Genome editing of preimplantation embryos for research and potential clinical use

Nada Kubikova
Junior Research Fellow
University of Oxford

NUFFIELD DEPARTMENT OF
WOMEN'S &
REPRODUCTIVE HEALTH
Medical Sciences Division

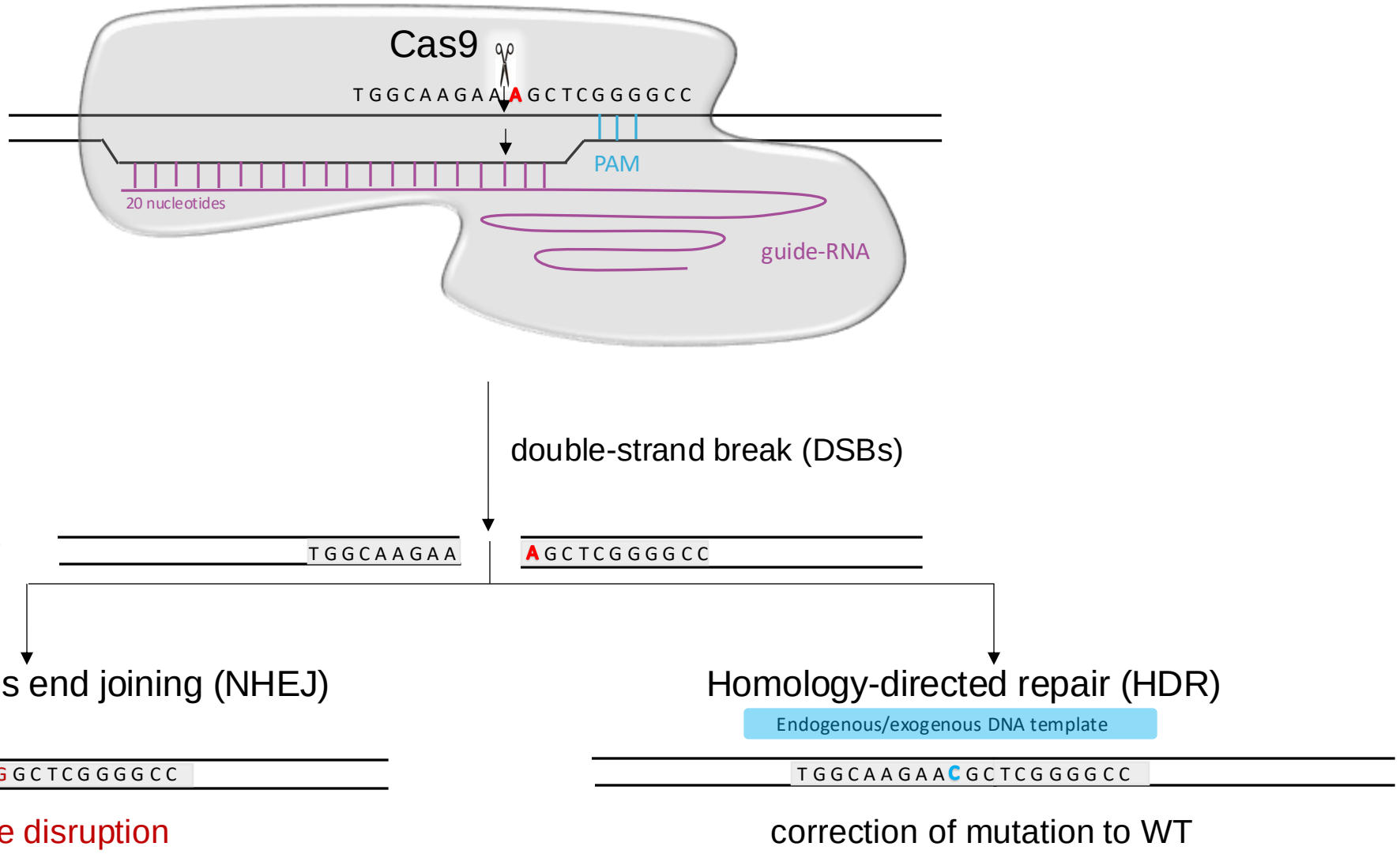
Genome editing

- Genome editing (GE) harnesses programmable nucleases to modify genetic information in a targeted manner

1. Possibility of correcting disease-causing mutations in the germline

2. Enhance the understanding of cellular mechanisms taking place during the first days post-fertilization.

Genome editing revolutionised by the CRISPR-Cas9 system



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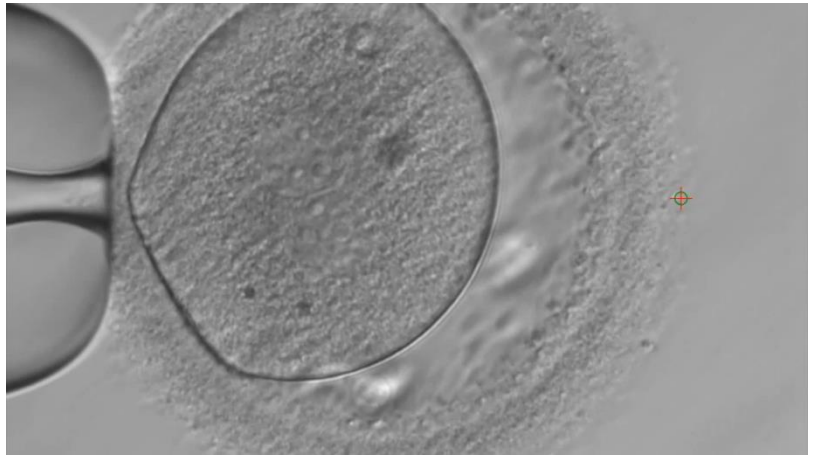
nature

