Summer Review 4

CRISPR

CRIPSR toolkit for genome editing and beyond

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[1]Department of Computer Science, University of Virginia
`https://qdata.github.io/deep2Read/`

CRISPR

- Clustered Regulatory Interspaced Short Palindromic Repeat DNA sequences
- Originally, E.coli's immune emchanism

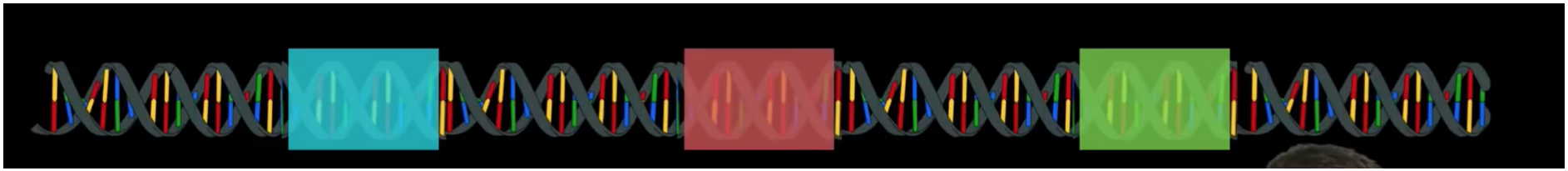

Figure: CRISPR

CRISPR

- The Non repeating sequences: **spacers**
- Spacers were recognized to belong to viruses.
- CRISPR elements are adjacent to CRISPR Associated(Cas) genes
- Cas enzyme blocks the DNA transfer from bacterial plasmids

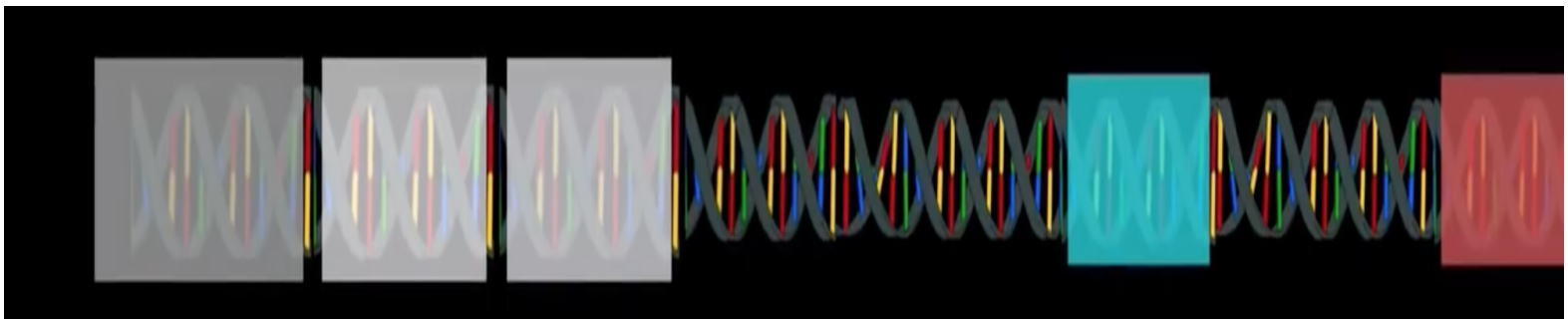

Figure: CRISPR

CRISPR Mechanism

- When virus attacks, bacteria integrate new spacers derived from attacker's genome into genome
- Spacer sequences dictate targeting specificity of the Cas enzymes: defense
- RNAs: crispr RNA (crRNA) and transacivating RNA (tracrRNA)
- crRNA: guiding sequence
- tracrRNA: base pairs with the sequence
- Both required for RNA-Cas9 complex for Double Strand Breaks(DSBs) at sites
- Cas9 can be used to modify any desired genomic target provided that sequence is unique and located just upstream of a Protospacer Adjacent Motif (PAM)

