CRISPR/Cas based technology : Taking Gene Editing to the next level

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NCSR "D"

BioHIT Group meeting

CRISPR - CRISPR/Cas

- **CRISPR** : **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats
- **Cas** : **C**RISPR **as**sociated genes

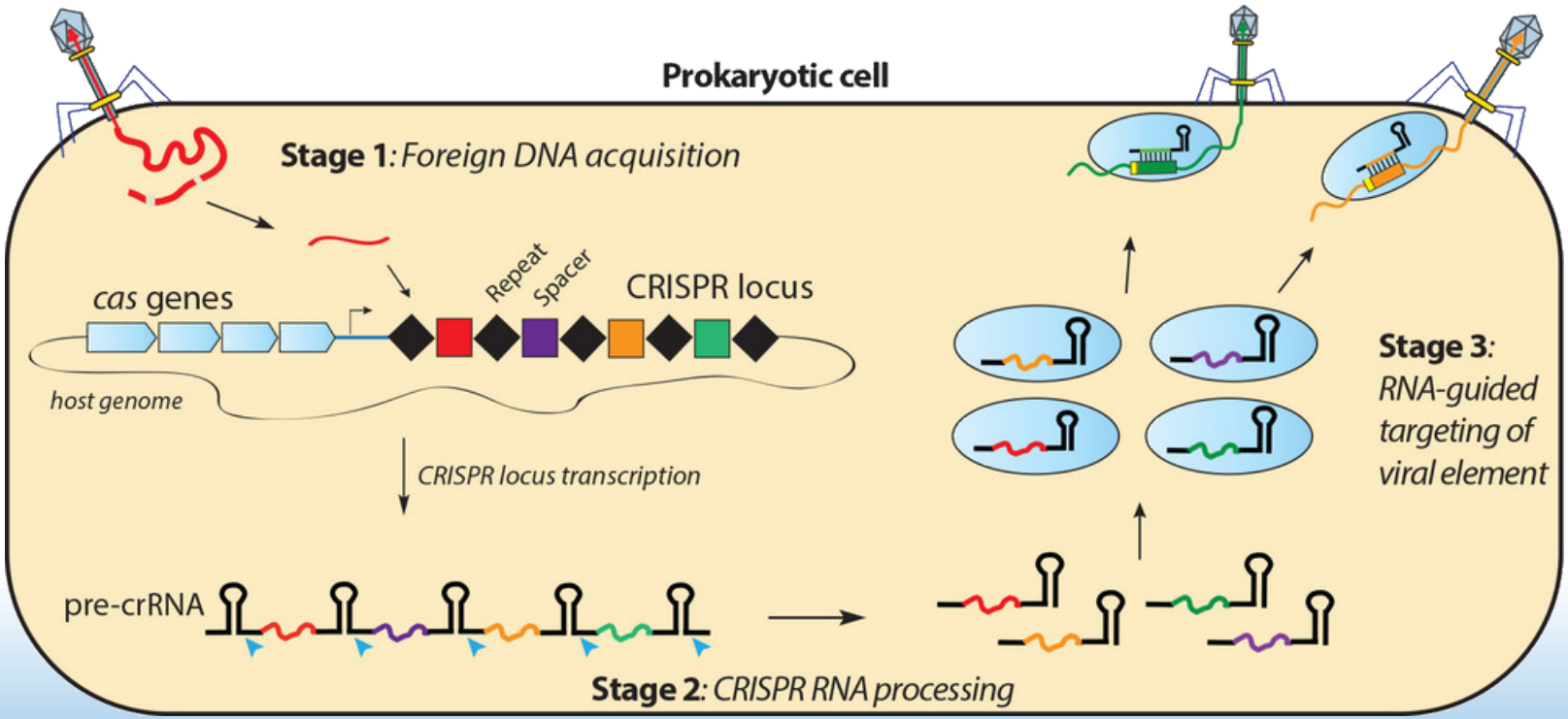
CRISPR Milestones

- **1987** : CRISPR Repeat Sequences Identified (Ishino et al.)
- **2007** : CRISPR Identified as an Adaptive Immune System (Barrangou et al.)
- **2012** : CRISPR Repurposed for Targeted DNA Cleavage (Jinek et al.)
- **2013** : First CRISPR Genome Engineering Applications (Cong et al., Mali et al.)
- Less than three years later, PubMed lists more than 2600 CRISPR-related publications

CRISPR Origins

Kurzgesagt, 2016.

