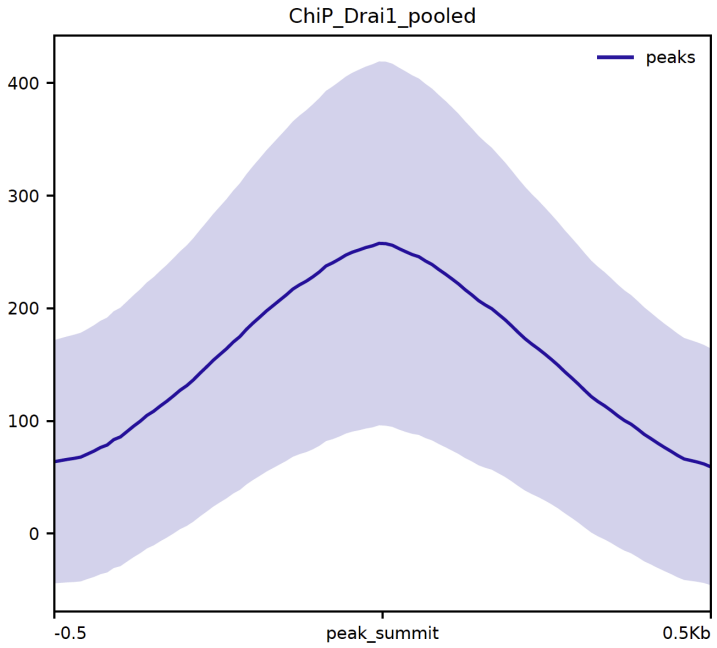


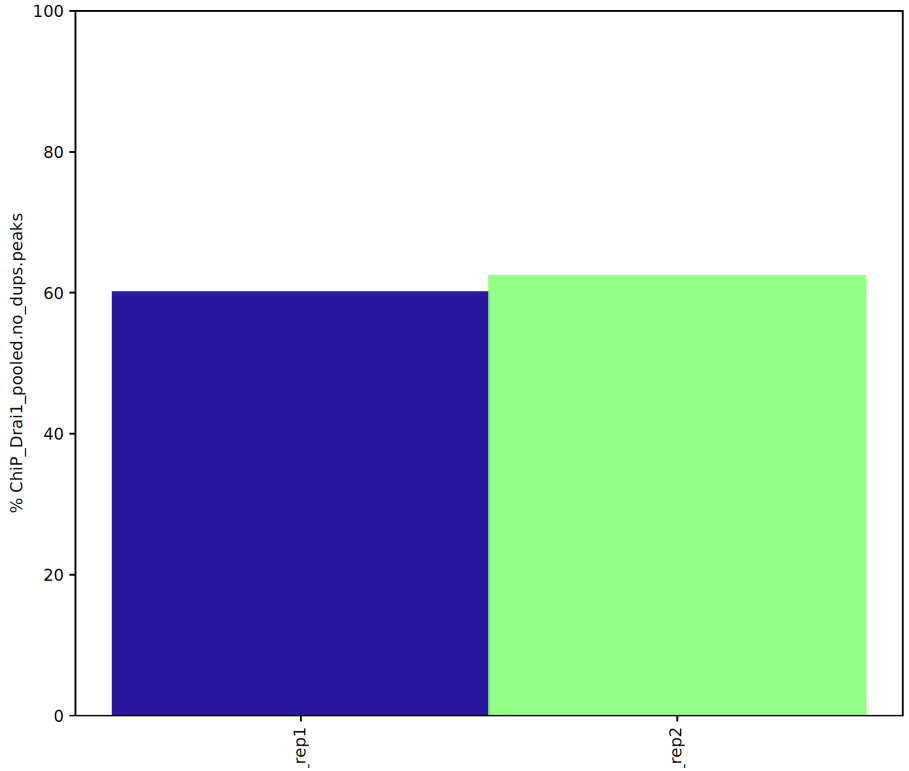
- Peak calling annotation
- Differential peak calling

Post peak calling QC



- FRIP score = fraction of reads in peaks
  - High number is good
  - However can be low in specific experiments and still the experiment be OK
- Average peak profile





