CERGENTIS

COMPLETE GENE SEQUENCING

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Introduction

- Spin-off KNAW/Hubrecht Institute
- Founded July 2012
- Based in Utrecht, the Netherlands
- Scientific Advisory Board:
  - Prof. Han Brunner
  - Prof. Edwin Cuppen
  - Prof. Sabine Linn

Hubrecht Institute

Agentschap NL
Ministerie van Economische Zaken, Landbouw en Innovatie

KONINKLIJKE NEDERLANDSE AKADEMIE VAN WETENSCHAPPEN

Cancer GENOMICS CENTRE
Improving cure rates for cancer patients

Netherlands Genomics Initiative
Excellence in genomics: for a healthy, sustainable and safe future

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Cergentis Businessmodel

- Services (through service providers)
- Kits

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TLA Technology

- Targeted Locus Amplification
- Targeted, low-cost sequencing
- Requires 2x20bp sequence information
- Physical proximity as basis of selection
- Compatible with all NGS Technologies
- Suitable for multiplexing

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TLA Technology

- Critical advantages:
  - Highly flexible
  - Complete
  - Hypothesis neutral
  - Enables haplotyping

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Applications

- Targeted academic & industrial genetic research
  - Human
  - Animal
  - Plant
  - Microbial
- Genetic diagnostics
- Oncogenetics
  - Development & implementation personalized medicine

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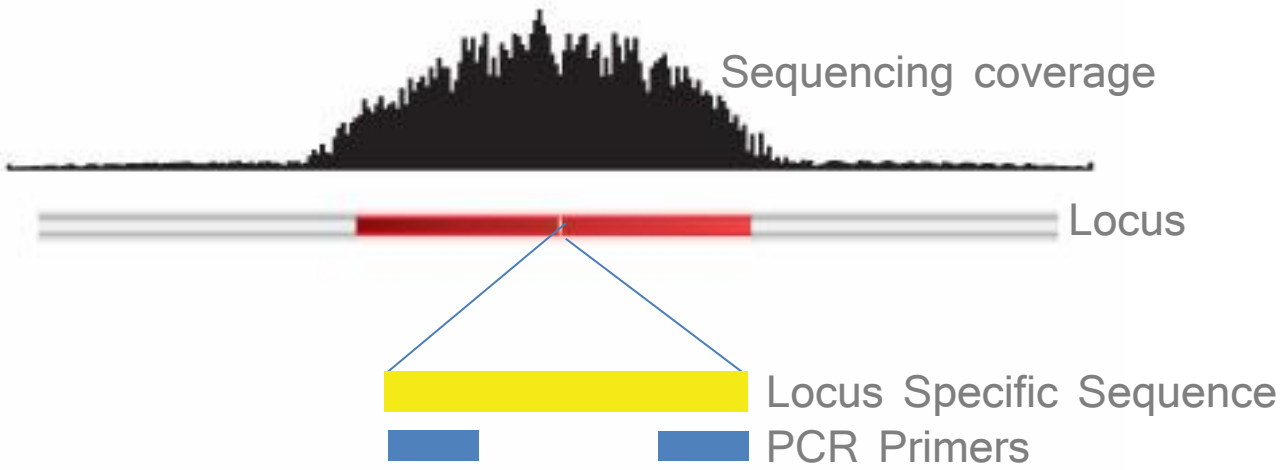
TLA Technology



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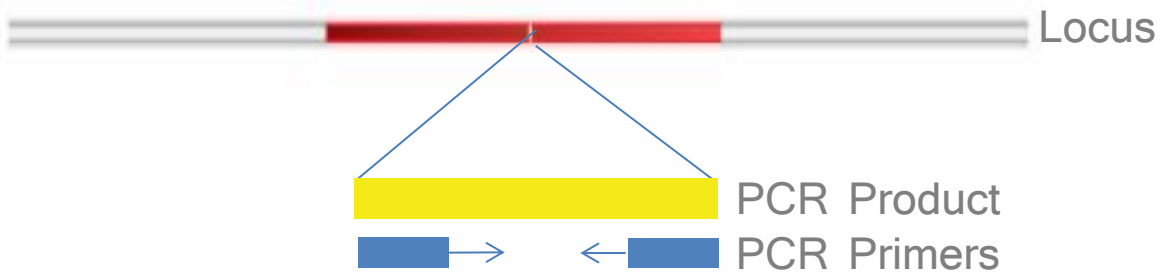
Conventional PCR



