Genes targeted by the estrogen and progesterone receptors in human endometrium

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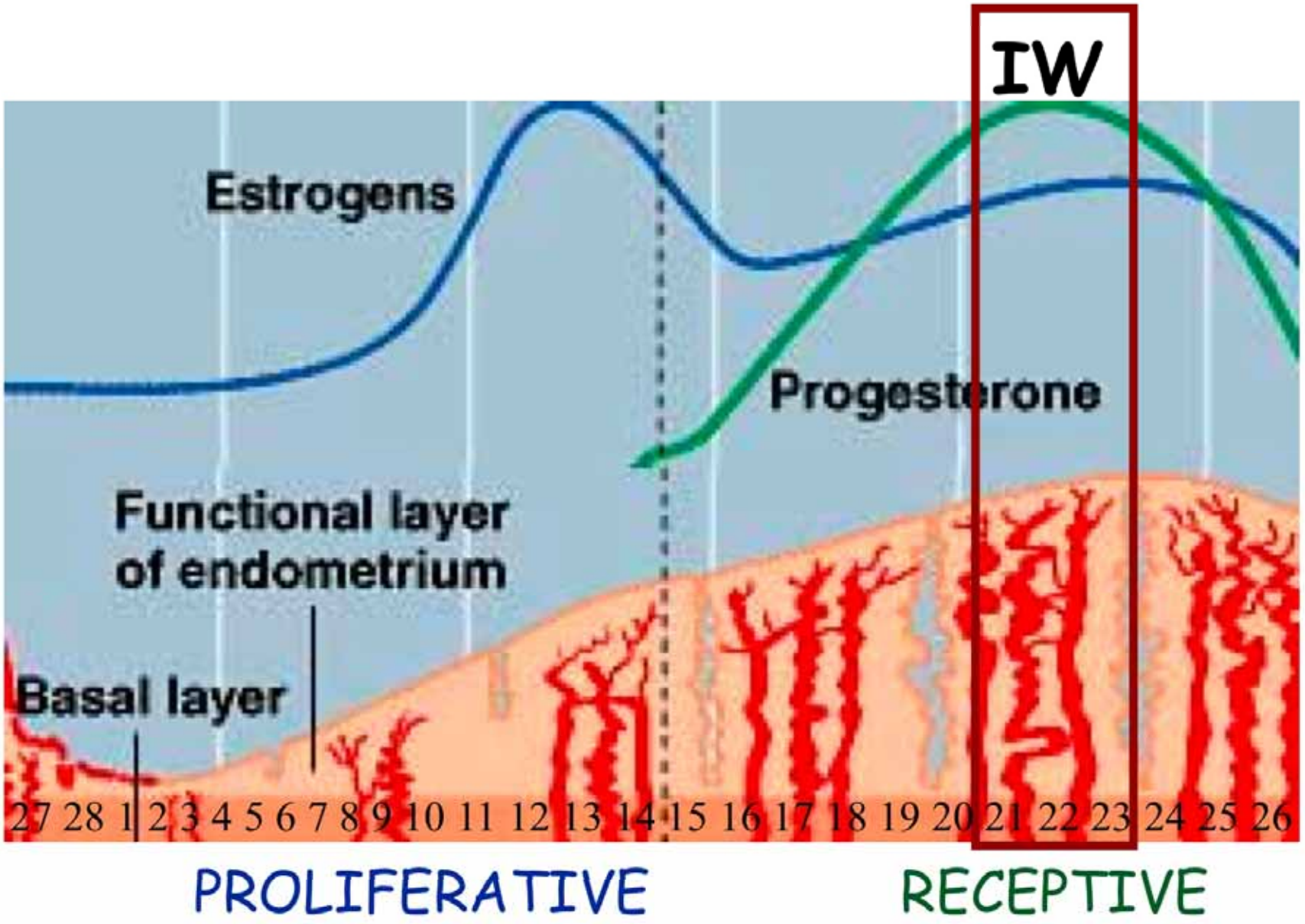
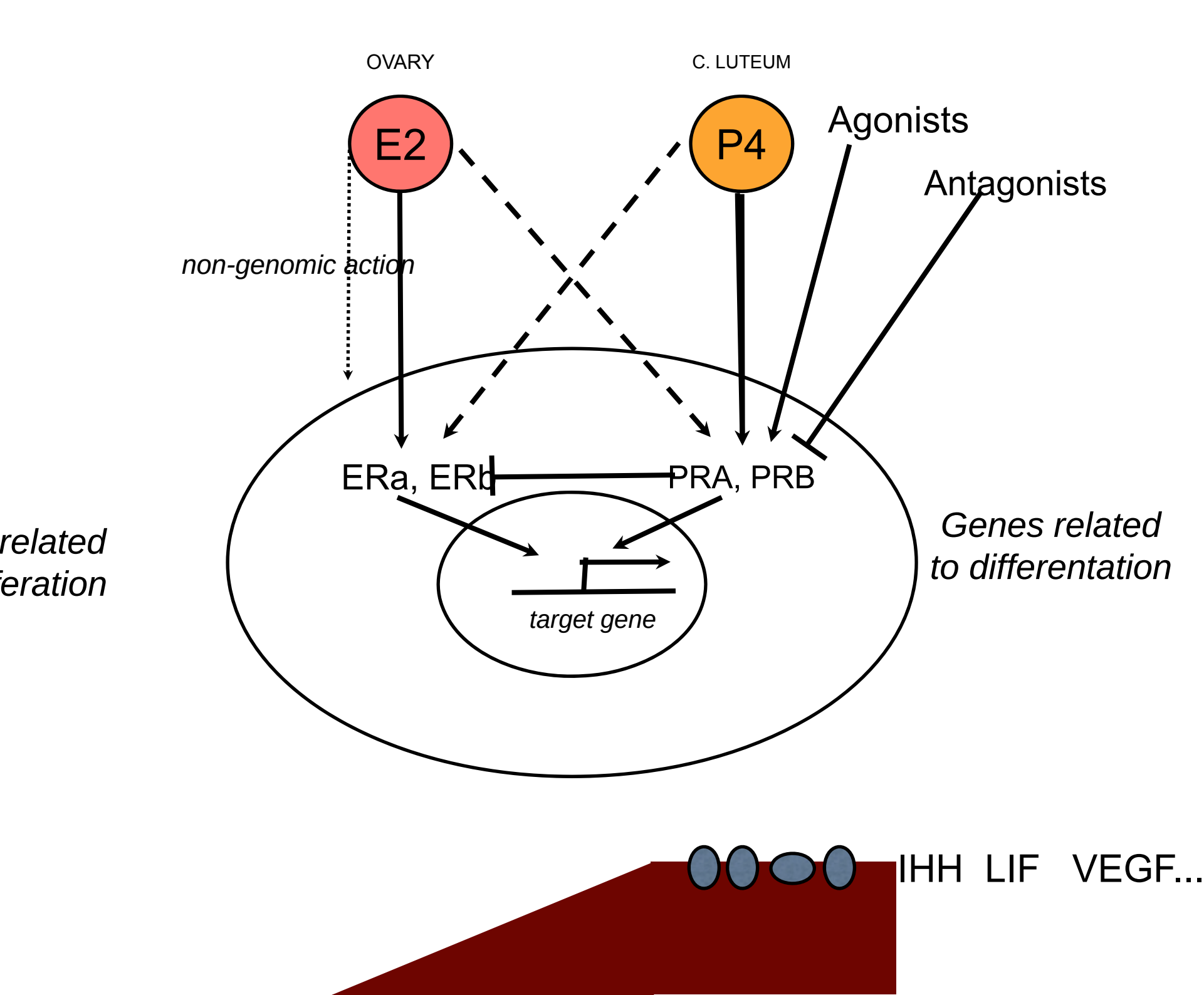
Human Endometrium




OVARY
C. LUTEUM
E2
P4
Agonists
Antagonists
non-genomic action
ERa, ERb
PRA, PRB
target gene
Genes related
to differentation
related
feration
IHH LIF VEGF...