Genome editing reveals a role for OCT4 in human embryogenesis

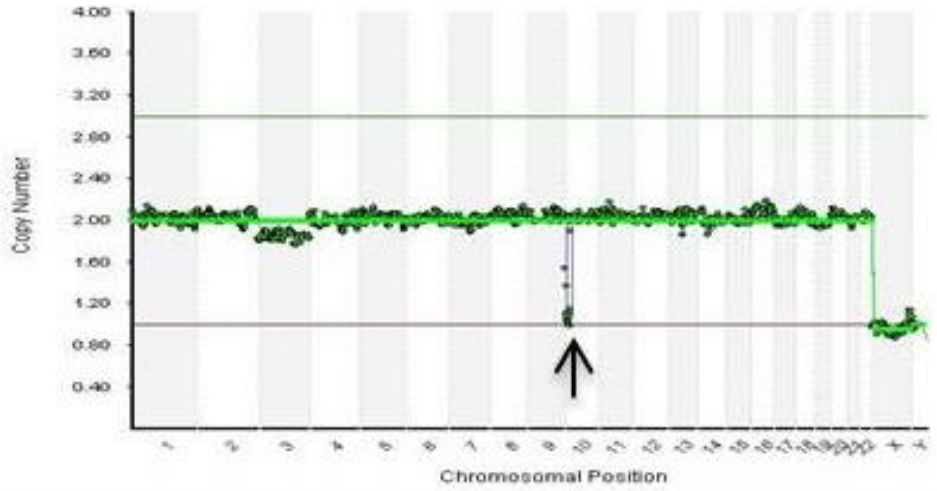
Norah M. E. Fogarty[1], Afshan McCarthy[1], Kirsten E. Snijders[2], Benjamin E. Powell[3], Nada Kubikova[4], Paul Blakeley[1], Rebecca Lea[1], Kay Elder[5], Sissy E. Wamaitha[1], Daesik Kim[6], Valdone Maciulyte[3], Jens Kleinjung[7], Jin-Soo Kim[6,8], Dagan Wells[4], Ludovic Vallier[2,9,10], Alessandro Bertero[10]†, James M. A. Turner[3] & Kathy K. Niakan[1]

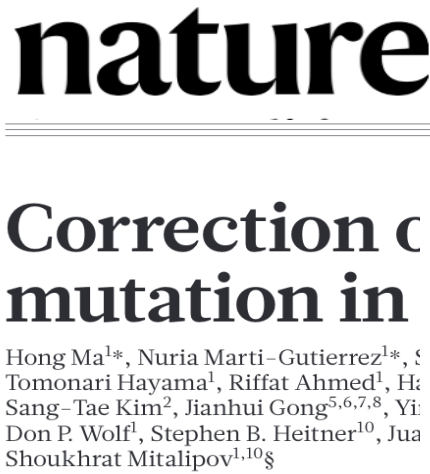


PNAS
Proceedings of the National Academy of Sciences of the United States of America www.pnas.org

Frequent loss of heterozygosity in CRISPR-Cas9–edited early human embryos

Gregorio Alanis-Lobato[a], Jasmin Zohren[b], Afshan McCarthy[a], Norah M. E. Fogarty[a,c], Nada Kubikova[d,e], Emily Hardman[a], Maria Greco[f], Dagan Wells[d,g], James M. A. Turner[b], and Kathy K. Niakan[a,h,1]




nature

doi:10.1038/nature23305

Correction c
mutation in

Hong Ma[1]*, Nuria Marti-Gutierrez[1]*, S
Tomonari Hayama[1], Riffat Ahmed[1], Ha
Sang-Tae Kim[2], Jianhui Gong[5,6,7,8], Yi
Don P. Wolf[1], Stephen B. Heitner[10], Jua
Shoukhrat Mitalipov[1,10]§


nature communications

Article

https://doi.org/10.1038/s41467-023-36820-6

Limitations of gene editing assessments in human preimplantation embryos

Liang et al. 2023

- Potential for repair as well as disruption of genes (Ma et al., 2017)
- Remove and replace specific pieces of DNA (via homology directed repair)
- Loss of heterozygosity (many embryos homozygous for normal gene copy)
- Attributed to HDR using the normal copy of the gene

Clinical Application…how far are we in terms of technology?

For clinical application to be considered:

1. Rate of HDR must be increased (normally <10%)

2. There must be no unintentional alterations of the genome and (sub)chromosomal aberrations

3. Methodology for gene editing assessment must be unified

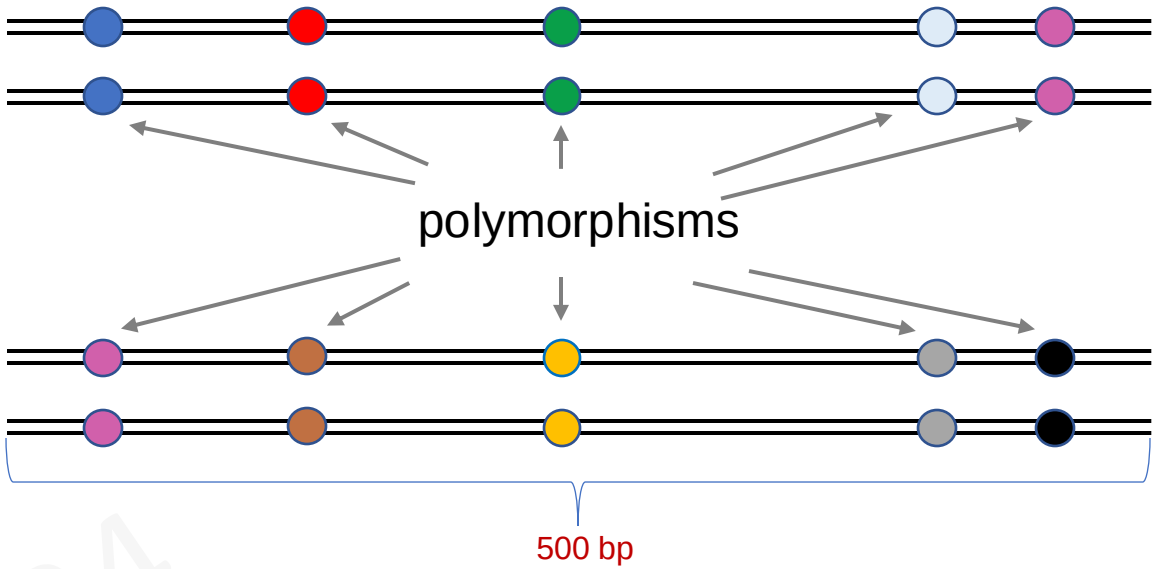
What can CRISPR tell us about embryo biology?

1. How do early human embryos resolve DNA damage?
2. Are cellular repair mechanisms fully functional prior to embryonic genome activation (EGA)?
3. Is there a risk that the therapeutic use of CRISPR-Cas9 to correct mutations could produce damage that embryonic cells fail to repair?

Repair pathways are compromised in the early human embryos prior to the EGA and the application of GE at this stage induces genomic instability

The study harnessed CRISPR-Cas9 to induce DNA damage in embryo cells in a highly controlled manner, allowing a critical evaluation of DNA repair capacity.