CRISPR-mediated immunity

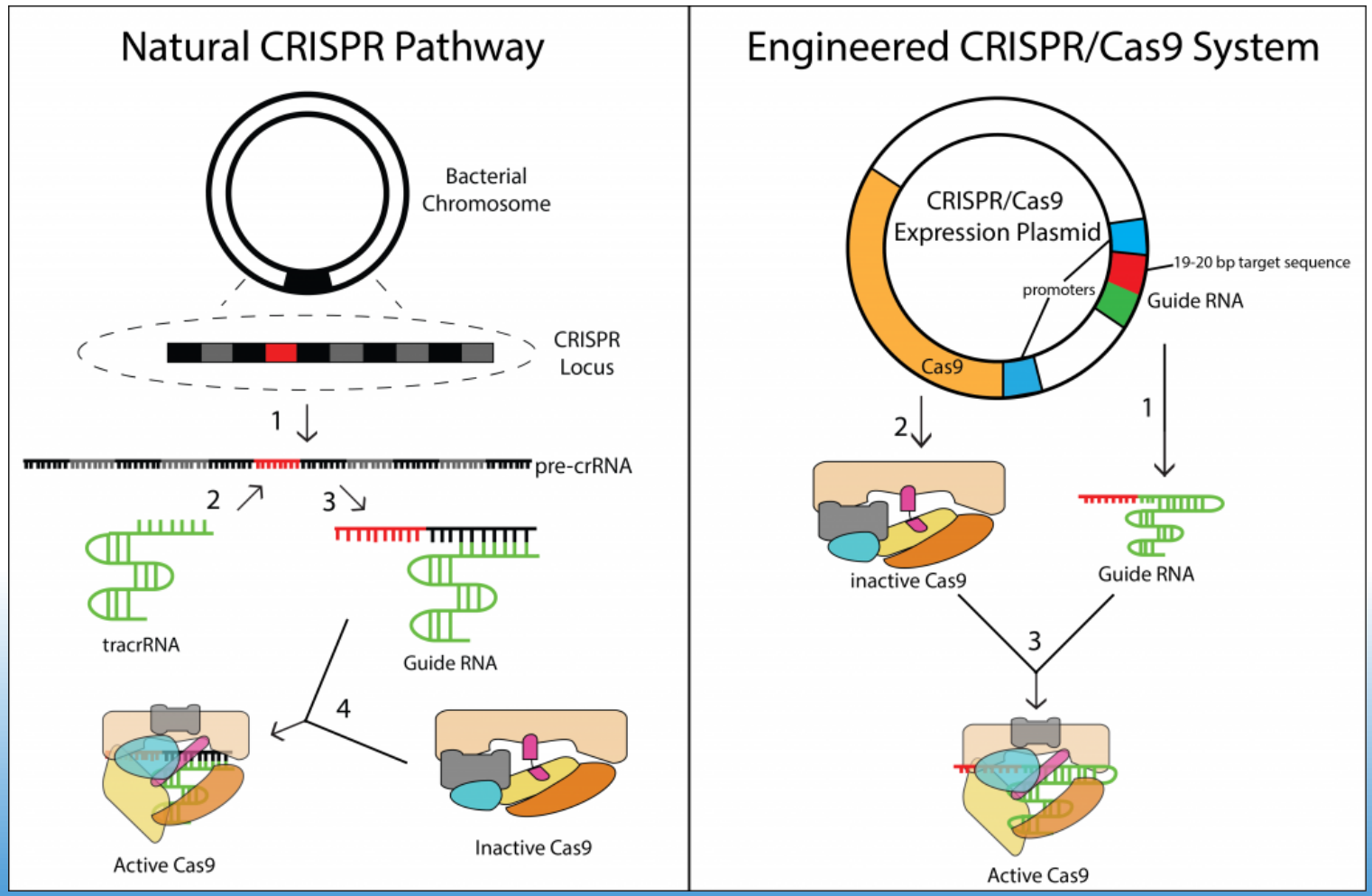


Adapted from Doudna Lab's website

CRISPR Systems' Diversity

- CRISPR systems are highly diverse and have been classified in 5 types and 16 subtypes based on shared characteristics and evolutionary similarity (Makarova et al., 2015).
- **Type II CRISPR/Cas system** was the first harnessed for genome engineering, as it is thought to be the simplest