E2, P4 10^{-8}M 45`

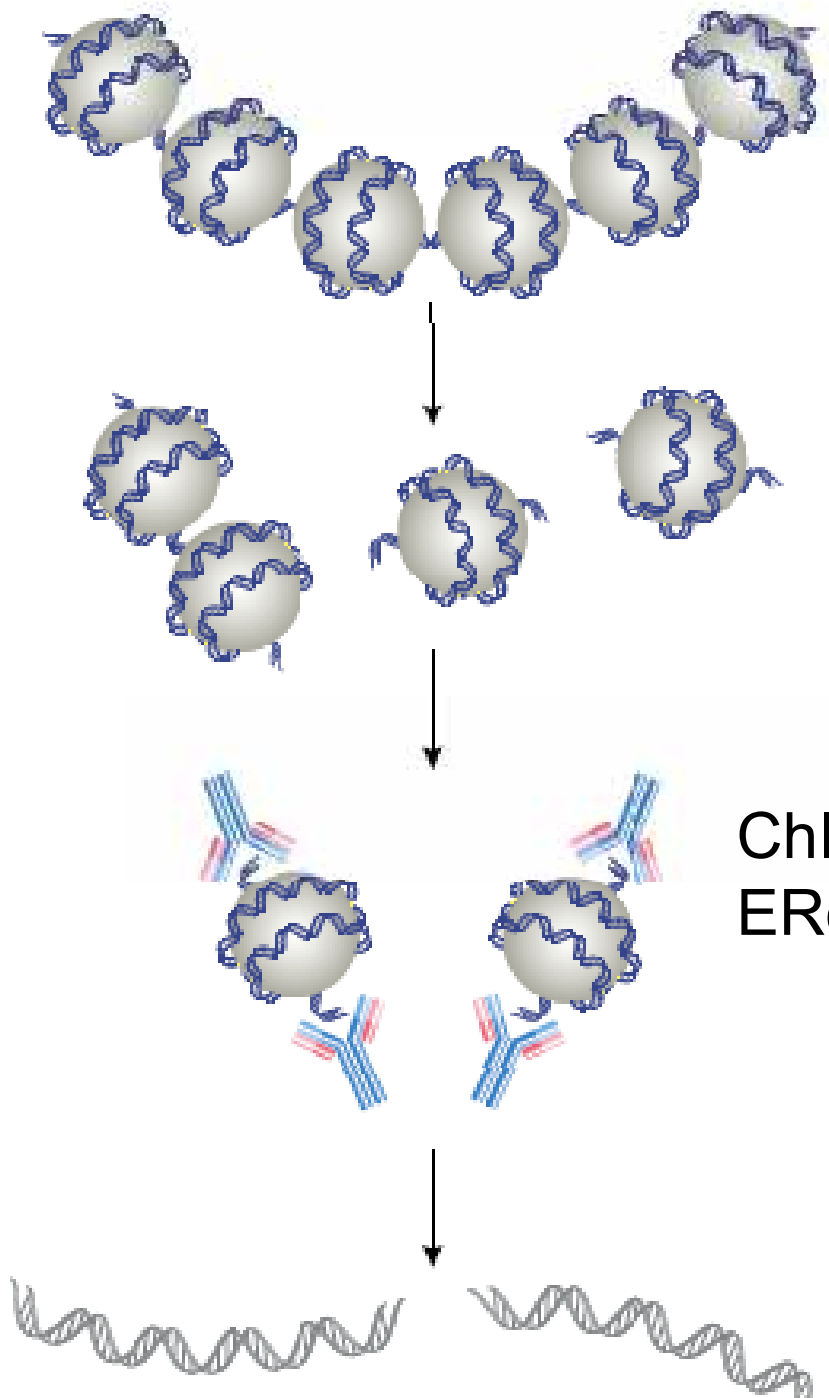
Method Overview

ells are fixed with formaldehyde to cross-link histone and on-histone proteins to DNA.

hromatin is digested with Micrococcal Nuclease into 150-900 o DNA/protein fragments.

ntibodies specific to histone or non-histone proteins are added nd the complex co-precipitates and is captured by Protein G garose or Protein G magnetic beads.

ross-links are reversed, and DNA is purified and ready for nalysis.



ChIP using Ab-s a
ERα, ERβ, PRAB,

ChIP enriched

and RL95-2

Pre-
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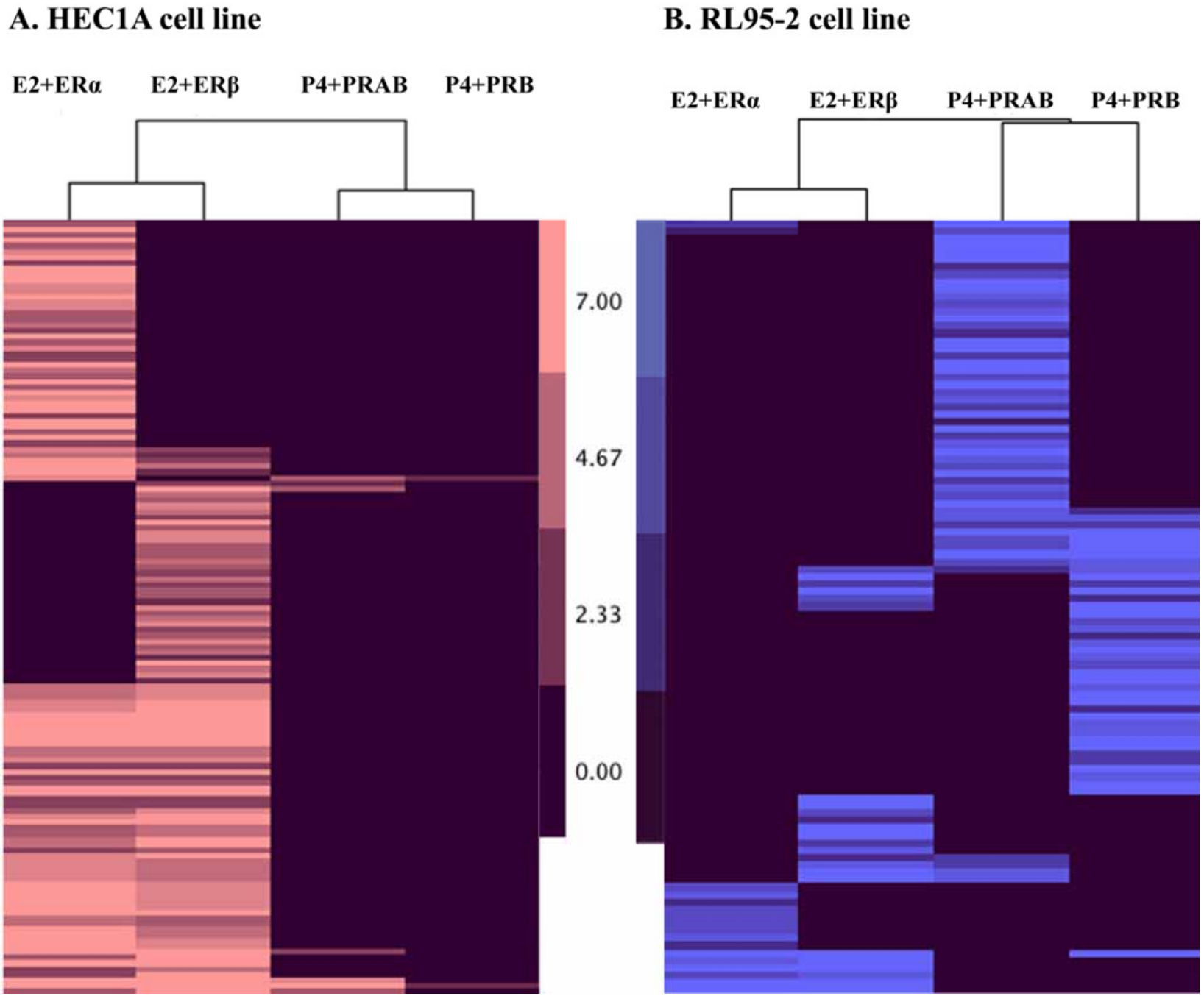
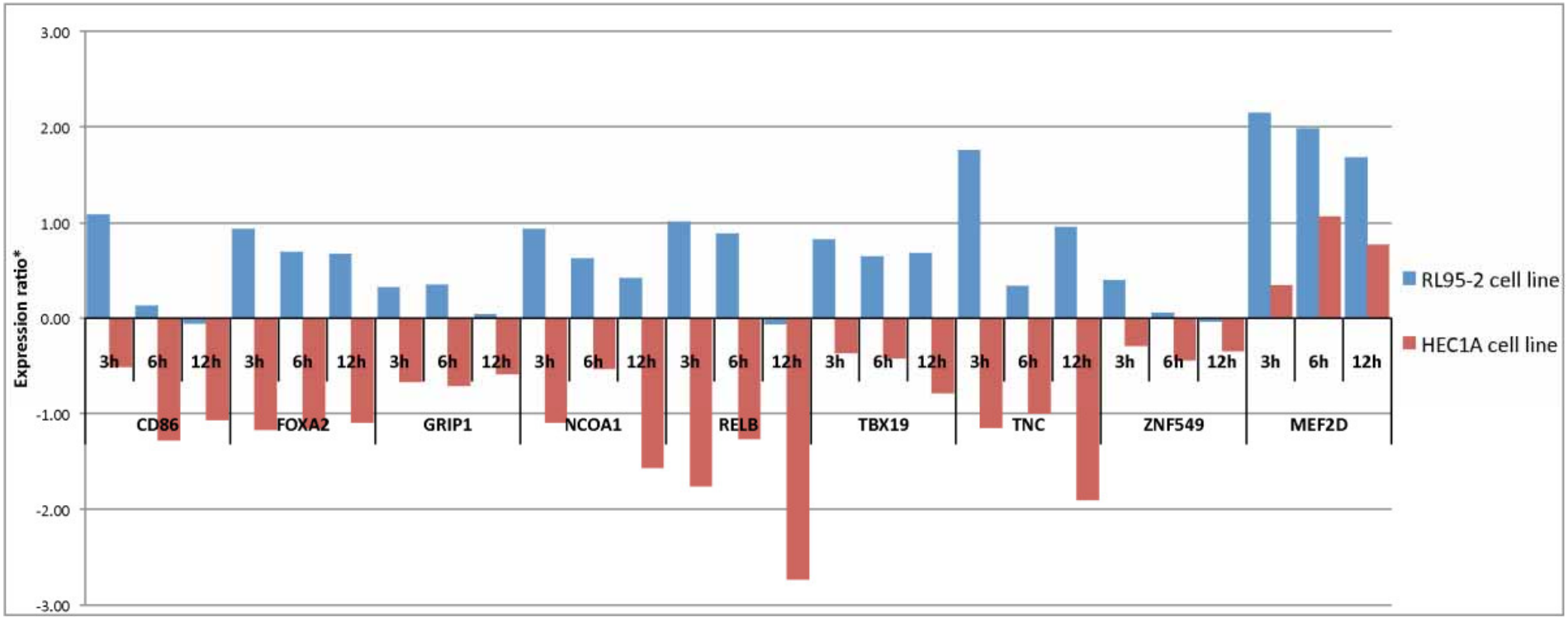
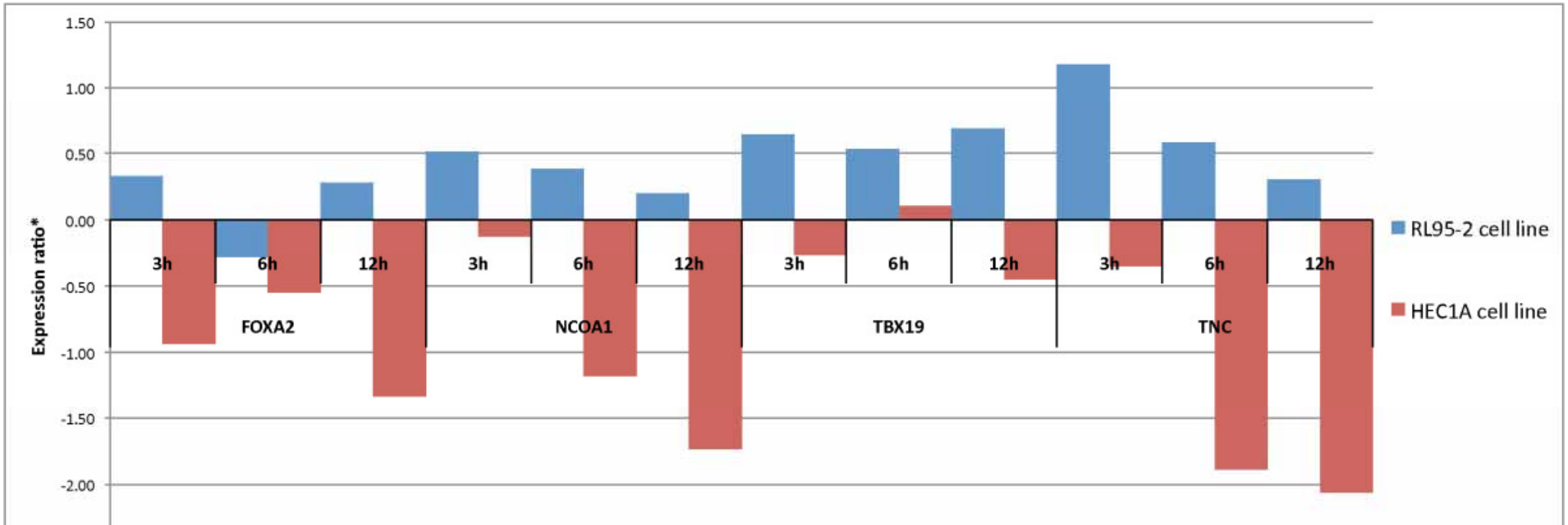
nes