Sperm donor



All polymorphisms homozygous

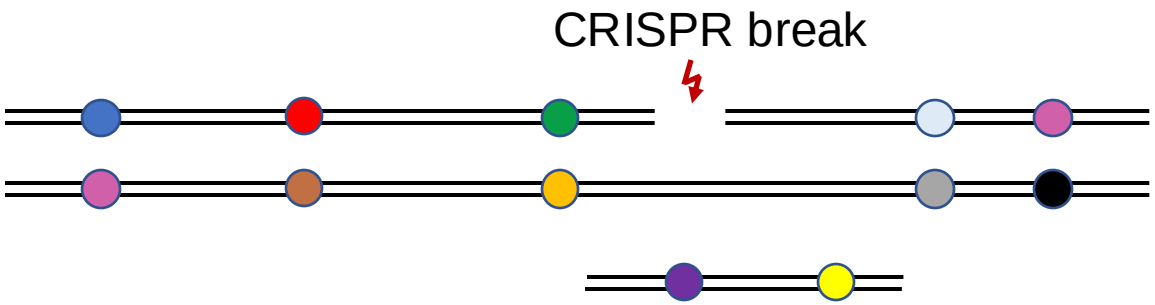
Oocyte donor

All polymorphisms homozygous

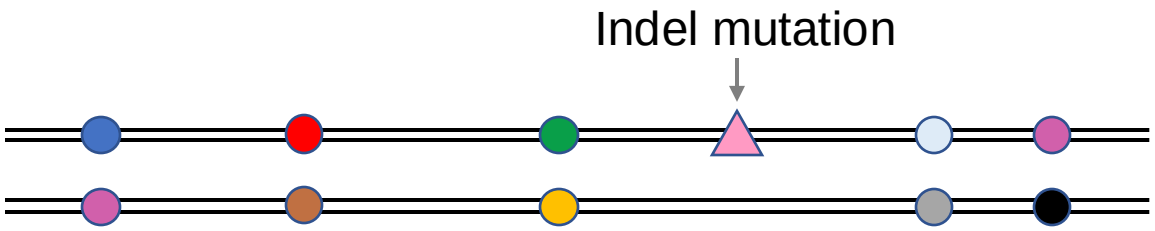
Embryo

All polymorphisms heterozygous

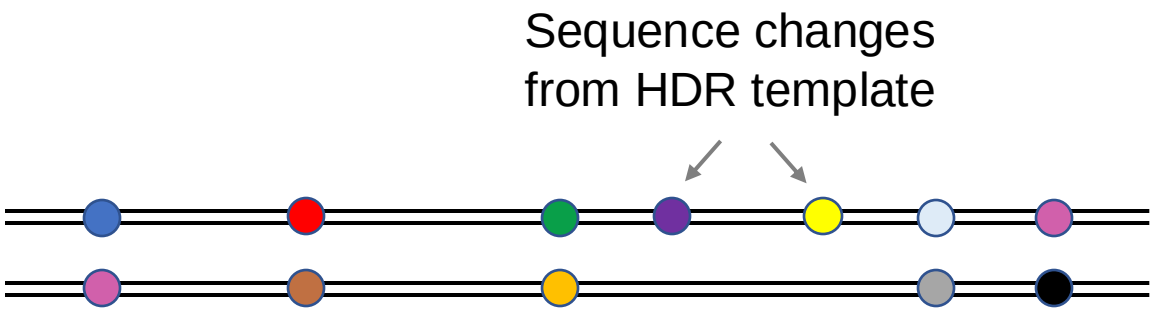
Embryo



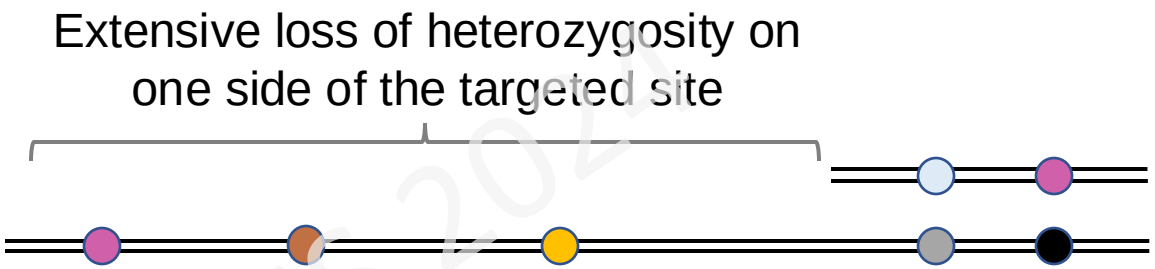
Possible outcomes after CRISPR-Cas9 targeting in human embryos



Non-homologous end joining
Detected by sequencing



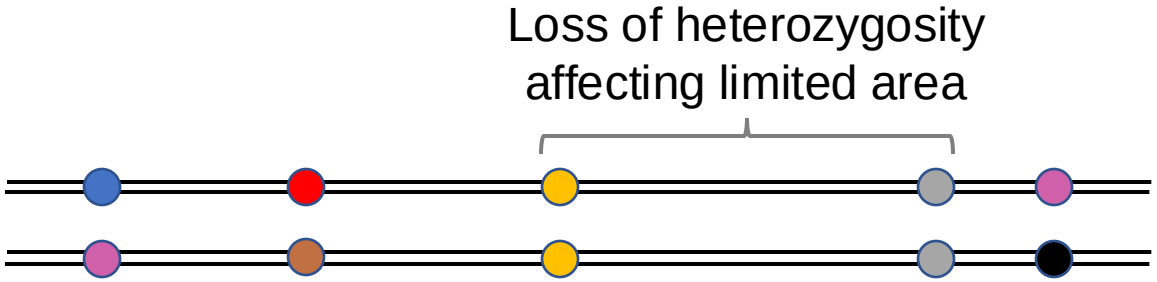
HDR using artificial template
Detected by sequencing



Large deletion
Revealed by electrophoresis and long-read sequencing

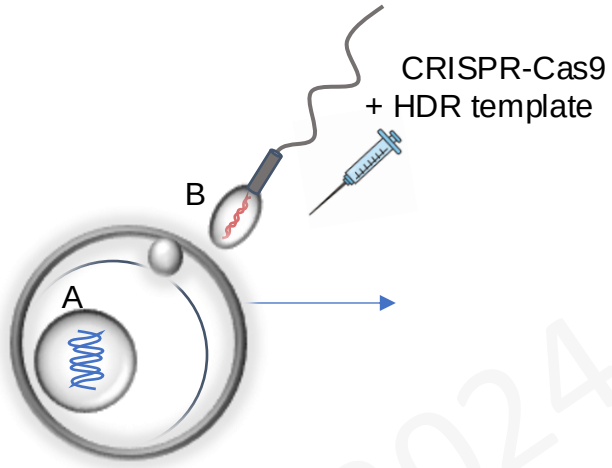
Unresolved DSB
Revealed by cytogenetic analysis (low pass genome sequencing)