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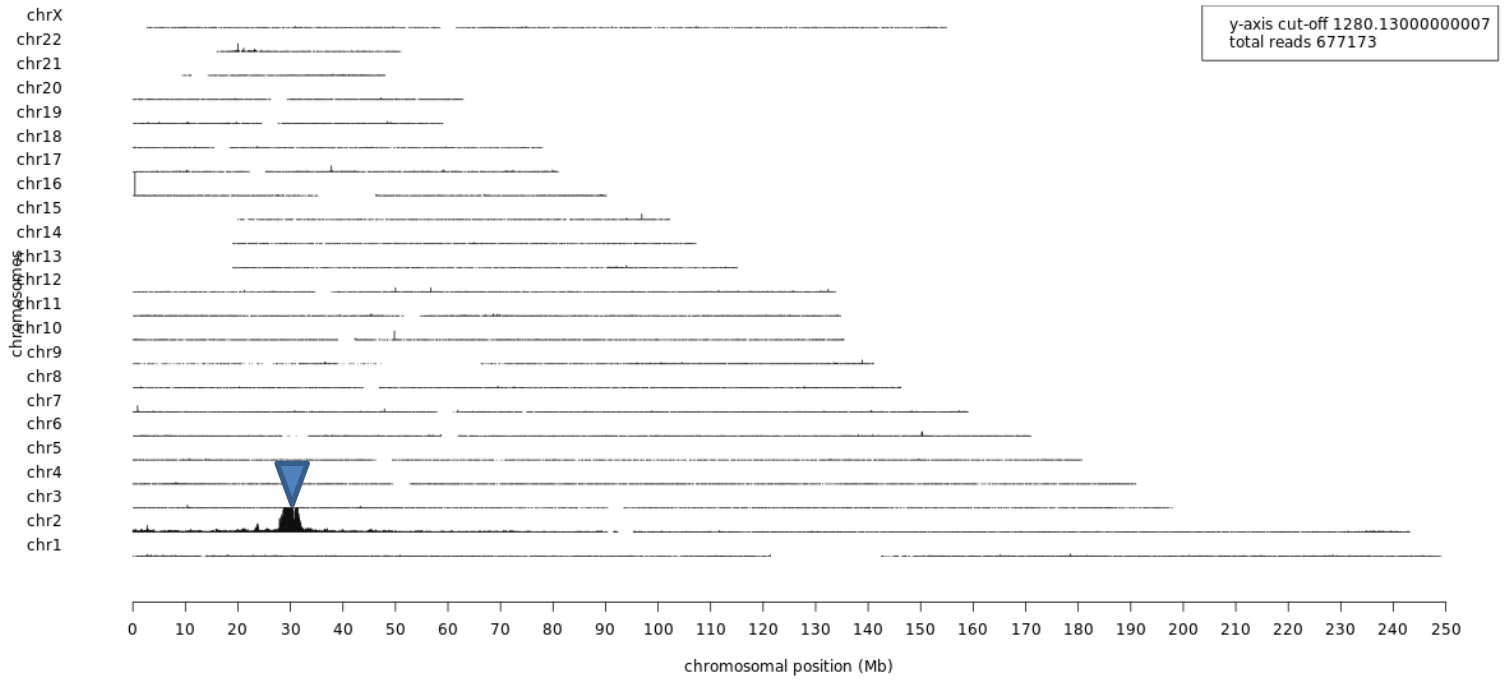
TLA Technology: ALK gene – human genome



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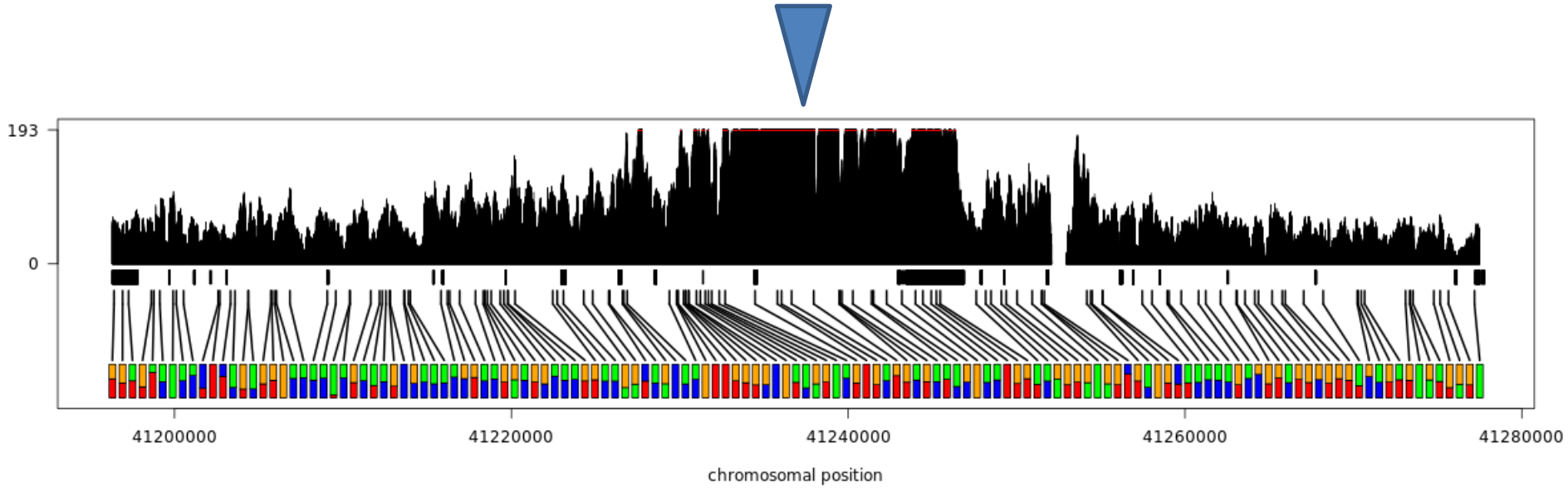
TLA Technology: BRCA1 gene



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TLA: complete gene seqeuncing and genetic diagnostics

- Diagnosed mutations have therapeutic impact
- Absence provides certainty

ORIGINAL CONTRIBUTION

Spectrum of Mutations in *BRCA1*, *BRCA2*, *CHEK2*, and *TP53* in Families at High Risk of Breast Cancer

Tom Walsh, PhD
Silvia Casadei, PhD
Kathryn Hale Coats, BS
Elizabeth Swisher, MD
Sunday M. Stray, BS
Jake Higgins, BS
Kevin C. Roach, BS

Context Genetic testing for inherited mutations in *BRCA1* and *BRCA2* has become integral to the care of women with a severe family history of breast or ovarian cancer, but an unknown number of patients receive negative (ie, wild-type) results when they actually carry a pathogenic *BRCA1* or *BRCA2* mutation. Furthermore, other breast cancer genes generally are not evaluated.

Objective To determine the frequency and types of undetected cancer-predisposing mutations in *BRCA1*, *BRCA2*, *CHEK2*, *TP53*, and *PTEN* among patients with breast cancer from high-risk families with negative (wild-type) genetic test results for *BRCA1* and *BRCA2*.

Conclusions The mutational spectra of *BRCA1* and *BRCA2* include many high-penetrance, individually rare genomic rearrangements. Among patients with breast cancer and severe family histories of cancer who test negative (wild type) for *BRCA1* and *BRCA2*, approximately 12% can be expected to carry a large genomic deletion or duplication in one of these genes, and approximately 5% can be expected to carry a mutation in *CHEK2* or *TP53*. Effective methods for identifying these mutations should be made available to women at high risk.