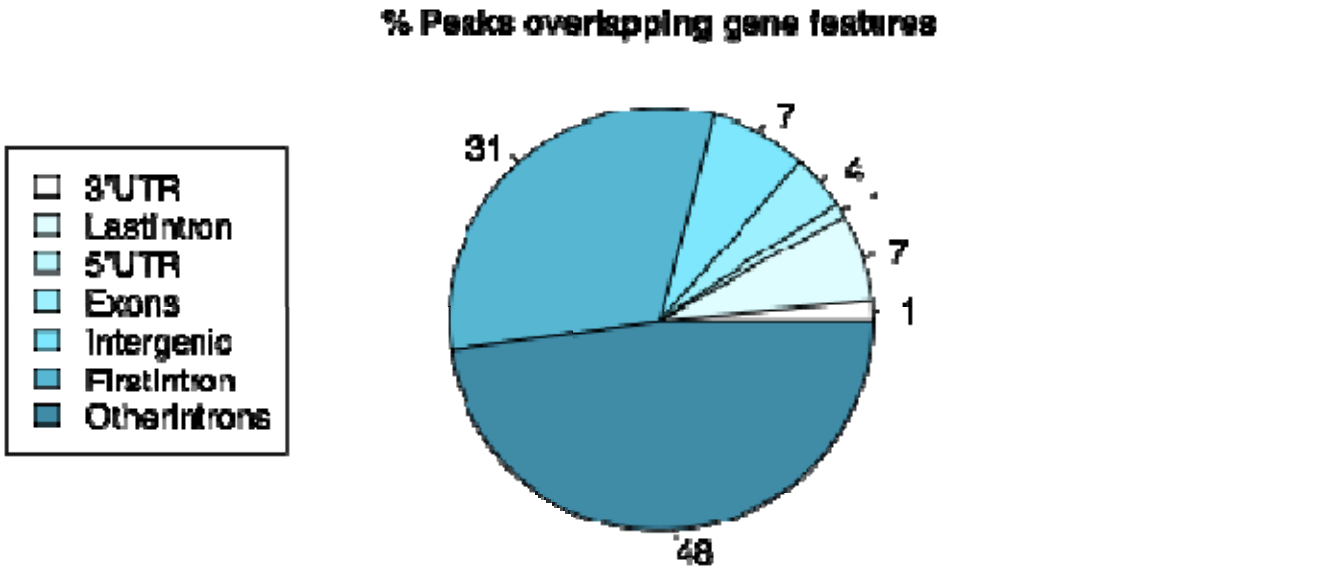
mediated time-dependant gene expression compared to non-treated samples.

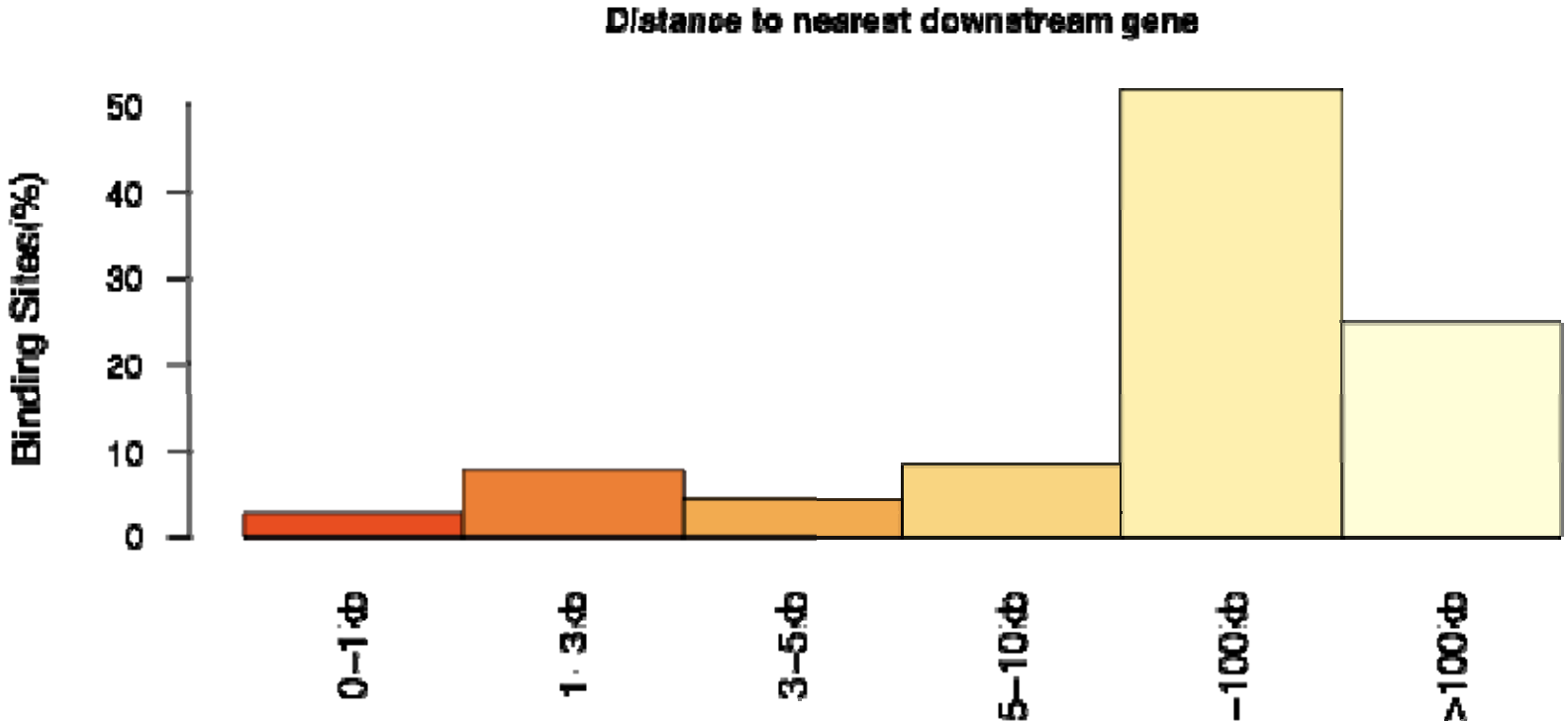


Identifying ER and PR targets and action on entire human genome

- ChIP-Seq: identifying ER and PR targets in whole genome using *Illumina Genome Analyzer IIe*
  - *in vitro:* Ishikawa cell line
  - *in vivo:* Endometrial biopsy samples
- RNA-Seq: describing the entire transcriptome of human endometrium.
  - *in vitro*
  - *in vivo*