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Peak calling results

chr	start	end	peak_ID	overall_score	strand	fold_change	-log(pavalue)	-log(qvalue)	relative_peak_summit	gene_name	gene_id
I	31479	33115	ChiP_BY_WT_pooled.no_dups_peak_1	429	.	1.88871	44.3616	42.9998	1232	GDH3	YAL062W
I	33537	34528	ChiP_BY_WT_pooled.no_dups_peak_2	610	.	2.08354	62.5989	61.0757	507	BDH2	YAL061W
I	35137	36342	ChiP_BY_WT_pooled.no_dups_peak_3	556	.	2.04075	57.1526	55.6747	425	BDH1	YAL060W
I	45839	46698	ChiP_BY_WT_pooled.no_dups_peak_4	126	.	1.43949	13.7207	Dec.75	433	FLC2	YAL053W
I	57192	60004	ChiP_BY_WT_pooled.no_dups_peak_5	854	.	2.40168	87.1869	85.4642	1022	BOL3,NA,BOL1,GCV3,PTA1	YAL046C,YAL045C,YAL044W-A,YAL044C,YAL043C
I	60315	63277	ChiP_BY_WT_pooled.no_dups_peak_6	704	.	2.15353	72.018	70.4181	1323	PTA1,YAL042C-A,ERV46,CDC24	YAL043C,YAL042C-A,YAL042W,YAL041W
I	66666	67791	ChiP_BY_WT_pooled.no_dups_peak_7	889	.	2.43399	90.6587	88.9061	755	CLN3	YAL040C
I	68347	69671	ChiP_BY_WT_pooled.no_dups_peak_8	820	.	2.25923	83.6998	82.0078	696	CYC3	YAL039C
I	71610	73588	ChiP_BY_WT_pooled.no_dups_peak_9	1183	.	2.44018	120.4	118.351	905	CDC19,NA	YAL038W,YAL037C-B,YAL037C-A
I	75651	76970	ChiP_BY_WT_pooled.no_dups_peak_10	860	.	2.41056	87.8185	86.0902	304	RBG1,FUN12	YAL036C,YAL035W
I	77324	77856	ChiP_BY_WT_pooled.no_dups_peak_11	398	.	1.93424	41.1426	39.8111	250	FUN12	YAL035W
I	79039	79494	ChiP_BY_WT_pooled.no_dups_peak_12	332	.	1.91858	34.5251	33.2593	247	FUN12,YAL034C-B	YAL035W,YAL034C-B
I	82712	84482	ChiP_BY_WT_pooled.no_dups_peak_13	113	.	1.42908	Dec.21	Nov.74	1469	POP5,PRP45	YAL033W,YAL032C
I	100120	100713	ChiP_BY_WT_pooled.no_dups_peak_14	807	.	2.47086	82.3941	80.7135	299	MAK16	YAL025C
I	106226	107428	ChiP_BY_WT_pooled.no_dups_peak_15	358	.	1.77564	37.1375	35.8455	501	PMT2	YAL023C
I	107973	109870	ChiP_BY_WT_pooled.no_dups_peak_16	771	.	2.26481	78.8375	77.1854	381	PMT2,FUN26	YAL023C,YAL022C
I	112214	114585	ChiP_BY_WT_pooled.no_dups_peak_17	520	.	Jan.32	53.4499	52.0045	840	CCR4,ATS1,NA	YAL021C,YAL020C,YAL019W-A
I	114751	116392	ChiP_BY_WT_pooled.no_dups_peak_18	350	.	1.78626	36.2902	35.0066	329	NA,FUN30	YAL019W-A,YAL019W
I	128402	132575	ChiP_BY_WT_pooled.no_dups_peak_19	1155	.	2.44079	117.568	115.556	2887	SYN8,DEP1,CYS3,SWC3	YAL014C,YAL013W,YAL012W,YAL011W
I	139243	139805	ChiP_BY_WT_pooled.no_dups_peak_20	69	.	1.34572	7.85378	6.90849	332	TRN1,SSA1	tP(UGG)A,YAL005C
I	142057	143930	ChiP_BY_WT_pooled.no_dups_peak_21	1811	.	3.16946	184.166	181.135	753	EFB1,SNR18,VPS8	YAL003W,snR18,YAL002W