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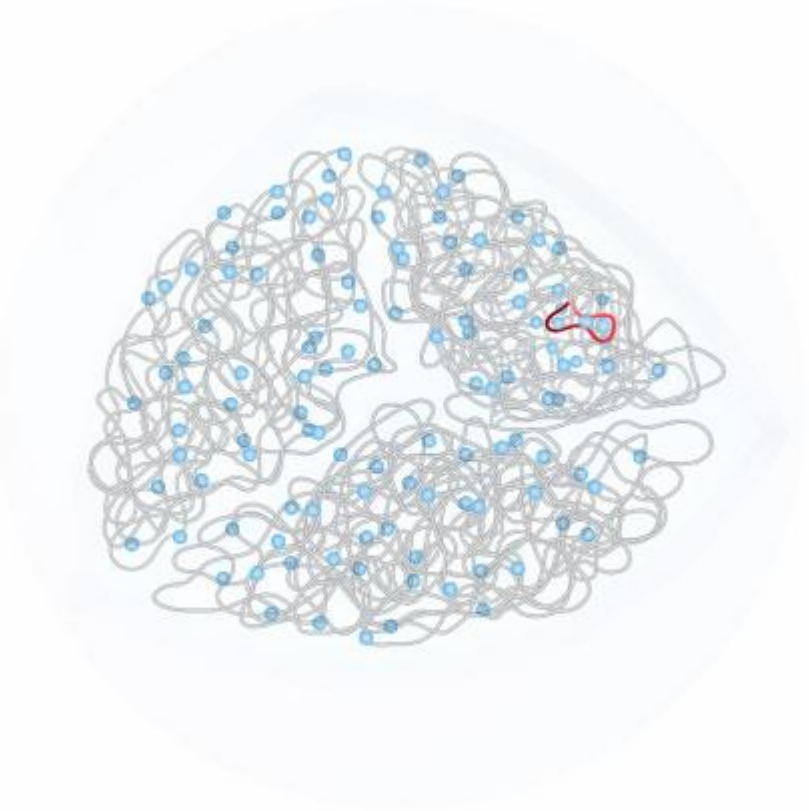

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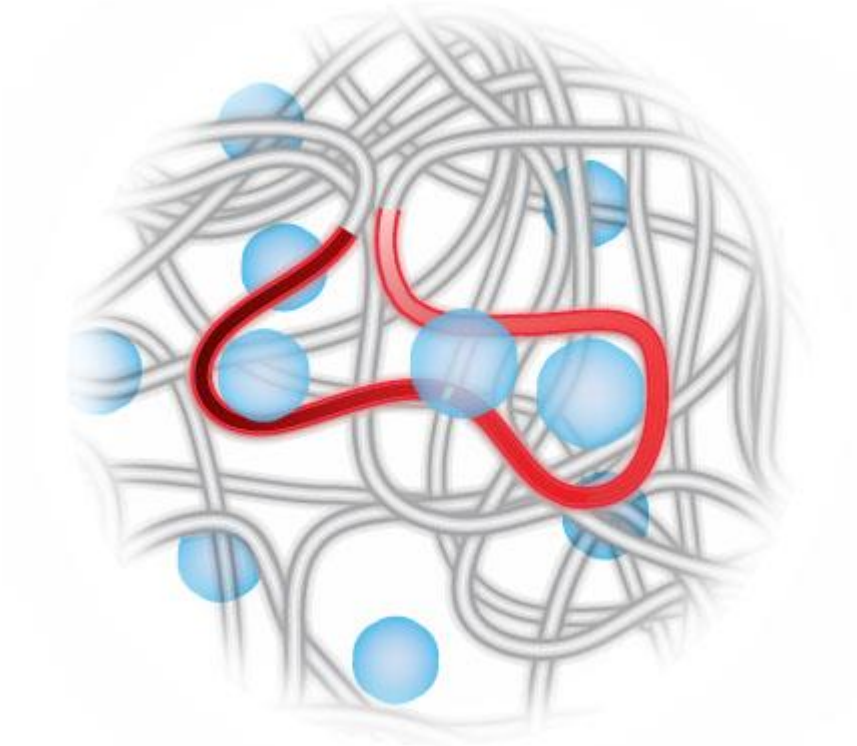

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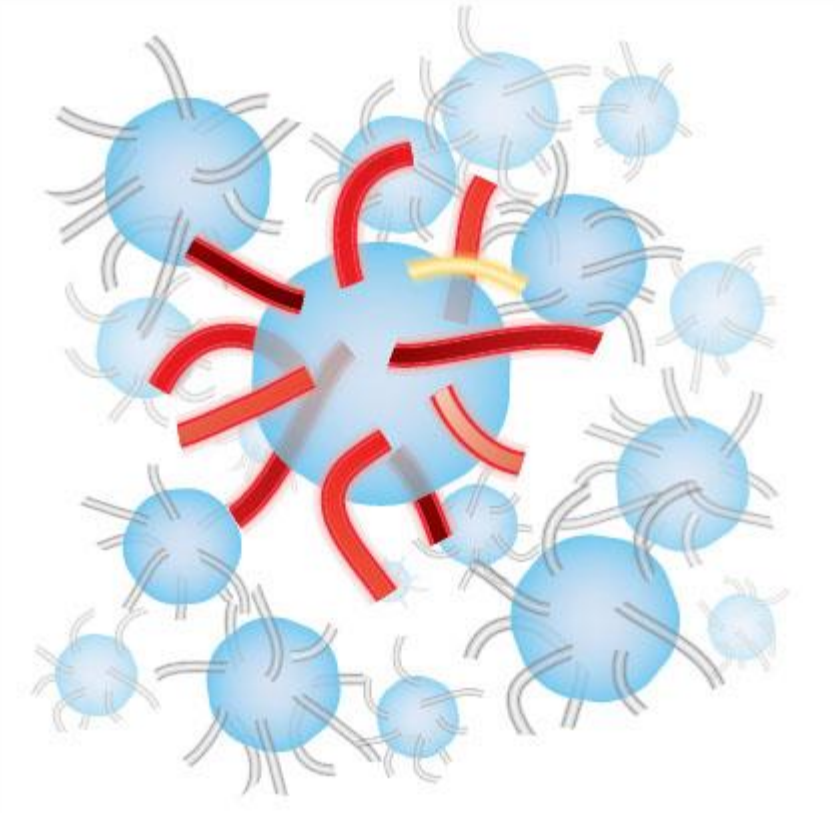