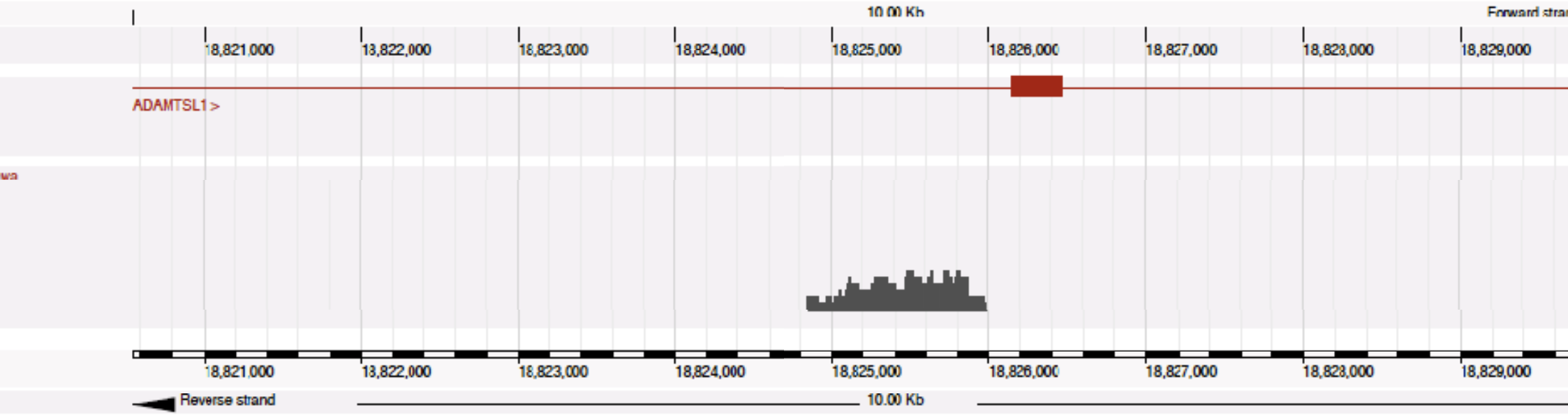
CESSED 8486736
PED 6248832 (73.63%)





ER target in *ADAMTSL1* after E2 treatment

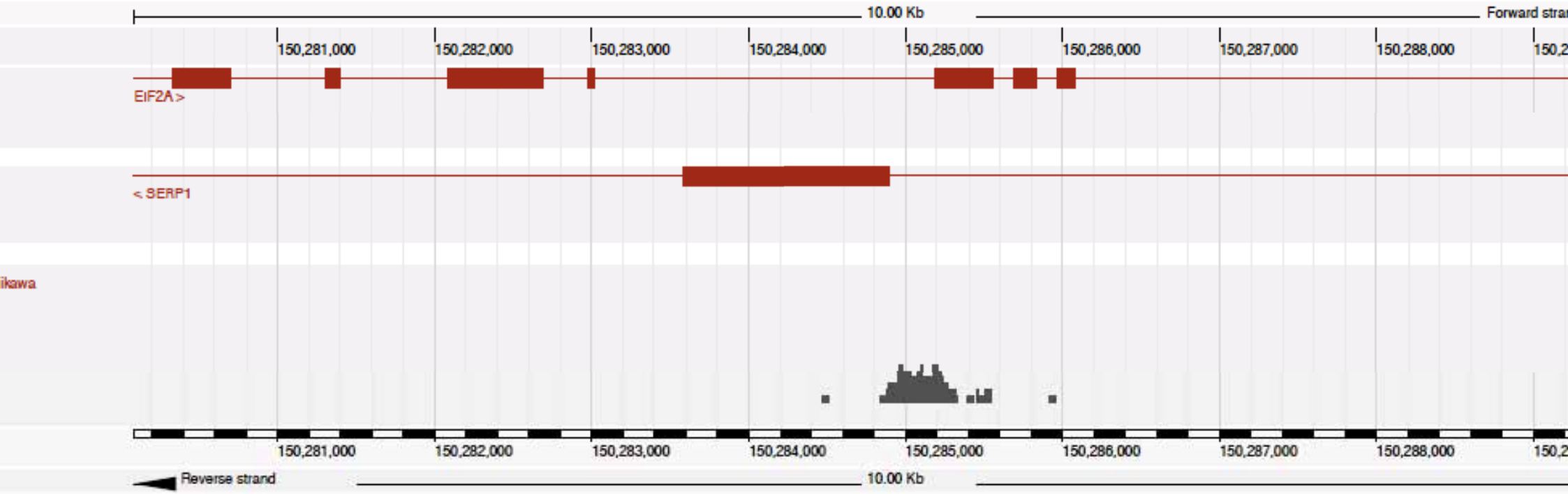
ncodes a secreted protein and member of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motif) fam
have important functions in the extracellular matrix. Alternative splicing results in multiple transcript variants encoding distinct p