I	166101	166567	ChiP_BY_WT_pooled.no_dups_peak_22	282	.	2.06498	29.4684	28.2548	244	TGA1	tA(UGC)A
I	169591	170278	ChiP_BY_WT_pooled.no_dups_peak_23	629	.	2.12958	64.5023	62.9641	326	ADE1	YAR015W
I	192608	193905	ChiP_BY_WT_pooled.no_dups_peak_24	384	.	1.78426	39.7611	38.4434	499	SWH1	YAR042W
II	36867	38491	ChiP_BY_WT_pooled.no_dups_peak_25	784	.	2.23904	80.1294	78.4676	482	NA,ATP1	YBL100C,YBL099W
II	43225	43782	ChiP_BY_WT_pooled.no_dups_peak_26	384	.	1.966	39.7836	38.4657	250	NA,MRX3	YBL096C,YBL095W,YBL094C
II	44181	44769	ChiP_BY_WT_pooled.no_dups_peak_27	596	.	2.15493	61.1141	59.6037	280	ROX3	YBL093C
II	45344	46996	ChiP_BY_WT_pooled.no_dups_peak_28	1527	.	3.1313	155.299	152.752	1118	RPL32,SCS22	YBL092W,YBL091C-A
II	59655	60610	ChiP_BY_WT_pooled.no_dups_peak_29	2000	.	4.40847	203.402	200.072	398	RPL23A	YBL087C
II	69809	71227	ChiP_BY_WT_pooled.no_dups_peak_30	260	.	1.65314	27.2126	26.0225	1005	NA,ALG3	YBL083C,YBL082C
II	72314	73020	ChiP_BY_WT_pooled.no_dups_peak_31	673	.	2.15795	68.9081	67.3341	344	NA	YBL081W
II	75150	75665	ChiP_BY_WT_pooled.no_dups_peak_32	520	.	2.1439	53.5167	52.0704	285	NUP170	YBL079W
II	87930	90492	ChiP_BY_WT_pooled.no_dups_peak_33	780	.	Feb.06	79.7143	78.0556	2285	NA,SNR56,RPS8A,KTI11	YBL073W,snR56,YBL072C,YBL071C-B,YBL071W-A,YBL071C
II	90761	91443	ChiP_BY_WT_pooled.no_dups_peak_34	314	.	1.84847	32.6653	31.4181	399	NA,AST1	YBL070C,YBL069W
II	111556	113226	ChiP_BY_WT_pooled.no_dups_peak_35	287	.	1.72466	30.0068	28.7874	851	SHP1,PTH2	YBL058W,YBL057C
II	113627	114157	ChiP_BY_WT_pooled.no_dups_peak_36	638	.	2.54546	65.4125	63.8668	279	PTC3	YBL056W
II	114710	115219	ChiP_BY_WT_pooled.no_dups_peak_37	388	.	2.13721	40.139	38.8175	233	PTC3	YBL056W
II	116347	117229	ChiP_BY_WT_pooled.no_dups_peak_38	231	.	1.81604	24.3357	23.1769	670	YBL055C	YBL055C
II	117472	118286	ChiP_BY_WT_pooled.no_dups_peak_39	1023	.	2.56761	104.225	102.348	446	TOD6	YBL054W
II	139205	140163	ChiP_BY_WT_pooled.no_dups_peak_40	327	.	1.91809	33.9732	32.7132	700	FUI1	YBL042C
II	141409	142016	ChiP_BY_WT_pooled.no_dups_peak_41	589	.	2.29474	60.4957	58.9904	258	PRE7	YBL041W
II	158759	159747	ChiP_BY_WT_pooled.no_dups_peak_42	692	.	2.11947	70.7935	69.2036	467	RIB1	YBL033C
II	160083	160915	ChiP_BY_WT_pooled.no_dups_peak_43	450	.	1.87536	46.4682	45.0864	409	HEK2	YBL032W

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Thank you for your attention!

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