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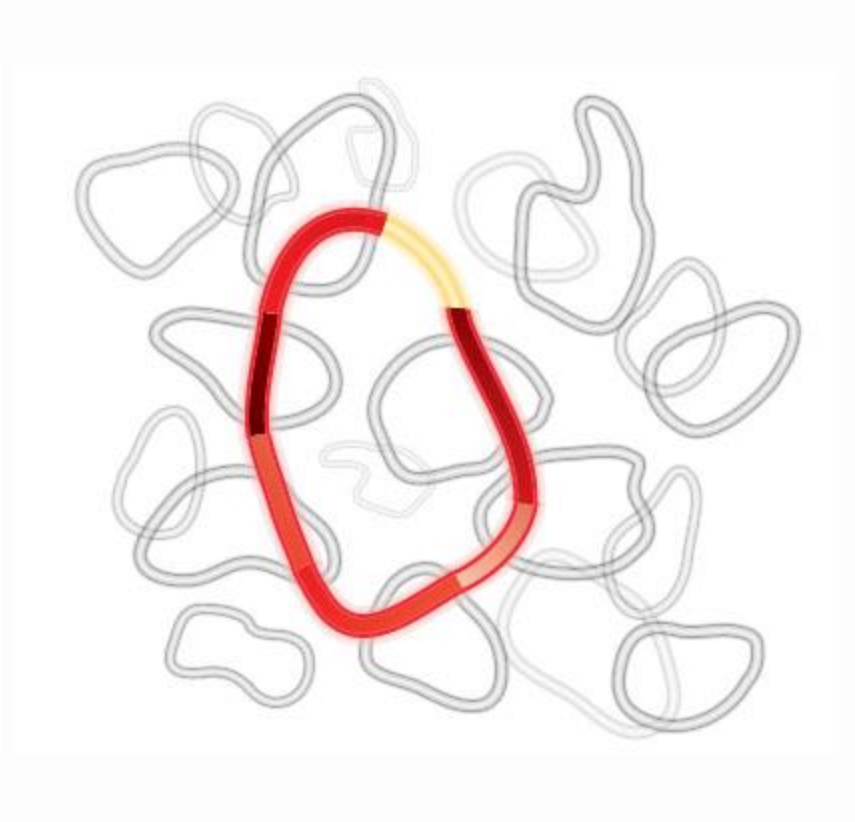

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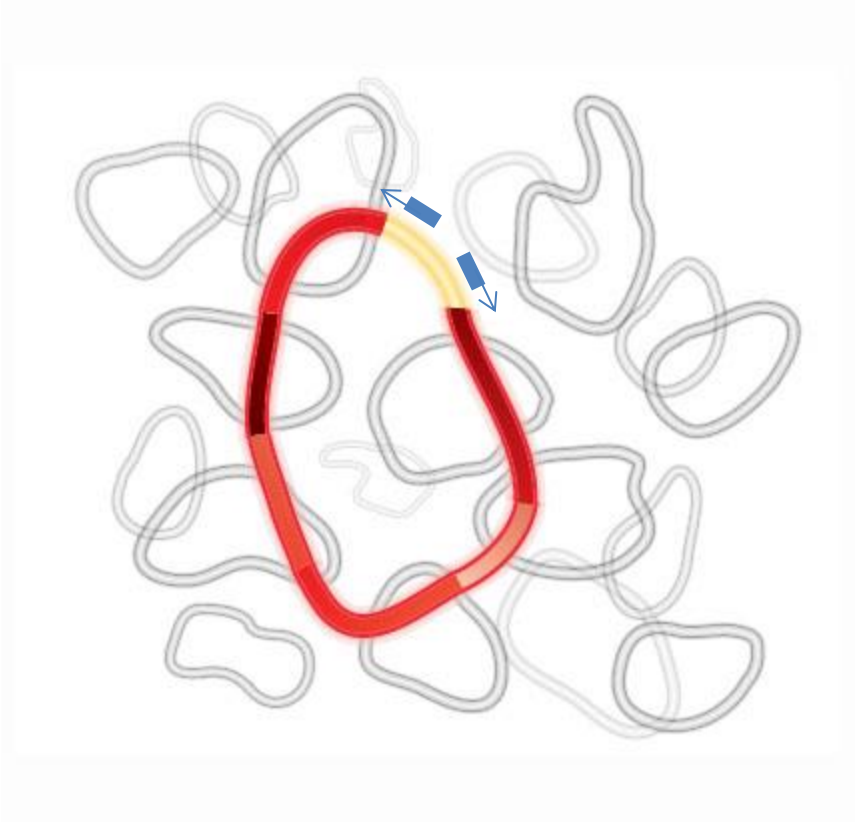

