CATCH

In vitro utility of cas9 cleavage

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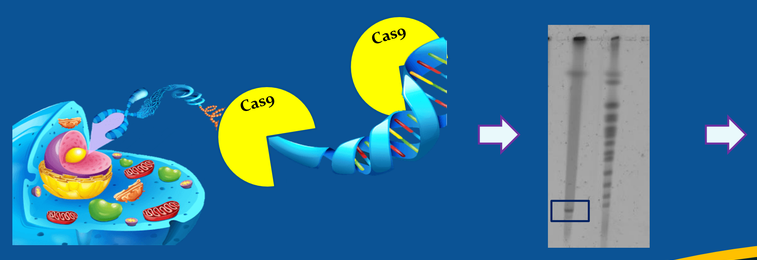
Abstract:

Here we describe a technique that allows the targeted digestion of near-arbitrary, long genomic sequences. Target genome segment is excised from chromosomes in vitro by the RNA-guided Cas9 nuclease at two designated loci. This technique can be an effective molecular tool for the targeted cloning of large gene clusters that are often expensive to synthesize by gene synthesis or difficult to obtain directly by traditional PCR and restriction-enzyme-based methods. CATCH can be combined with ultra-deep NGS sequencing, high coverage long read nanopore sequencing and optical genome mapping, to provide a fast and unique way to detect and isolate gene clusters and antibiotic resisters genes.

Nano-pore "On demand" long read sequencing

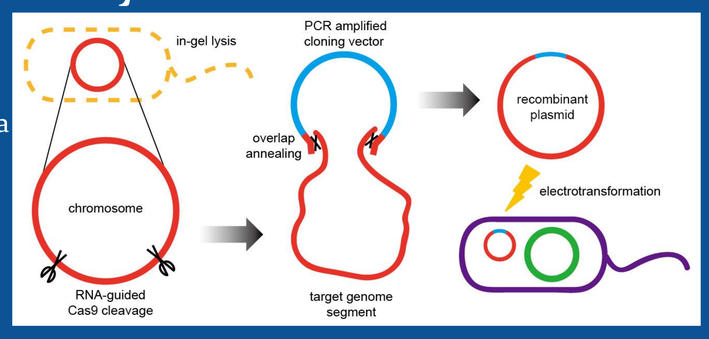
200kb segment was digested by cas9 and extracted from PFEG gel.
For long read sequencing we used the Oxford minION nanopore.
This enable the detection of structural variation and complex genomic regions.



Oxford Nanopore minION

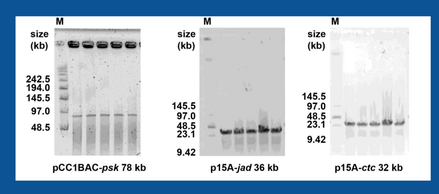
Single step **cloning** of large gene clusters by CATCH

• Chromosomes after cell lysis are digested by Cas9 at target sites in agarose gel.
•The cloning vectors ligate to the target DNA through sequence complementary in a Gibson assembly mix.
•The recombinant plasmids can be than introduced to a new host.



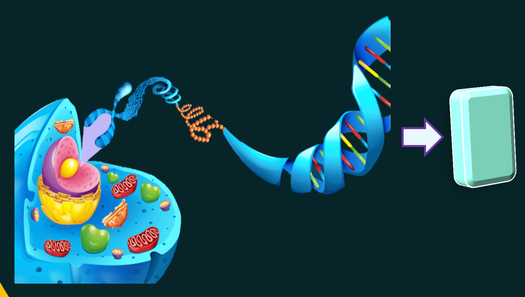
Gene clusters cloning from other bacteria

- *Bacillus subtilis* - PSK gene cluster (78 kb)
- *Streptomyces venezuelae*-jad 36 kb jadomycin-producing
- *Streptomyces aureofaciens*- ctc 32-kb chlortetracycline