Chromosome rearrangement
Detected by sequence capture and long-read sequencing

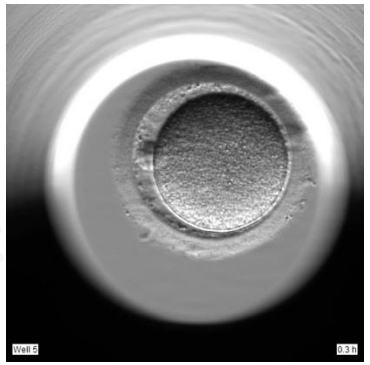


HDR using endogenous allele
Detected by sequencing

Workflow



MII oocyte and sperm



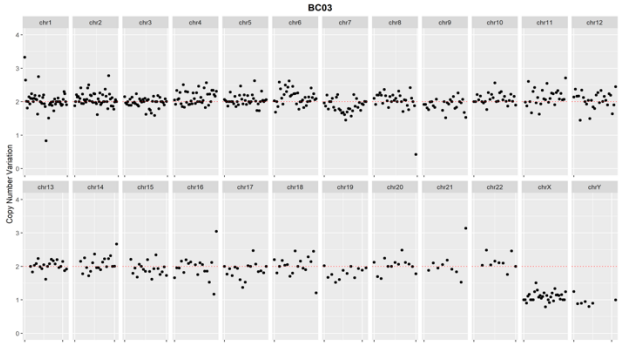
Blastomere disaggregation

ONT sequencing workflows

Genotype analysis

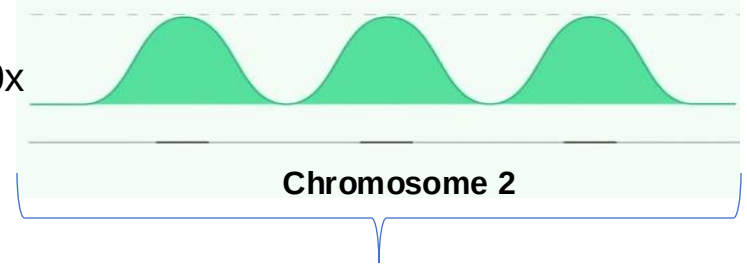
Repair by HDR using template

Cytogenetic analysis



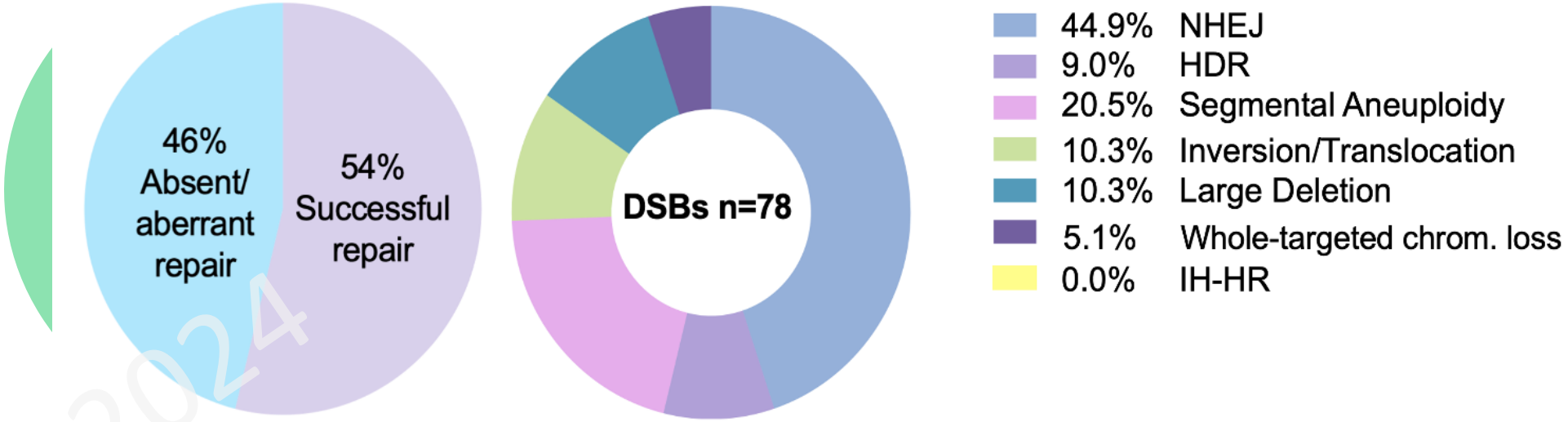
Sequence Capture – target enrichment

3000x



CRISPR-Cas9 targeting is highly efficient in microinjected human zygotes

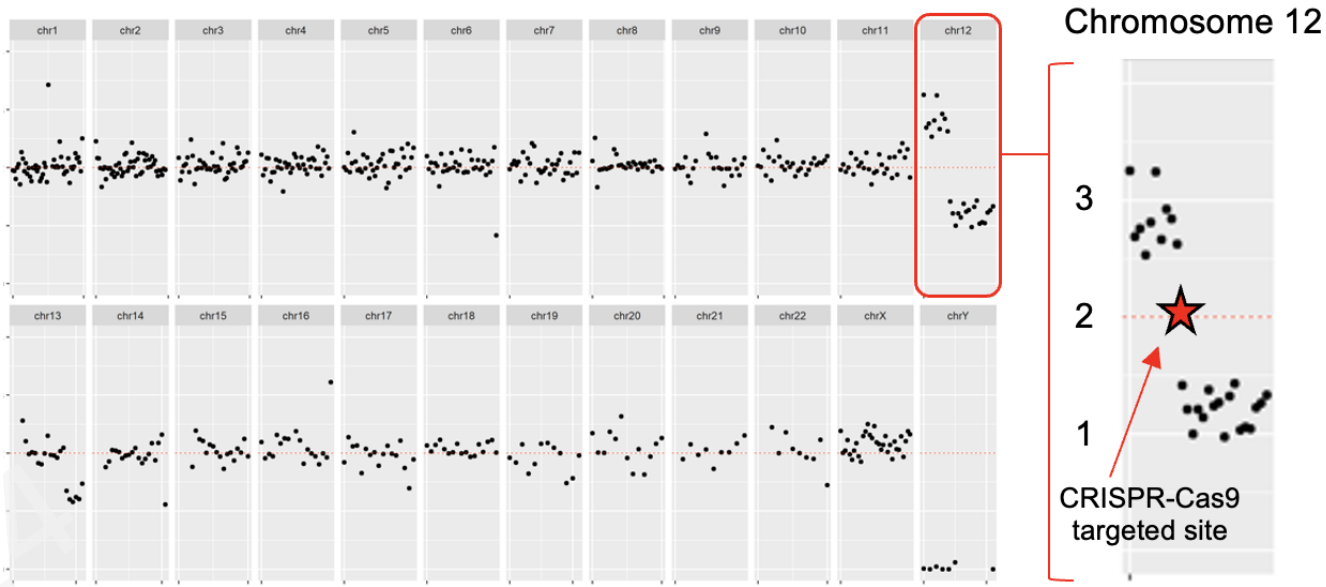
Targeting efficiency extremely high (100%) 27/27 embryos



…However, they are deficient in repair!