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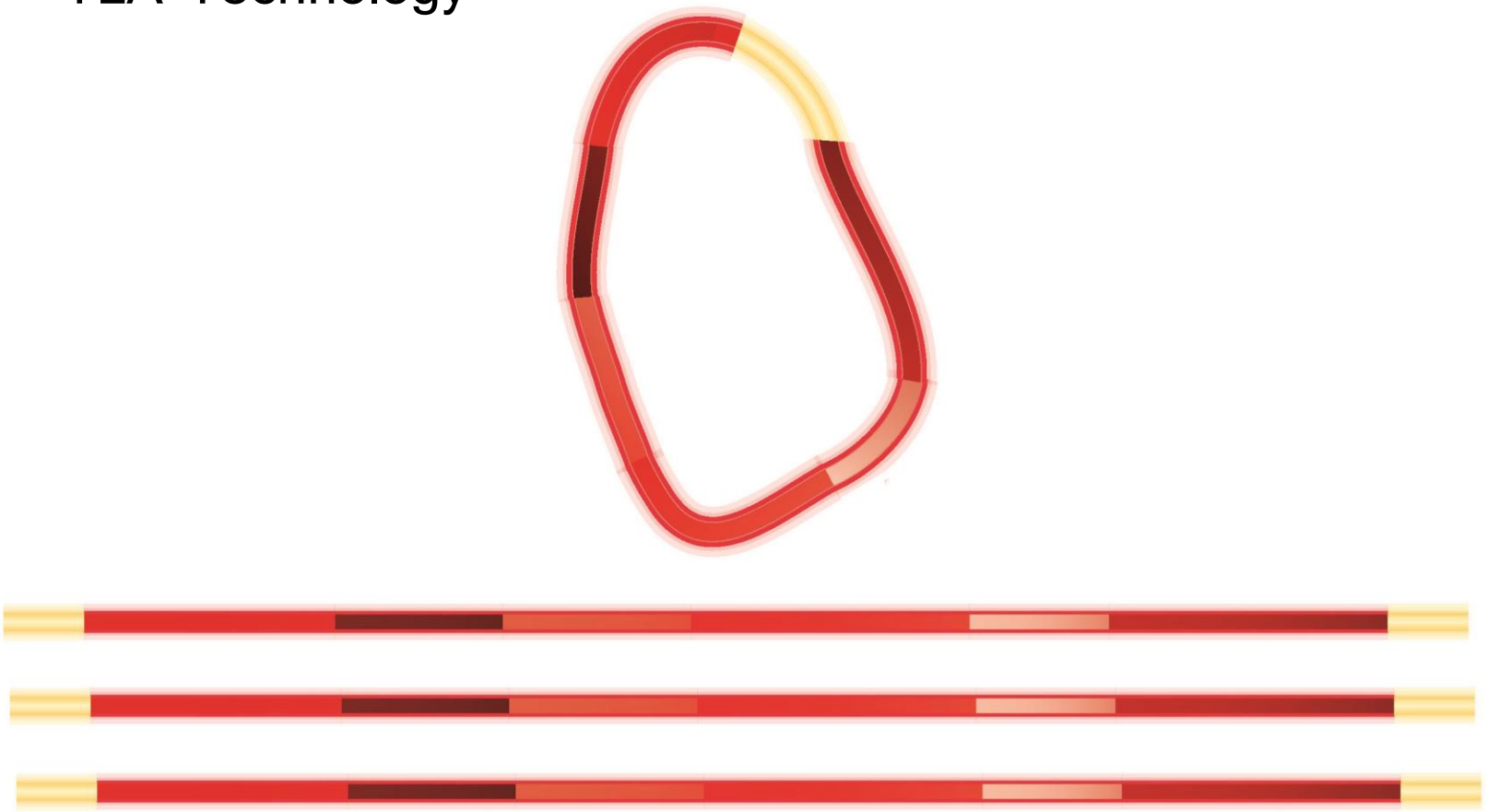

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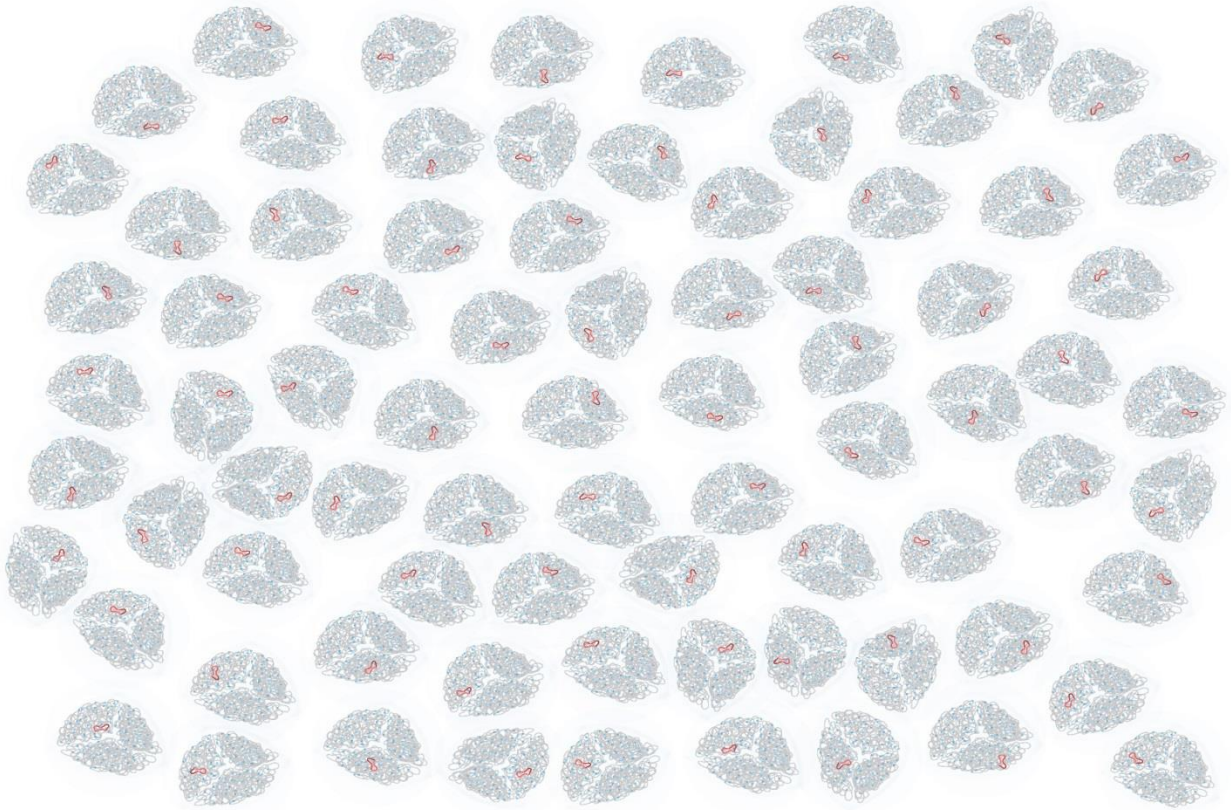