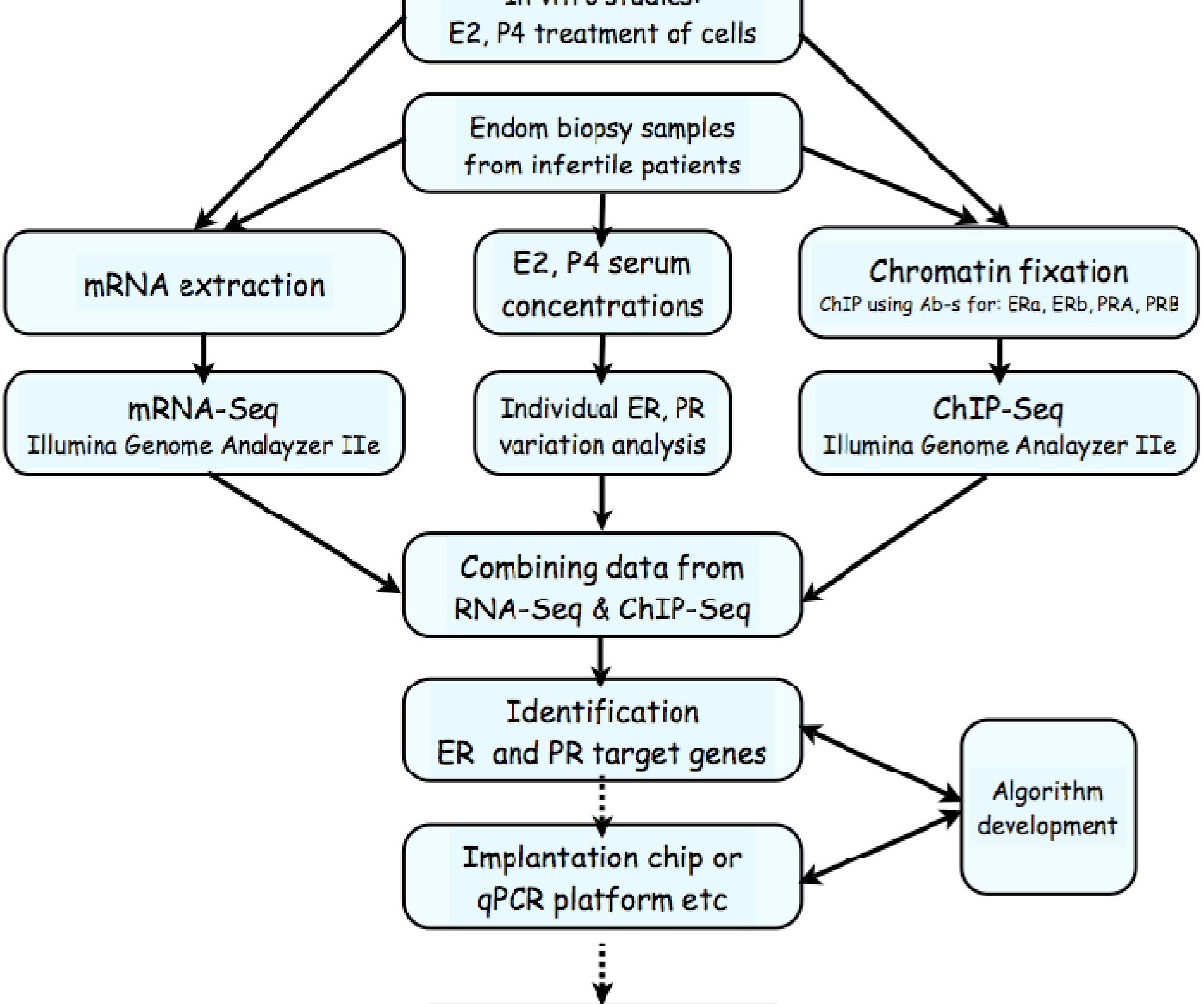
PeakAnaly

ER target in *EIF2A* without E2 treatment

aryotic Translation Initiation Factor 2



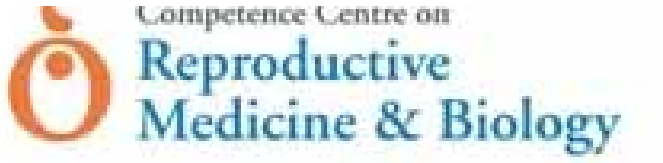
PeakAnaly


E2, P4 treatment of cells
Endom biopsy samples from infertile patients
mRNA extraction
E2, P4 serum concentrations
Chromatin fixation
ChIP using Ab-s for: ERa, ERb, PRA, PRB
mRNA-Seq
Illumina Genome Analayzer IIe
Individual ER, PR variation analysis
ChIP-Seq
Illumina Genome Analayzer IIe
Combining data from RNA-Seq & ChIP-Seq
Identification ER and PR target genes
Algorithm development
Implantation chip or qPCR platform etc

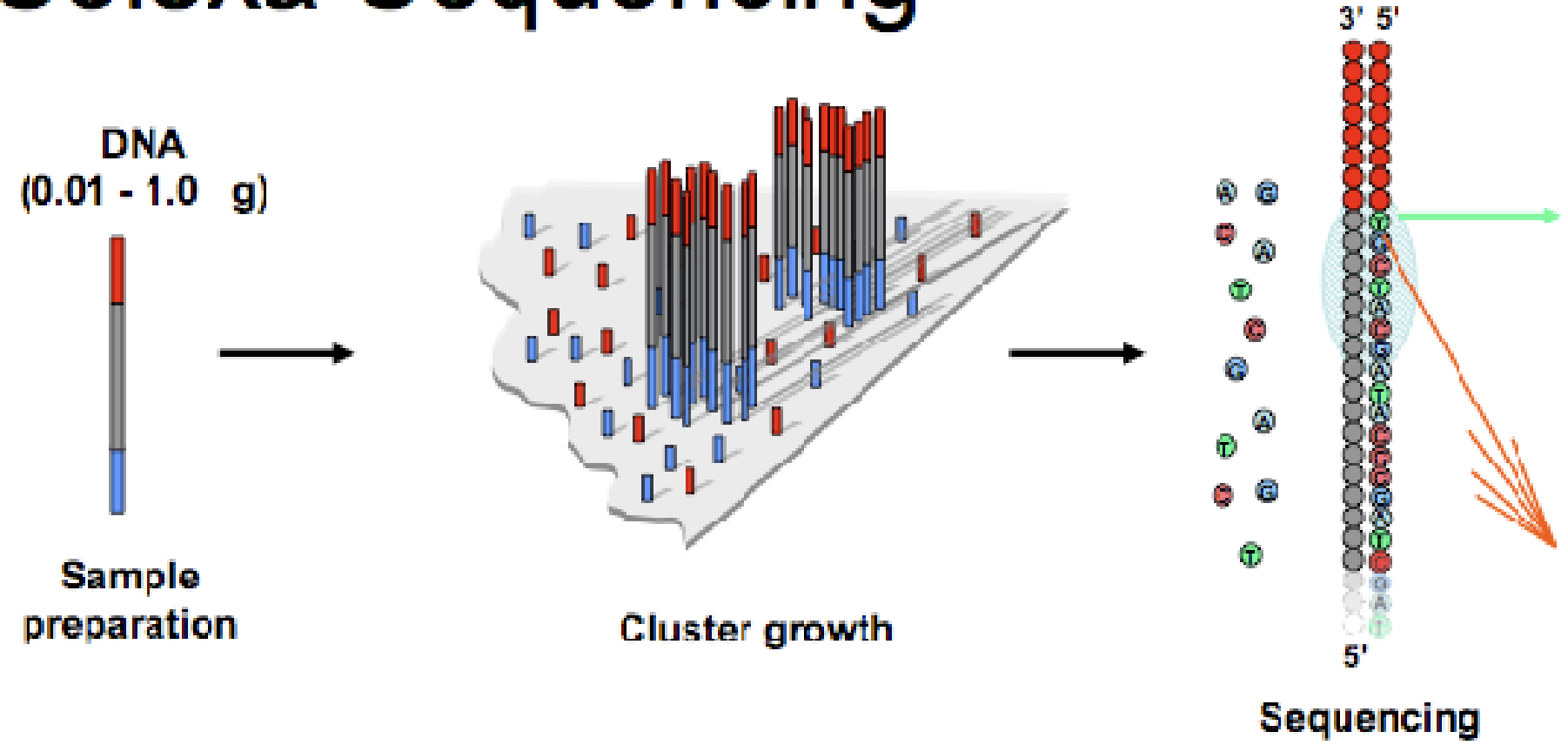
Many thanks

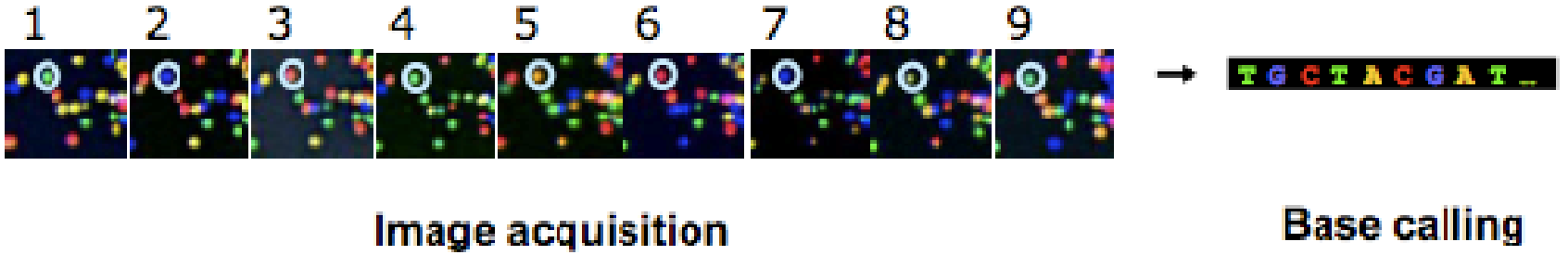
- Miia Rõõm
- Jaak Simm
- Kairi Tammoja

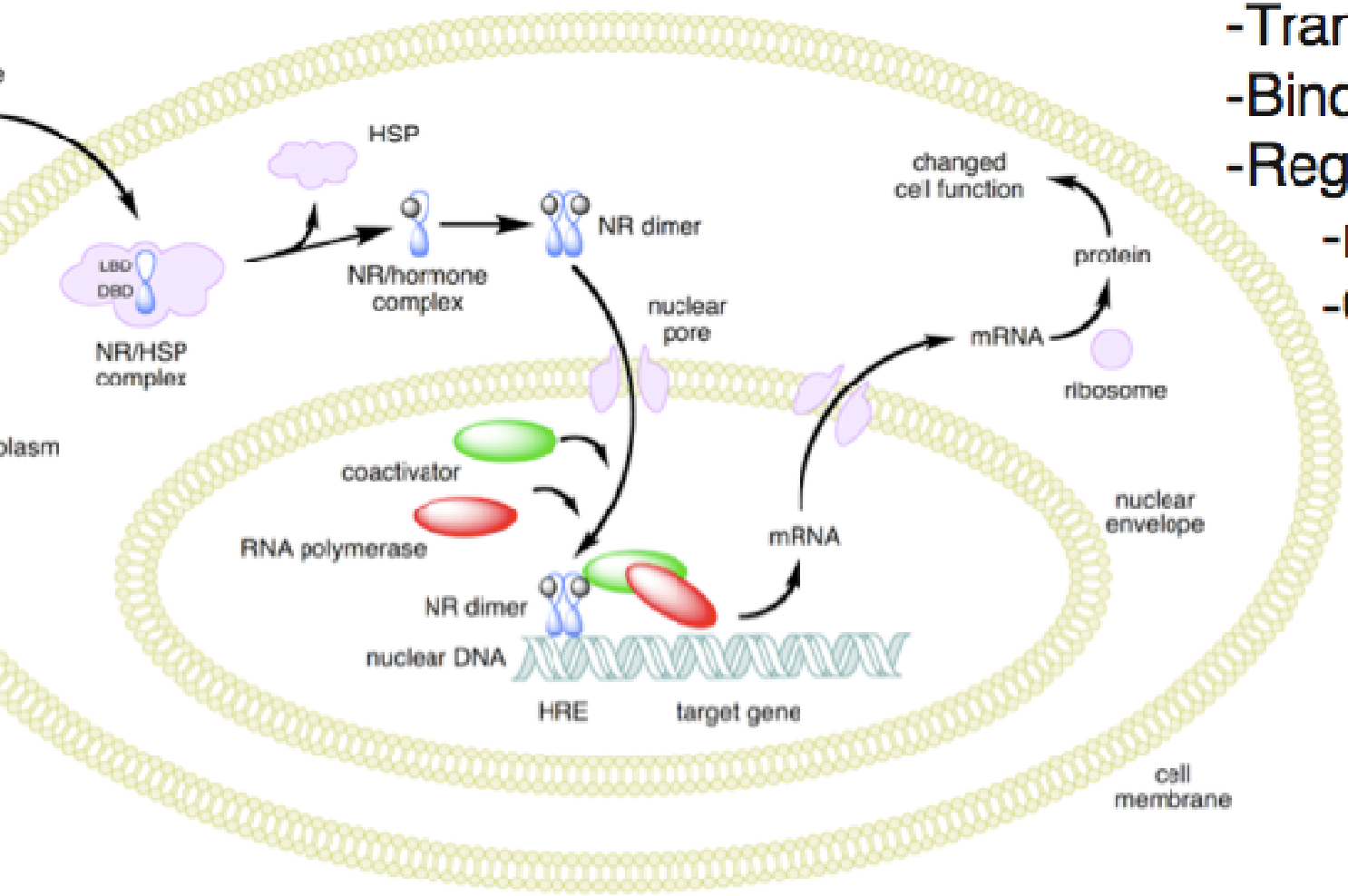
- Madis Metsis
- Andres Salumets
- Sten Linnarson