and the easiest to adapt, and is the basis for the current genome engineering technology available.
- **The Cas9 nuclease** is the active enzyme for the Type II CRISPR system.

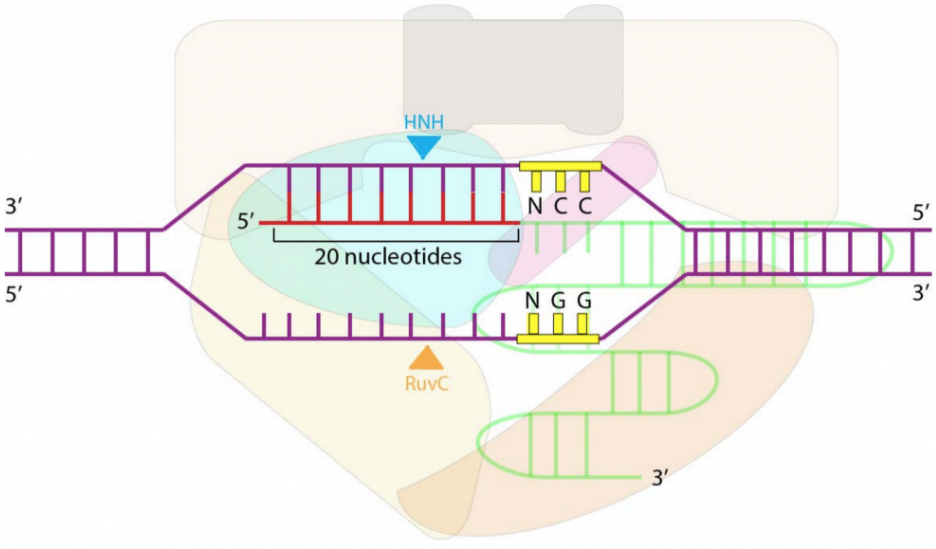
Natural VS Engineered CRISPR/Cas9 activation



Cavanagh & Garrity, CRISPR/Cas9, Tufts University, 2014.

CRISPR/Cas based technology : Taking Gene Editing to the next level, NCSR Demokritos, 2016.