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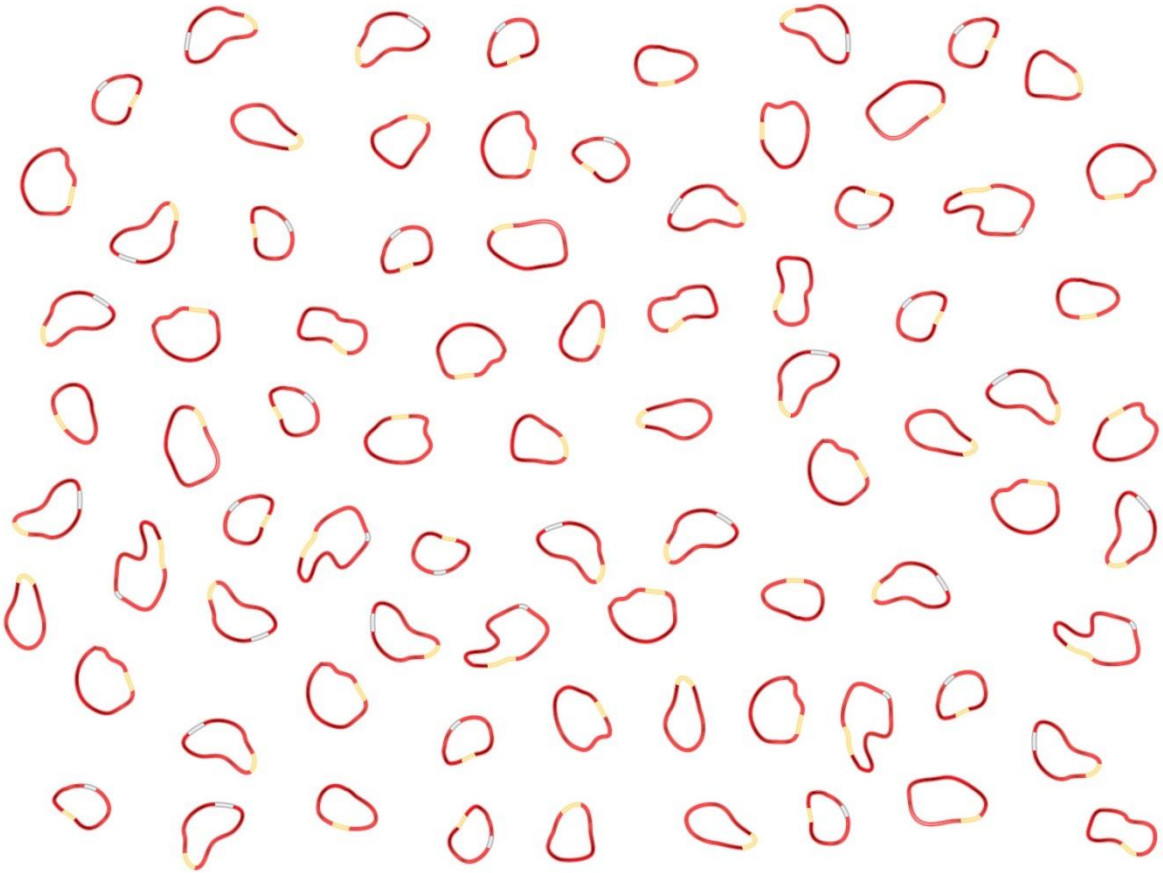

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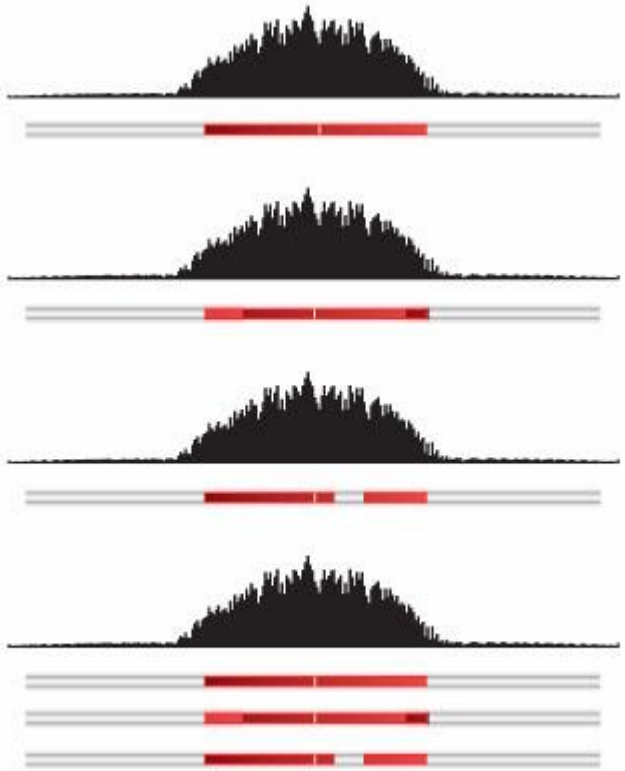

