



CERGENTIS

COMPLETE GENE SEQUENCING

NOVEMBER
26TH 2013
HOLIDAY INN
LEIDEN



LIFE SCIENCE
TECHNOLOGY
EVENT 2013

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

NOVEMBER
26TH 2013
HOLIDAY INN
LEIDEN



**LIFE SCIENCE
TECHNOLOGY
EVENT 2013**

Cergentis Businessmodel

- Services (through service providers)
- Kits



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



TLA Technology

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

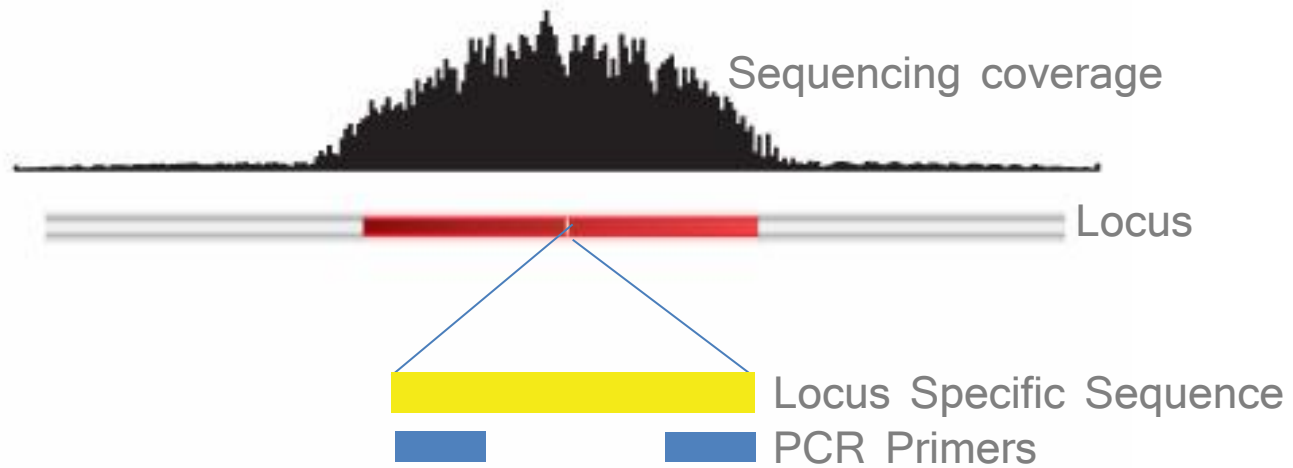


Applications

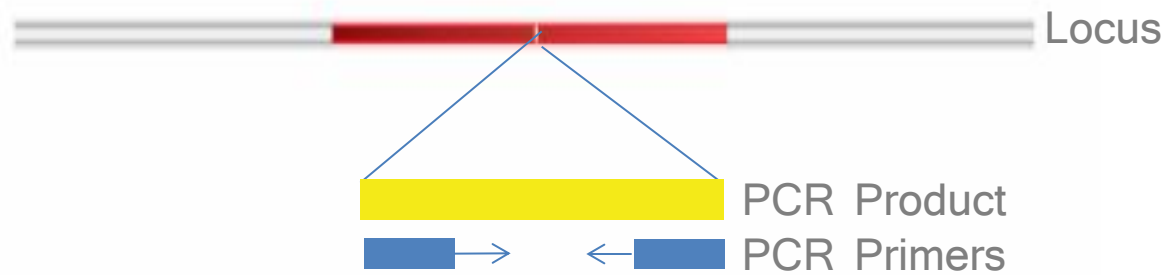
- Targeted academic & industrial genetic research
 - Human
 - Animal
 - Plant
 - Microbial
- Genetic diagnostics
- Oncogenetics
 - Development & implementation personalized medicine