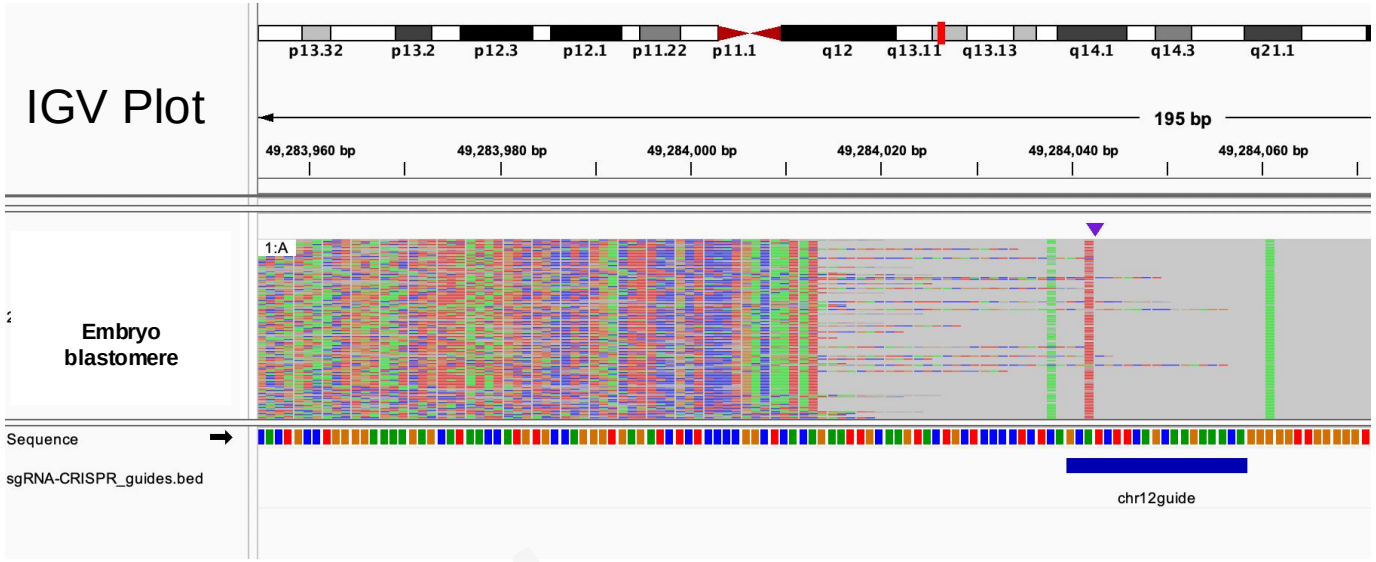
Double-stranded break repair is deficient in human embryos prior to activation of the embryonic genome

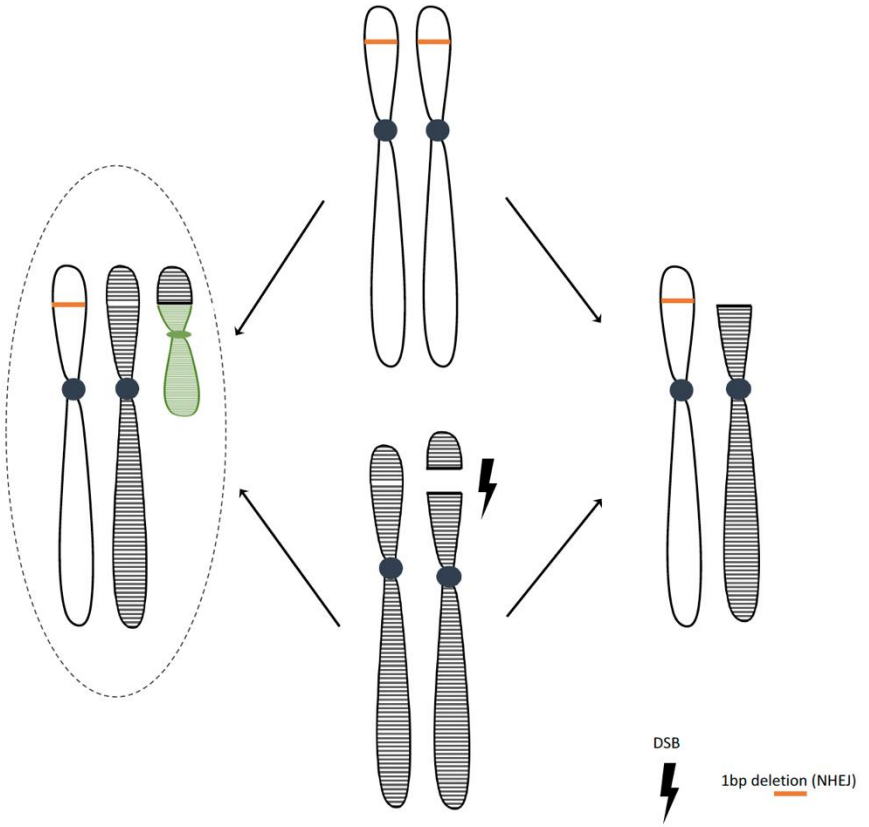
Low-pass genome sequencing analysis to reveal segmental aneuploidy associated with CRISPR-Cas9



- 46% (36/78) DSBs failed to be appropriately repaired affecting two-thirds of embryos
- Failure of DSB repair led to abnormalities affecting the chromosome containing the target site
- Breakpoints contained within the target site

Sequence capture reveals large-scale genomic rearrangements in microinjected human zygotes