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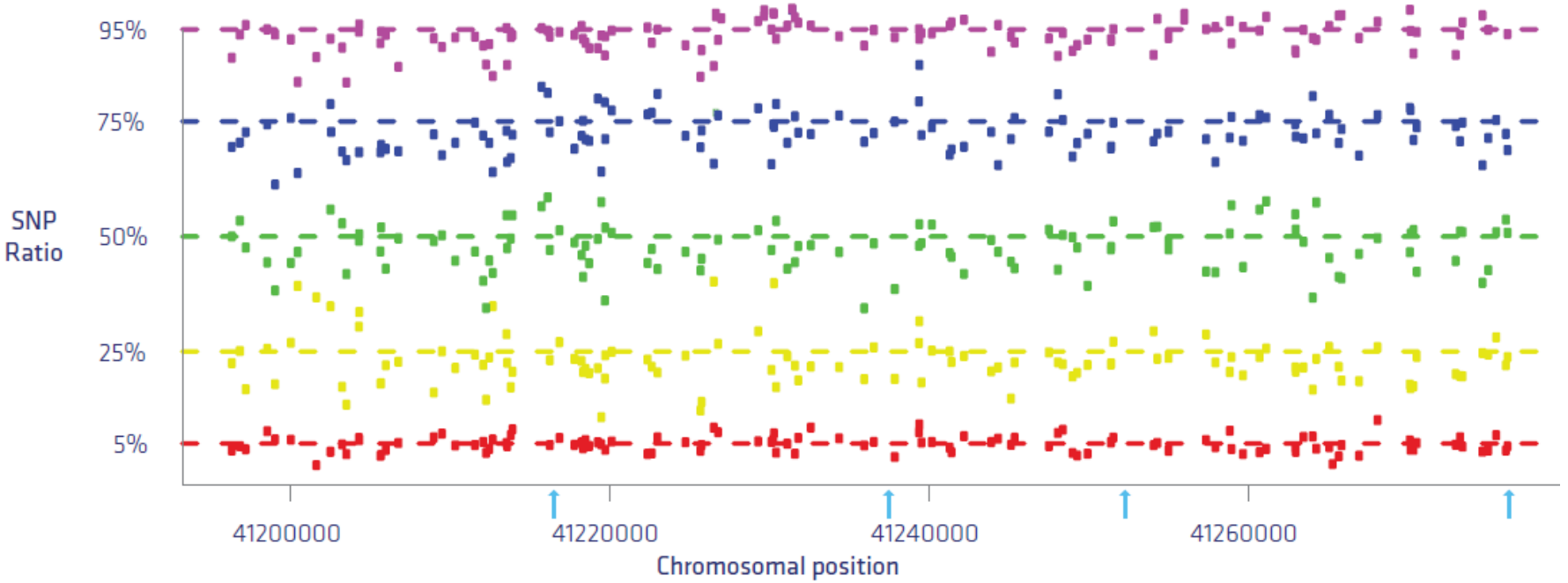
Complex cell mixtures

- Cell lines in ratios; 95/5, 75/25, 50/50, 25/75, 5/95



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TLA Technology & haplotyping

- Paired-end sequencing
- Paired ends from same originate from allele

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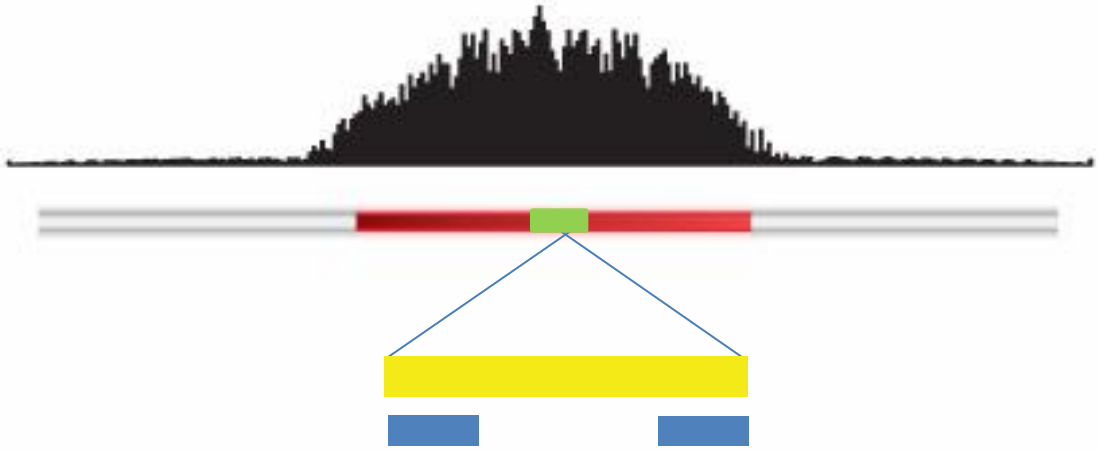
TLA Technology & Transgene sequencing



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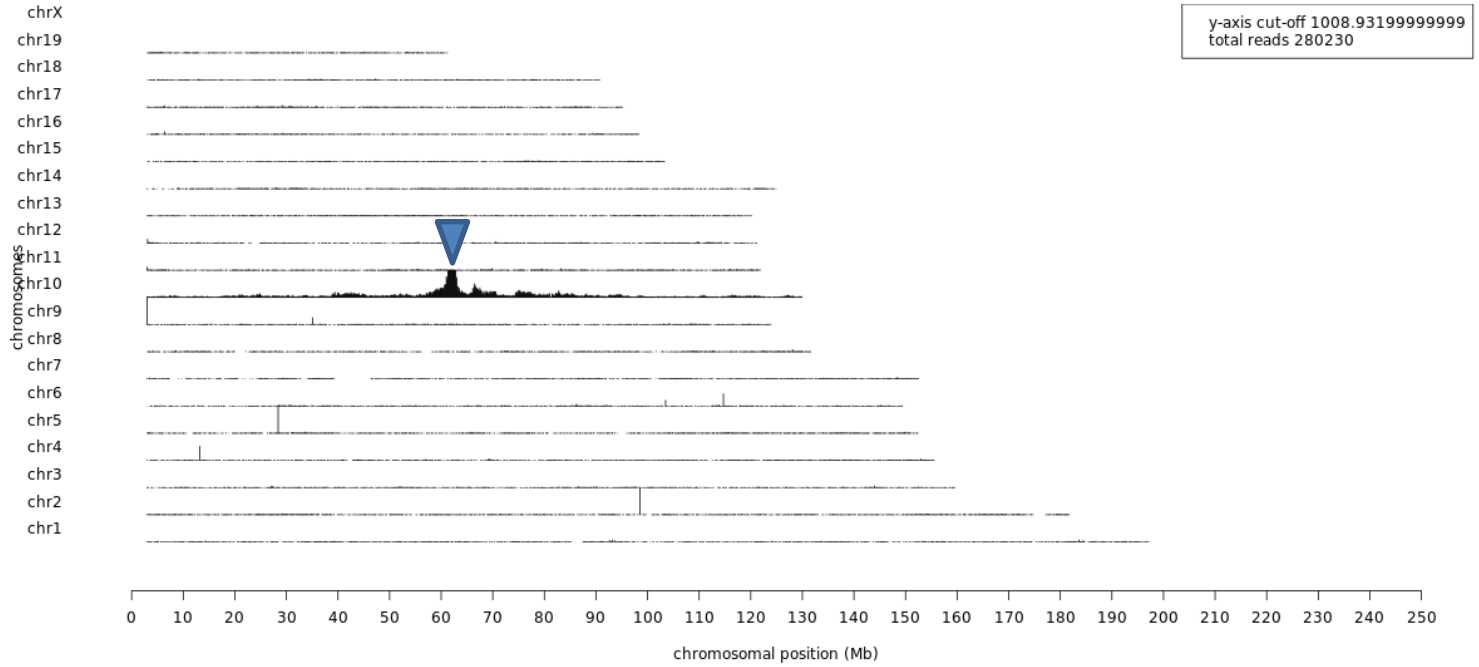
TLA Technology: transgene integration in mouse genome