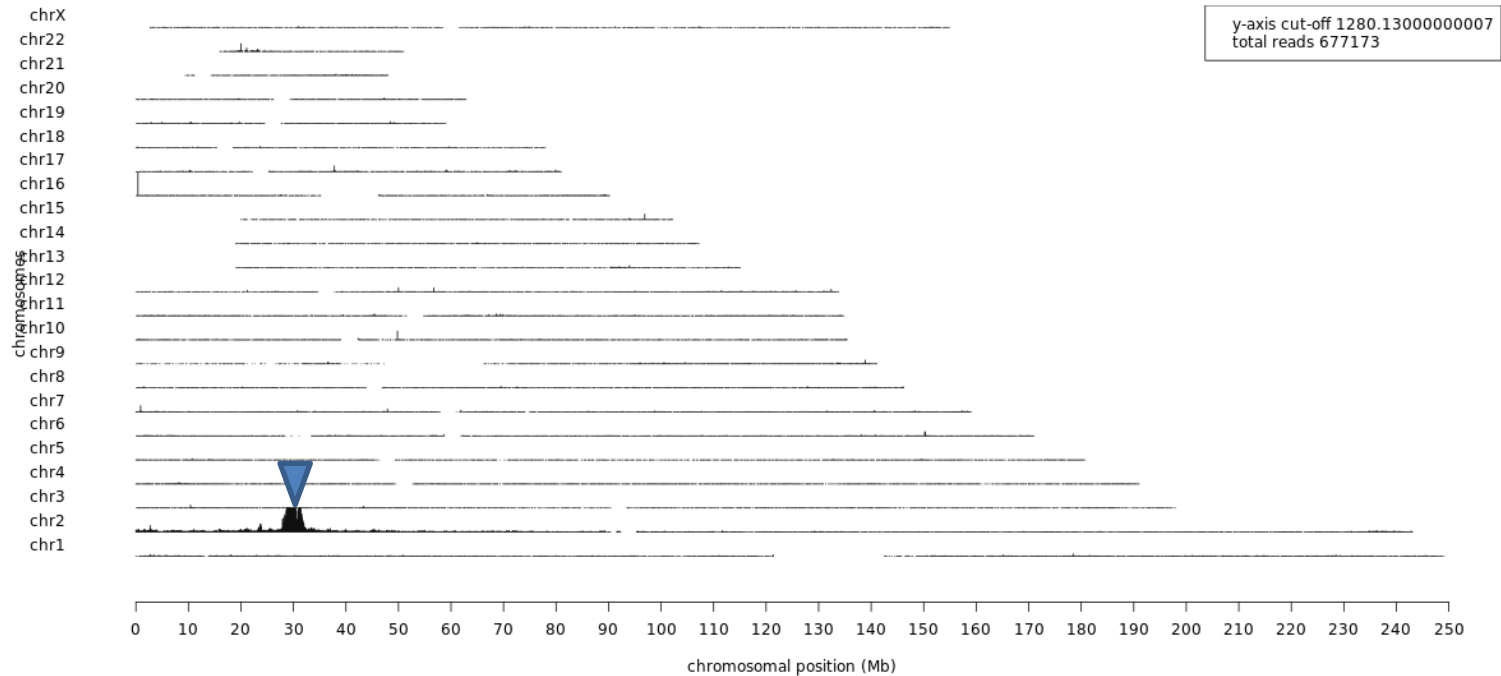
TLA Technology



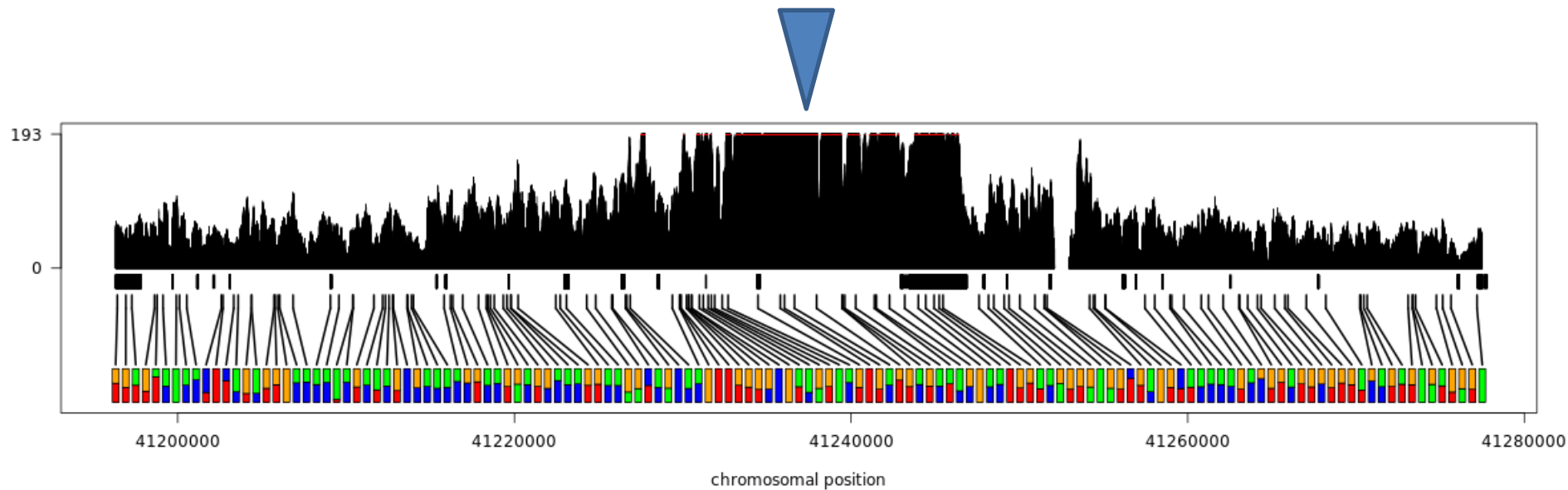
Conventional PCR



TLA Technology: ALK gene – human genome



TLA Technology: BRCA1 gene



TLA: complete gene sequencing 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
Shira Casadei, PhD
Kathryn Hale Gault, BS
Elizabeth Swabor, MD
Sunday M. Sway, BS
Jake Higgins, BS
Kevin C. Beach, BS
From: *Mol Cell Biol*, 2011

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*.

Results: Between 2002-2005, probands from 300 US breast or ovarian cancer but with negative (wild-type) *BRCA1* and *BRCA2* were screened by multiple DNA-based methods to detect genomic rearrangements in *BRCA1* and *BRCA2* loci in *CHEK2*, *TP53*, and *PTEN*. Evidently undetected germline mutations in *BRCA1*, that predispose to breast cancer, frequencies of these negative genetic test results.

52 (17%) carried previously undetected mutations, rearrangements of *BRCA1* or *BRCA2* (44 (8%)) with *TP53* mutations. All *BRCA1* and *BRCA2* 22 alterations found, of size less than 1 kb to greater than previously described and all were individually rare. All deletion was discovered in 2 families of Czechoslovak descent in 8 of 631 (1.3%) patients with breast cancer in the Czech and Slovak Republics. For all resequencing were determined and diagnostic, primary mutations included cases of childhood sarcoma or late cases of breast cancer.

Spectra of *BRCA1* and *BRCA2* include many high-frequency rearrangements. Among patients with breast 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.

www.jco.org

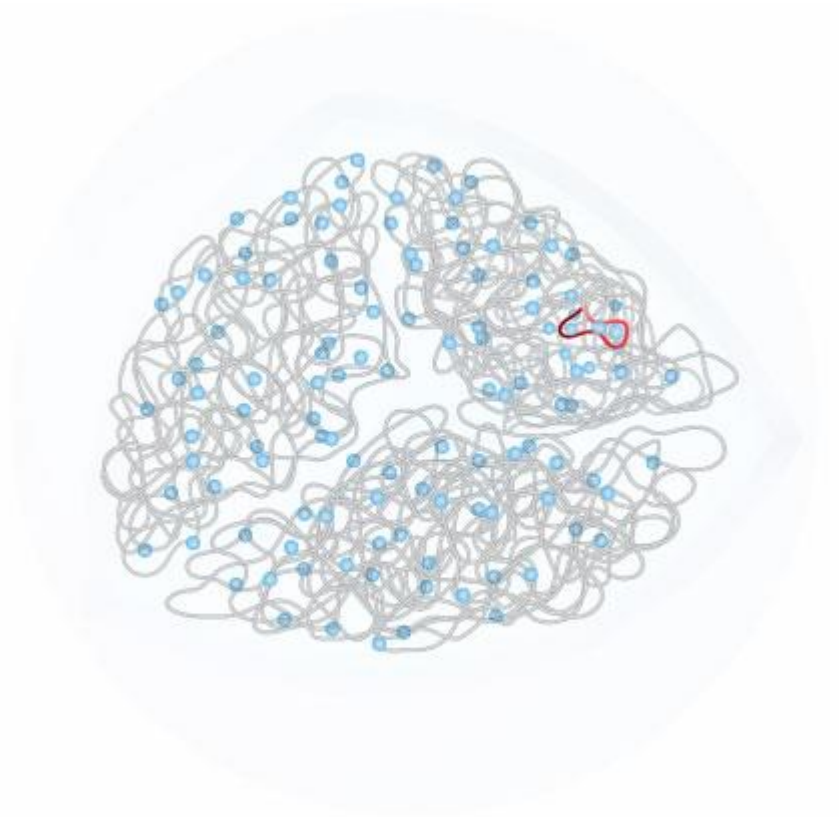
Author Affiliations are listed at the end of this article.
Corresponding Author: Tom Walsh, PhD, Center for Genome Sciences and Policy, University of Pennsylvania, 3737 Locust Walk, Philadelphia, PA 19104-6218.
E-mail: walsh@genetics.upenn.edu

Received JAMA, March 22, 2011; 205(12):1671-1679. DOI: 10.1001/jama.2011.1111

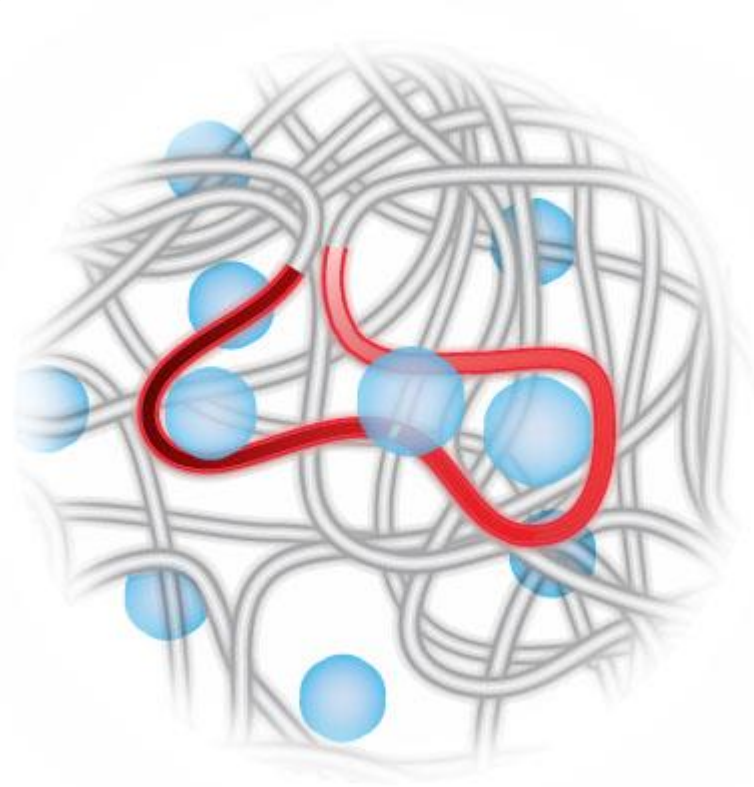
Conclusions The mutational spectra of *BRCA1* and *BRCA2* include many high-penetrance, individually rare genomic rearrangements. Among patients with breast cancer and severe family histories of cancer who test negative (wild type) for *BRCA1* and *BRCA2*, approximately 12% can be expected to carry a 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.



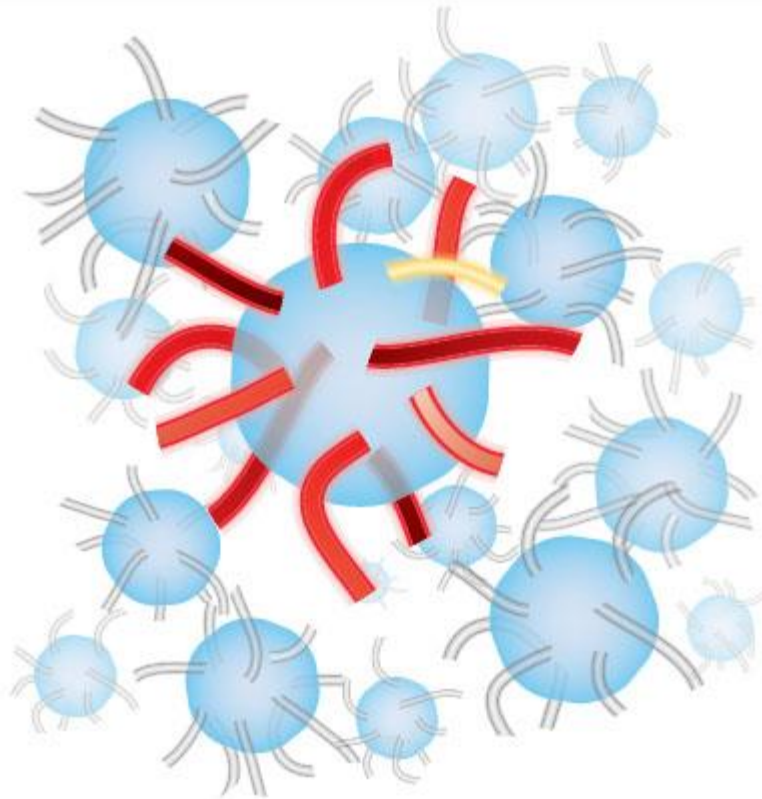
TLA Technology



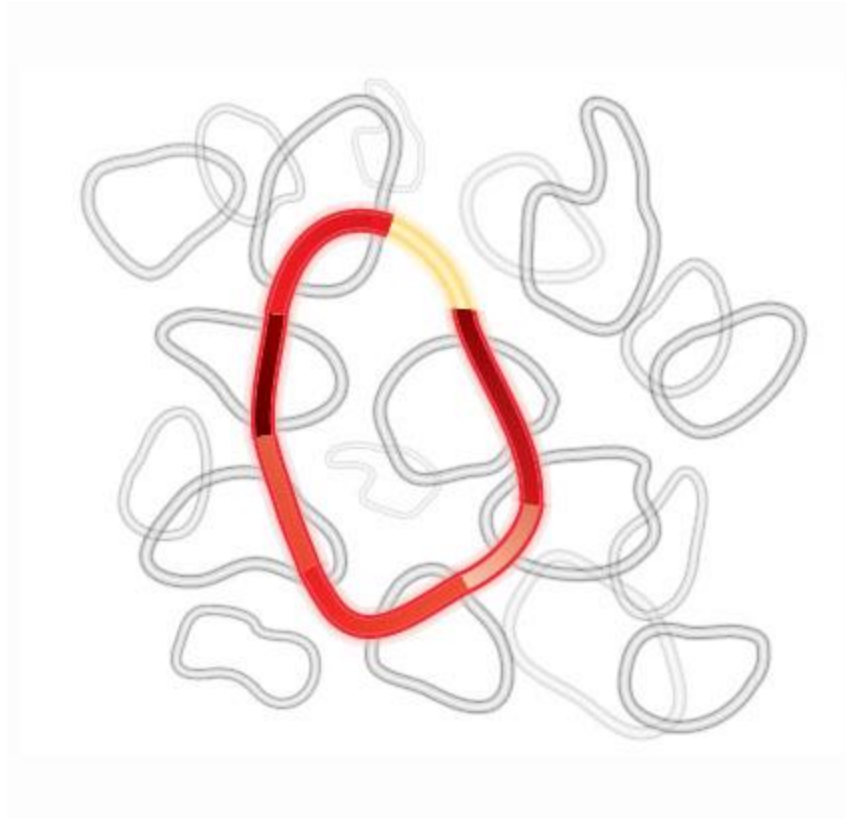
TLA Technology



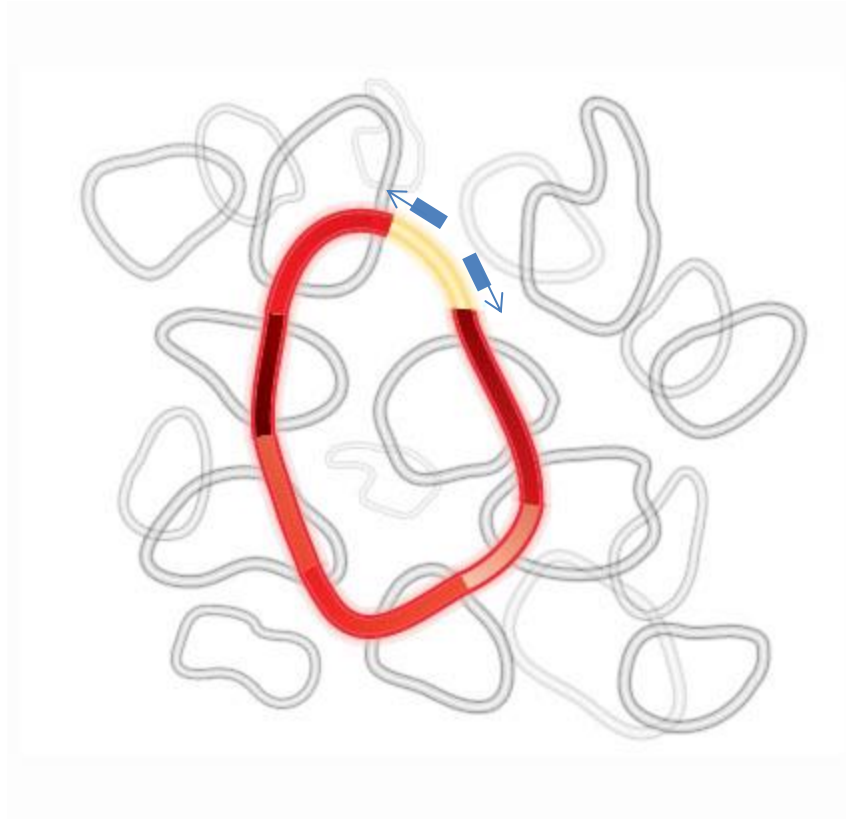
TLA Technology



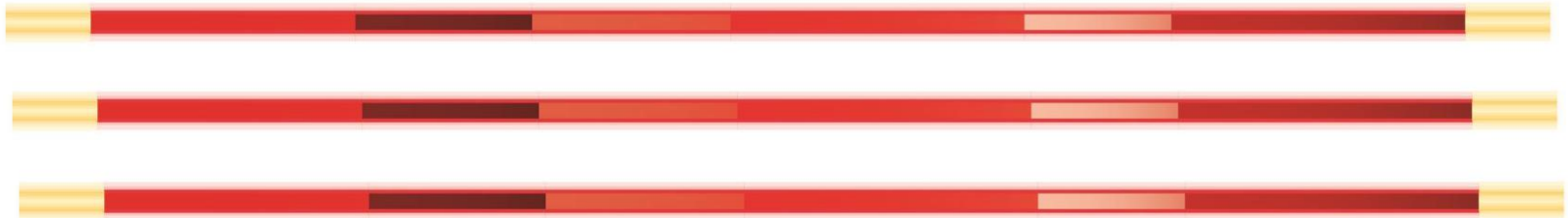
TLA Technology



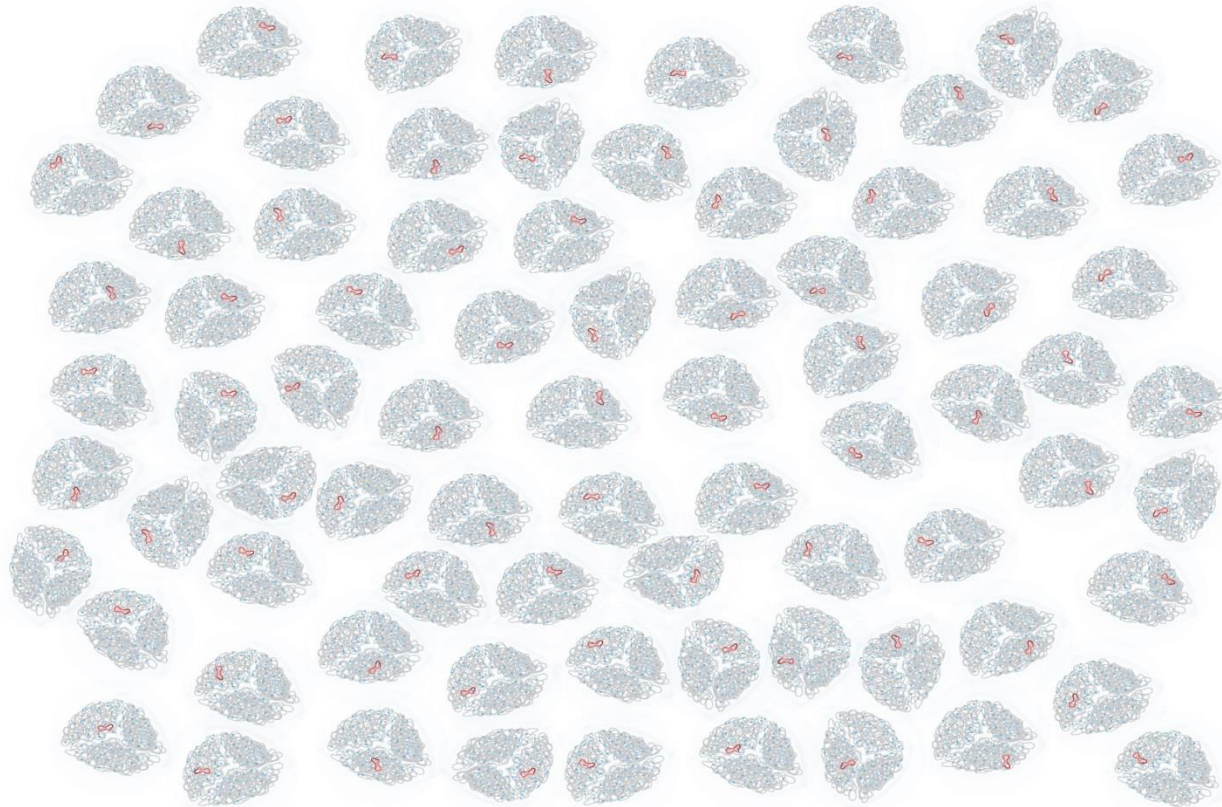
TLA Technology



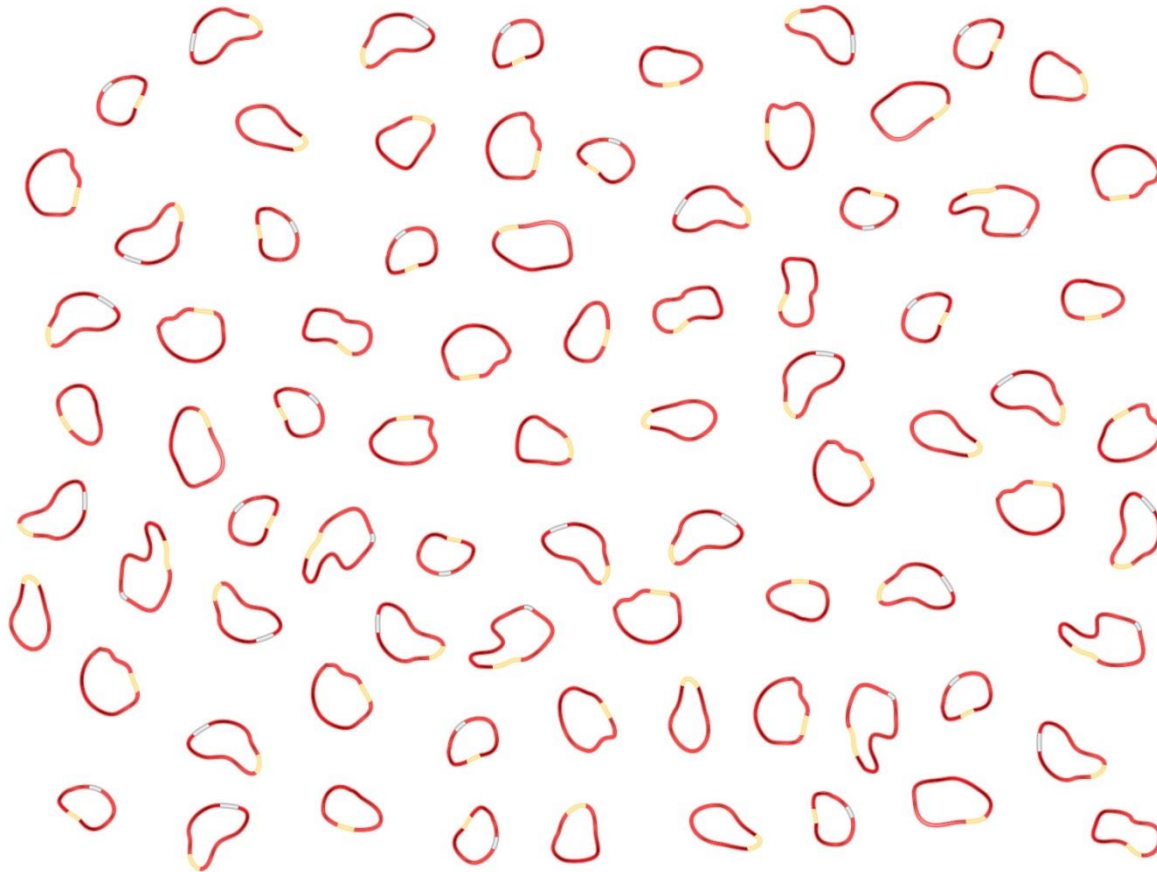
TLA Technology



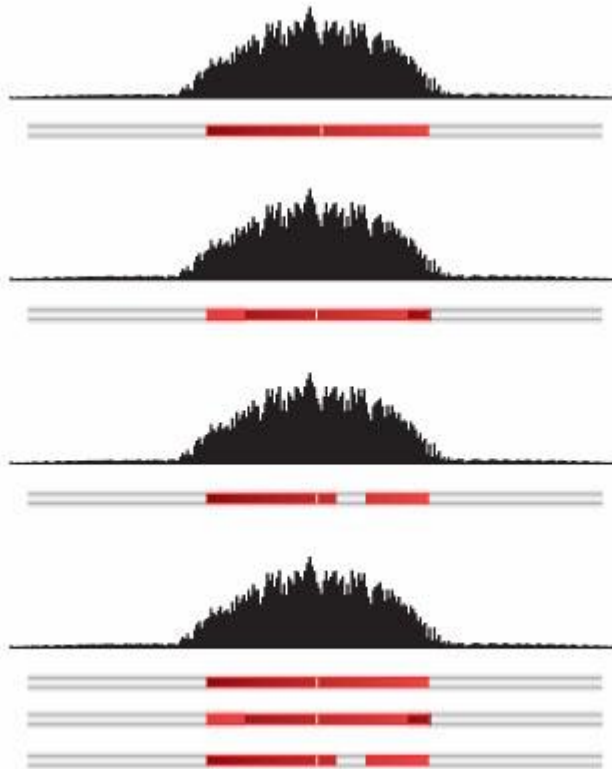
TLA Technology



TLA Technology

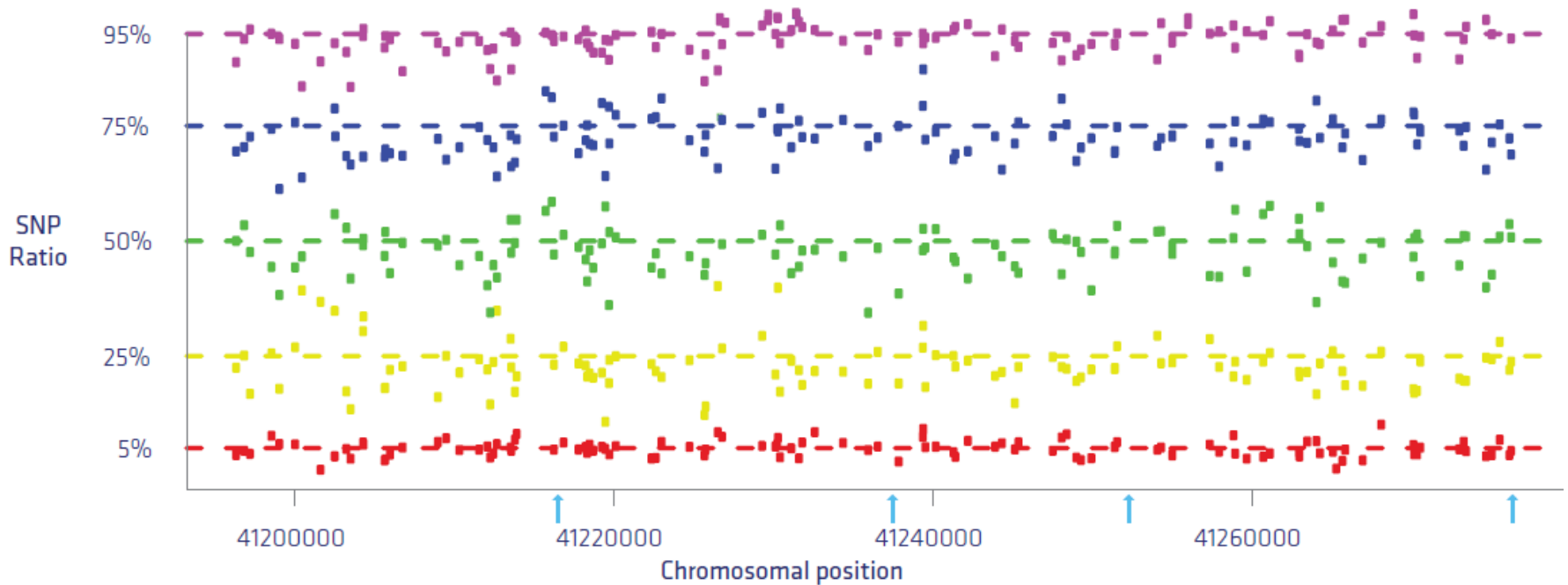


TLA Technology



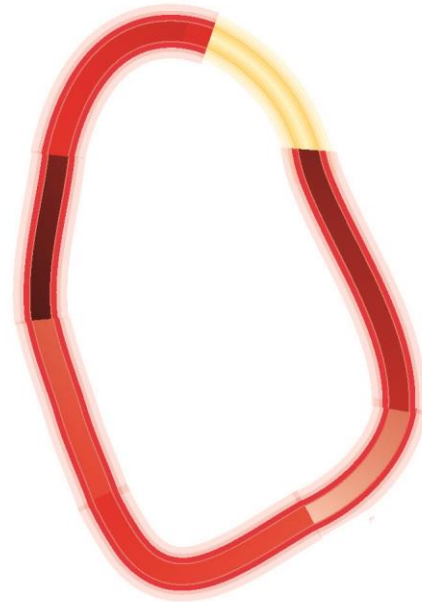
Complex cell mixtures

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