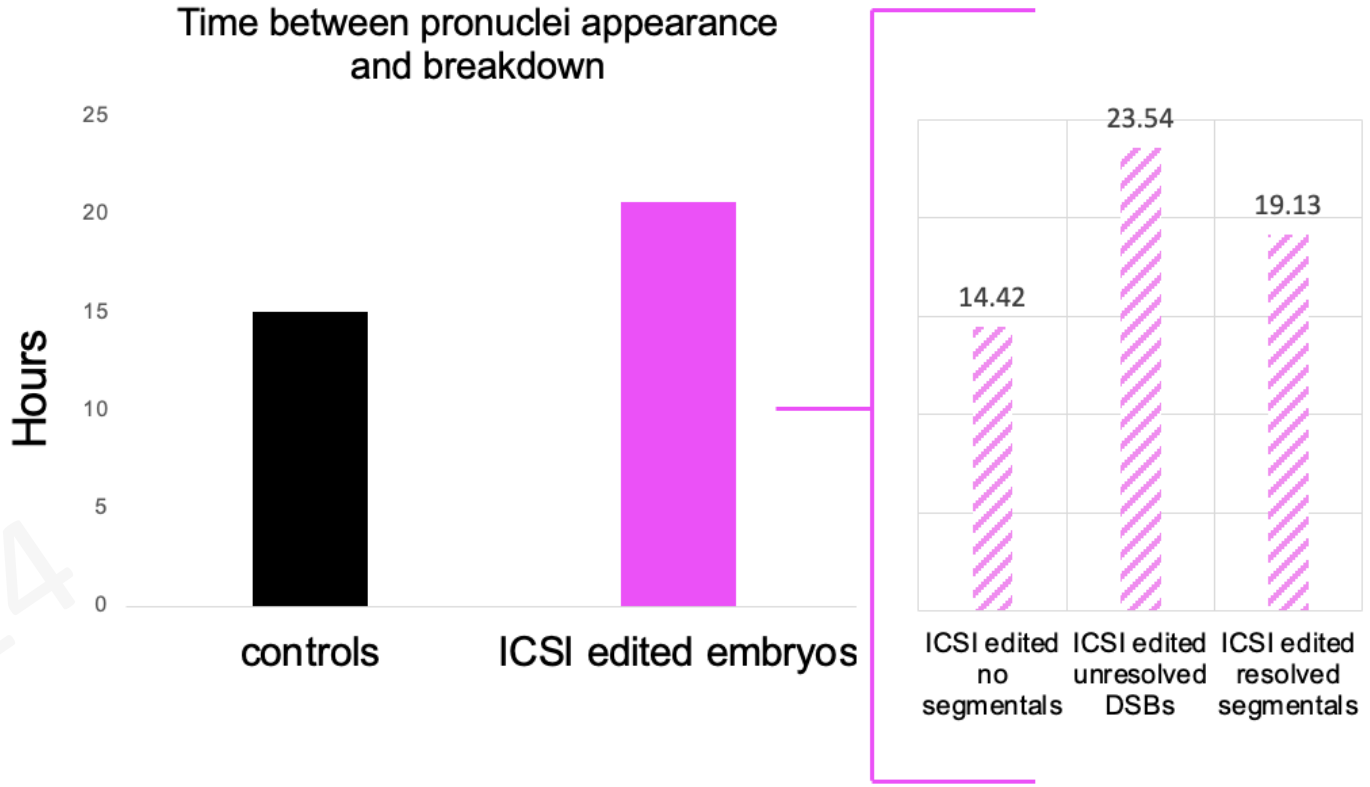




10.3% of all targeting events culminated in large-scale genomic rearrangements (translocations and inversions)

Carries a possibility of stable transmission through mitosis and therefore a risk of congenital abnormalities in the offspring

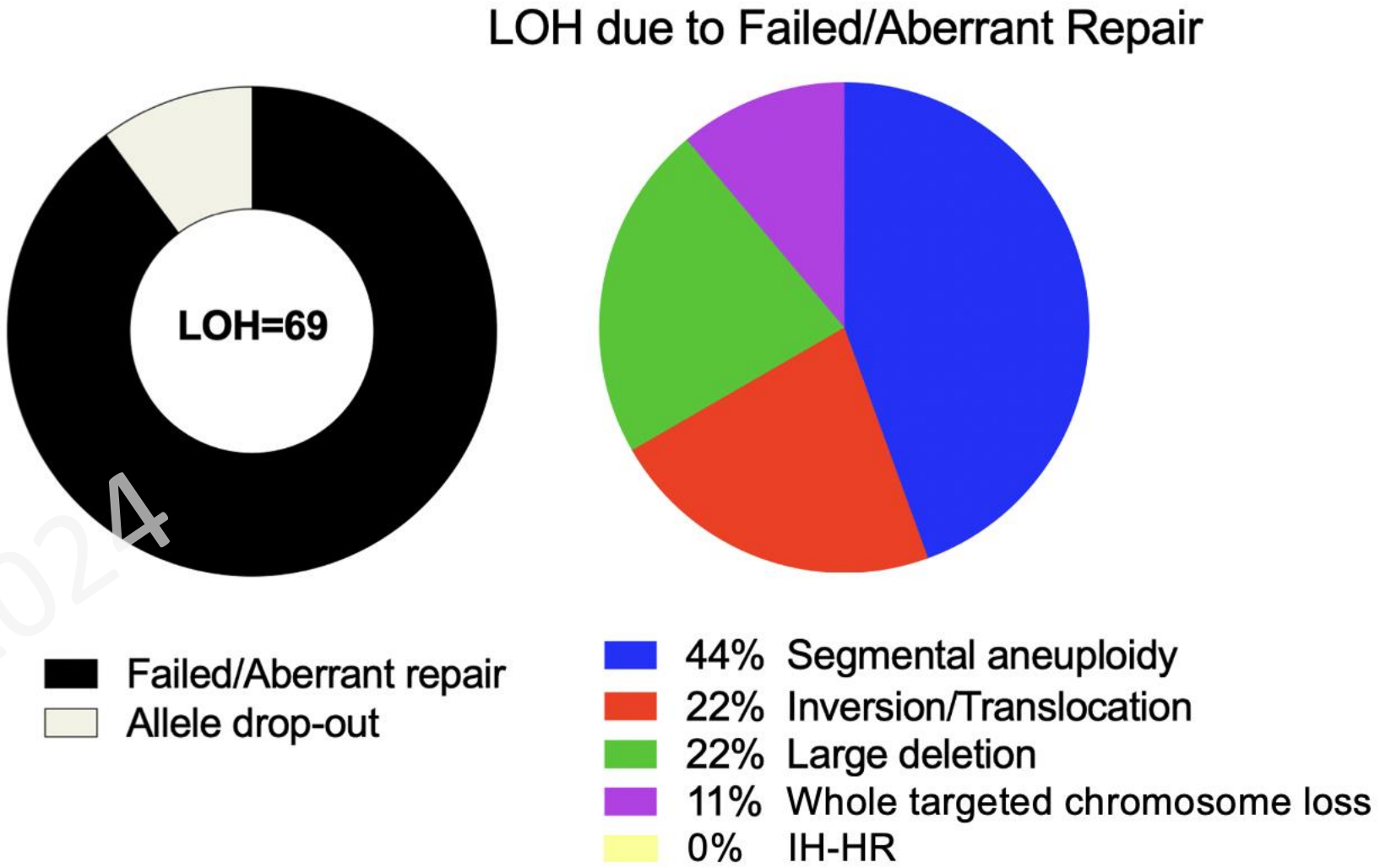
Delay in mitotic progression of blastomeres with unresolved DSBs is consistent with checkpoint activity in preimplantation embryos

