# Structural and functional characterization of five bovine cell lines

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# Cell Lines in Animal Research

- Cell lines from farmed animal species are used as *in vitro* surrogates for *in vivo* experiments, but they lack functional annotation

- Cell lines with cell aneuploidy and heteroploidy often show functional consequences[1,2] → molecular characterization of cell lines is important to gain insight into possible alterations

## Objective

**Characterization of cell lines frequently used in bovine research at functional and structural level**

(1) de Vos et al. (2023) iScience 26(3):106252
(2) Williams et al. (2008) Science 322(5902): 703–709.

# The Five Bovine Cell Lines

(1) Rutter and Luther (1984) Vet Rec 114(16):393-396
(2) Hambruch et al. (2010) Placenta 31(1):67-74
(3) Madin and Darby (1958) Proc Soc Exp Biol Med 98(3):574-576
(4) Huynh et al. (1991) Exp Cell Res 197(2):191–199
(5) Sharma et al., unpublished

- **EBL**(1)

embryonic bovine lung cells: established from a *lung* of a 7-month old bovine fetus, spontaneously immortalized

- **F3**(2)

*Trophoblast* cells isolated from a bovine cotyledon of a male fetus (approx. 5 month of gestation), spontaneously immortalized

- **MDBK**(3)

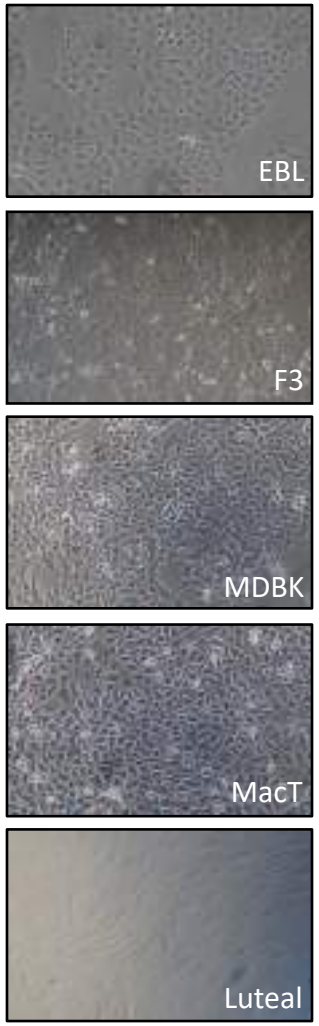
Madin-Darby Bovine Kidney cells: derived from a *kidney* of an adult steer, spontaneously immortalized

- **MacT**(4)

generated from *mammary alveolar cells* using SV40 large T antigen

- **Luteal cells**(5)

generated from ovary tissue from lactating Holstein cow



# Omics Data

- **Whole-genome data**
  - Genomic DNA of cell lines was sequenced on a NovaSeq6000
  - Data was analyzed using the nf-core Sarek workflow(1)
  - SNPs and small indels were called with HaplotypeCaller (GATK)(2)

- **Whole-transcriptome data**
  - polyA+ libraries from EBL, MacT, MDBK and Luteal cells were sequenced on a HiSeq2500 (Illumina), the F3 RNA-Seq library was sequenced on a NextSeq2000 (Illumina)
  - Data was analyzed using the nf-core rnaseq-3.3 and 3.4 pipelines

- **Epigenomic data**
  - Cell pellets distributed to partners
  - ATACseq libraries sequenced on NovaSeq, peak calling with BovReg nf-core ATACseq pipeline (ULIEGE)

(1) Garcia et al. (2020) F1000Res 9:63
(2) McKenna et al. (2010) Genome Res 20(9):1297–1303

# Genomic Features – SNPs and small Indels

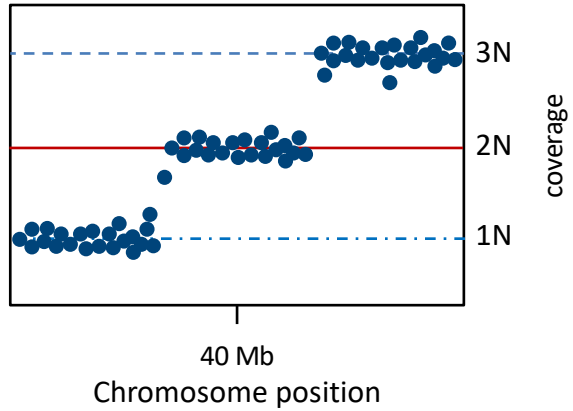
| | EBL | F3 | MacT | MDBK | Luteal |
|---|---|---|---|---|---|
| SNPs | 7,361,455 | 8,870,406 | 7,327,940 | 7,804,410 | 8,295,578 |
| Indels | 1,399,610 | 1,402,483 | 1,513,700 | 1,507,973 | 1,529,942 |
| **Total** | 8,761,065 | 10,272,889 | 8,841,640 | 9,312,386 | 9,825,520 |