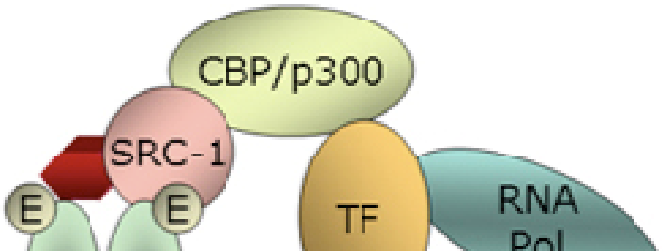
Solexa Sequencing



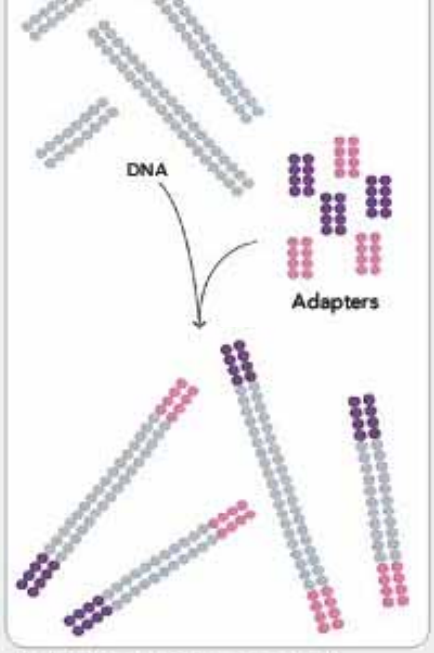




-Hormone binding to the NR
-Translocation to the nucleus
-Binding to the HRE
-Regulation transcription of targ
-mRNA translated into prote
-Change in cell function

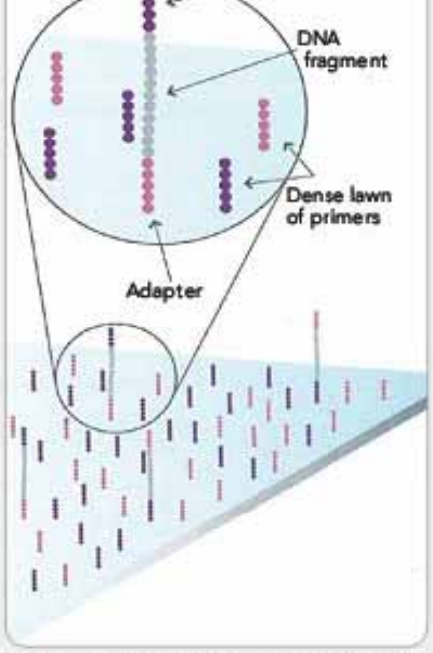


uclear receptor DNA complex recruits



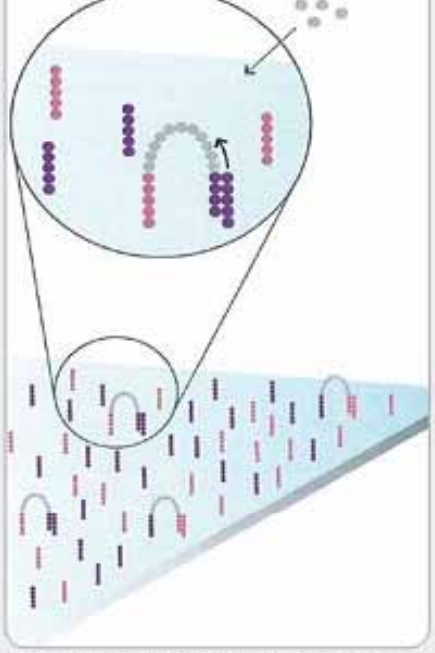
Randomly fragment genomic DNA and ligate adapters to both ends of the fragments.

4. FRAGMENTS BECOME DOUBLE STRANDED



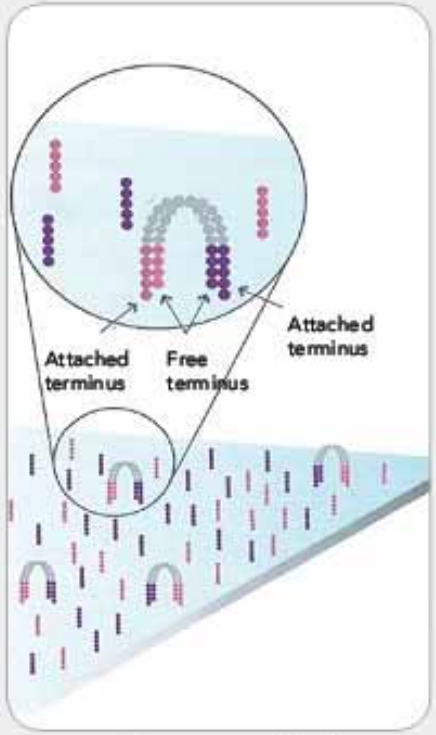
Bind single-stranded fragments randomly to the inside surface of the flow cell channels.

5. DENATURE THE DOUBLE-STRANDED MOLECULES

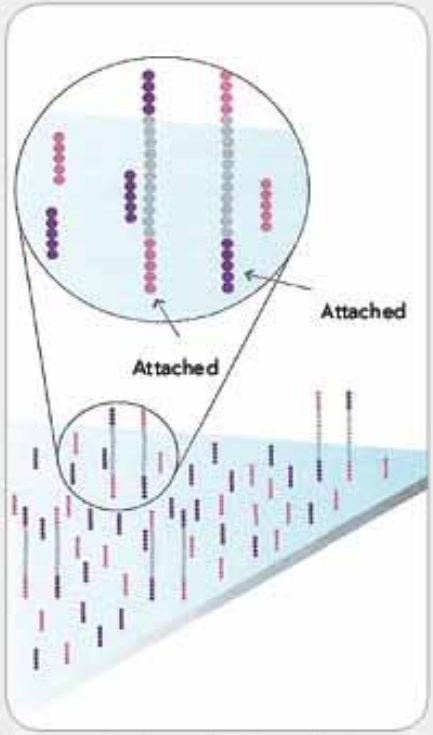


Add unlabeled nucleotides and enzyme to initiate solid-phase bridge amplification.