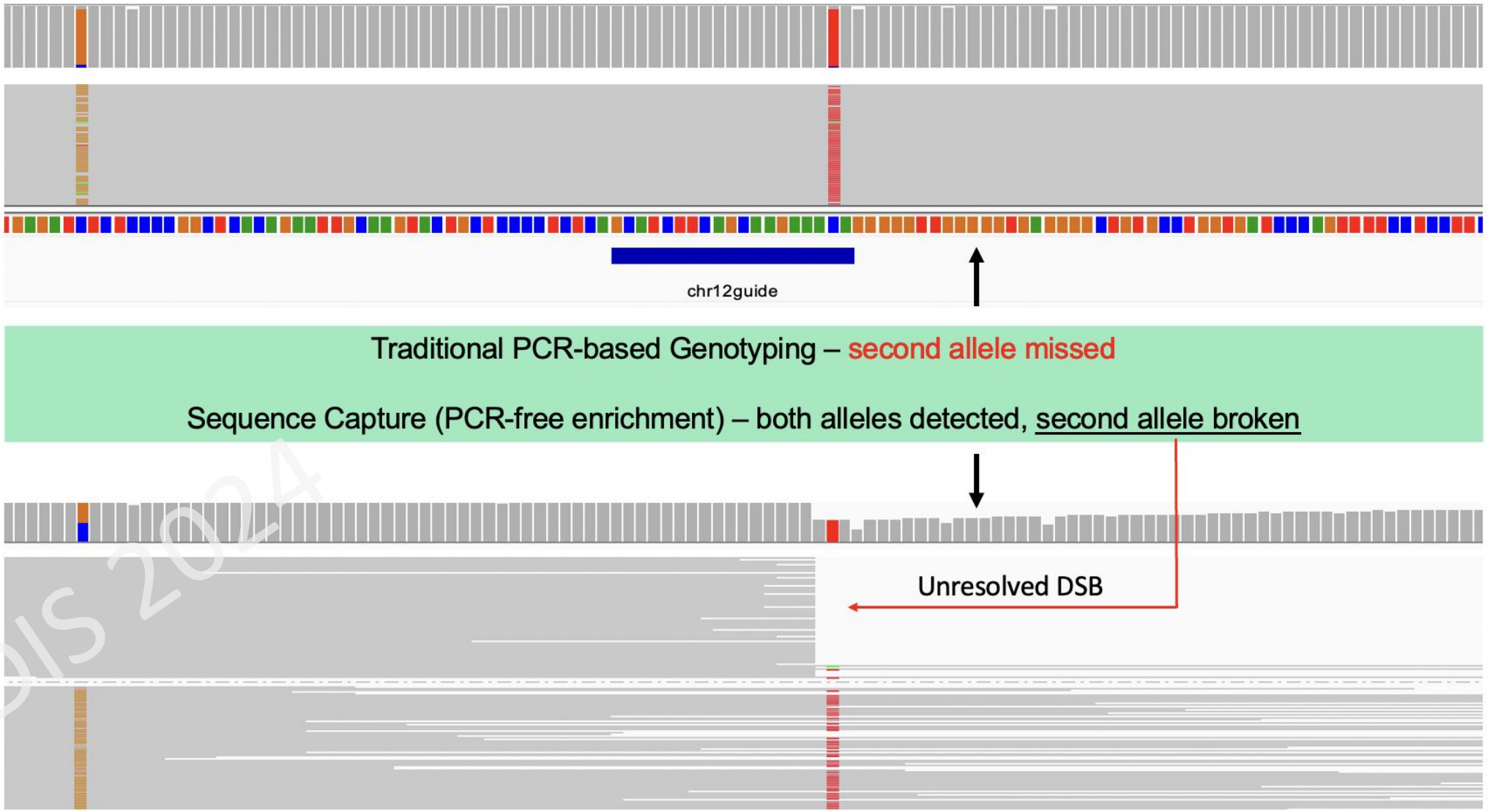


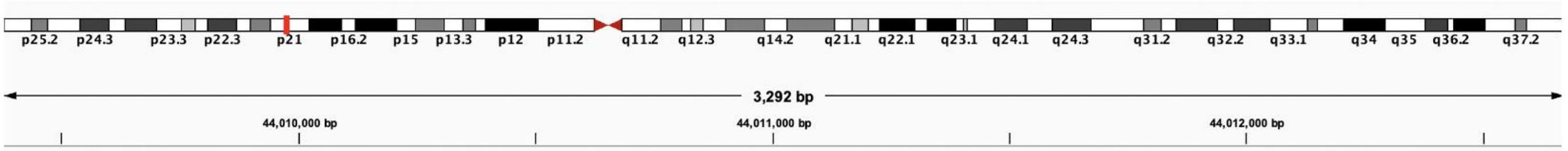
Significant delay in mitotic progression indicates checkpoint activity

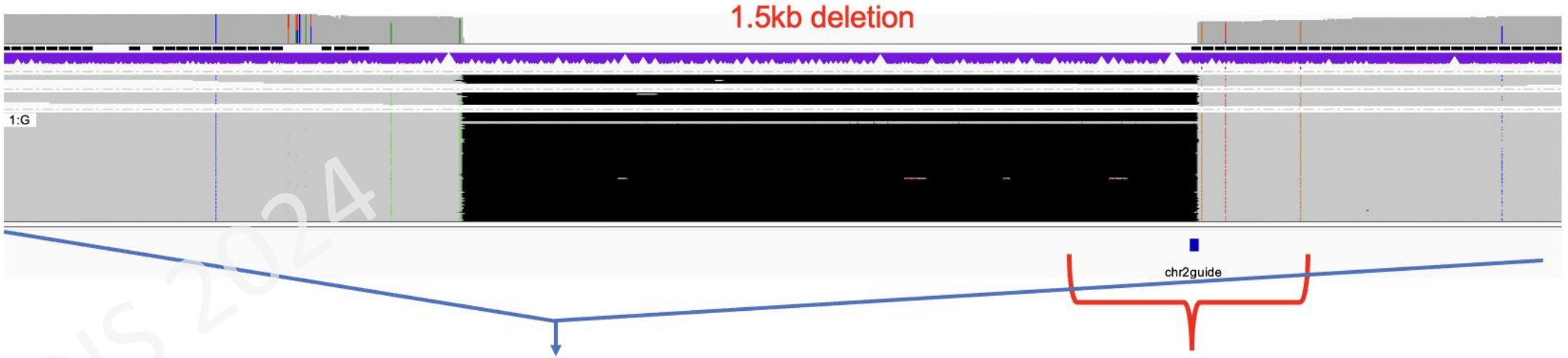
>1/3 embryos with seg. aneuploidy progressed, indicating relaxed checkpoint control