- **Ø 7.8 million single nucleotide polymorphisms (SNPs) and 1.45 million small indels**
- **No clear difference between spontaneously immortalized and transfected cell lines**

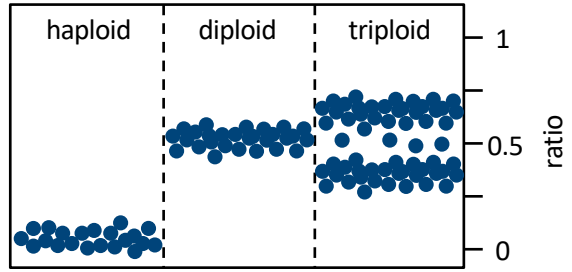
# Genomic Features – large structural aberrations

## Criteria

- **Read depth:** coverage of each chromosome should be similar to the average coverage




- **Allele support for heterozygous SNPs:** ratio of reads that support the alternative allele for heterozygous SNPs should be around 0.5 for diploid chromosomes

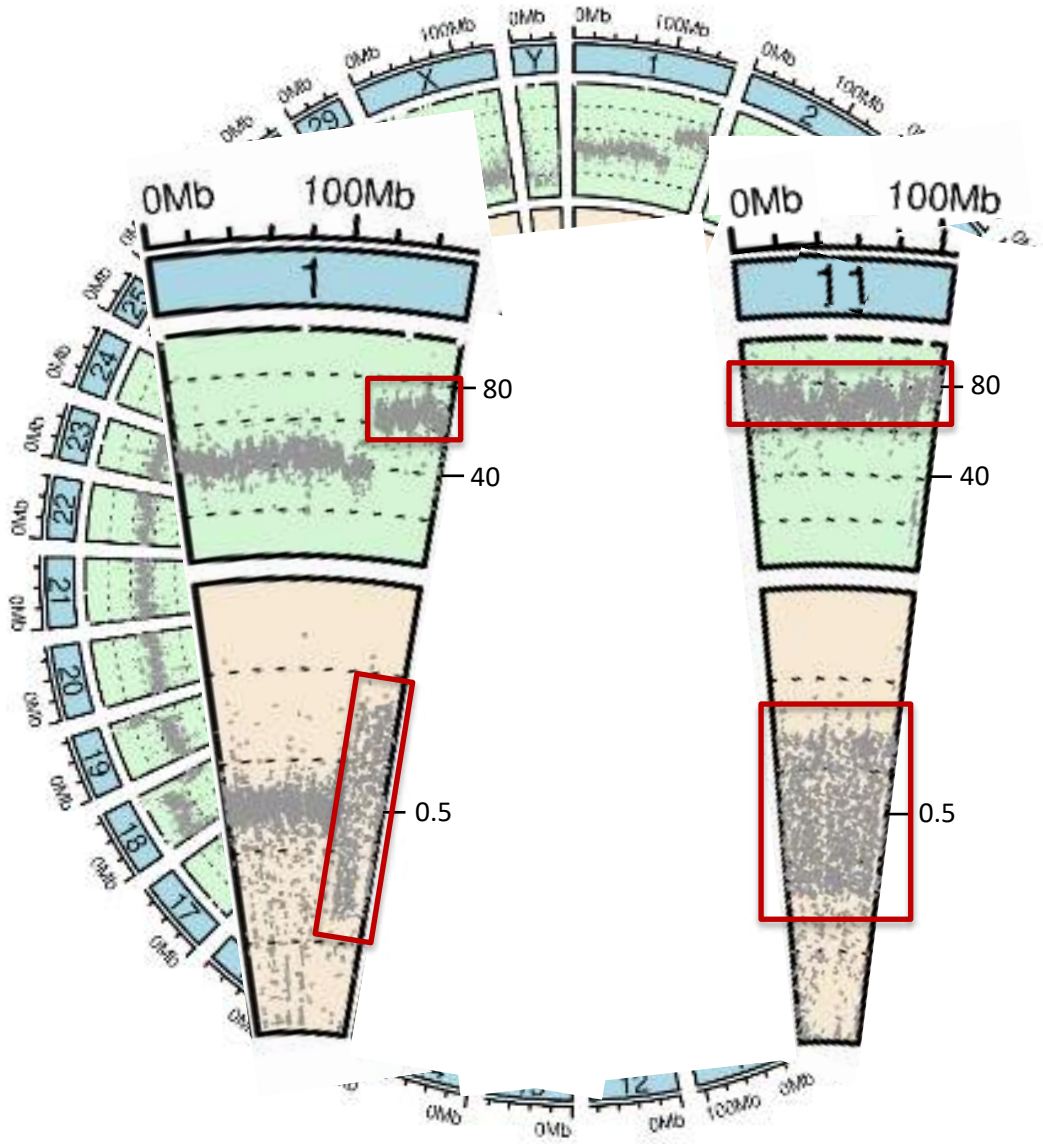


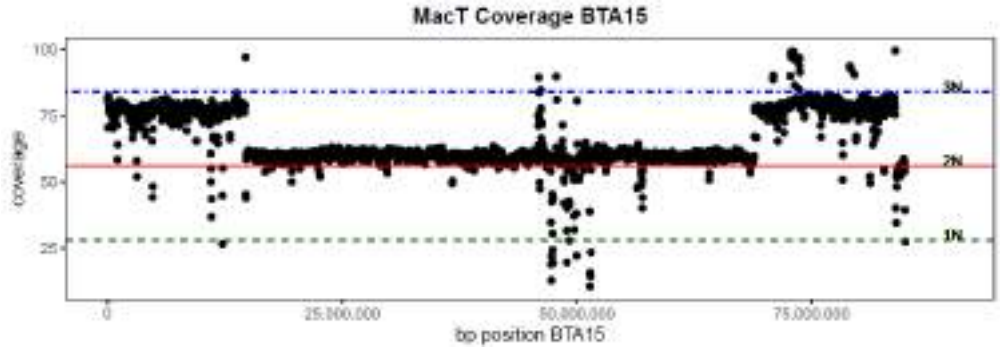
Read depth

Allele support ratio

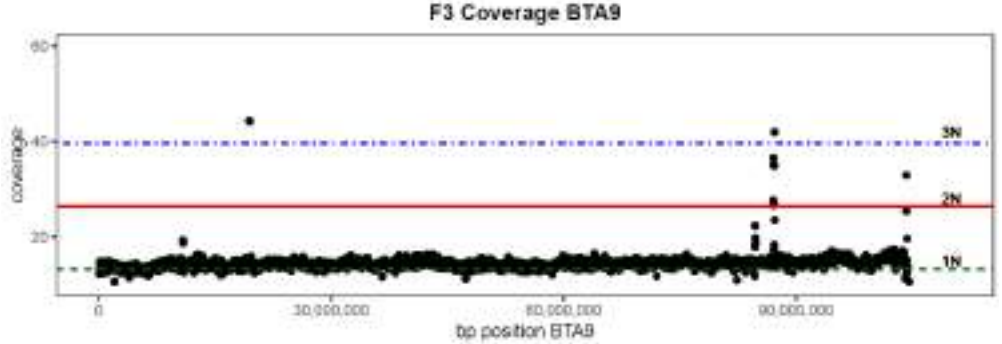
(in 50kb bins)

EBL Ø coverage = 49.8X

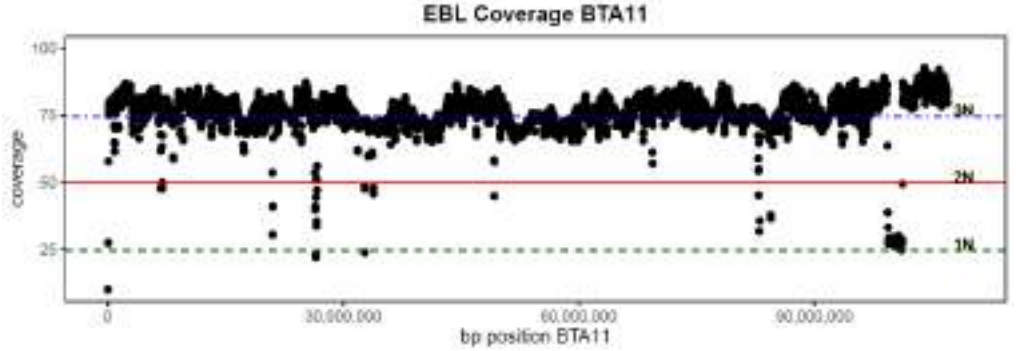





MacT BTA15 partly triploid

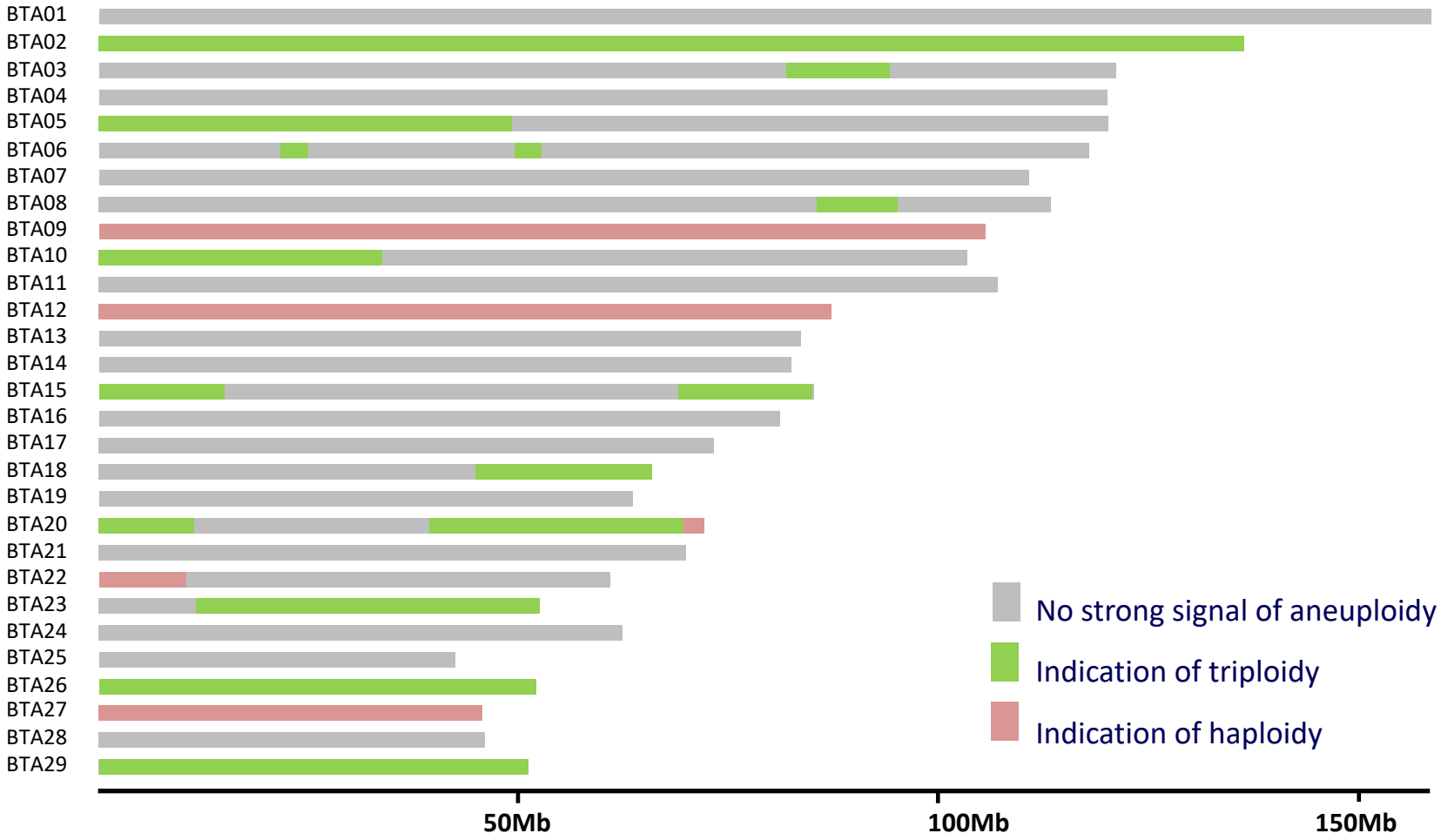



F3 BTA09 haploid




EBL BTA11 triploid

# MacT - large structural aberrations



# Genomic Features – large structural aberrations

| Cell Line | Aneuploidy (whole chromosome) | Structural variation (part of the chromosome) |
|---|---|---|
| EBL | 1 | 11 |
| F3 | 6 | 2 |
| MacT | 6 | 11 |
| MDBK | 2 | 7 |
| Luteal | 0 | 7 |

- **Aneuploidy and structural variations are common in all characterized cell lines**

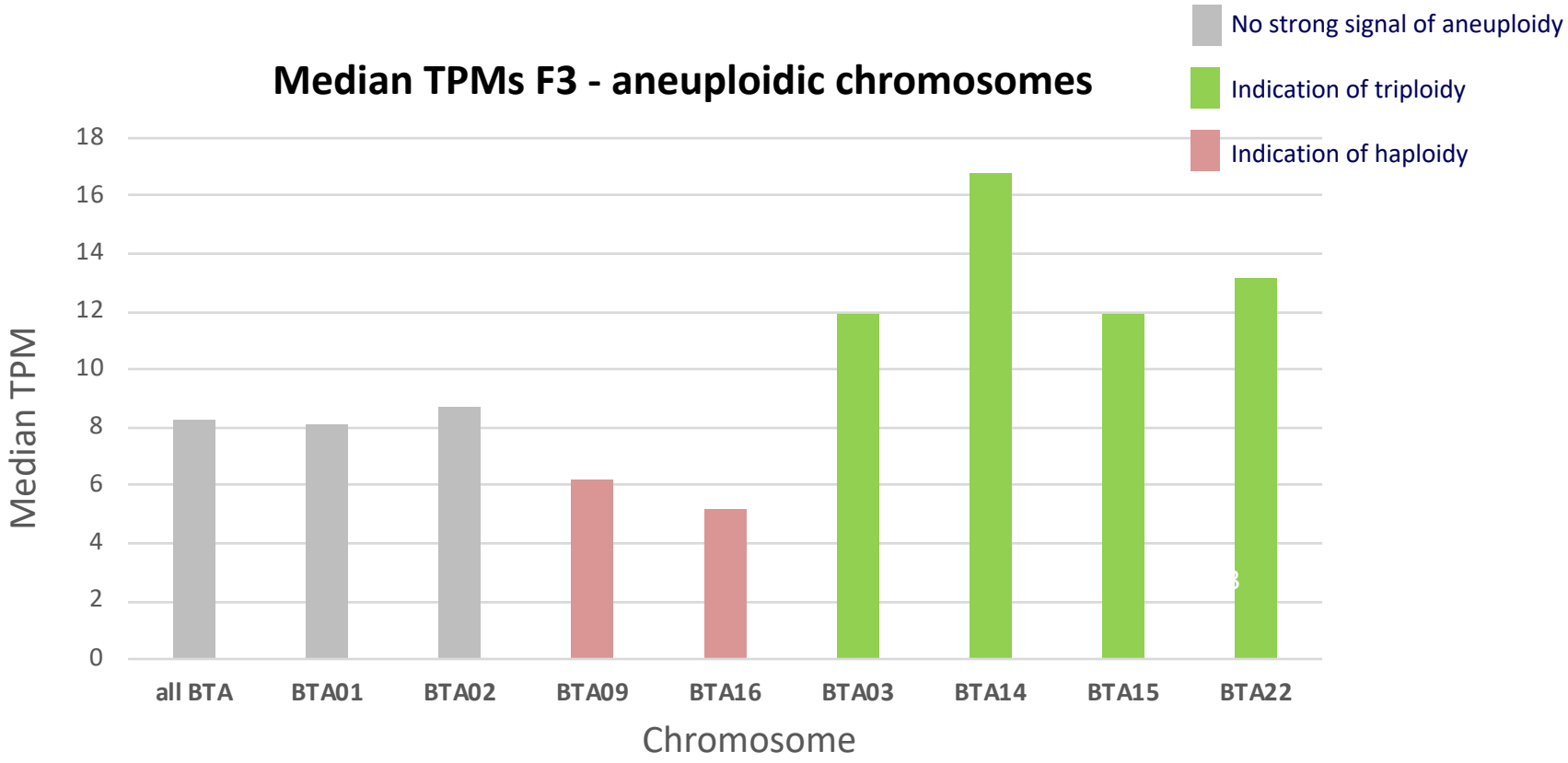
# Expression Activity

| | TPM > 1[a] | TPM > 0.1[b] | Median TPM[c] |
|---|---|---|---|
| **EBL** | 12,205 | 14,423 | 12.26 |
| **F3** | 11,348 | 13,280 | 8.28 |
| **MacT** | 11,478 | 13,709 | 9.90 |