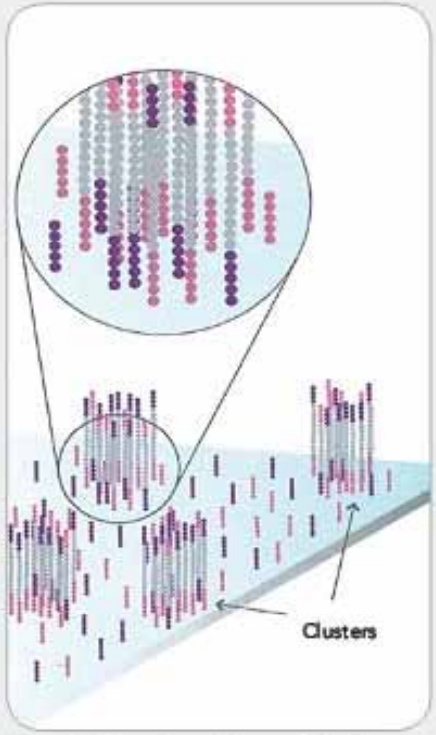
6. COMPLETE AMPLIFICATION



The enzyme incorporates nucleotides to

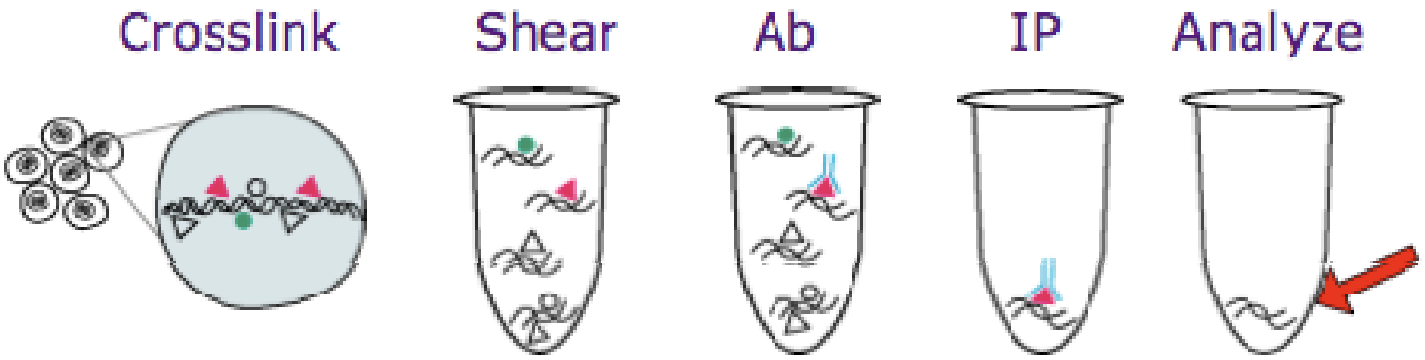


Denaturation leaves single-stranded



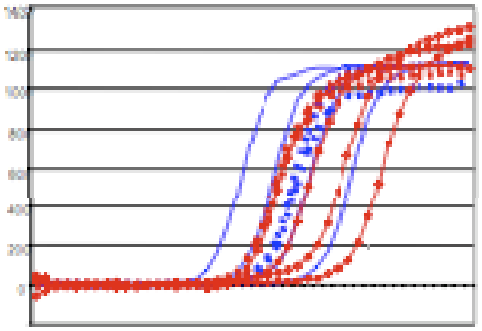
Several million dense clusters of double-

Transcription factor binding site assays (ChIP)

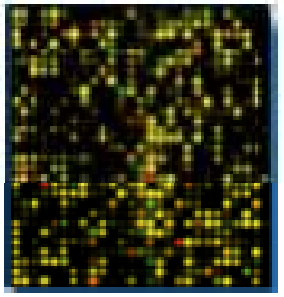


Enriches for DNA fragments bound by the transcription factor

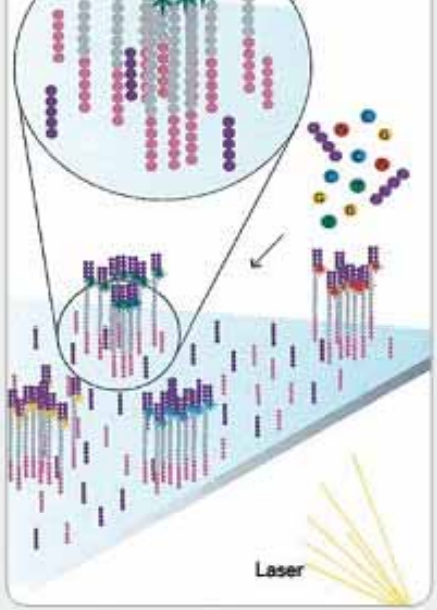
ChIP-QPCR



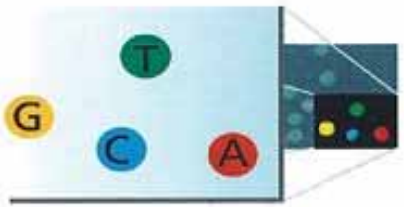
ChIP-chip



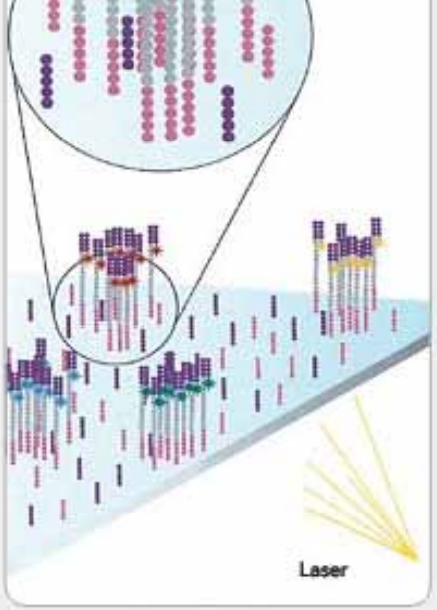
Resolution is about ~500bp



First chemistry cycle: to initiate the first sequencing cycle, add all four labeled reversible terminators, primers and DNA polymerase enzyme to the flow cell.



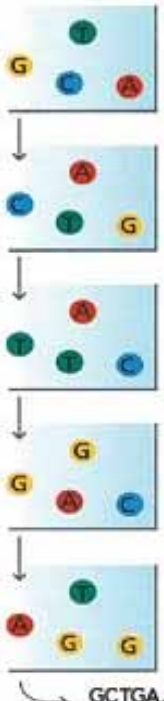
After laser excitation, capture the image of emitted fluorescence from each cluster on the flow cell. Record the identity of the first base for each cluster.



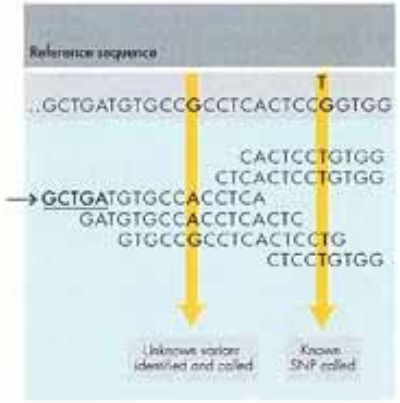
Second chemistry cycle: to initiate the next sequencing cycle, add all four labeled reversible terminators and enzyme to the flow cell.

10. IMAGE SECOND CHEMISTRY CYCLE

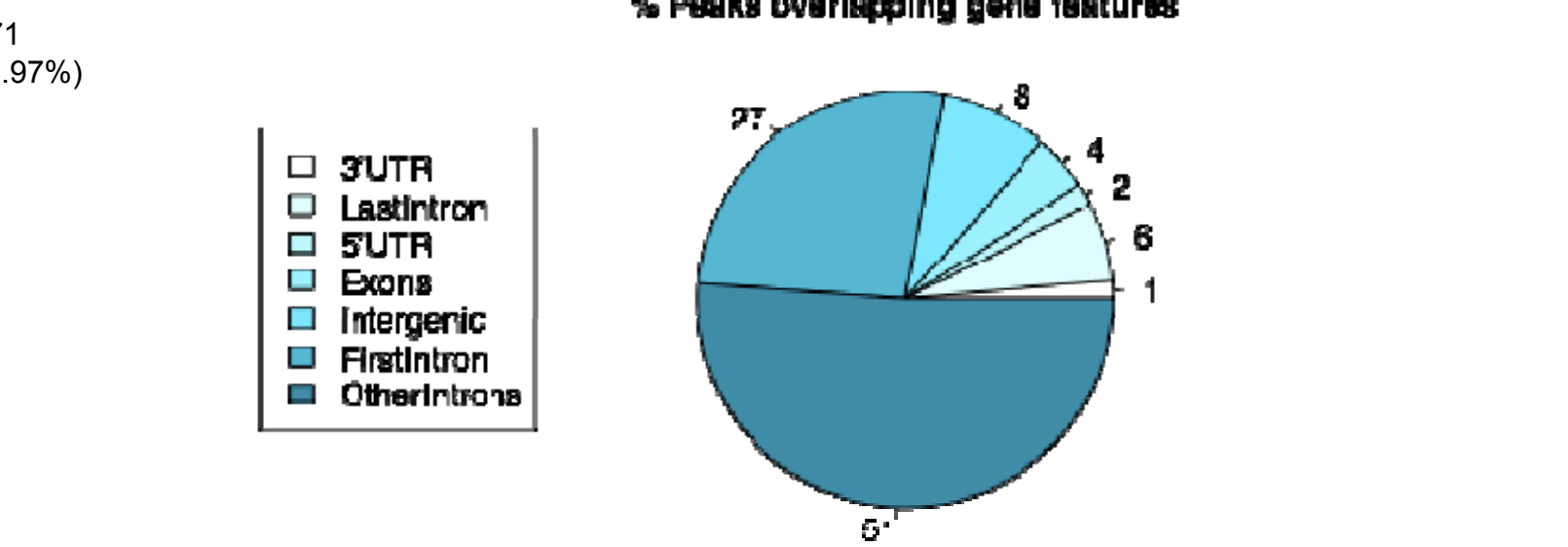
11. SEQUENCE READS OVER MULTIPLE CHEMISTRY CYCLES