CRISPR Mechanism

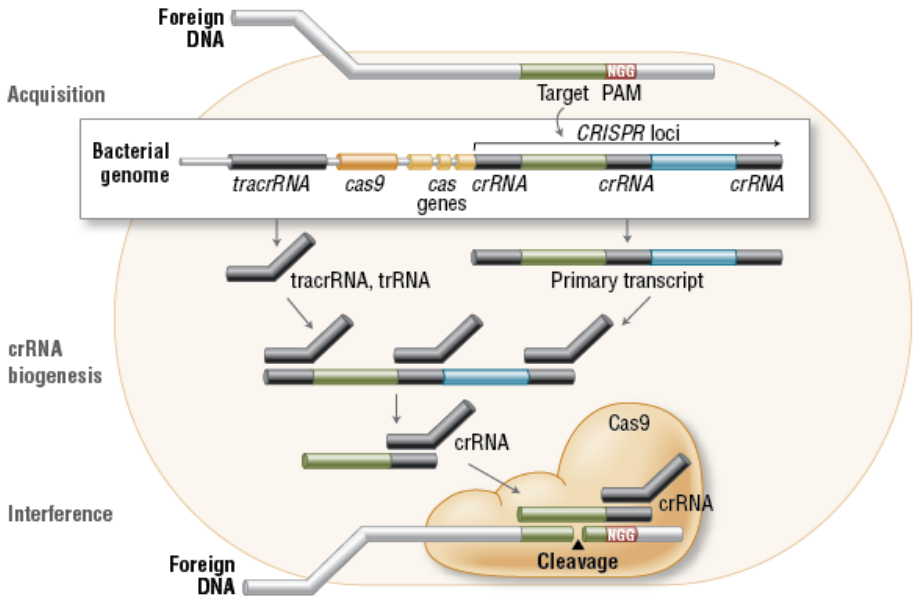

Figure: CRISPR Mechanism

n the acquisition phase, foreign DNA is incorporated into the bacterial genome at the CRISPR loci. CRISPR loci is then transcribed and processed into crRNA during crRNA biogenesis. During interference, Cas9 endonuclease complexed with a crRNA and separate tracrRNA cleaves foreign DNA containing a 20-nucleotide crRNA complementary sequence adjacent to the PAM sequence

CRISPR-Cas9

- single RNA instead of two: sgRNA
- Cas9: targeting specificity and cutting activity can be programmed with sgRNA

Desired Characteristics

- reducing size of Cas9 nucleases
- increasing fidelity: specific mutations, 2 sgRNAs,etc.
- expanding targeting scope of Cas9
- off-target effects:
  - Cas9 binding sites + DNase Seq information: improved prediction of off-target effects
  - But binding does not mean cleavage
  - BLESS, GUIDESeq, Digenome-Seq : map DSB sites

second generation CRISPR gene editing tools

- base editing technology
- precisely convert a single base into another without DNA DSBs
- foundation: Nickase Cas9[C to T, A to G]
- Can be used for
  - early STOP Codons into genes

CRISPR mediated gene expression regulation

- CRISPRi
  - dCas9 strongly binds to target sequence
  - interferes with activity of other DNA binding proteins(TFs, RNA polymerase II[1])
- can also be used to increase gene expression
- dCas9-VP64 can recruit transcriptional activators

[1]RNA polymerase II (RNAP II and Pol II) is a multiprotein complex. It is one of the three RNAP enzymes found in the nucleus of eukaryotic cells.[1] It catalyzes the transcription of DNA to synthesize precursors of mRNA and most snRNA and microRNA.

CRISPR mediated epigenome editing

- functional roles of histone modifications poorly understood.
- CRISPR can give locus specific modifications: causal links between chromatin modification and gene expression.
- Use the programmable Cas9.
- DNA methylation: dCas9+DNMT3A/MQ3: deposition of DNA methylation lead to altered gene expression
- dCas9 to recruit for histone modifications to observe downstream effects

CRISPR: Cell chromatin imaging and manipulation of chromatin topology

- reengineering of chromatin loops
- new enhancer-promoter connections/ inhibit aberrant connections: gene expression

large scale genetic and epigenetic CRISPR screenings

- Instead of single sgRNA, use multiple sgRNA to guide Cas9 variant
- to identify genes that influence a particular phenomenon: can functionally screen thousands of genes at the same time
- say a gene is responsible for cell proliferation
- cells with sgRNA targeting that gene will deplete over time