| **MDBK** | 12,273 | 14,568 | 11.88 |
| **Luteal** | 11,780 | 14,186 | 8.98 |

- **Between 13,280 (F3) to 14,568 (MDBK) genes were expressed per cell line**

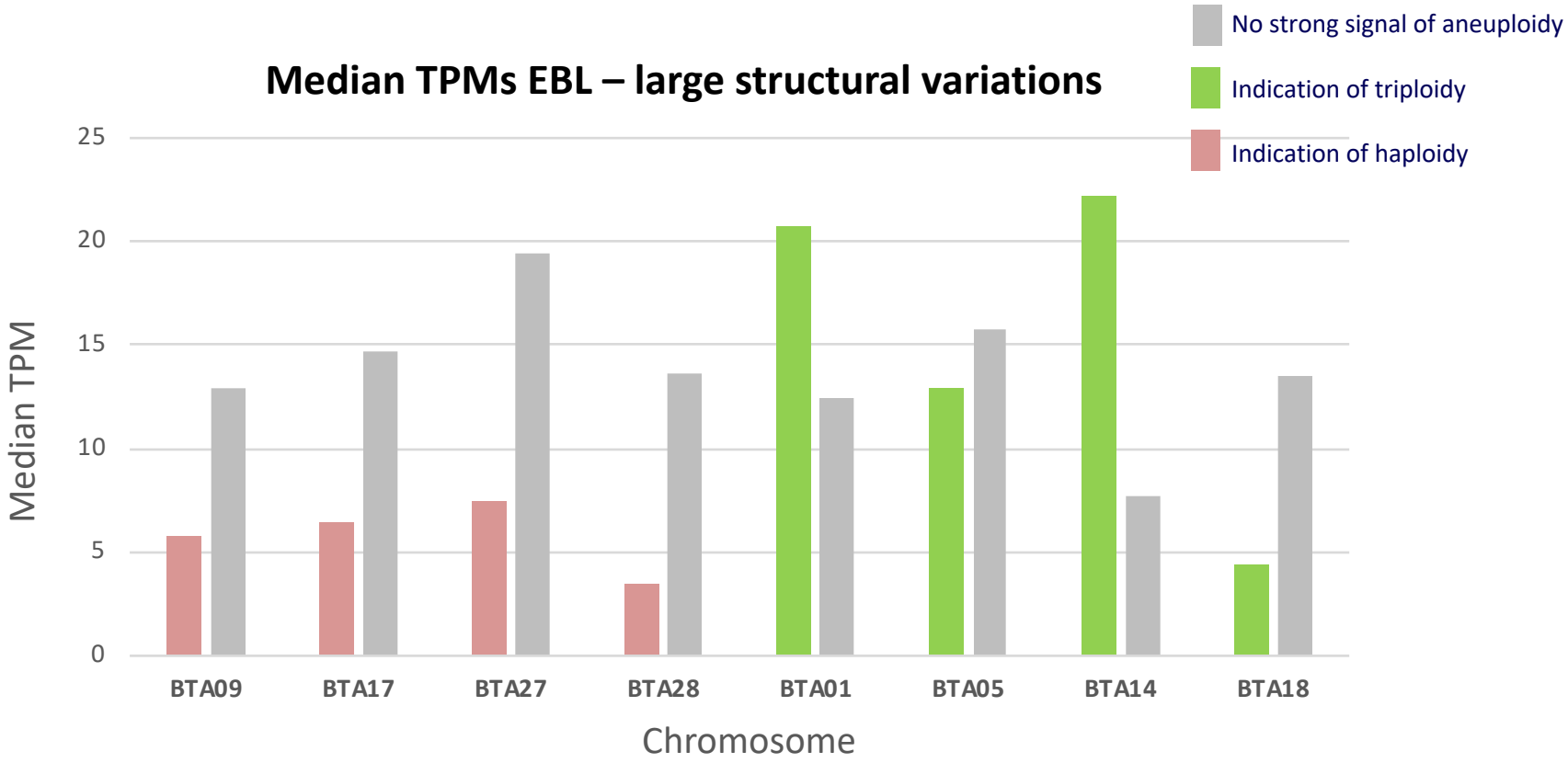
a: number of genes with TPM > 1
b: number of genes with TPM > 0.01
c: median TPM (only genes with TPM>0)