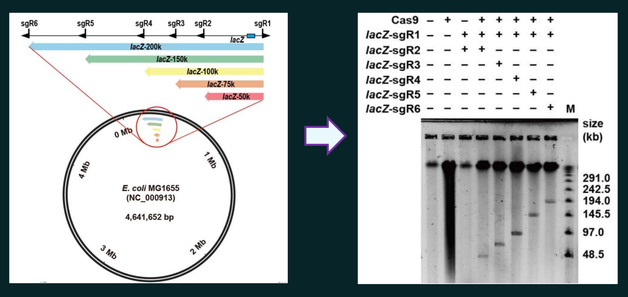


Cas9-Assisted Targeting of CHromosome segments

High molecular DNA is extracted by embedding cells in agarose gel and the lysis is performed in gel plugs



Six sgRNA pairs were designed to target genome segments of different lengths 50, 75, 100, 150 and 200 kb, respectively
This DNA segments can be further used for different application



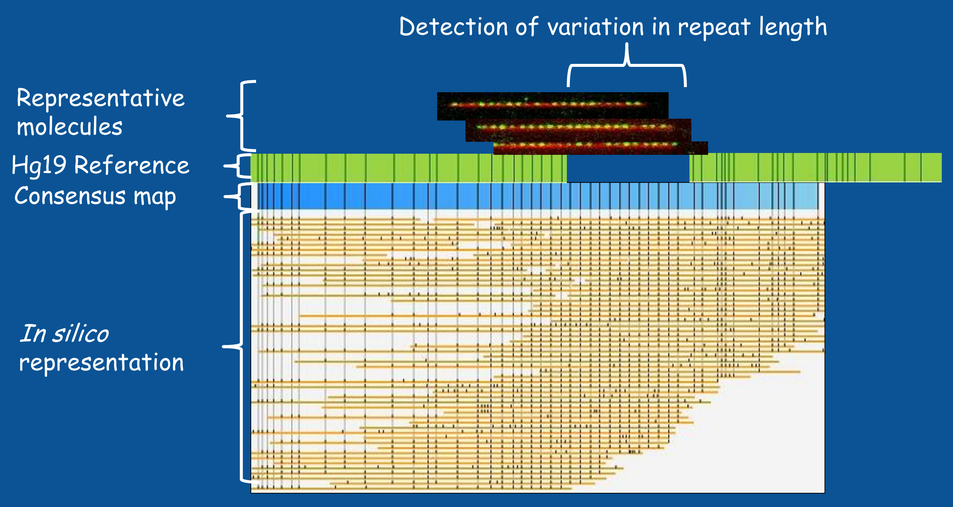
Optical mapping of large structural variations

Genetic labeling by nick translation (NLRS)

We incorporate fluorescent nucleotides by Taq polymerase in nicking sites of Nt. BspQI and create a barcode of the DNA sequence. This allow us to detect structural variations as repeats.

For example Chromosome 9- repeat variation

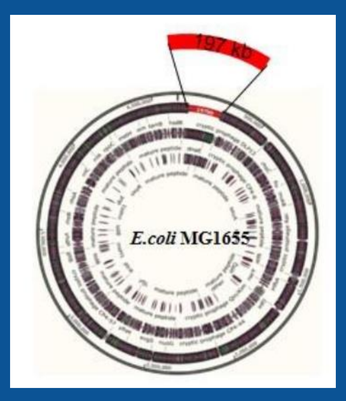
The expected number of repeats as predicted by sequencing is lower then what observed by optical mapping

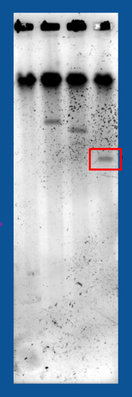


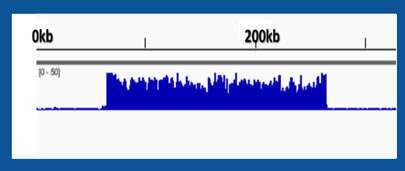
Targeted **NGS** allowing ultra deep sequencing of selected regions and mutation analysis

200kb segment was digested by cas9 and extracted from PFEG gel sent to deep sequencing coverage was detected only in the target sequence.

Illumina Hiseq2000 deep-sequencing







Funding: