# TLA technology for targeted complete Next Generation Sequencing of (trans)genes and gene editing events in plants

**Judith Bergboer, Scientific Account Manager Cergentis**

**Rotterdam, May 14, 2018**

**6th Plant Genomics & Gene Editing Congress**

CERGENTIS

COMPLETE GENE SEQUENCING

CERGENTIS
COMPLETE GENE SEQUENCING

# Outline

- Intro Cergentis
- Next Generation Sequencing
- TLA Technology
- Transgene, Integration Site and Gene-editing Sequencing
- Haplotyping
- Services and Kits

Cergentis

CERGENTIS
COMPLETE GENE SEQUENCING

# Cergentis

- Founded July 2012
- Based in Utrecht, the Netherlands

# Cergentis' business model

- Services

- Kits

CERGENTIS
COMPLETE GENE SEQUENCING

# Cergentis' business model

- Services
  - Routine or Tailored

- Kits

CERGENTIS
COMPLETE GENE SEQUENCING

# Cergentis' business model

- Services
  - Routine or Tailored
- Kits
  - Manual or Automate

CERGENTIS
COMPLETE GENE SEQUENCING

# Applications of TLA

Transgenes & Gene editing

Leukaemia & Oncogenetics

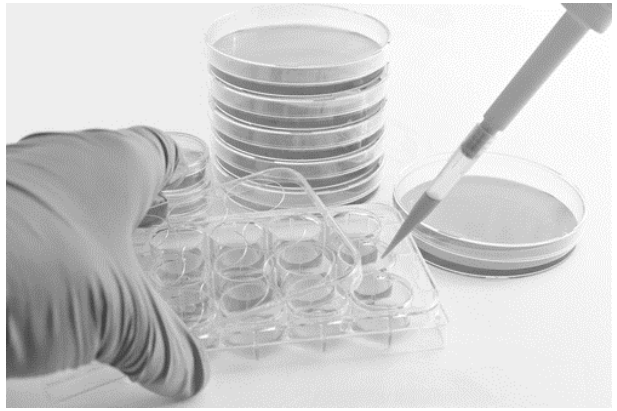
Genetic diagnostics

Agrigenomics

CERGENTIS
COMPLETE GENE SEQUENCING

# Applications of TLA

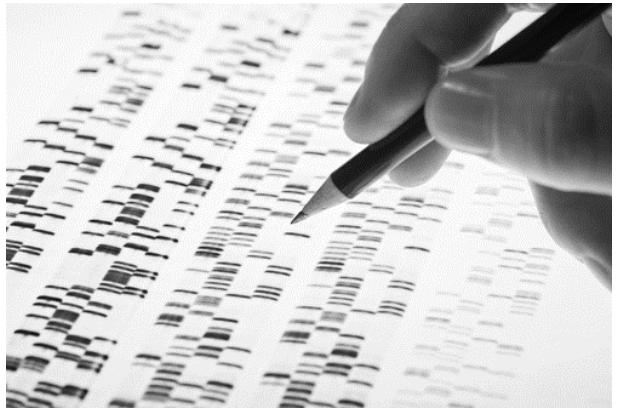
Co-funded by the Horizon 2020 programme of the European Union



Transgenes & Gene editing

Leukaemia & Oncogenetics

Cergentis awarded Horizon 2020 grant to advance TLA-based targeted complete NGS for cancer companion diagnostics



Genetic diagnostics

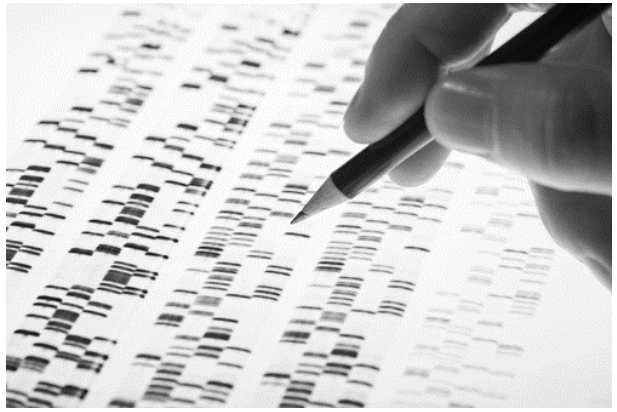
Agrigenomics

CERGENTIS
COMPLETE GENE SEQUENCING

# Applications of TLA

Transgenes & Gene editing

Leukaemia & Oncogenetics



Genetic diagnostics

Agrigenomics

# Next Generation Sequencing (NGS)

CERGENTIS
COMPLETE GENE SEQUENCING

CERGENTIS
COMPLETE GENE SEQUENCING

# Next Generation Sequencing

- Generates millions of (short) “reads” from input DNA.
- https://en.wikipedia.org/wiki/DNA_sequencing

CERGENTIS
COMPLETE GENE SEQUENCING

# Next Generation Sequencing

ACGTCGGTATCGTATCGTACGTATTGCACGTACGTACGTTTGGCAAACCCTGTTGTACACACTGTGATAGCTACGAGCATGACGATCAGCGATCGAG Read

CERGENTIS
COMPLETE GENE SEQUENCING

# Next Generation Sequencing

Read

CERGENTIS
COMPLETE GENE SEQUENCING

# Next Generation Sequencing

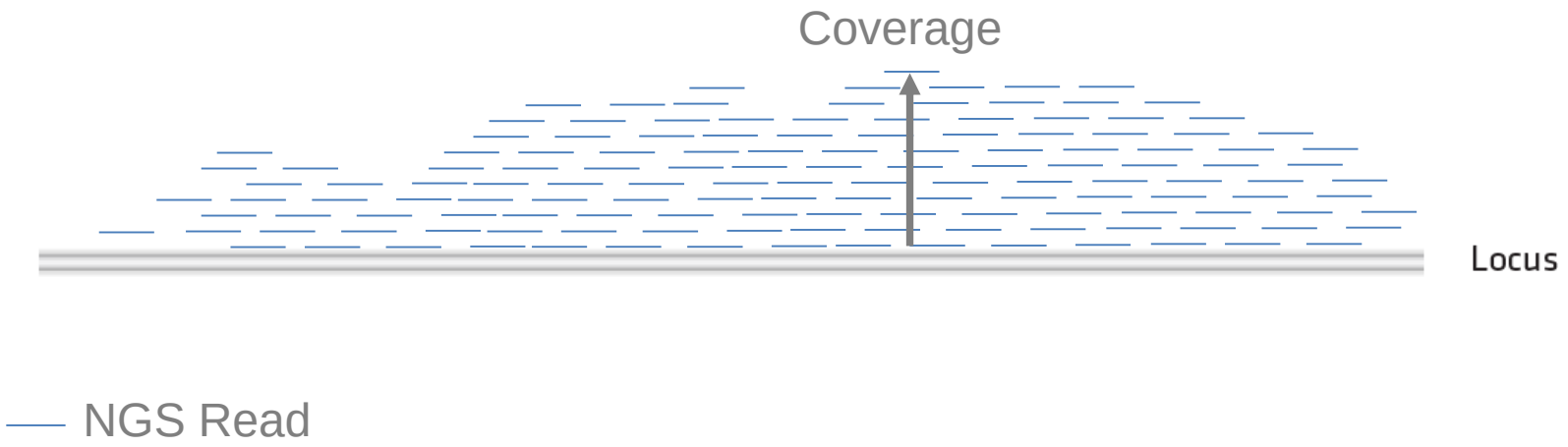
CERGENTIS
COMPLETE GENE SEQUENCING

# Next Generation Sequencing


NGS Read

CERGENTIS
COMPLETE GENE SEQUENCING

# Next Generation Sequencing



# TLA Technology

CERGENTIS
COMPLETE GENE SEQUENCING

CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology

- Targeted Locus Amplification
- Targeted, low-cost Next Generation Sequencing
- Cell-based and Genomic DNA protocols
- Requires 2x20bp sequence information
- Physical proximity as basis of selection
- Compatible with all NGS Technologies
- Suitable for multiplexing
- Critical advantages:
  - Highly flexible
  - Complete
  - Hypothesis neutral
  - Enables haplotyping


nature biotechnology

ARTICLES

Targeted sequencing by proximity ligation for comprehensive variant detection and local haplotyping

Paula J P de Vree[1,16], Elzo de Wit[1,2,16], Mehmet Yilmaz[2], Monique van de Heijning[2], Petra Klous[2], Marjon J A M Verstegen[1], Yi Wan[1], Hans Teunissen[1], Peter H L Krijger[1], Geert Geeven[1], Paul P Eijk[3], Daoud Sie[3], Bauke Ylstra[3], Lorette O M Hulsman[4], Marieke F van Dooren[4], Laura J C M van Zutven[4], Ans van den Ouweland[4], Sjef Verbeek[5,6], Marion Cornelissen[7], Atze T Das[7], Ben Berkhout[7], Birgit Sikkema-Raddatz[8], Eva van den Berg[8], Pieter van der Vlies[8], Desiree Weening[8], Johan T den Dunnen[9], Magdalena Matusiak[10,11], Mohamed Lamkanfi[10,11], Marjolijn J L Ligtenberg[12], Petra ter Brugge[13], Jos Jonkers[13], John A Foekens[14], John W Martens[14], Rob van der Luijt[15], Hans Kristian Ploos van Amstel[15], Max van Min[2], Erik Splinter[2] & Wouter de Laat[1,2]



Despite developments in targeted gene sequencing and whole-genome analysis techniques, the robust detection of all genetic variation, including structural variants, in and around genes of interest and in an allele-specific manner remains a challenge. Here we present targeted locus amplification (TLA), a strategy to selectively amplify and sequence entire genes on the basis of the crosslinking of physically proximal sequences. We show that, unlike other targeted re-sequencing methods, TLA works without detailed prior locus information, as one or a few primer pairs are sufficient for sequencing tens to hundreds of kilobases of surrounding DNA. This enables robust detection of single nucleotide variants, structural variants and gene fusions in clinically relevant genes, including *BRCA1* and *BRCA2*, and enables haplotyping. We show that TLA can also be used to uncover insertion sites and sequences of integrated transgenes and viruses. TLA therefore promises to be a useful method in genetic research and diagnostics when comprehensive or allele-specific genetic information is needed.

Current methods in genetic diagnostics and research are limited in their ability to uncover all possible genetic variation in genes of interest[1]. Clinical genetic tests, for example, often focus only on exons and therefore miss variants in the noncoding regulatory sequences (promoters and enhancers) of genes that can also disrupt gene function[2]. In addition, structural variants—such as copy number variants, translocations that are not accompanied by a loss or gain of sequences). Also, balanced structural variants can cause disease through the disruption of genes, creation of gene fusions or position effects—i.e., when a gene is placed under the control of different regulatory DNA elements[3,4]. Editable detection of structural variants is hampered by the hypothesis-driven nature of current methods for targeted re-sequencing, in which the sequences to be analyzed are determined by the set of probes used in hybridization-based capture methods[5] or the primers in polymerase or ligase-based re-sequencing approaches[6]. Unknown sequences, such as those introduced by chromosomal rearrangements, are difficult to capture and re-sequence with these methods[7,8]. In addition, none of the existing targeted sequencing methods allow haplotyping, the allelic phasing of genetic variation. This information is useful, for example, when a person carries multiple recessive genetic variants that may coexist on one allele (giving carrier status) or be divided over both alleles (giving disease status).

In chromosome-conformation capture (3C)[9] and related methods such as 3C on chip or combined with sequencing[10,11] (4C, which enables searching of the genome for sequences contacting a site of interest), chromatin is crosslinked, fragmented and re-ligated to identify on the basis of their ligation efficiency, genomic loci that are in close spatial proximity in the nucleus. Although these methods are used to detect genome folding inside cells, the resulting contact profiles typically show high enrichment of sequences directly neighboring

[1]Hubrecht Institute-KNAW and University Medical Center Utrecht, Utrecht, the Netherlands. [2]Cergentis B.V., Utrecht, the Netherlands. [3]Department of Pathology, VU University Medical Center, Amsterdam, the Netherlands. [4]Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, the Netherlands. [5]Department of Human Genetics, Leiden University Medical Center, Leiden, the Netherlands. [6]Department of Experimental Virology, Department of Medical Microbiology, Center for Infectious Diseases, Leiden University Medical Center, Leiden, the Netherlands. [7]Laboratory of Experimental Virology, Department of Medical Microbiology, Center for Infection and Immunity Amsterdam (CINIMA), Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands. [8]Department of Genetics, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands. [9]Leiden Genome Technology Center, Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, the Netherlands. [10]Department of Biochemistry, Ghent University, Ghent, Belgium. [11]Department of Molecular Biology, Ghent University, Ghent, Belgium. [12]Department of Pathology and Medical Genetics, Radboud University Medical Center, Nijmegen, the Netherlands. [13]Department of Molecular Pathology and Cancer Genetics, Erasmus MC Cancer Institute, Erasmus University Medical Center, Rotterdam, the Netherlands. [14]Department of Medical Oncology, Erasmus MC Cancer Institute, Erasmus University Medical Center, Rotterdam, the Netherlands. [15]Department of Medical Genetics, University Medical Center Utrecht, Utrecht, the Netherlands. [16]These authors contributed equally to this work. Correspondence should be addressed to E.S. (erik.splinter@cergentis.com) or W.d.L. (w.delaat@hubrecht.eu).

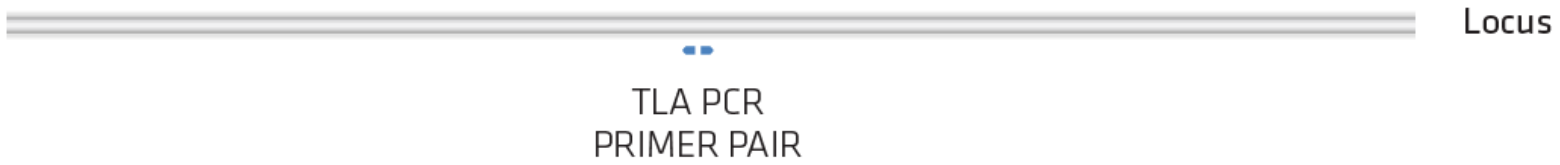
Received 26 November 2013; accepted 16 June 2014; published online 17 August 2014; doi:10.1038/nbt.2971

CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology

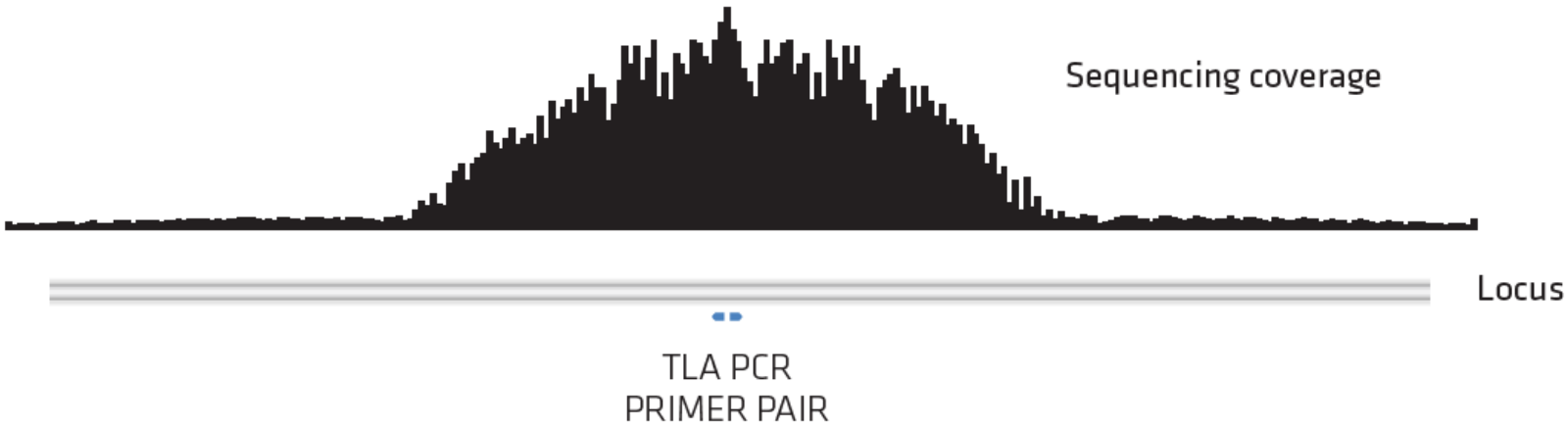
CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology



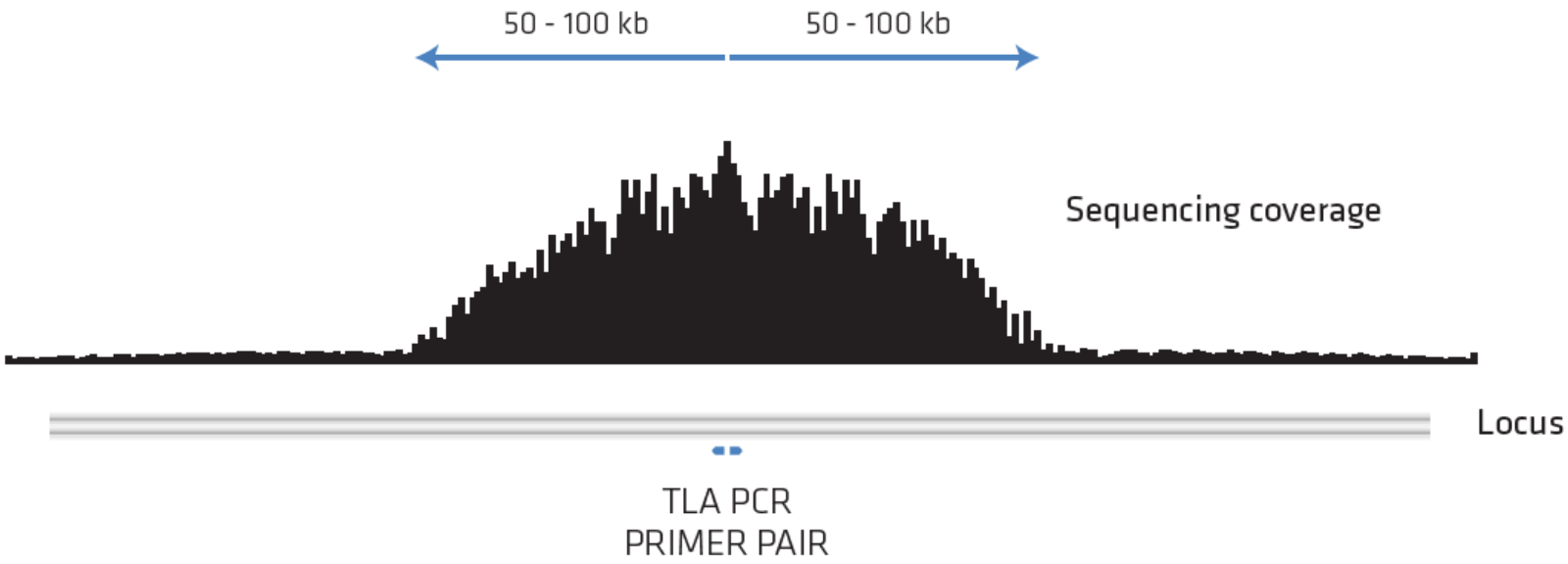
CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology



CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology



CERGENTIS
COMPLETE GENE SEQUENCING

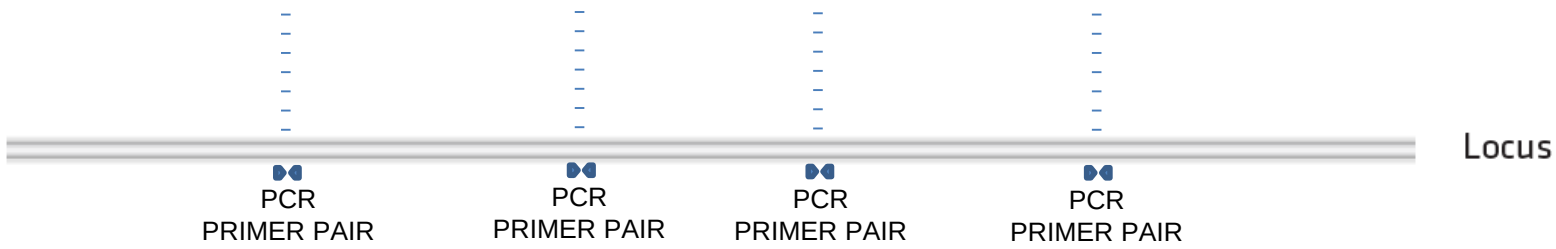
# Alternative: Whole Genome Sequencing

- Single genes represent 0.001% of genome
- Not cost effective
- Data difficult to interpret
- Relatively low coverage

CERGENTIS
COMPLETE GENE SEQUENCING

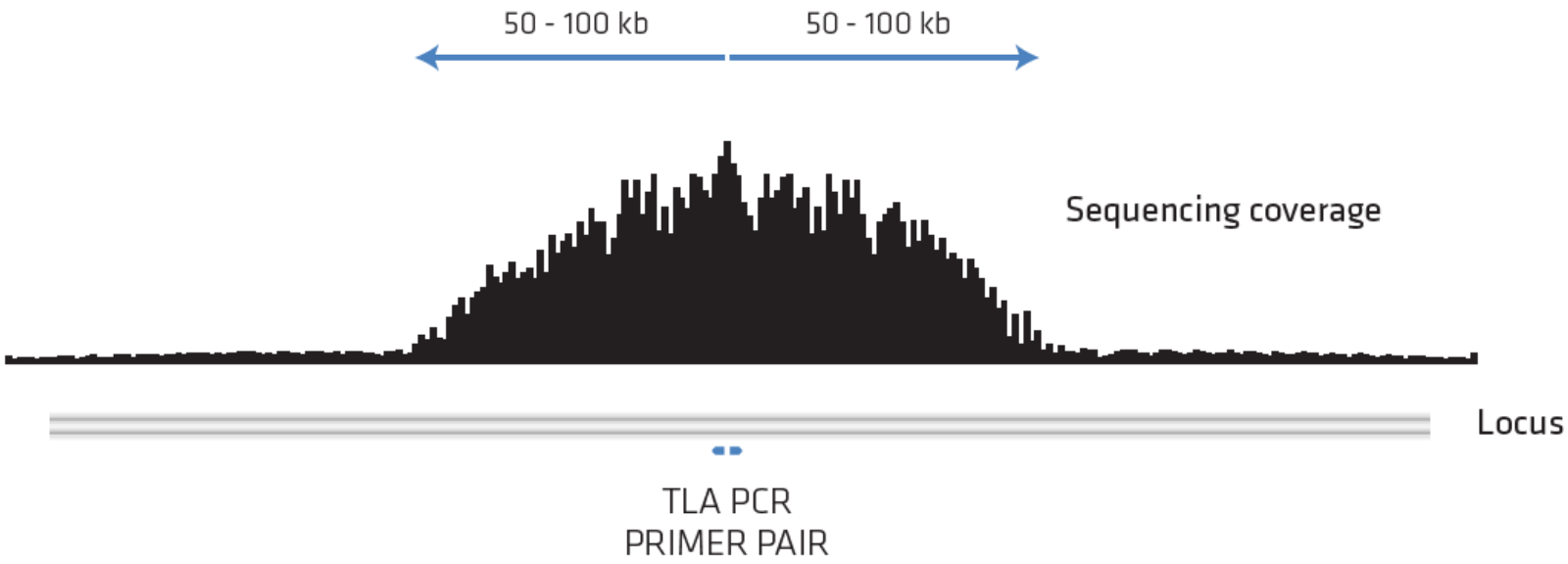
# Alternative: PCR/Capture

- Individual short pieces of sequence information
- Inherently, only known sequences are amplified



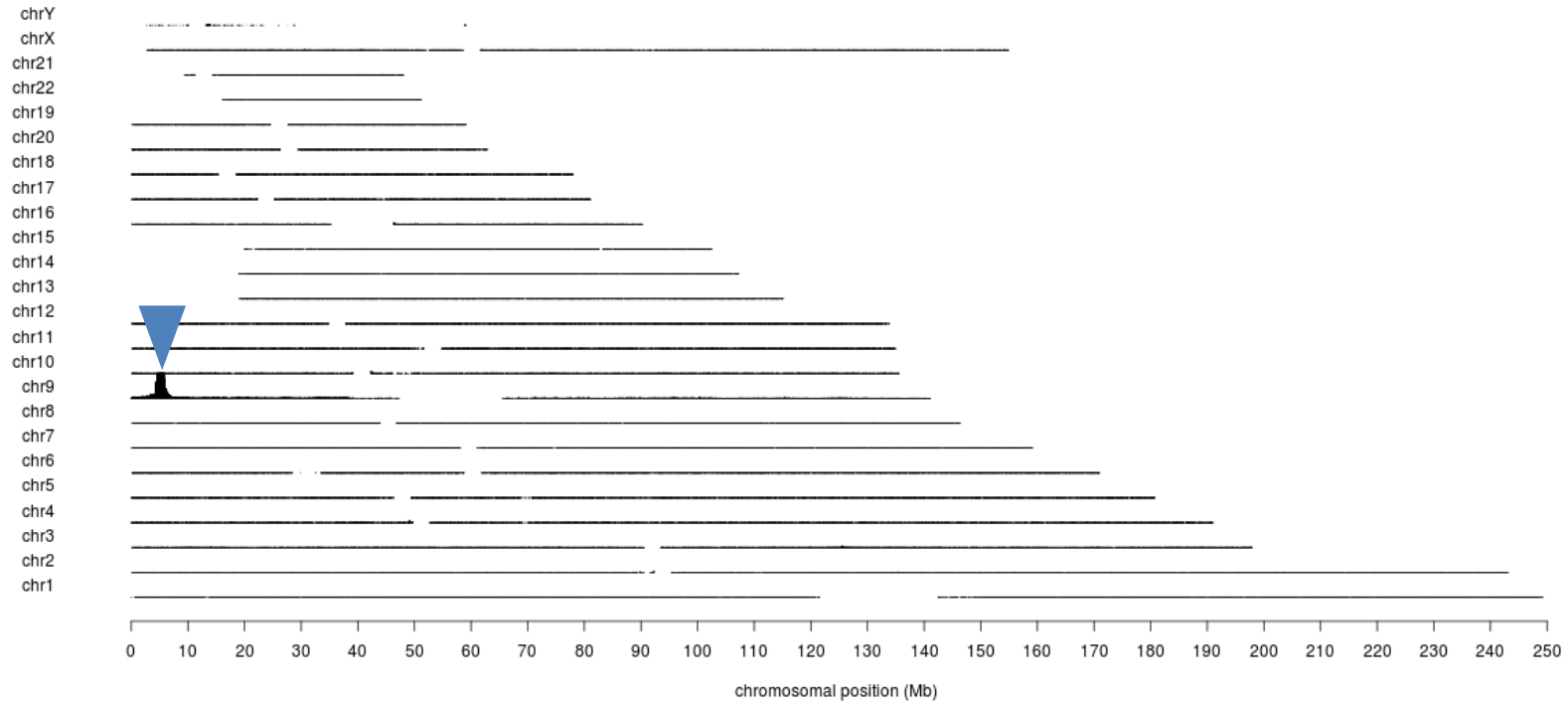
CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology



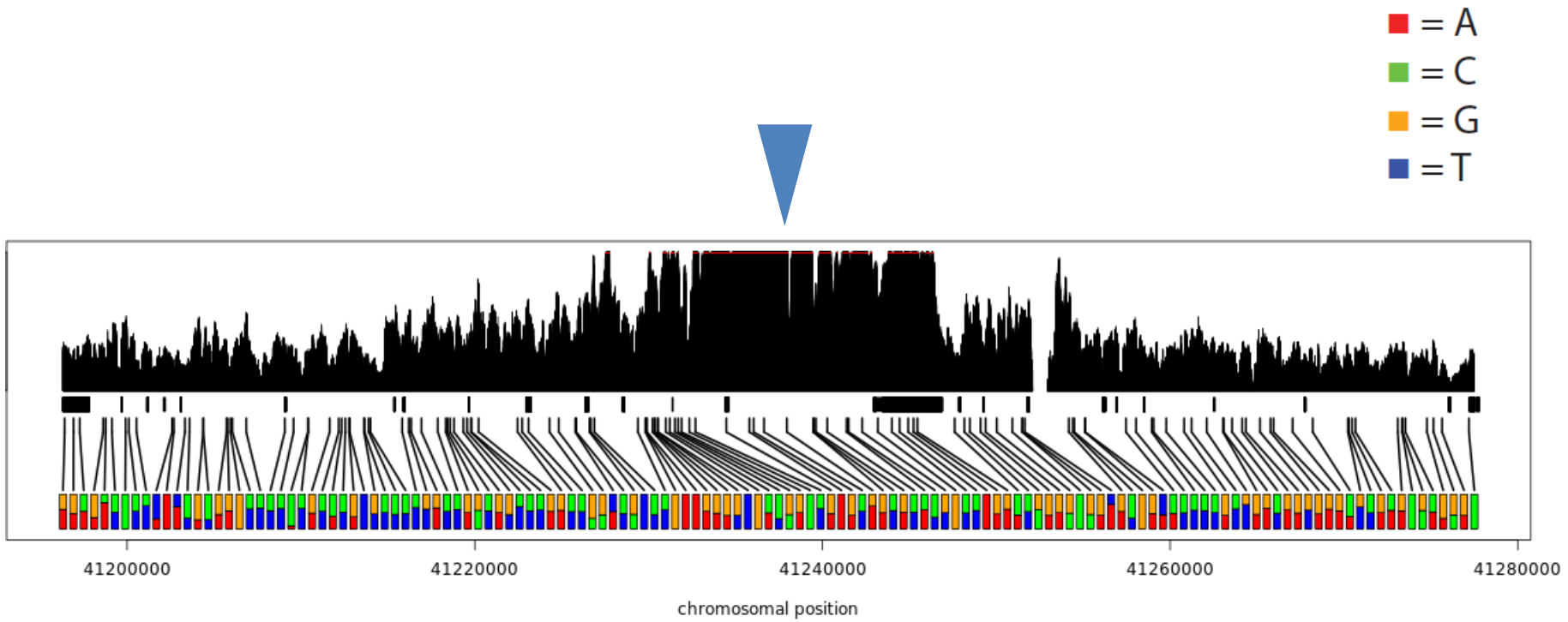
CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology: JAK gene – human genome



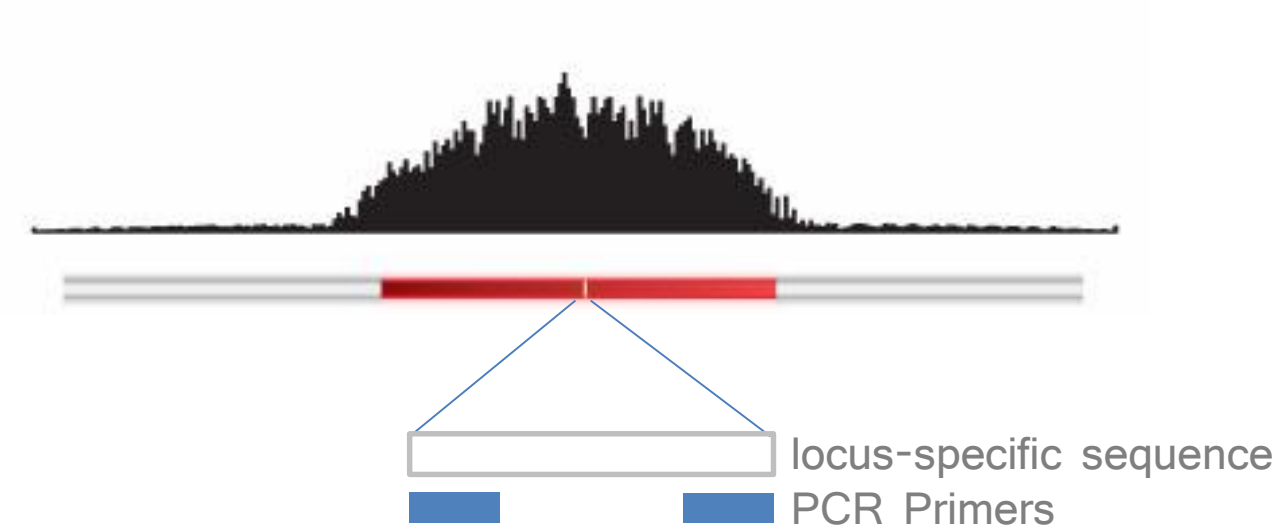
CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology: BRCA1 gene



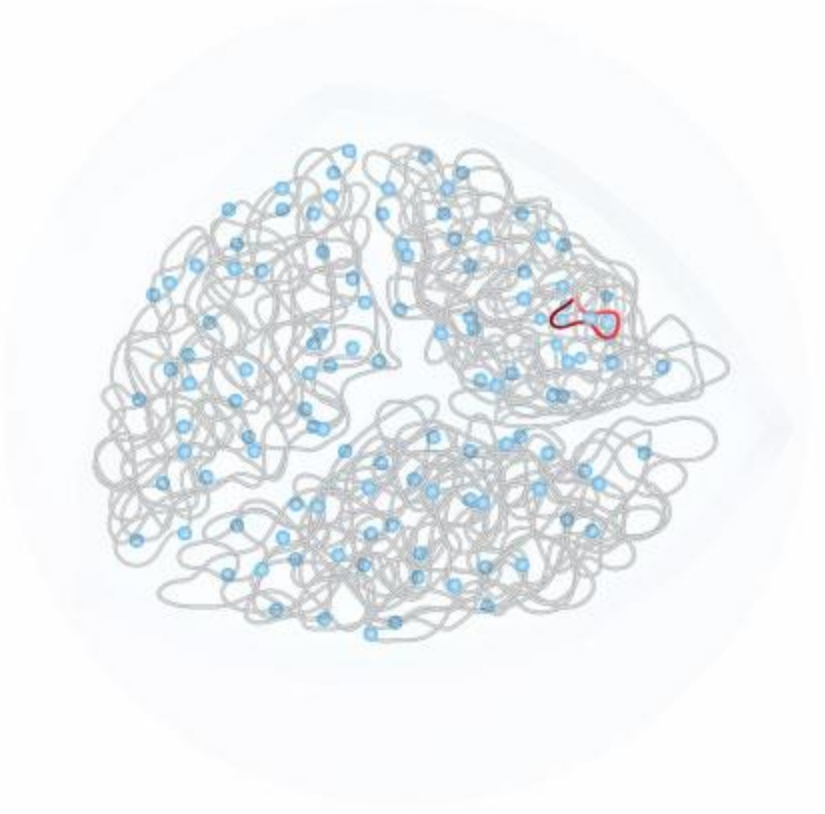
CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology



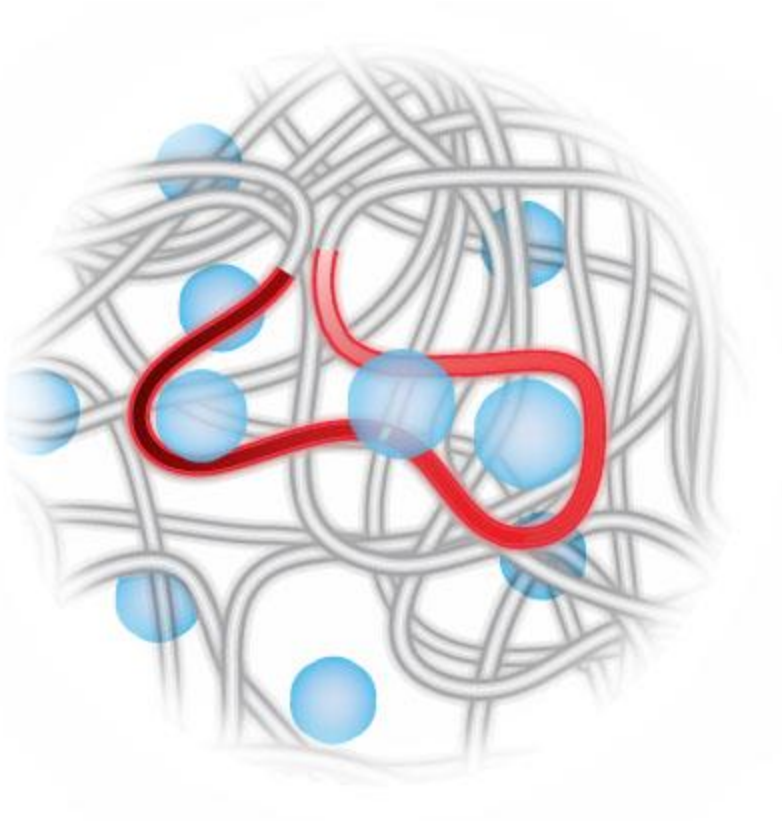
CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology



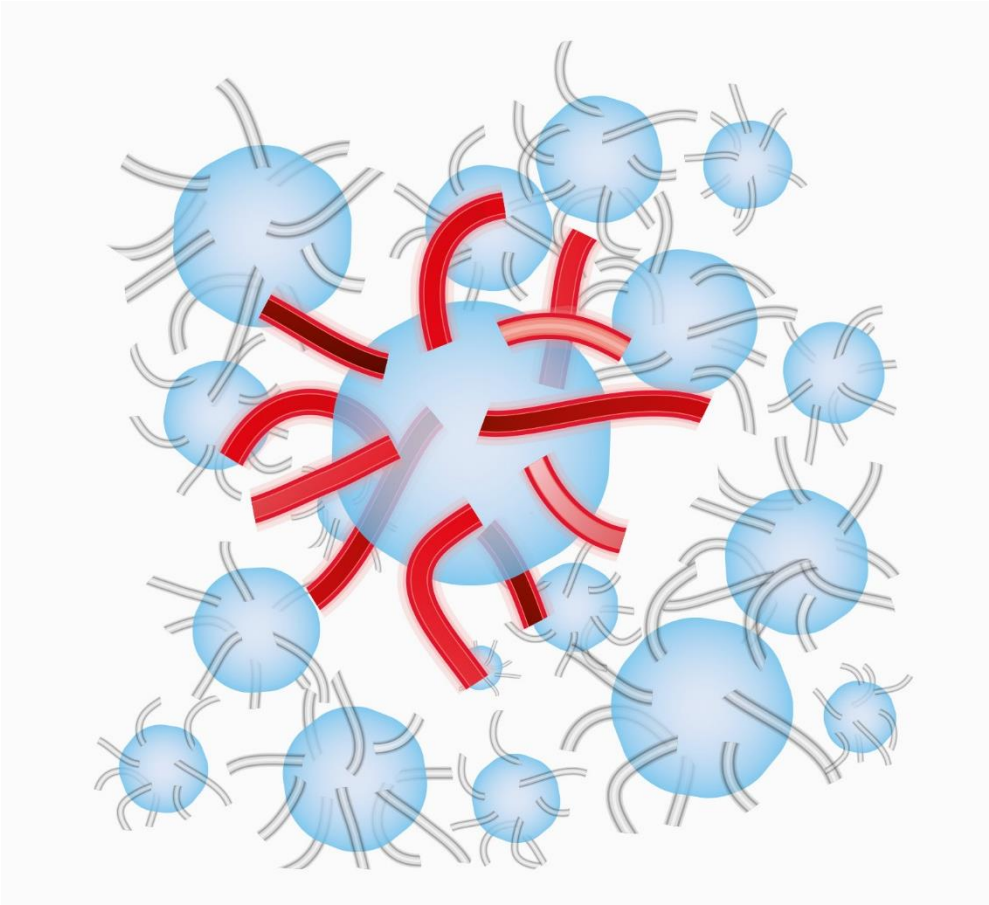
CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology



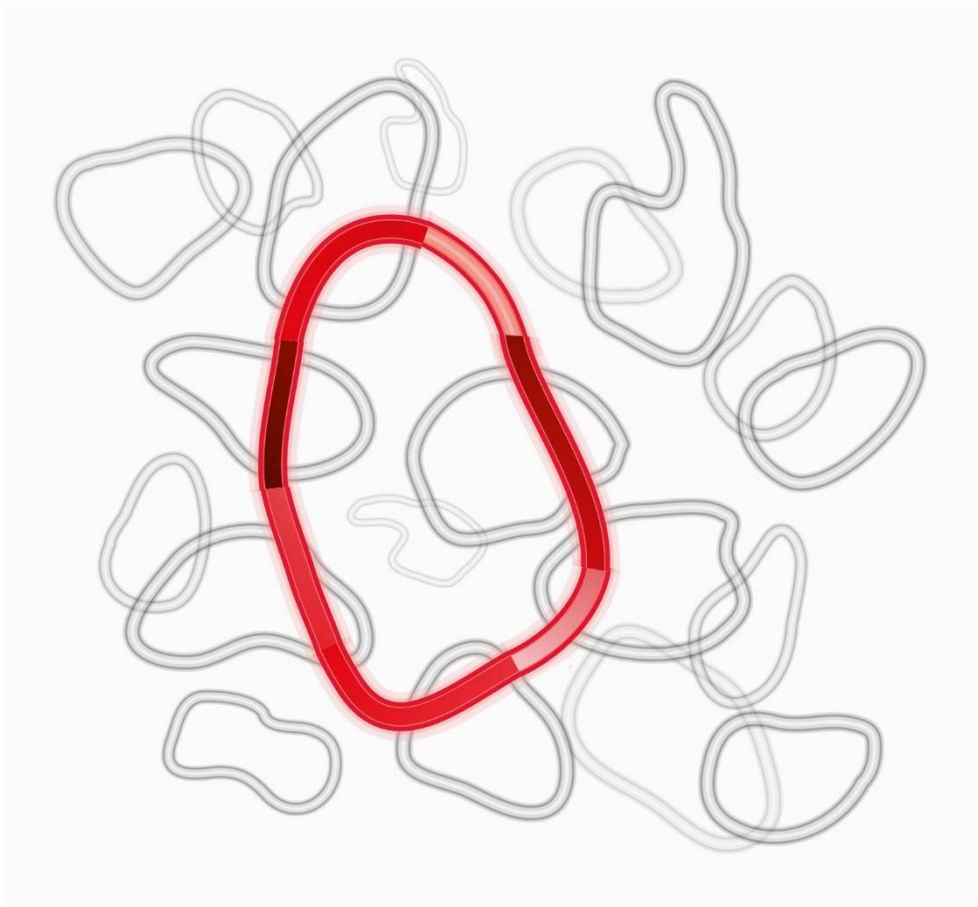
CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology



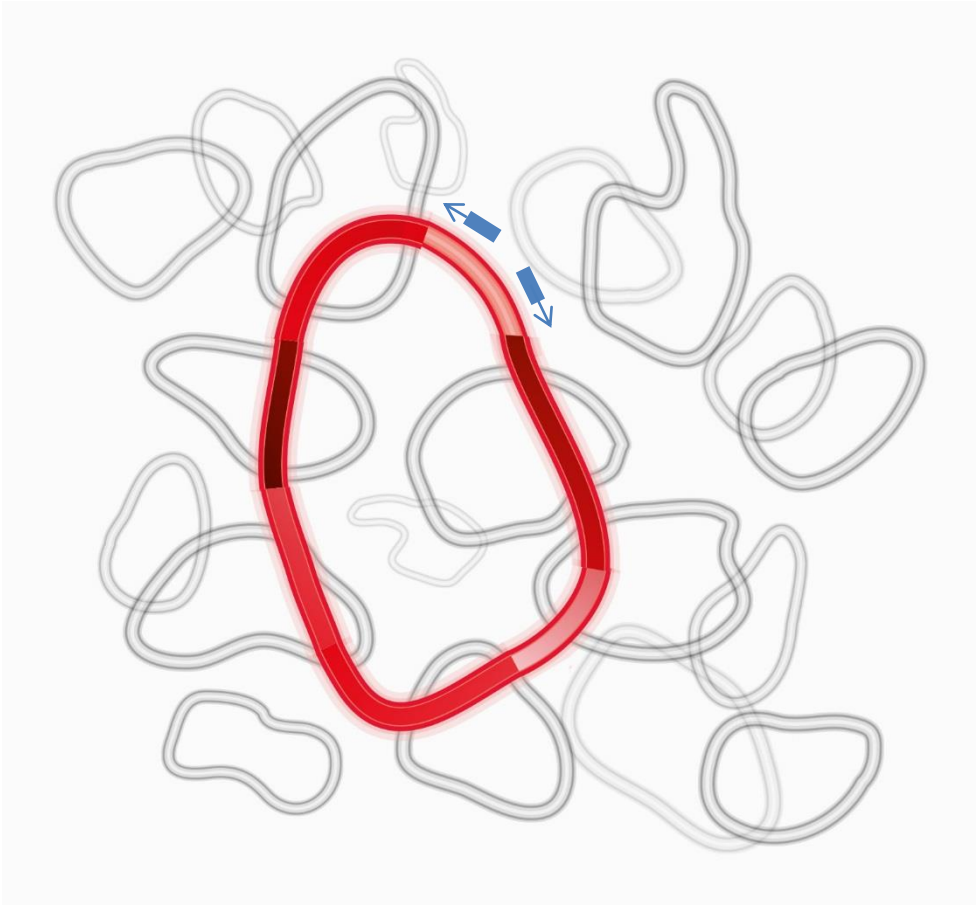
CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology



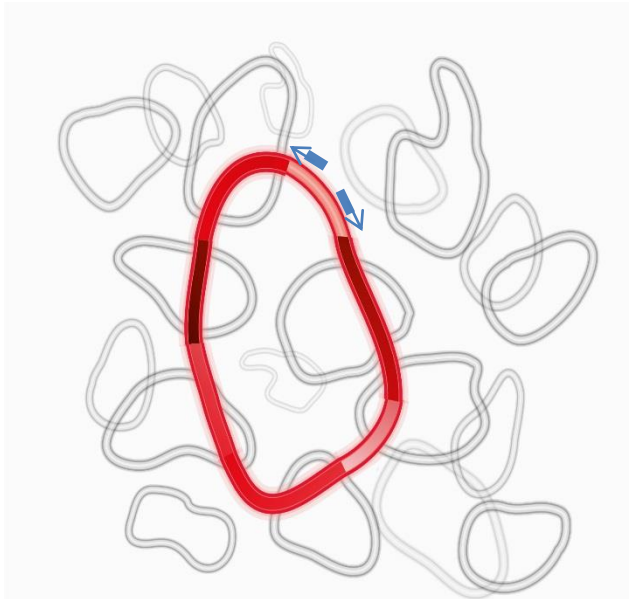
CERGENTIS
COMPLETE GENE SEQUENCING

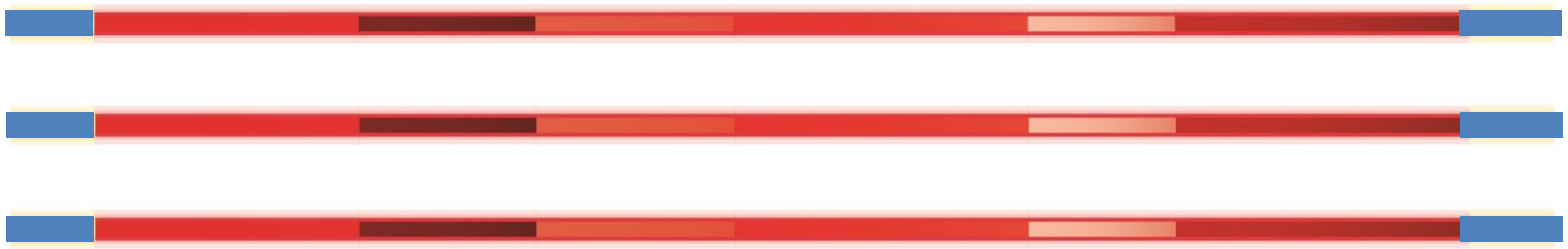
# TLA Technology



CERGENTIS
COMPLETE GENE SEQUENCING

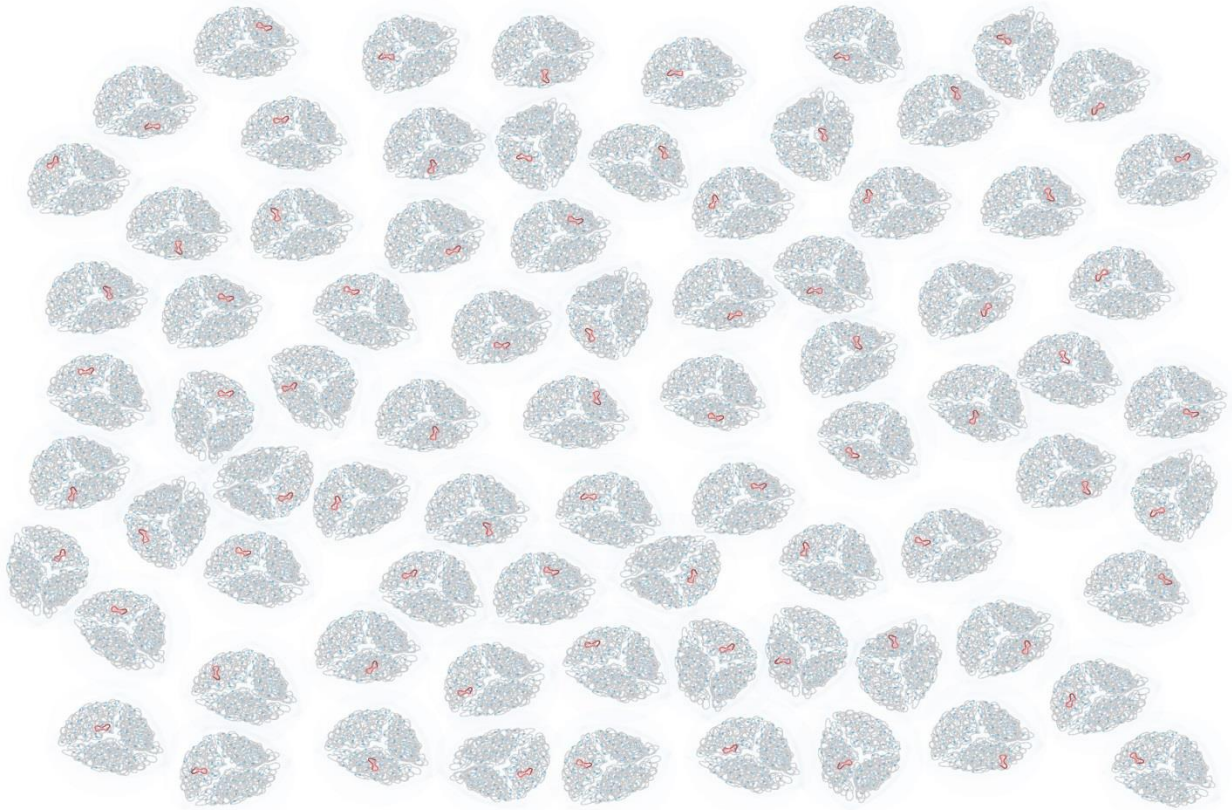
# TLA Technology





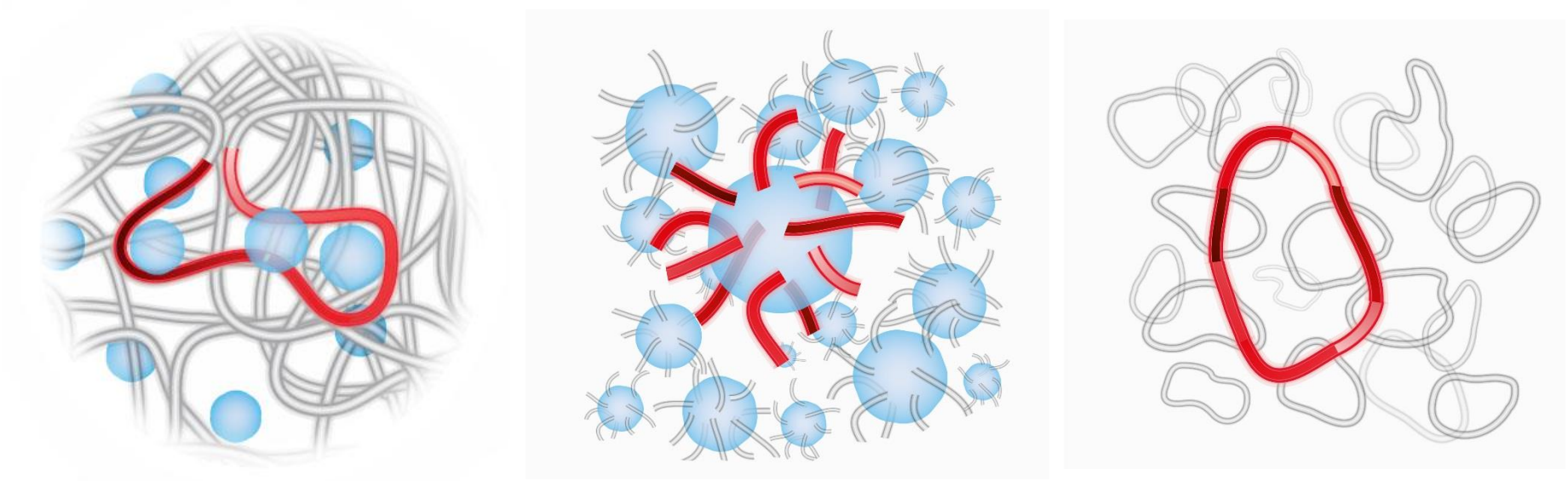
CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology



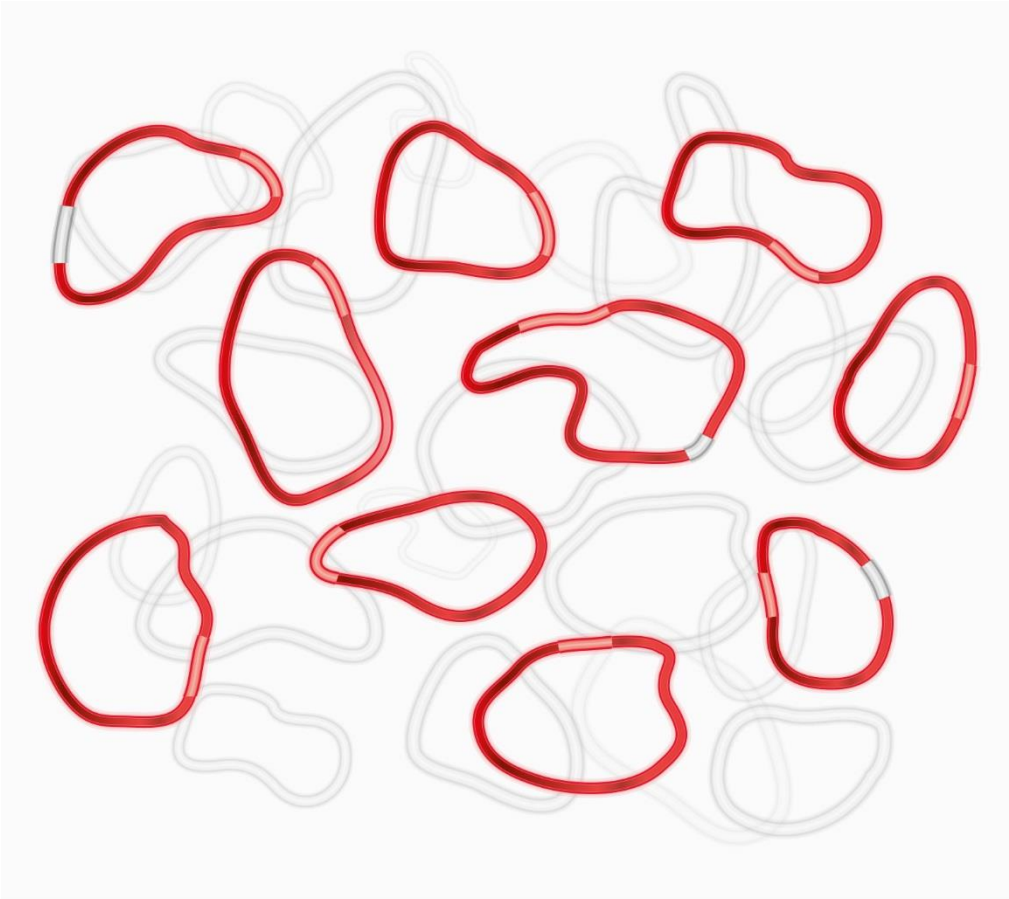
CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology



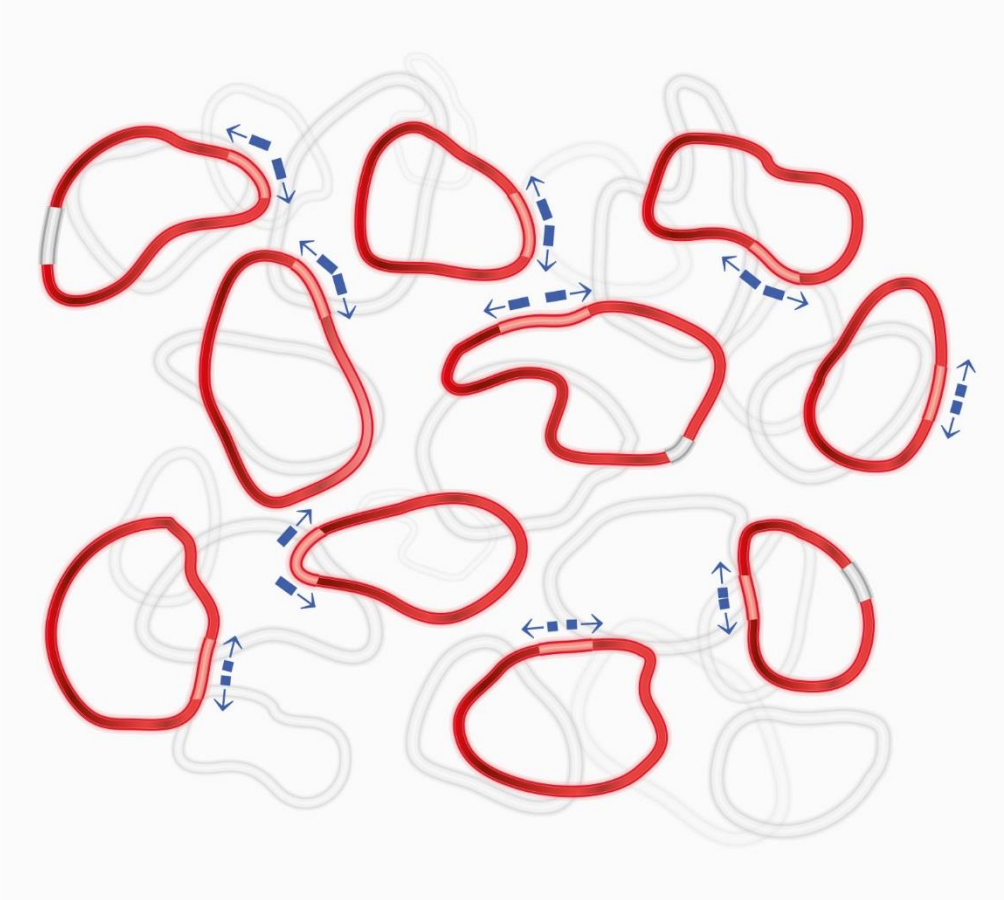
CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology



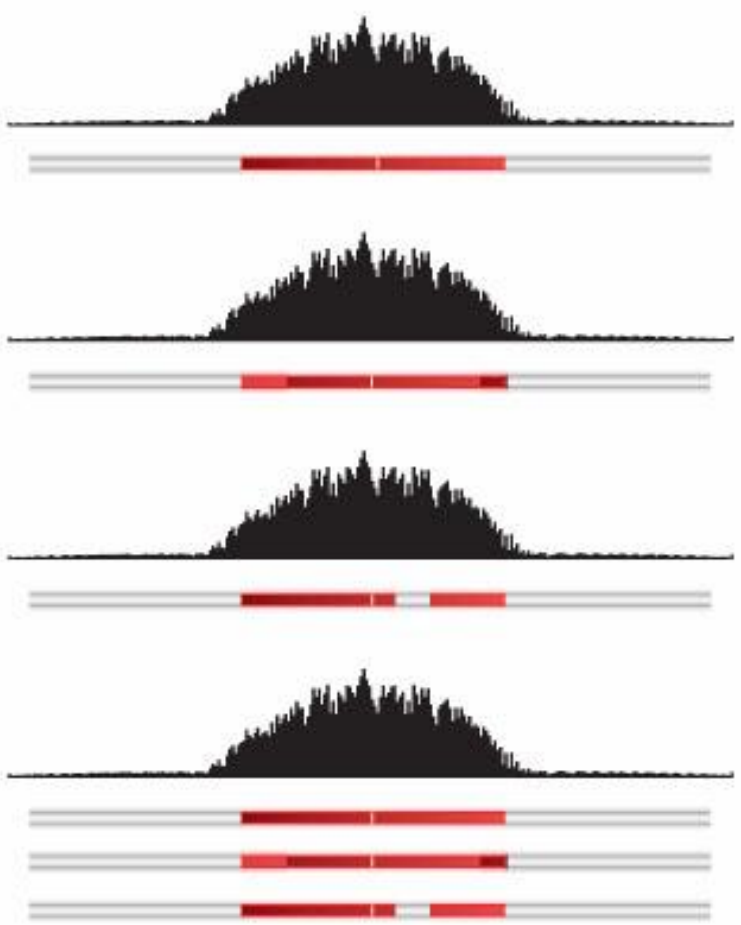
CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology



CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology



# Transgenes, integration sites and gene editing events

CERGENTIS
COMPLETE GENE SEQUENCING

CERGENTIS
COMPLETE GENE SEQUENCING

# Transgenes, integration sites and gene editing events

- CHO cell lines
- CAR-T cells
- Human cell lines
- Animal models
- Viral integrations (e.g. AAV, HIV, HBV)
- Transgenic plants
- Etc.

CERGENTIS
COMPLETE GENE SEQUENCING

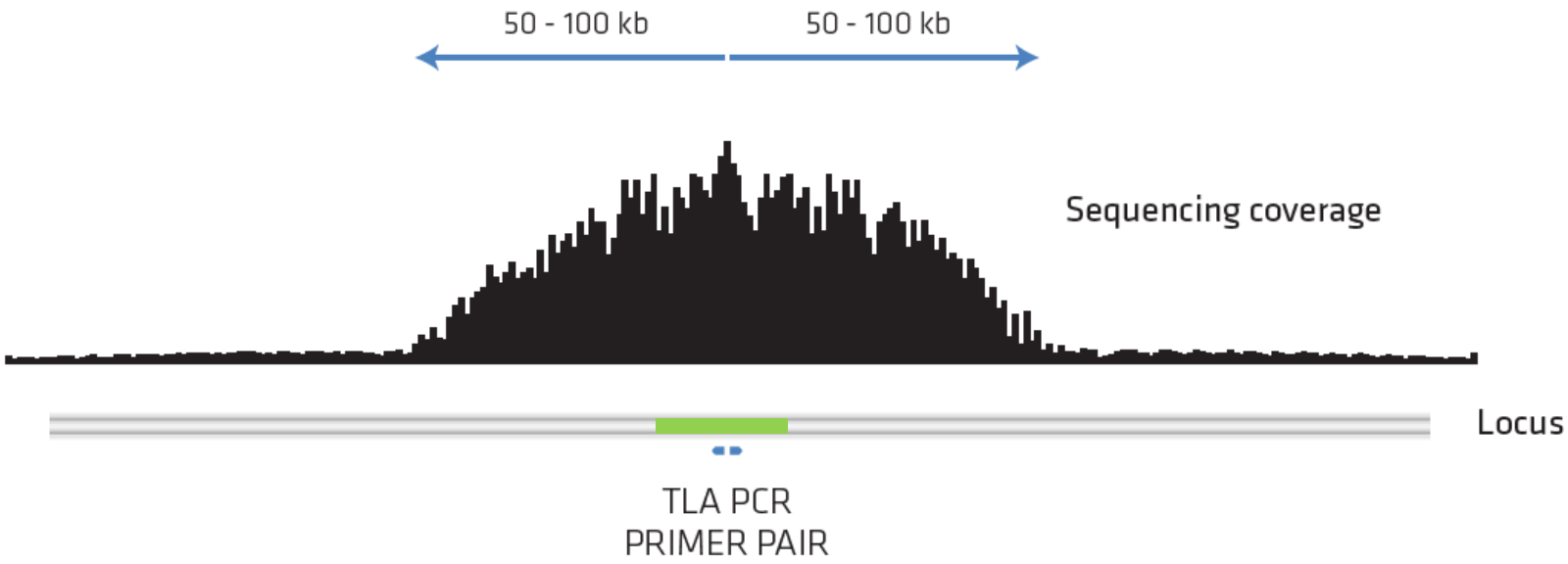
# TLA Technology | Transgene sequencing

- Transgene sequencing
  - Integration site(s)
  - Structural changes surrounding integration site
  - Single Nucleotide Variants in transgene
  - Structural changes in transgene

- Targeted sequencing of locus of interest
  - Targeted integrations
  - Knock outs

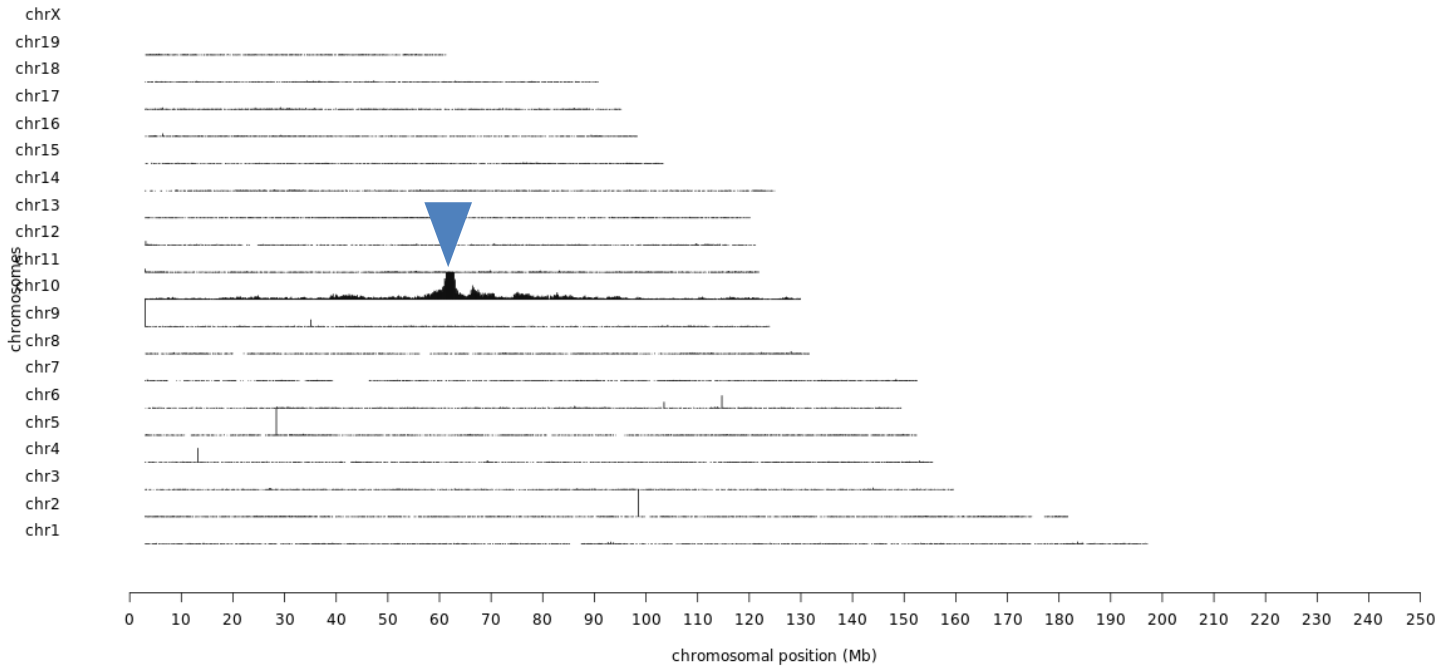
CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology | Transgene and integration site(s)

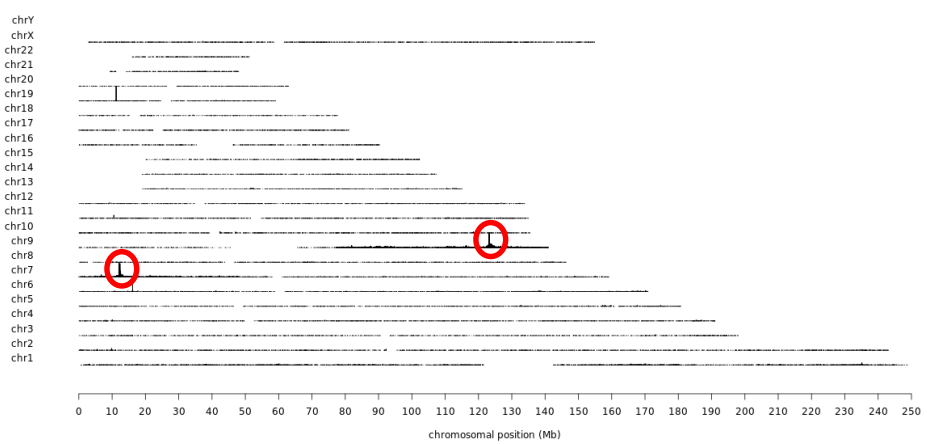


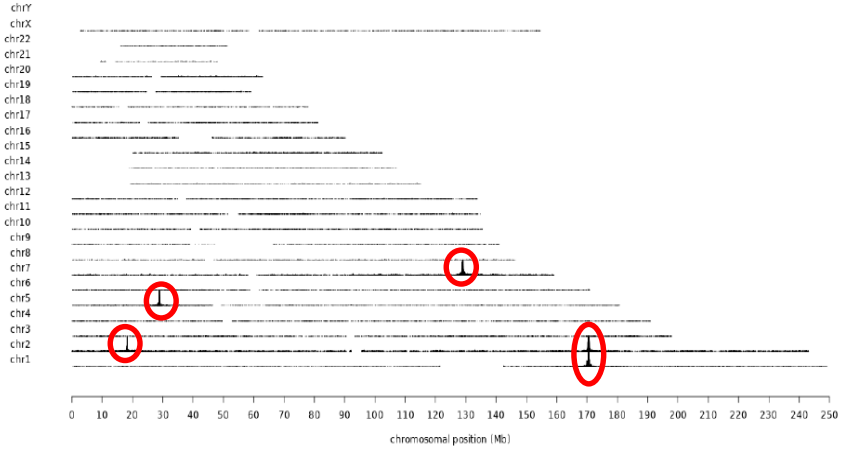
CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology | Transgene integration in mouse genome



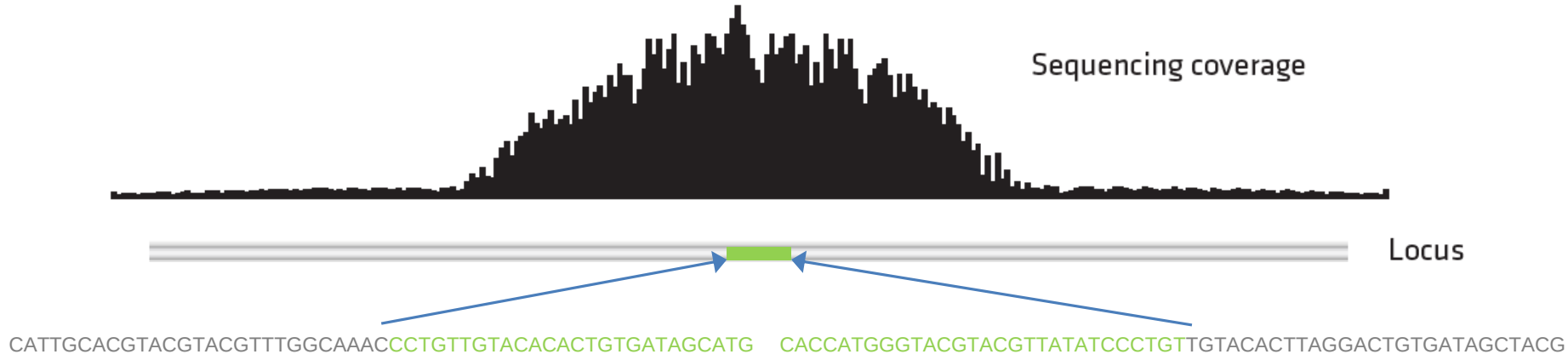
# TLA Technology | Detection of multiple integration sites



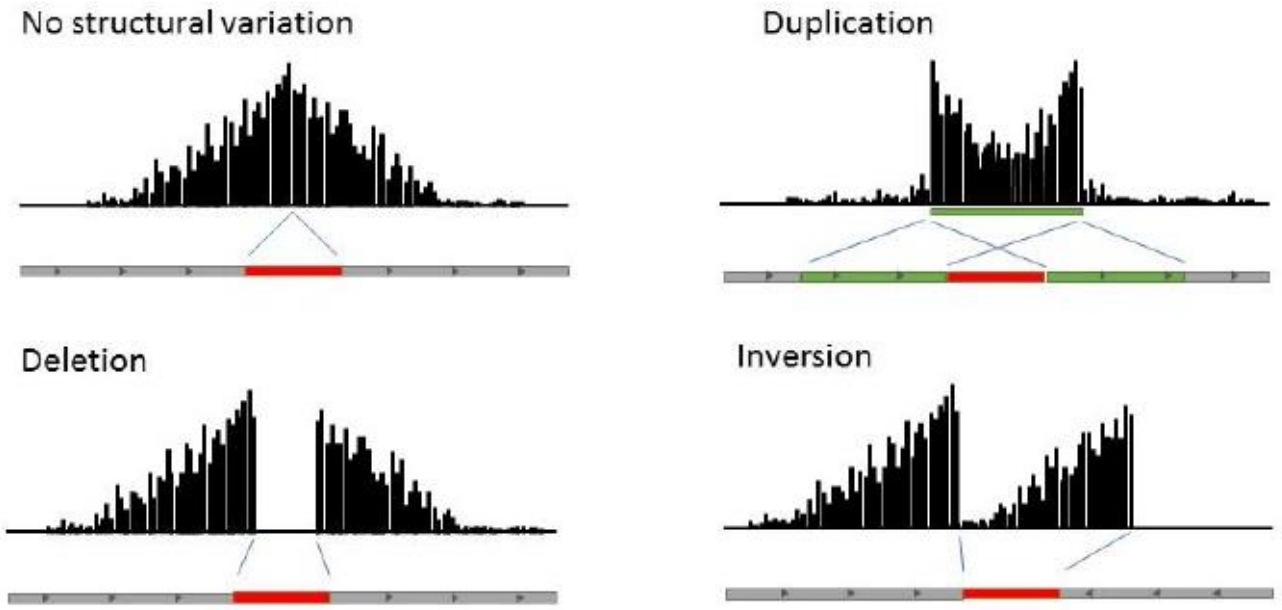
# TLA Technology | integration site breakpoint reads

- TLA provides sequence coverage across breakpoints resulting from integration



CERGENTIS
COMPLETE GENE SEQUENCING

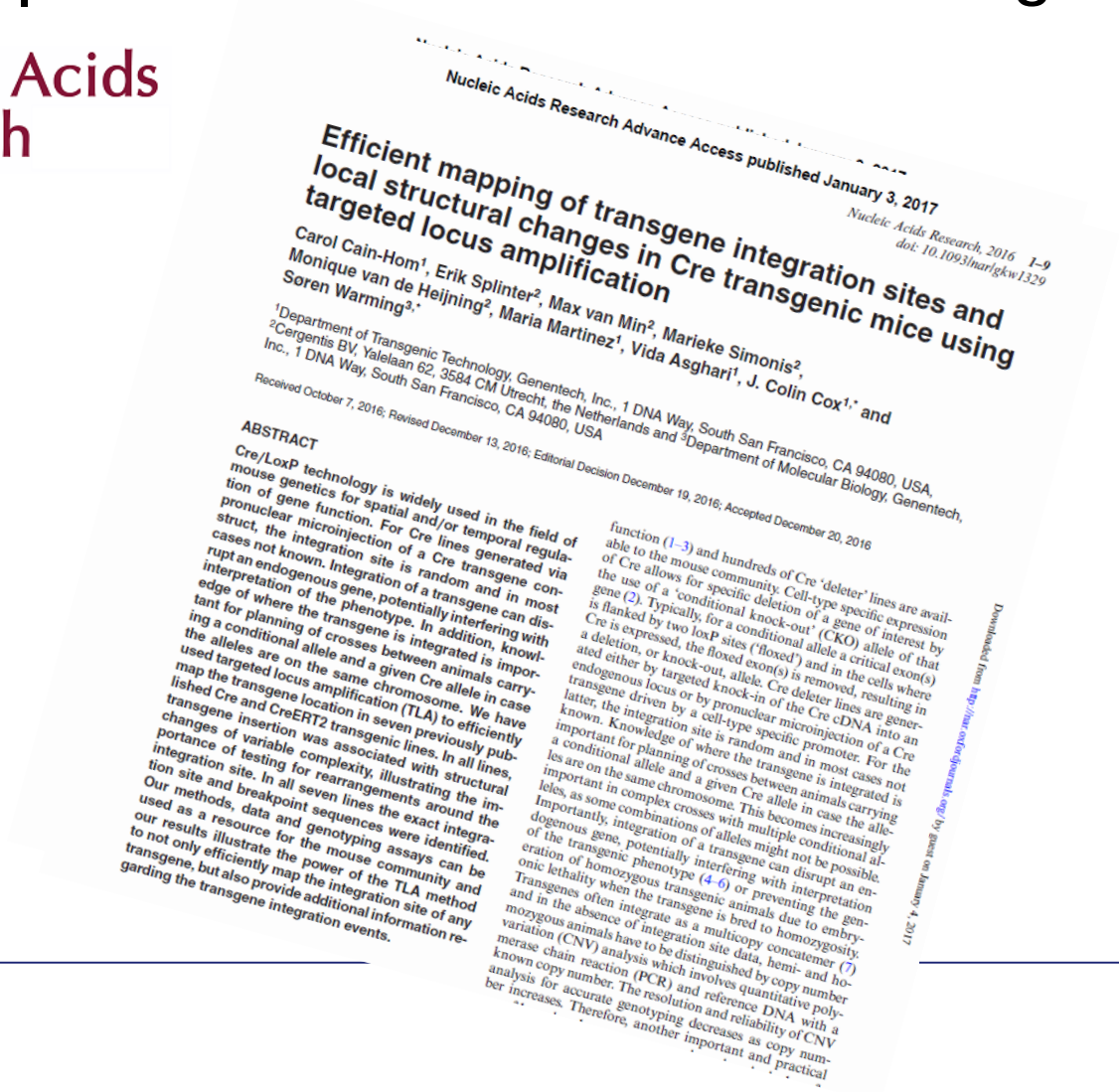
# TLA technology | Structural variantions in host genome



CERGENTIS
COMPLETE GENE SEQUENCING

# TLA technology | Structural variantions in host genome

Nucleic Acids Research



Nucleic Acids Research Advance Access published January 3, 2017

Nucleic Acids Research, 2016 1–9
doi: 10.1093/nar/gkw1329

## Efficient mapping of transgene integration sites and local structural changes in Cre transgenic mice using targeted locus amplification

Carol Cain-Hom[1], Erik Splinter[2], Max van Min[2], Marieke Simonis[2], Monique van de Heijning[2], Maria Martinez[1], Vida Asghari[1], J. Colin Cox[1,*] and Søren Warming[3,*]

[1]Department of Transgenic Technology, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080, USA, [2]Cergentis BV, Yalelaan 62, 3584 CM Utrecht, the Netherlands and [3]Department of Molecular Biology, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080, USA

Received October 7, 2016; Revised December 13, 2016; Editorial Decision December 19, 2016; Accepted December 20, 2016

ABSTRACT

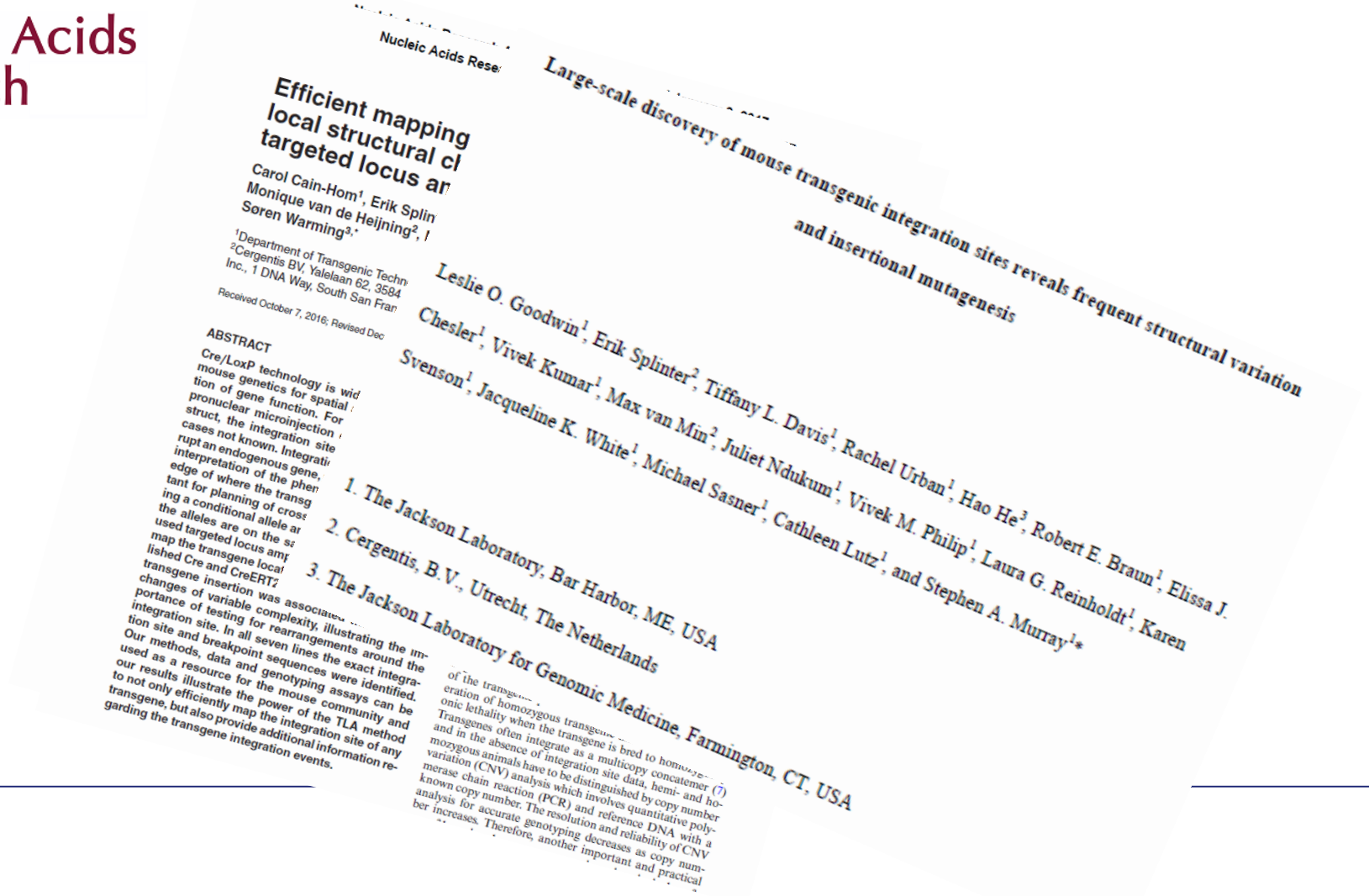
Cre/LoxP technology is widely used in the field of mouse genetics for spatial and/or temporal regulation of gene function. For Cre lines generated via pronuclear microinjection of a Cre transgene construct, the integration site is random and in most cases not known. Integration of a transgene can disrupt an endogenous gene, potentially interfering with interpretation of the phenotype. In addition, knowledge of where the transgene is integrated is important for planning of crosses between animals carrying a conditional allele and a given Cre allele in case the alleles are on the same chromosome. We have used targeted locus amplification (TLA) to efficiently map the transgene location in seven previously published Cre and CreERT2 transgenic lines. In all lines, transgene insertion was associated with structural changes of variable complexity, illustrating the importance of testing for rearrangements around the integration site. In all seven lines the exact integration site and breakpoint sequences were identified. Our methods, data and genotyping assays can be used as a resource for the mouse community and to not only efficiently map the integration site of any transgene, but also provide additional information regarding the transgene integration events.

function (1–3) and hundreds of Cre 'deleter' lines are available to the mouse community. Cell-type specific expression of Cre allows for specific deletion of a gene of interest by the use of a 'conditional knock-out' (CKO) allele of that gene (2). Typically, for a conditional allele a critical exon(s) is flanked by two loxP sites ('floxed') and in the cells where Cre is expressed, the floxed exon(s) is removed, resulting in a deletion, or knock-out, allele. Cre deleter lines are generated either by targeted knock-in of the Cre cDNA into an endogenous locus or by pronuclear microinjection of a Cre transgene driven by a cell-type specific promoter. For the latter, the integration site is random and in most cases not known. Knowledge of where the transgene is integrated is important for planning of crosses between animals carrying a conditional allele and a given Cre allele in case the alleles are on the same chromosome. This becomes increasingly important in complex crosses with multiple conditional alleles, as some combinations of alleles might not be possible. Importantly, integration of a transgene can disrupt an endogenous gene, potentially interfering with interpretation of the transgenic phenotype (4–6) or preventing the generation of homozygous transgenic animals due to embryonic lethality when the transgene is bred to homozygosity. Transgenes often integrate as a multicopy concatemer (7) and in the absence of integration site data, hemi- and homozygous animals have to be distinguished by copy number variation (CNV) analysis which involves quantitative polymerase chain reaction (PCR) and reference DNA with a known copy number. The resolution and reliability of CNV analysis for accurate genotyping decreases as copy number increases. Therefore, another important and practical

CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology | Transgene Sequencing: structural variants

Nucleic Acids Research



Nucleic Acids Rese

Efficient mapping
local structural cl
targeted locus ar

Carol Cain-Hom[1], Erik Splin
Monique van de Heijning[2], I
Søren Warming[3,*]

[1]Department of Transgenic Techn
[2]Cergentis BV, Yalelaan 62, 3584
Inc., 1 DNA Way, South San Fran

Received October 7, 2016; Revised Dec

ABSTRACT

Cre/LoxP technology is wid
mouse genetics for spatial
tion of gene function. For
pronuclear microinjection
struct, the integration site
cases not known. Integrati
rupt an endogenous gene,
interpretation of the phen
edge of where the transg
tant for planning of cross
ing a conditional allele ar
the alleles are on the sa
used targeted locus amp
map the transgene locat
lished Cre and CreERT2
transgene insertion was associated
changes of variable complexity, illustrating the im-
portance of testing for rearrangements around the
integration site. In all seven lines the exact integra-
tion site and breakpoint sequences were identified.
Our methods, data and genotyping assays can be
used as a resource for the mouse community and
our results illustrate the power of the TLA method
to not only efficiently map the integration site of any
transgene, but also provide additional information re-
garding the transgene integration events.

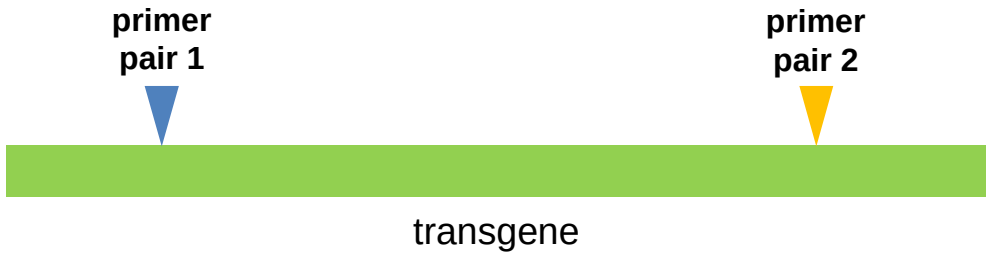
Large-scale discovery of mouse transgenic integration sites reveals frequent structural variation and insertional mutagenesis

Leslie O. Goodwin[1], Erik Splinter[2], Tiffany L. Davis[1], Rachel Urban[1], Hao He[3], Robert E. Braun[1], Elissa J. Chesler[1], Vivek Kumar[1], Max van Min[2], Juliet Ndukum[1], Vivek M. Philip[1], Laura G. Reinholdt[1], Karen Svenson[1], Jacqueline K. White[1], Michael Sasner[1], Cathleen Lutz[1], and Stephen A. Murray[1]*

1. The Jackson Laboratory, Bar Harbor, ME, USA
2. Cergentis, B.V., Utrecht, The Netherlands
3. The Jackson Laboratory for Genomic Medicine, Farmington, CT, USA

of the transgene
eration of homozygous transgenic (7)
onic lethality when the transgene is bred to homozygosity.
Transgenes often integrate as a multicopy concatemer
and in the absence of integration site data, hemi- and ho-
mozygous animals have to be distinguished by copy number
variation (CNV) analysis which involves quantitative poly-
merase chain reaction (PCR) and reference DNA with a
known copy number. The resolution and reliability of CNV
analysis for accurate genotyping decreases as copy num-
ber increases. Therefore, another important and practical

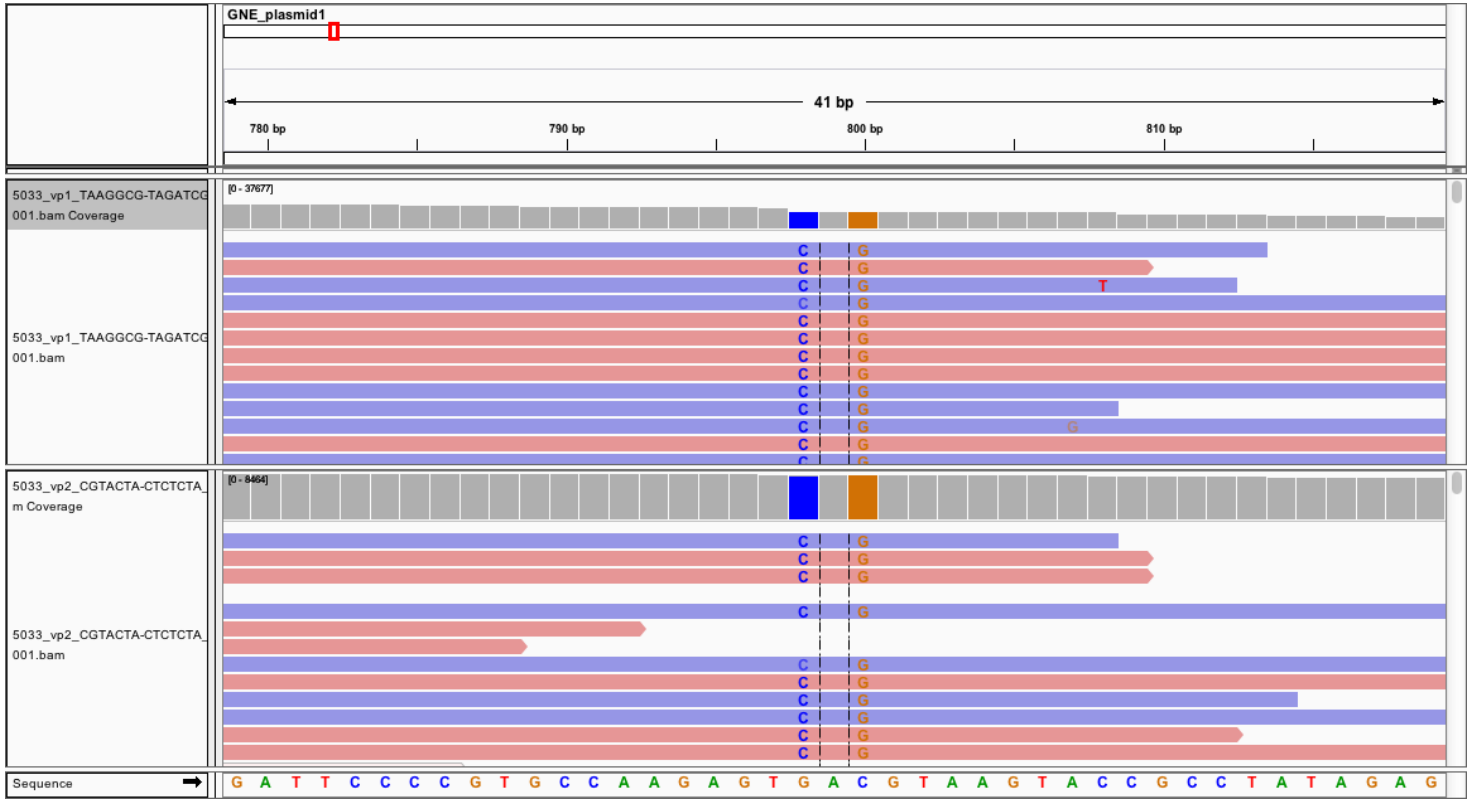
# 2 Individual primer pairs



CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology | Transgene Sequence

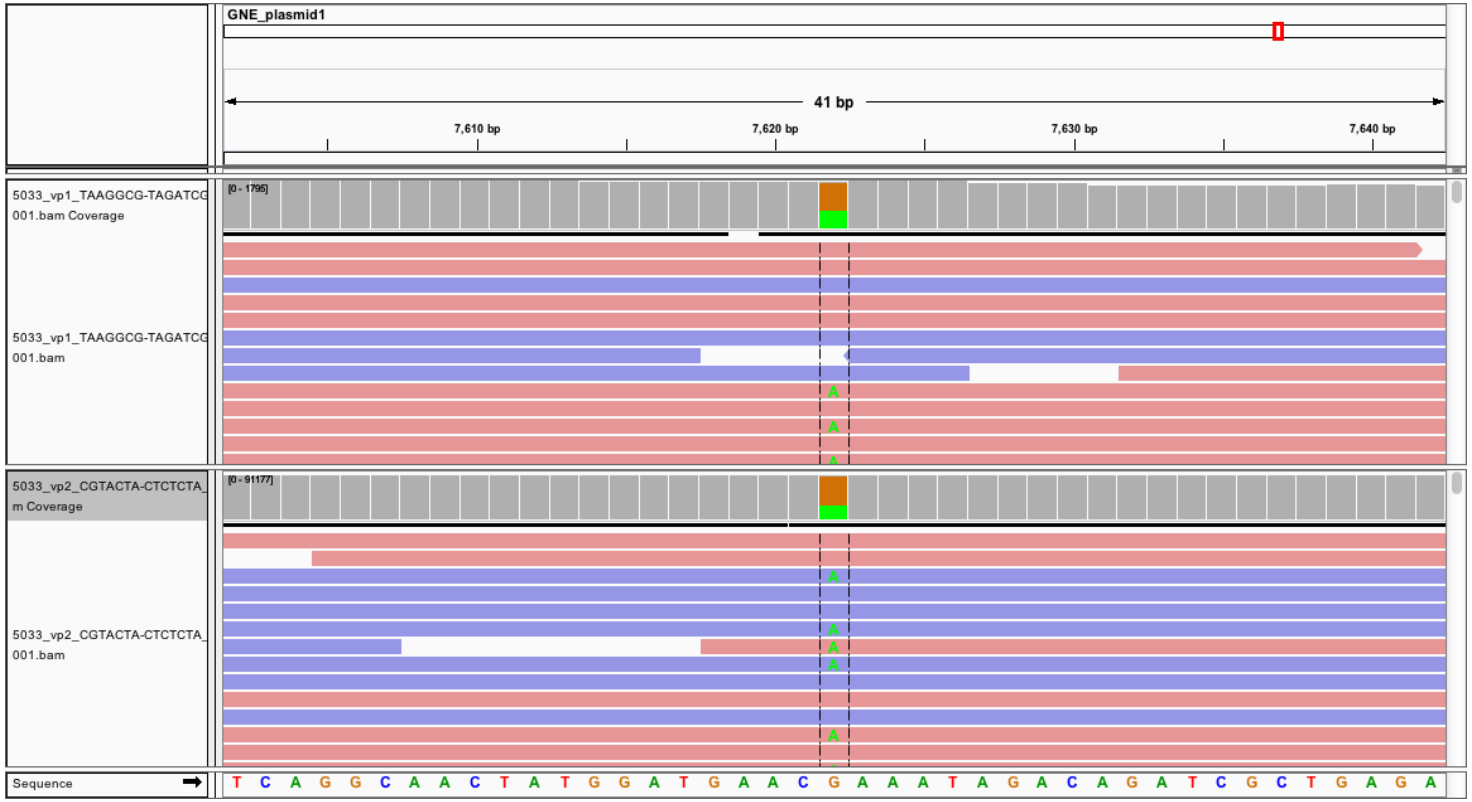
- Single Nucleotide Variants



CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology | Transgene Sequence

- Infrequent Single Nucleotide Variants



CERGENTIS
COMPLETE GENE SEQUENCING

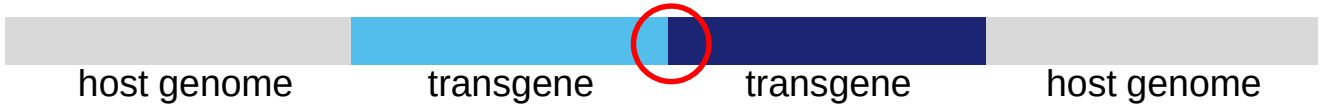
# TLA Technology | Transgene Sequence

- Single Nucleotide Variants reporting

| | | | | primer-set 1 | | primer-set 2 | |
|---|---|---|---|---|---|---|---|
| seq1 | pos | ref | alt | cov | SNV-freq(%) | cov | SNV-freq(%) |
| Transgene | 141 | A | C | 305 | 30 | 232 | 28 |
| Transgene | 489 | A | G | 370 | 1 | 285 | 1 |
| Transgene | 816 | T | G | 234 | 1 | 179 | 2 |
| Transgene | 1013 | T | C | 389 | 100 | 101 | 100 |
| Transgene | 1304 | A | C | 486 | 100 | 195 | 100 |
| Transgene | 1305 | G | C | 486 | 100 | 195 | 100 |
| Transgene | 2956 | T | C | 611 | 1 | 245 | 2 |
| Transgene | 3561 | C | A | 449 | 100 | 100 | 100 |
| Transgene | 4638 | G | A | 487 | 100 | 193 | 99 |
| Transgene | 5698 | A | C | 493 | 1 | 95 | 2 |
| Transgene | 8836 | T | G | 396 | 1 | 325 | 1 |
| Transgene | 9487 | T | C | 639 | 1 | 425 | 1 |
| Transgene | 11037 | A | G | 435 | 20 | 425 | 21 |

# TLA Technology | Transgene Sequence

- Structural variations

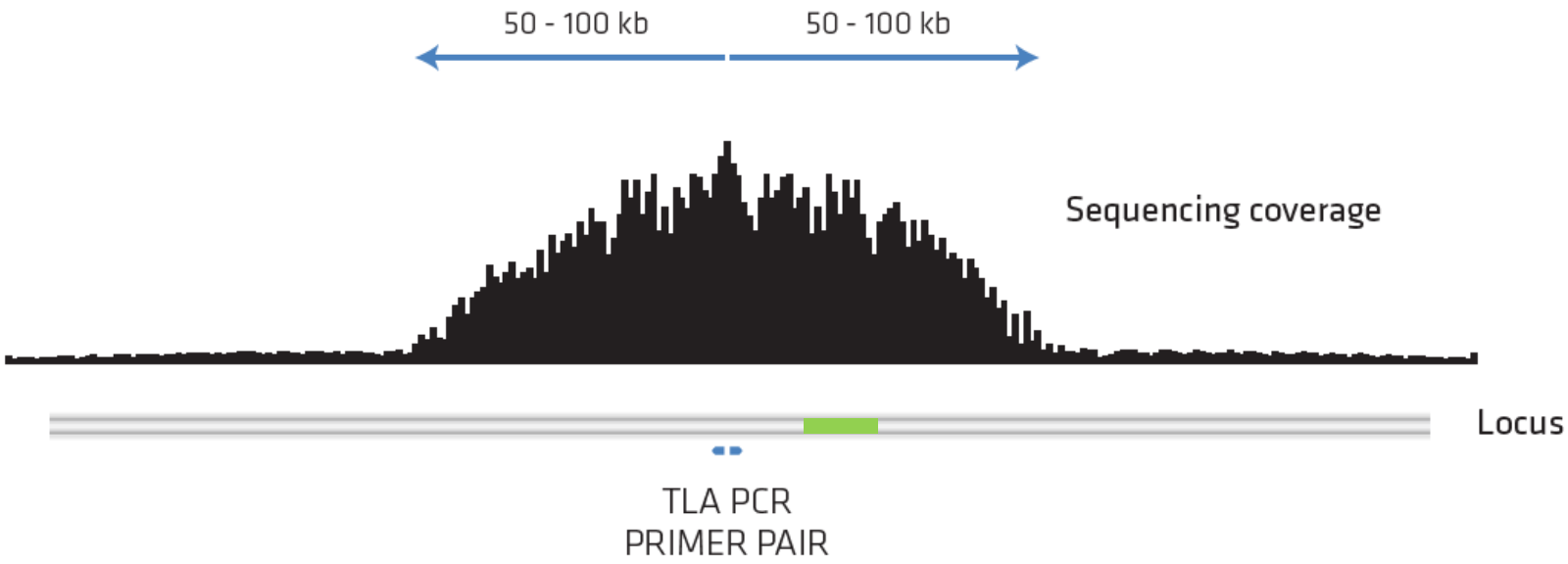



- Transgene-transgene fusions
- Partial deletions of transgene sequence
- Partial integrations that result in aberrant protein

| Fusion | Transgene | | Transgene | | Orientation of the fusion |
|---|---|---|---|---|---|
| 1 | → | 2500 | 5000 | → | tail to head |
| 2 | → | 2500 | 5000 | ← | tail to tail |
| 3 | ← | 2500 | 5000 | → | head to head |
| 4 | ← | 2500 | 5000 | ← | head to tail |

CERGENTIS
COMPLETE GENE SEQUENCING

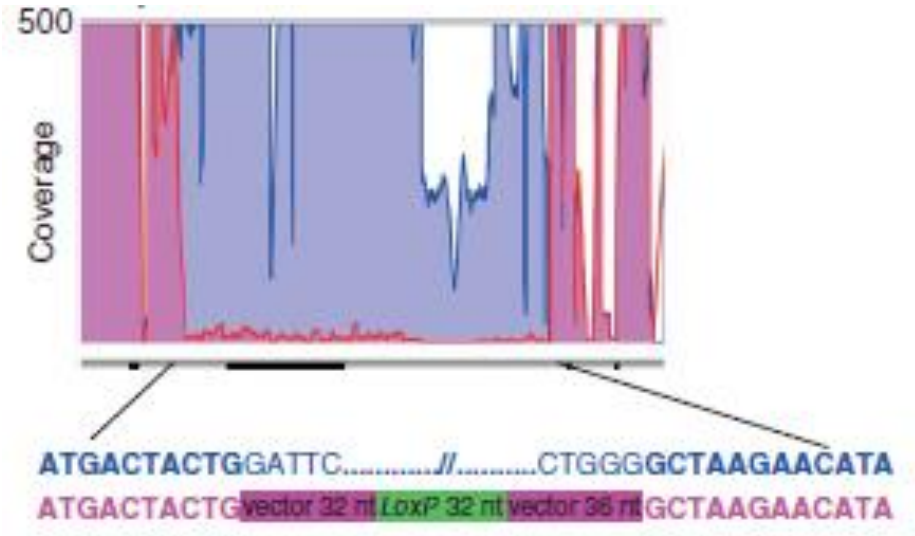
# TLA Technology | Targeted sequencing of gene editing events

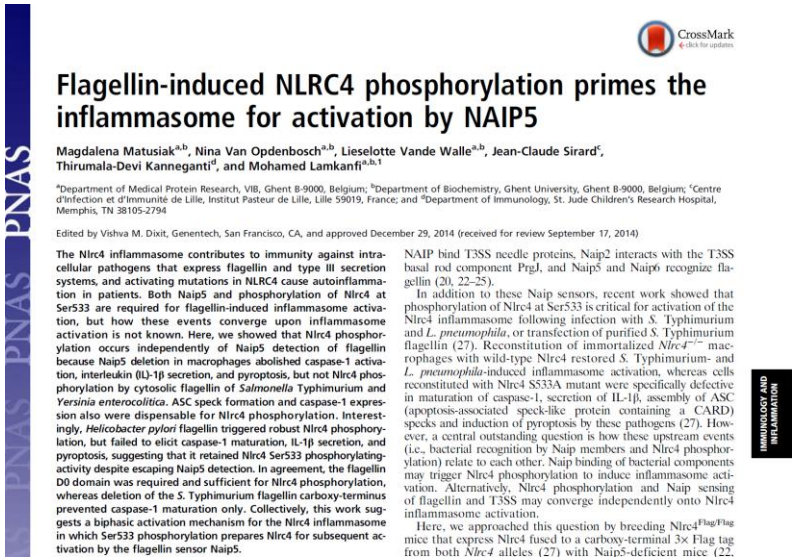


CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology | Targeted Mouse knock-out sequencing

- Knock out assumed to be in Naip5
- TLA enables targeted sequencing of each gene
- Knock out confirmed to be in the right position
- Additional SNP identified



PNAS PNAS PNAS

IMMUNOLOGY AND INFLAMMATION

CrossMark

## Flagellin-induced NLRC4 phosphorylation primes the inflammasome for activation by NAIP5

Magdalena Matusiak[a,b], Nina Van Opdenbosch[a,b], Lieselotte Vande Walle[a,b], Jean-Claude Sirard[c], Thirumala-Devi Kanneganti[d], and Mohamed Lamkanfi[a,b,1]

[a]Department of Medical Protein Research, VIB, Ghent B-9000, Belgium; [b]Department of Biochemistry, Ghent University, Ghent B-9000, Belgium; [c]Centre d'Infection et d'Immunité de Lille, Institut Pasteur de Lille, Lille 59019, France; and [d]Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN 38105-2794

Edited by Vishva M. Dixit, Genentech, San Francisco, CA, and approved December 29, 2014 (received for review September 17, 2014)

**The Nlrc4 inflammasome contributes to immunity against intracellular pathogens that express flagellin and type III secretion systems, and activating mutations in NLRC4 cause autoinflammation in patients. Both Naip5 and phosphorylation of Nlrc4 at Ser533 are required for flagellin-induced inflammasome activation, but how these events converge upon inflammasome activation is not known. Here, we showed that Nlrc4 phosphorylation occurs independently of Naip5 detection of flagellin because Naip5 deletion in macrophages abolished caspase-1 activation, interleukin (IL)-1β secretion, and pyroptosis, but not Nlrc4 phosphorylation by cytosolic flagellin of *Salmonella* Typhimurium and *Yersinia enterocolitica*. ASC speck formation and caspase-1 expression also were dispensable for Nlrc4 phosphorylation. Interestingly, *Helicobacter pylori* flagellin triggered robust Nlrc4 phosphorylation, but failed to elicit caspase-1 maturation, IL-1β secretion, and pyroptosis, suggesting that it retained Nlrc4 Ser533 phosphorylating-activity despite escaping Naip5 detection. In agreement, the flagellin D0 domain was required and sufficient for Nlrc4 phosphorylation, whereas deletion of the *S.* Typhimurium flagellin carboxy-terminus prevented caspase-1 maturation only. Collectively, this work suggests a biphasic activation mechanism for the Nlrc4 inflammasome in which Ser533 phosphorylation prepares Nlrc4 for subsequent activation by the flagellin sensor Naip5.**

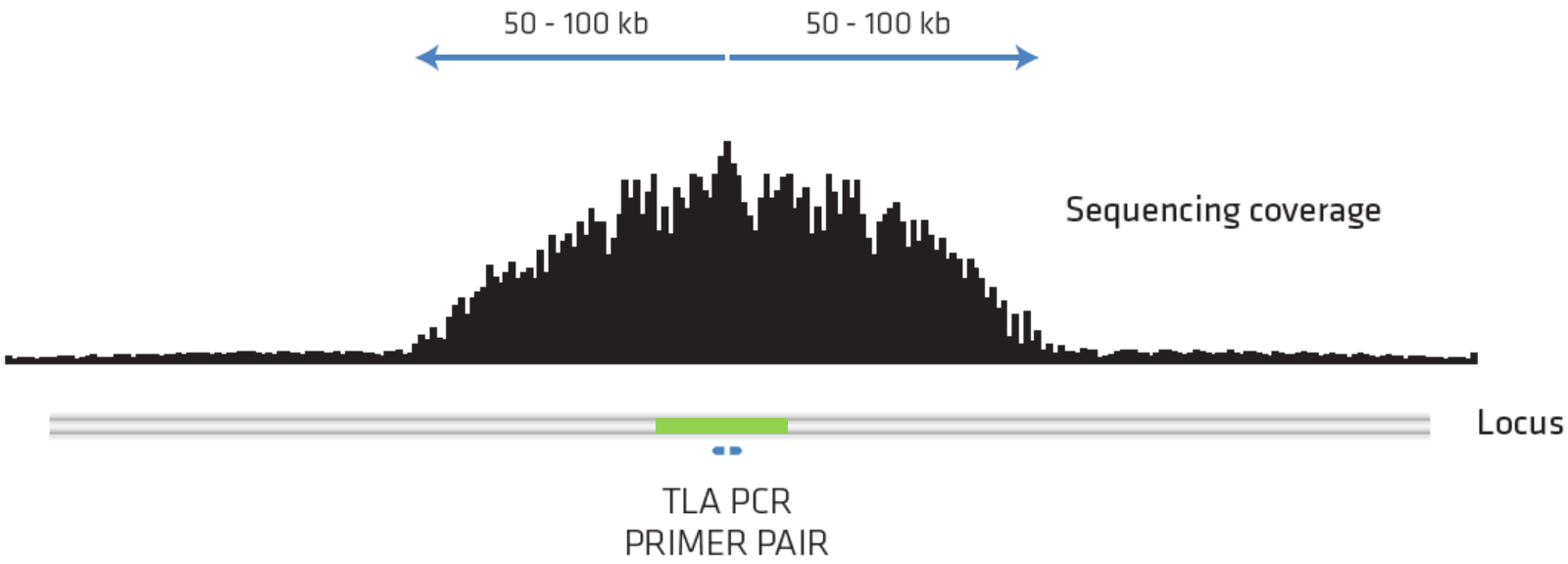
NAIP bind T3SS needle proteins, Naip2 interacts with the T3SS basal rod component PrgJ, and Naip5 and Naip6 recognize flagellin (20, 22–25).

In addition to these Naip sensors, recent work showed that phosphorylation of Nlrc4 at Ser533 is critical for activation of the Nlrc4 inflammasome following infection with *S.* Typhimurium and *L. pneumophila*, or transfection of purified *S.* Typhimurium flagellin (27). Reconstitution of immortalized *Nlrc4*$^{-/-}$ macrophages with wild-type Nlrc4 restored *S.* Typhimurium- and *L. pneumophila*-induced inflammasome activation, whereas cells reconstituted with Nlrc4 S533A mutant were specifically defective in maturation of caspase-1, secretion of IL-1β, assembly of ASC (apoptosis-associated speck-like protein containing a CARD) specks and induction of pyroptosis by these pathogens (27). However, a central outstanding question is how these upstream events (i.e., bacterial recognition by Naip members and Nlrc4 phosphorylation) relate to each other. Naip binding of bacterial components may trigger Nlrc4 phosphorylation to induce inflammasome activation. Alternatively, Nlrc4 phosphorylation and Naip sensing of flagellin and T3SS may converge independently onto Nlrc4 inflammasome activation.

Here, we approached this question by breeding Nlrc4$^{Flag/Flag}$ mice that express Nlrc4 fused to a carboxy-terminal 3× Flag tag from both *Nlrc4* alleles (27) with Naip5-deficient mice (22,

CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology | Targeted sequencing of transgenes

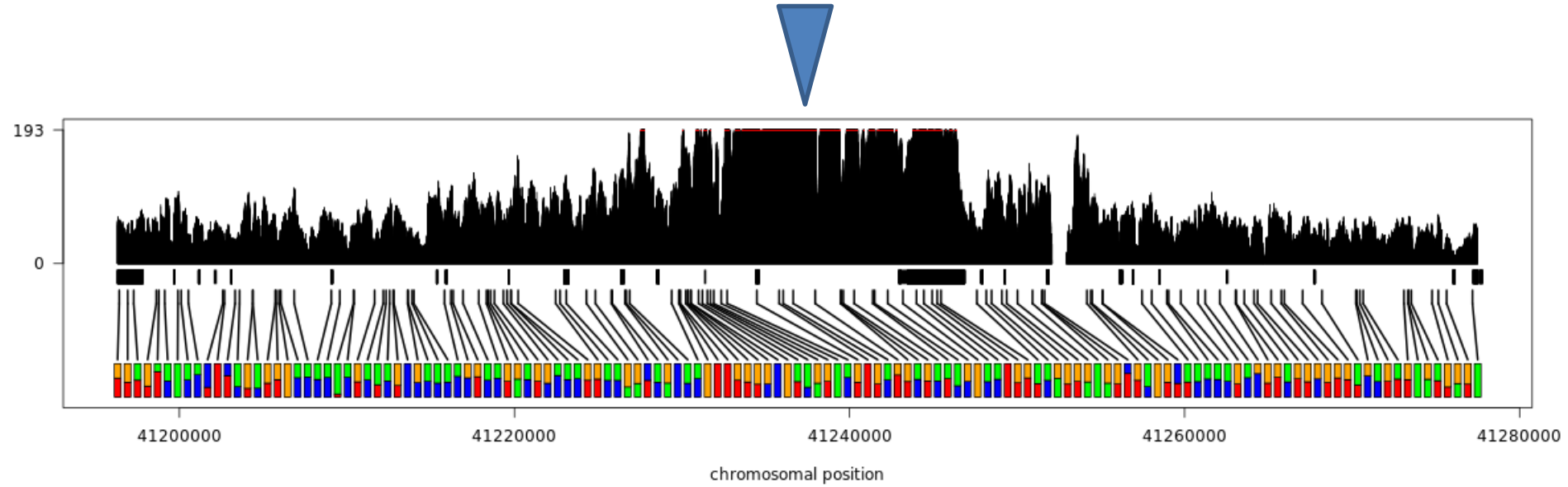


# Haplotyping

CERGENTIS
COMPLETE GENE SEQUENCING

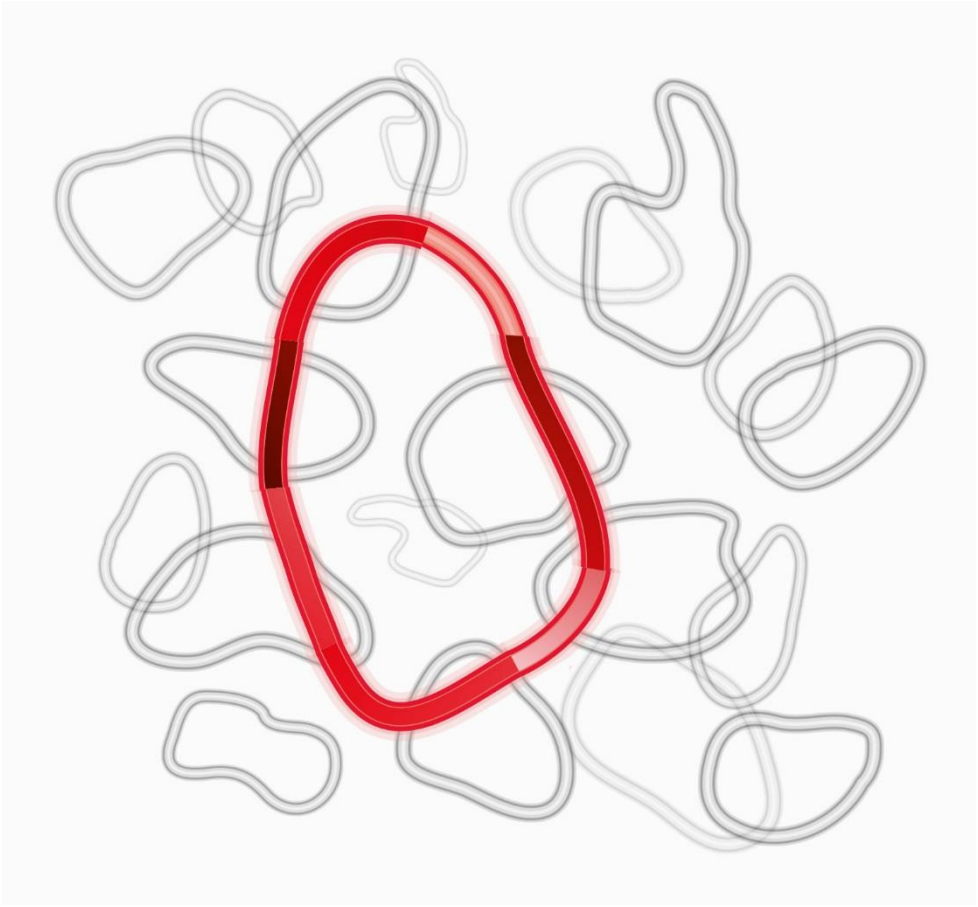
CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology: BRCA1 gene



CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology



CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology | Haplotyping

- DNA fragments in the same circle & amplicon originate from the same allele.

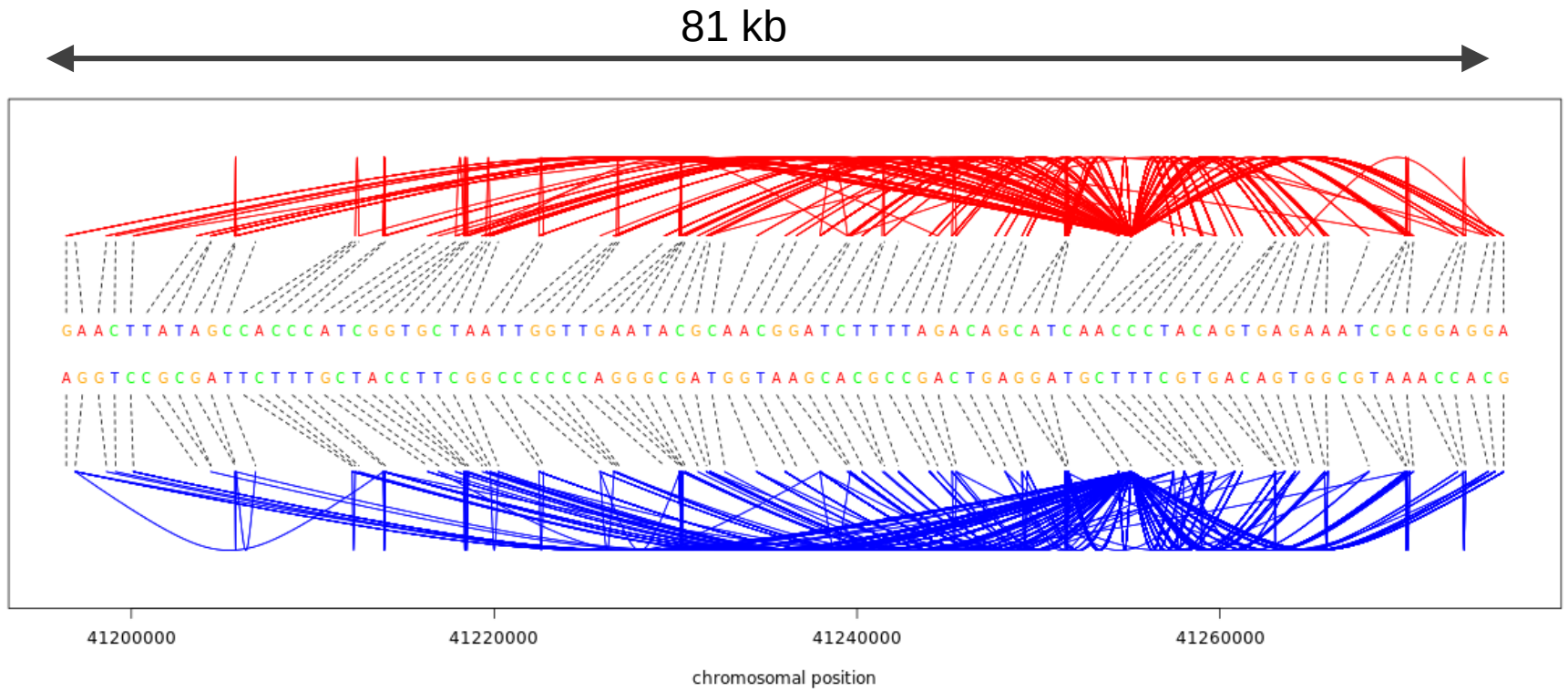
# TLA Technology | Haplotyping

- DNA fragments in the same circle & amplicon originate from the same allele.

CERGENTIS
COMPLETE GENE SEQUENCING

# TLA Technology | Haplotyping

- Full phasing of BRCA1 gene



# TLA Technology | Haplotyping

- DNA fragments in the same circle & amplicon originate from the same allele.

CERGENTIS
COMPLETE GENE SEQUENCING

PACBIO®

Application Note
Targeted Sequencing

# Targeted Sequencing and Chromosomal Haplotype Assembly Using Cergentis TLA Technology with SMRT® Sequencing

## Introduction

Conventional, PCR-based targeted sequencing methodologies are impractical for detecting and phasing single nucleotide and structural variants over regions that are tens of kilobases in length.

The Targeted Locus Amplification (TLA) Technology[1] from Cergentis enables the targeted, hypothesis-neutral, amplification of any genomic locus of interest over 50 kb using just one primer pair complementary to a short locus-specific sequence. TLA is a strategy to selectively amplify complete loci on the basis of crosslinking physically proximal sequences. Unlike other targeted sequencing methods, TLA works without prior detailed locus information, as one primer pair is sufficient to amplify tens to hundreds of kilobases of DNA surrounding that locus. In a separate application of TLA, the unamplified template can be used for genome-wide phasing and assembly. TLA enables targeted sequencing and detection of single nucleotide and structural variants in genes or regions of interest.

Single Molecule, Real-Time (SMRT®) Sequencing provides high consensus accuracy and long read lengths. As such, it enables end-to-end sequencing of multi-kilobase TLA amplicons or unamplified TLA templates. The combination of Cergentis' TLA and SMRT Sequencing technologies allows for sequencing and haplotyping of individual genes, chromosomes and genomes.

## TLA Method

In the TLA sample preparation method, the genomic DNA is first crosslinked. Because crosslinking occurs preferentially between sequences in close physical proximity, sequences predominantly from the same locus are crosslinked. The crosslinked DNA is then fragmented, religated and de-crosslinked.

The resulting TLA template consists of long fragments of DNA comprising religated fragments originating from the same locus (Figure 1). At this point the procedure changes depending upon the desired application.

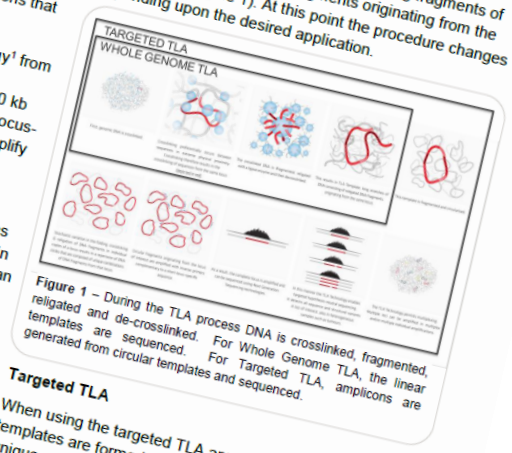



**Figure 1** – During the TLA process DNA is crosslinked, fragmented, religated and de-crosslinked. For Whole Genome TLA, the linear templates are sequenced. For Targeted TLA, amplicons are generated from circular templates and sequenced.

### Targeted TLA

When using the targeted TLA approach, the linear TLA templates are formed into DNA circles that are composed of unique combinations of DNA fragments from that locus. Inverse primers are designed for the locus of interest and only circles containing the complementary region are amplified. As a result, the complete locus is amplified and the resulting amplicons can be sequenced.

### Whole Genome TLA

When using the Whole Genome TLA approach, the linear fragments are not circularized. Instead, the linear TLA templates (Panel D in Figure 1), are directly converted into SMRTbell™ templates, size-selected and sequenced. Each individual TLA template contains fragments that may be ten to hundreds of kilobases apart in base space, but are all close enough to be cross-linked. Because it is not a targeted approach, this strategy results in long range information that covers the entire genome.

Experiment Design | Targeted Enrichment | Template Prep | Sequencing | Analysis

www.pacb.com/target

# More information

CERGENTIS
COMPLETE GENE SEQUENCING

CERGENTIS
COMPLETE GENE SEQUENCING

# More information

- info@cergentis.com
- www.cergentis.com