# Expression Activity – Aneuploidic chromosomes

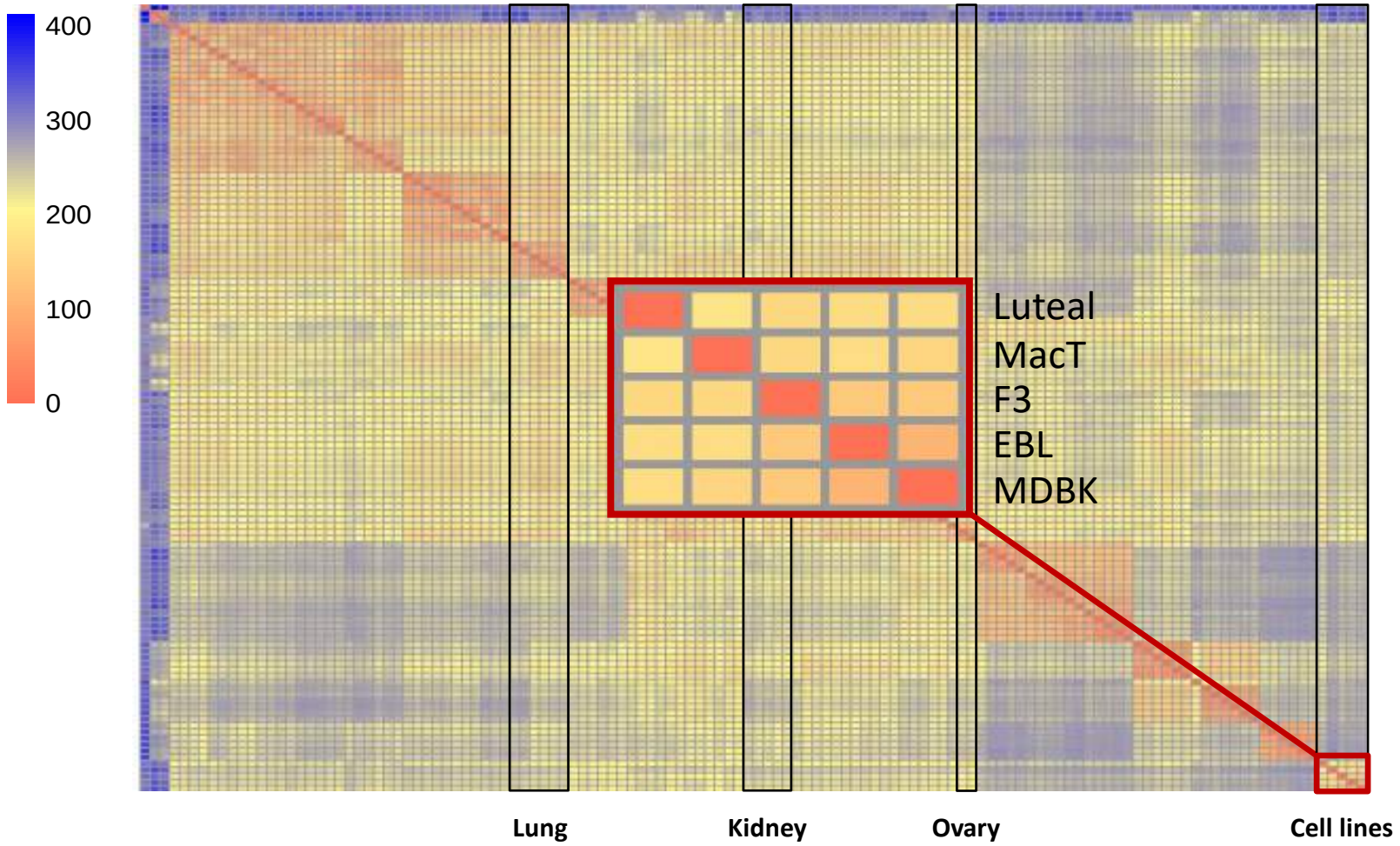



- **Aneuploidy is reflected in the transcriptome**

# Expression Activity – large structural variations



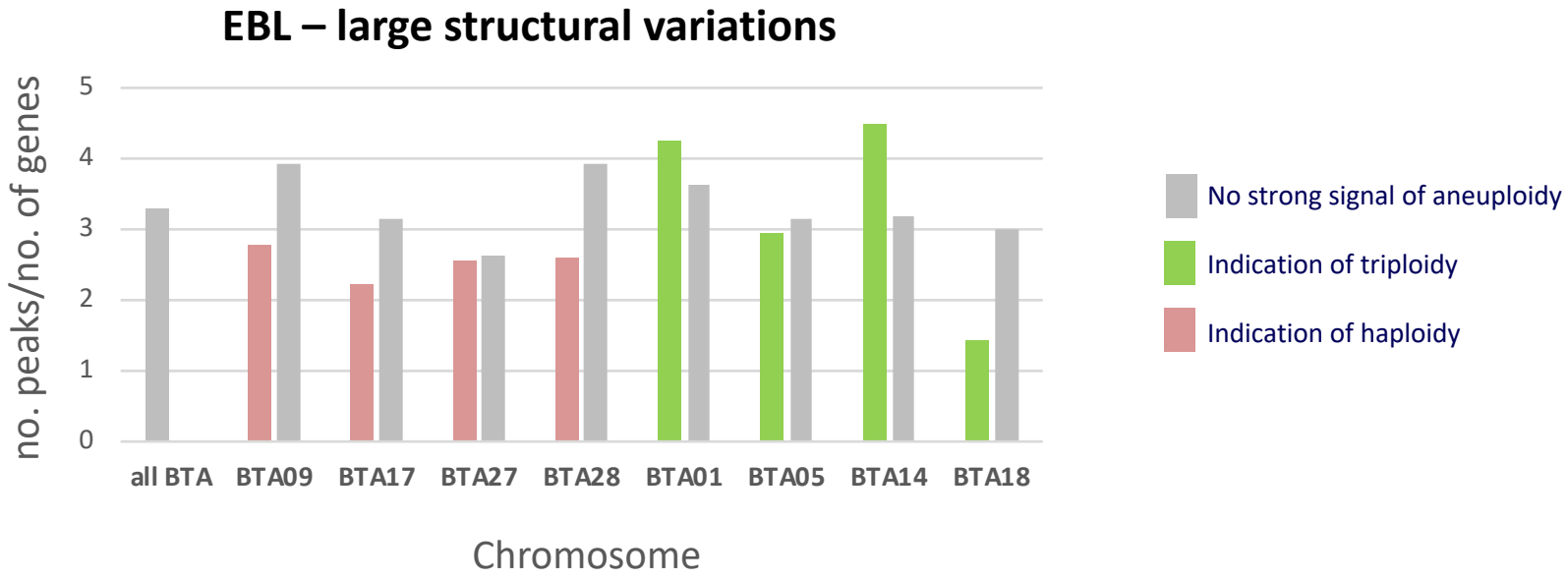

- **Structural variations are reflected in the transcriptome, but duplications do not necessarily lead to a higher expression, whereas deletions seem to decrease the expression activity**




- **Cell lines cluster together and only show low correlation with tissue of origin**

# Epigenomic data - ATACseq

| | EBL | F3 | MacT | MDBK | Luteal |
|---|---|---|---|---|---|
| Total Number of ATACseq peaks | 86,123 | 112,554 | 86,207 | 96,037 | 127,284 |




- **Chromosomal structural variation is partly reflected in chromatin accessibility**

# Summary

- **Aneuploidy and structural variations are common in all characterized cell lines**
  - Identification of various chromosomes that were either (partly) haploid or triploid

- **Different omics data provide information about the genome structure, transcriptome and chromatin accessibility of five bovine cell lines**
  - Reference of the genome architecture of these cell lines for future functional studies
  - Further omics data will deliver even more details (WGMS, CHiPseq, CAGE, HiC)

- **Chromosomal structural variation is reflected in transcriptomic profile and partly in the epigenomic profile**

*„All models are wrong, but some are useful.“*
G. Box, Statistician

# PARTNERS

*Thank you for your attention*

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