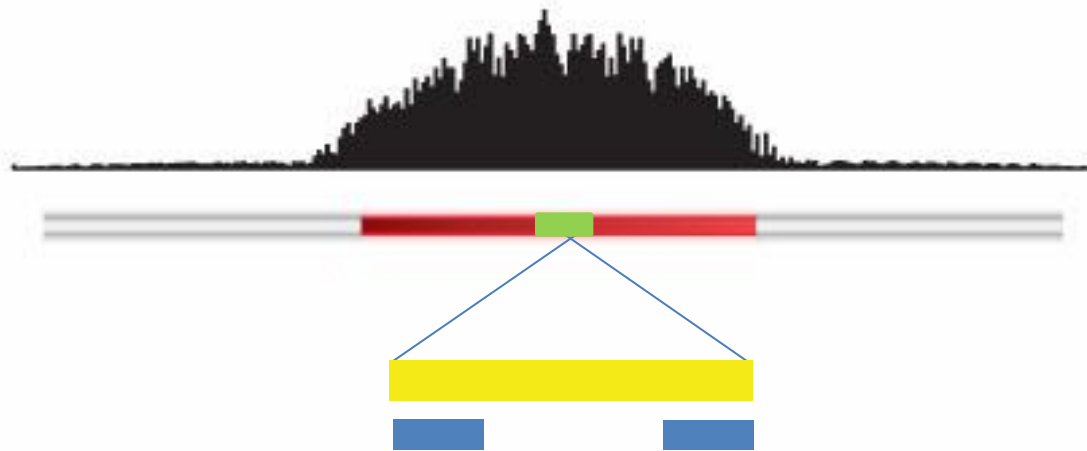


TLA Technology & haplotyping

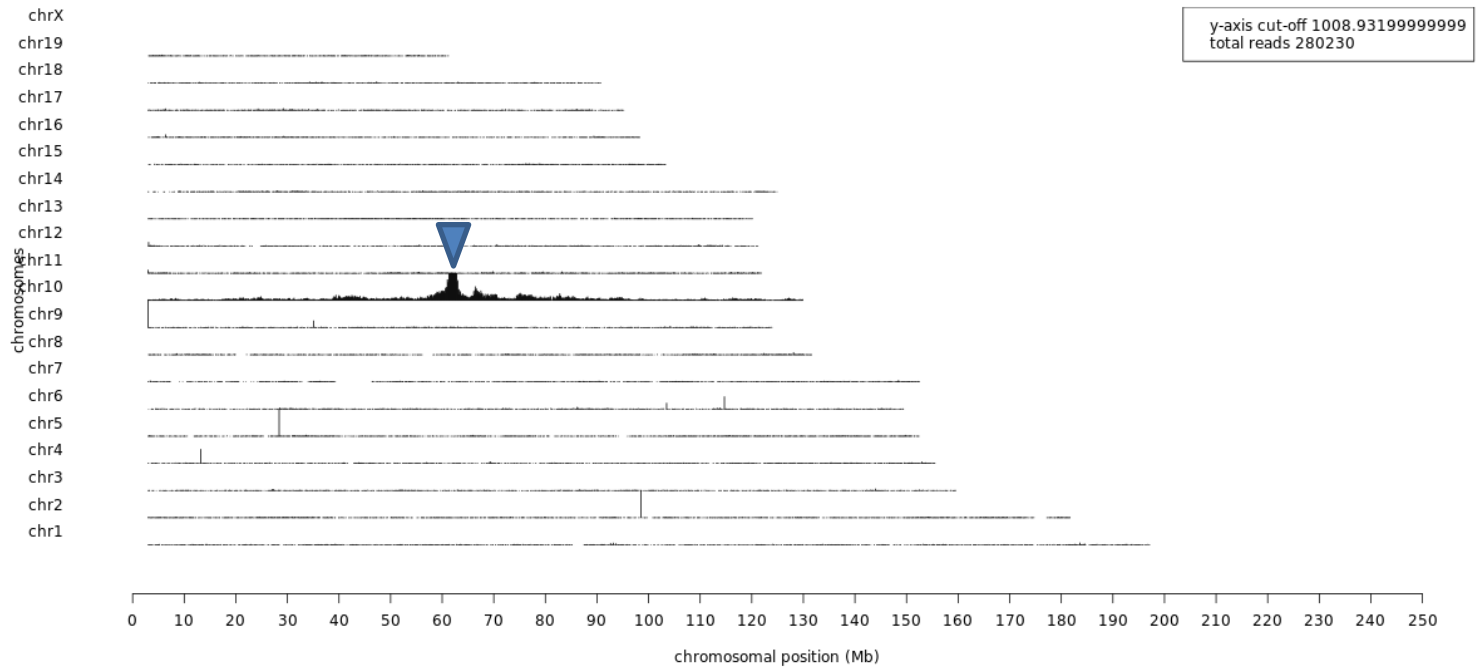
- Paired-end sequencing
- Paired ends from same originate from allele



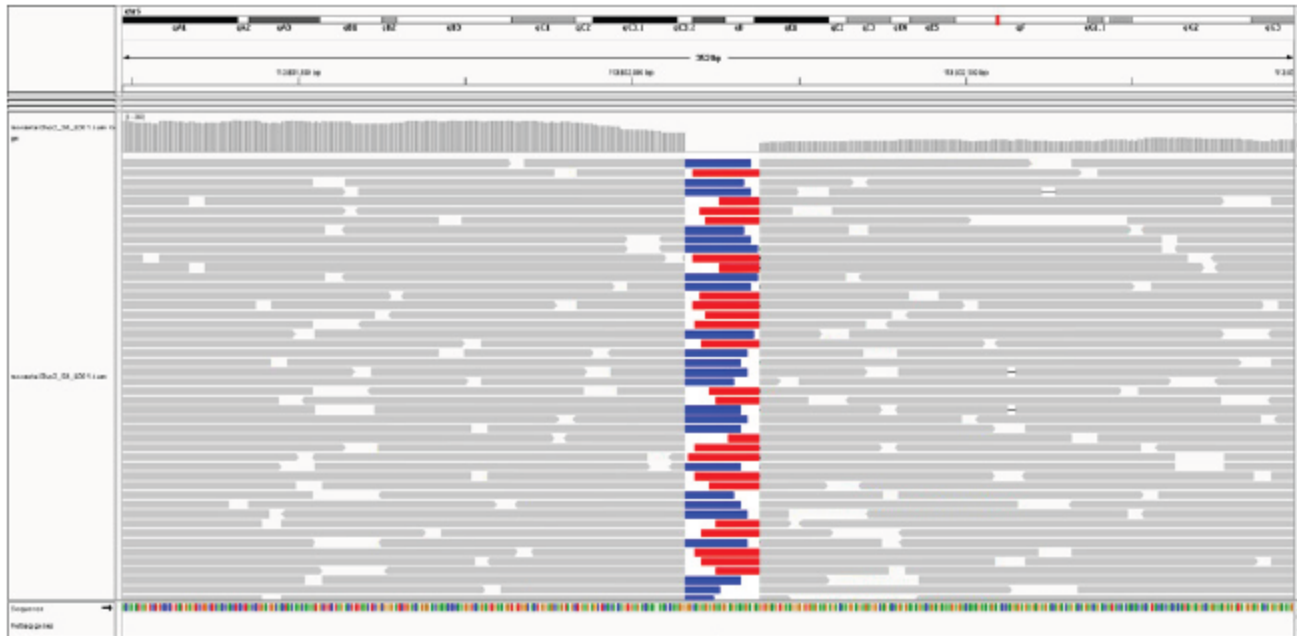
TLA Technology & Transgene sequencing



TLA Technology: transgene integration in mouse genome

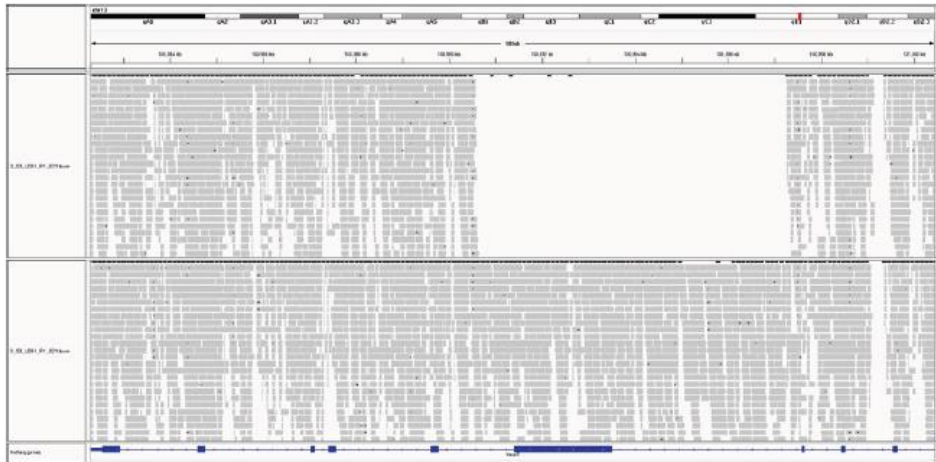


TLA Technology: transgene integration in mouse genome



TLA & Targeted Mouse knock-out sequencing

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



TLA & Gene-fusions

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



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.



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





CERGENTIS

COMPLETE GENE SEQUENCING

NOVEMBER
26TH 2013
HOLIDAY INN
LEIDEN



LIFE SCIENCE
TECHNOLOGY
EVENT 2013