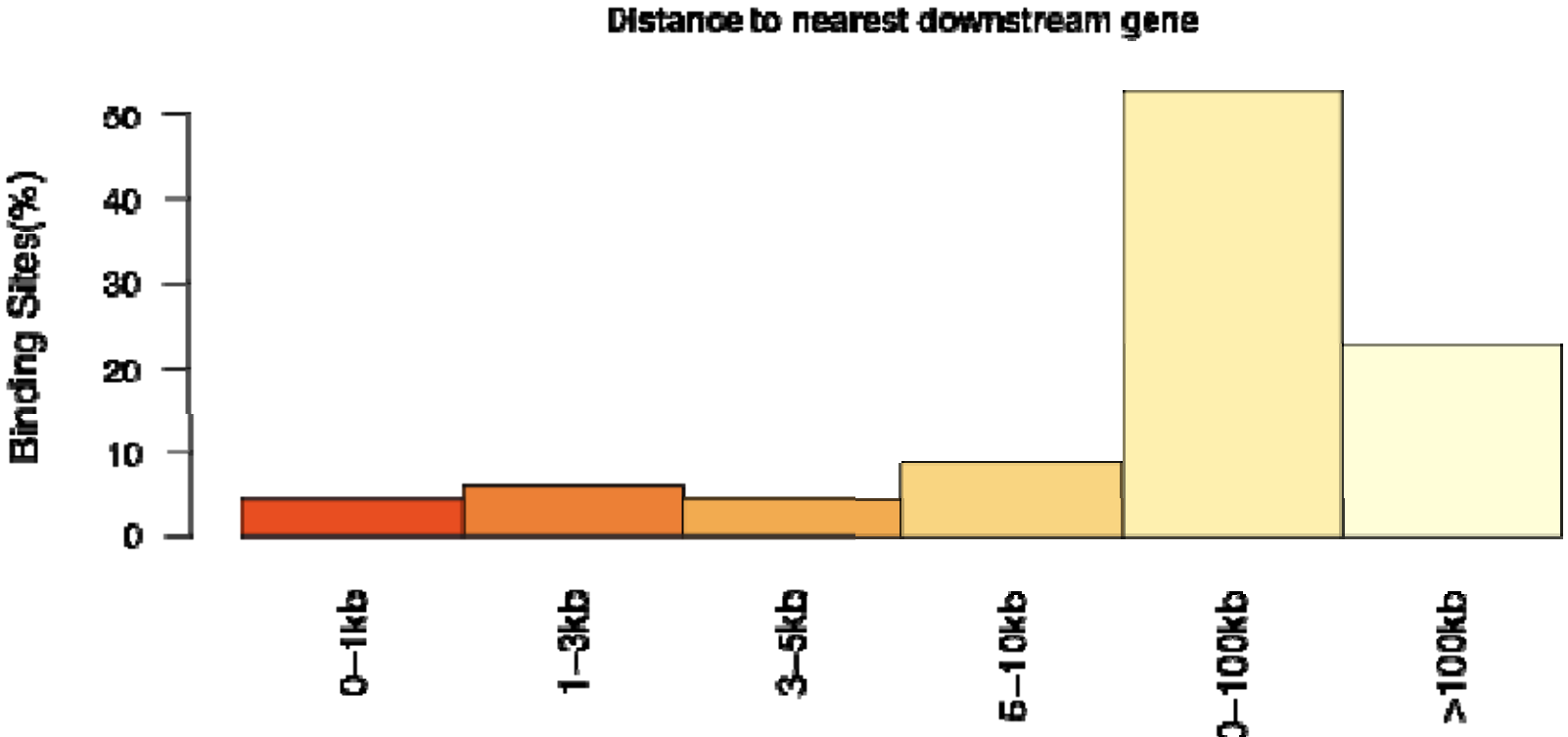


12. ALIGN DATA

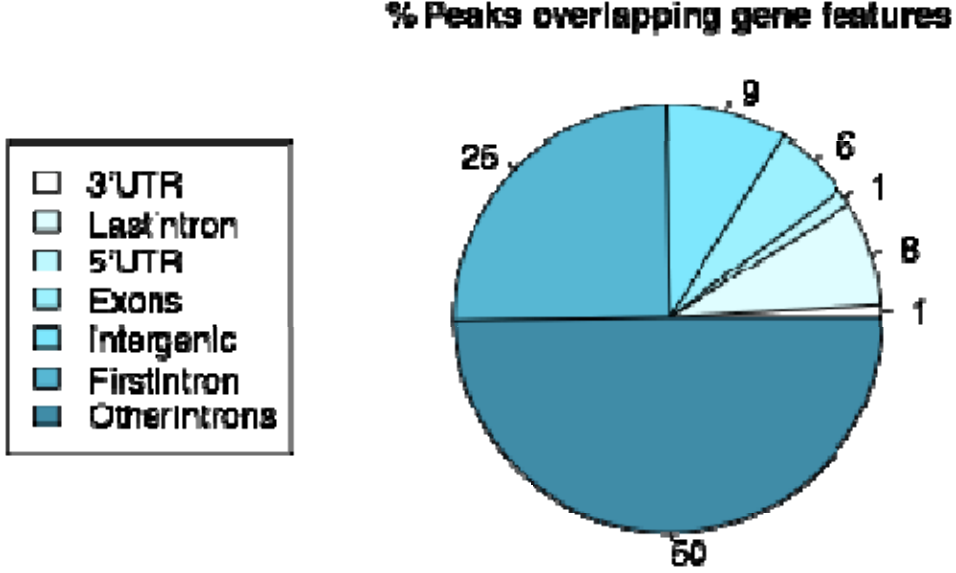


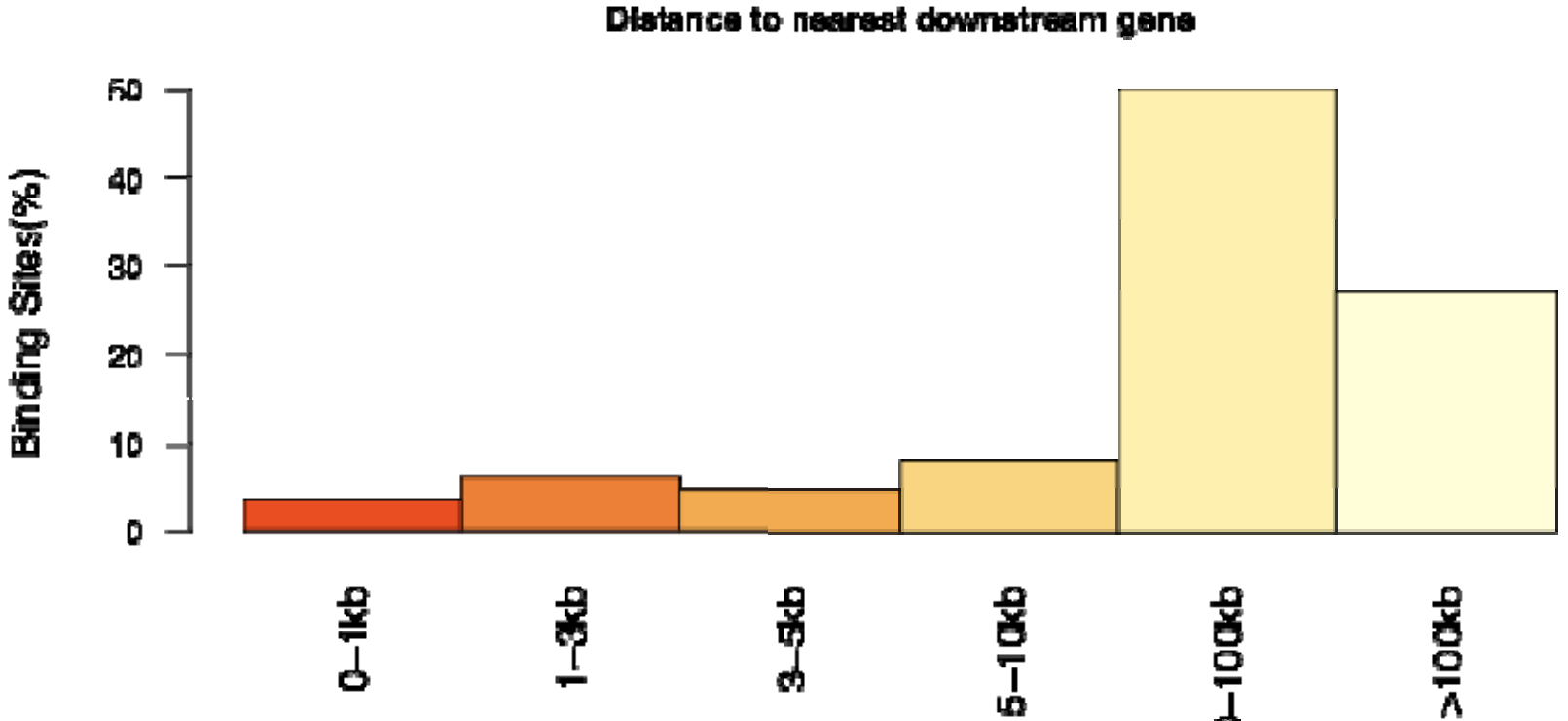
CESSED 4628571
PED 1942431 (41.97%)





CESSED 5283384
PED 3588174 (67.91%)





CESSED 9637206
PED 5475248 (56.81%)