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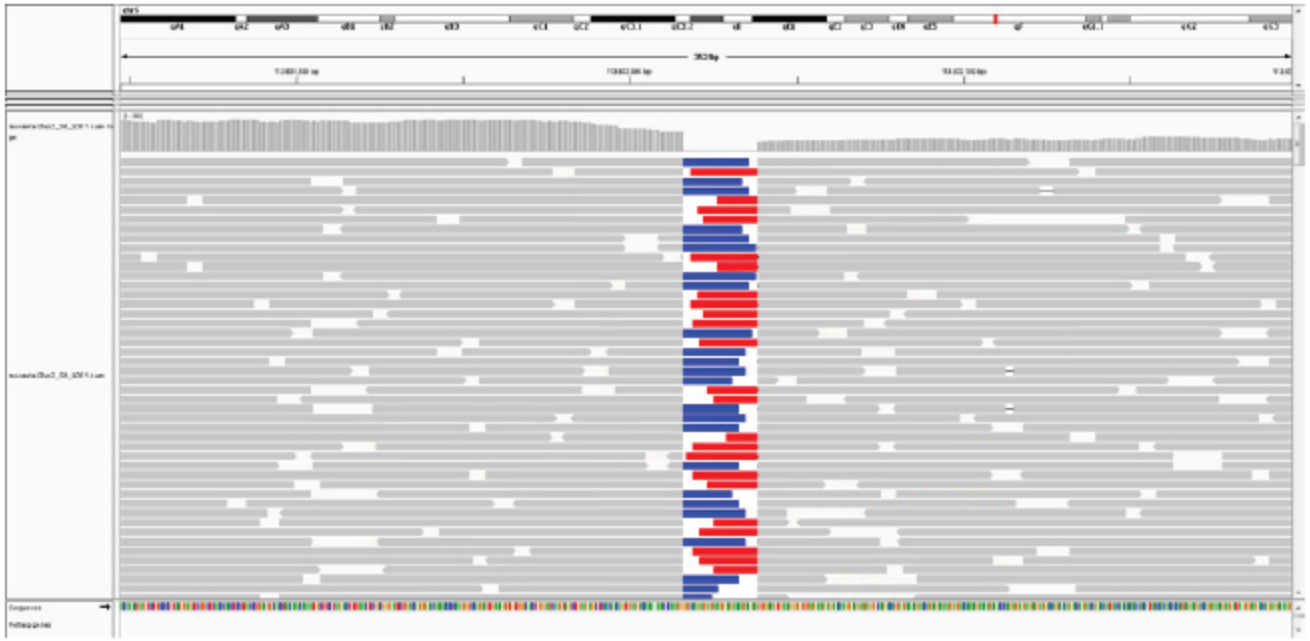

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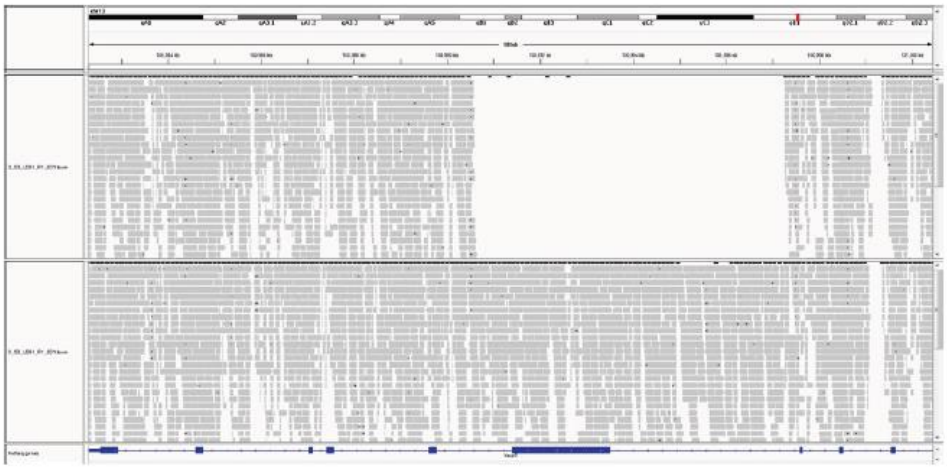
TLA & Targeted Mouse knock-out sequencing

- Different members gene family
- Knock out confirmed to be in the right position
- Additional relevant mutations identified



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TLA & Gene-fusions

- Automated protocol to replace incomplete & cumbersome FISH based assays
- TLA enables multiplexing
- Provides a complete diagnosis

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Automation TLA Technology

- One automated flexible protocol: only variable are the locus specific primers
- Generates sufficient TLA circles for 10's of (multiplex) amplifications: new amplifications can be performed in hours.

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Conclusion

- TLA Technology presents critical advantages in high quality targeted complete gene sequencing

Applications

- Candidate gene sequencing
- Diagnostic gene sequencing
- Targeted sequencing knock-outs & vector integration sites
- Haplotyping regions of interest
- Complete gene sequencing in tumours: somatic and structural variation
- Targeted analysis of gene fusions / fusion partners

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