Most loss of heterozygosity (LOH) events at the targeted sites are a consequence of aberrant DNA repair




chr12guide
Traditional PCR-based Genotyping – second allele missed
Sequence Capture (PCR-free enrichment) – both alleles detected, second allele broken
Unresolved DSB





Long-range Nanopore sequencing reveals ~1.5kb deletion in the edited locus

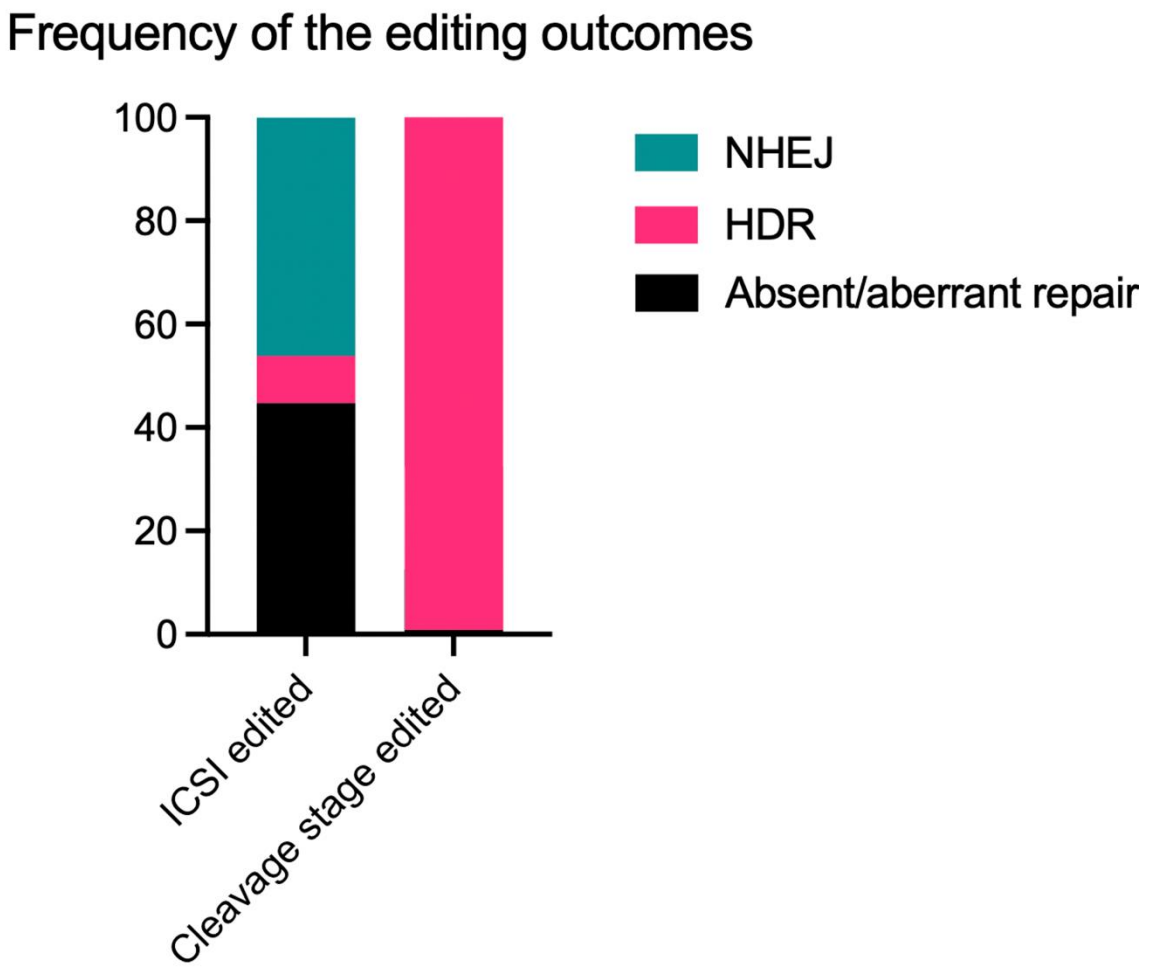
PCR-based amplicon genotyping reveals WT-homozygous genotype

Genome-edited cleavage stage embryos repair DSBs predominantly by HDR

A group of embryos (n=37) were allowed to develop into 6-cell stage (post-EGA) and then microinjected with CRISPR-Cas9

Only 16% were successfully targeted and <u>100% repaired the DSBs with HDR</u>

Rate of seg. aneuploidies greatly reduced, consistent with the notion that DNA repair is fully functional post EGA



…Perhaps clinical application can be considered at this stage?

Conclusions

Fertilization stage is the most optimal for targeting efficiency. It also appears to be the stage when human embryos are most susceptible to DNA damage.

Genome editing by CRISPR is highly efficient in producing DSBs, and these are predominantly repaired by NHEJ (84%).

- *HDR needed for correction of most mutations but seldom used by the cells (<10%)*

Segmental abnormalities and genomic rearrangements are a prominent unintended effect of application of CRISPR-Cas9 in human embryo cells.

Conclusions

Limited DNA repair capacity indicates that current GE tools cannot be safely applied at the earliest stage of development (but maybe okay later?)

HDR appears to be the predominant form of repair in the post-EGA human embryo

Significant implications for ART, potentially helping to guide the formulation new embryo culture systems

University of Oxford

Prof Dagan Wells
Munuse Savash
Jack Fagan
Columba Avila Perez

Juno Genetics UK

Dr Katharina Spath
Dr Clement Coudereau

IVI Barcelona

Dr Marga Esbert

The Francis Crick Institute and University of Cambridge

Dr Kathy Niakan

Foundation for Embryonic Competence

Dr Shiny Titus
Dr Richard Scott

Jesus College