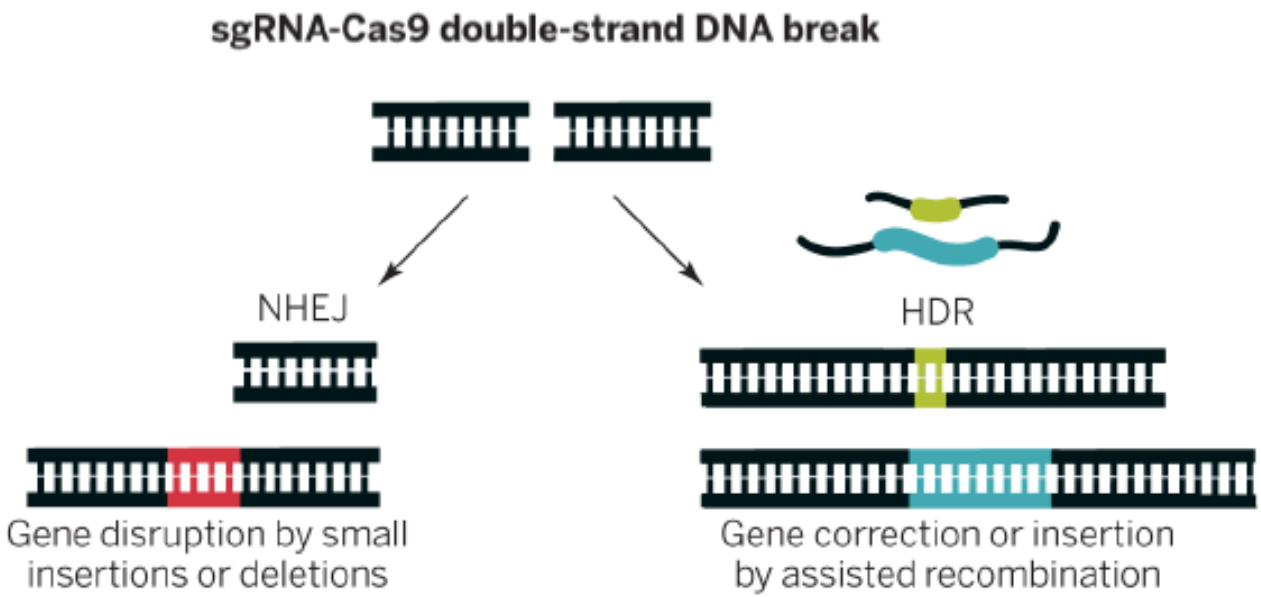
Cas9 cleaves double-stranded target DNA



Cavanagh & Garrity, 2014.

- CRISPR/Cas9 locates Protospacer Adjacent Motif (PAM) on target DNA
- Guide RNA (gRNA), also refered to as single guide RNA (sgRNA), binds on target next to the PAM sequence
- Cas9 cleaves both DNA strands with its HNH and RuvC domains

Double Strand DNA Break (DSB) Repair Pathways



Doudna et al., 2014.

Gene Knockout by Non-Homologous End-Joining (NHEJ) inducing small indels that often result in frameshift mutations. Gene Edit by Homology Directed Repair (HDR)

Genomic Manipulations with CRISPR

- **Knockout** : Permanently disrupt gene function in a particular cell type or organism without regard for specific mutation
- **Knock-In** or Edit : Generate a specific user-defined sequence change in a particular gene, such as generating a point mutation or inserting a tag
- Interference (**CRISPRi**) : Reduce expression of a particular gene(s) without permanently modifying the genome
- Activation (**CRISPRa**) : Increase expression of an endogenous gene(s) without permanently modifying the genome

Why Choose CRISPR?

- Cheap
- Fast
- Easy to implement
- Precise
- Scalable

CRISPR is not Perfect : Off-targets

Approaches to minimise/eliminate CRISPR off-target binding :

- **Biological**
  e.g. Cas9 nuclease Alternatives (Cas9 Nickase, Cas9 variants : eSpCas9 and SpCas9-HF1, Cpf1)
- **Computational**
  Several Software tools are being developed to improve CRISPR/Cas efficacy

CRISPR Experiment Design Steps

1. Biological Question?
2. Desired Manipulation? (e.g. Knockout)
3. Genome Wide or Single Gene Edit?
4. Expression System and Delivery? (e.g. Viral Vectors)
5. In Silico Design sgRNAs and Perform Cloning
6. Deliver CRISPR Components and Validate Genome Edit

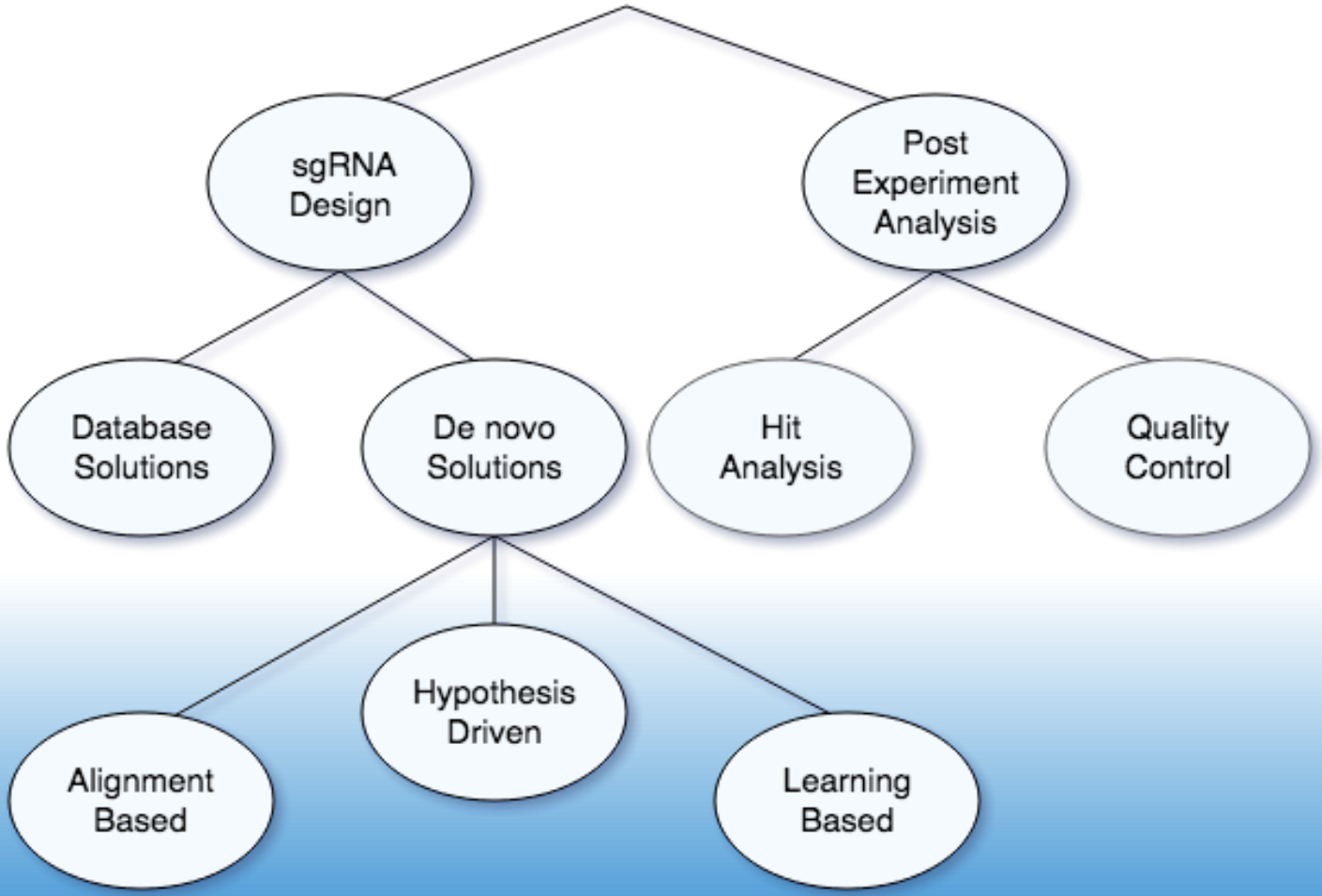
CRISPR Software : The Piñata effect

Cameron MacPherson for Addgene, 2015.

CRISPR Software Available

- 36 CRISPR Software tools published and documented by omictools, 11 new tools appear yearly.
- Some attempts to compare those tools : Graham & Root, Chuai et al.
- The CRISPR Software Matchmaker : an Excel-based tool-selection aid program (MacPherson et al.)
- **Lack of systematic tool evaluation.**
- **In need of well-curated benchmark data in different species and cell types.**

Areas for CRISPR Software Development



Curated from Chuai et al., MacPherson et al.

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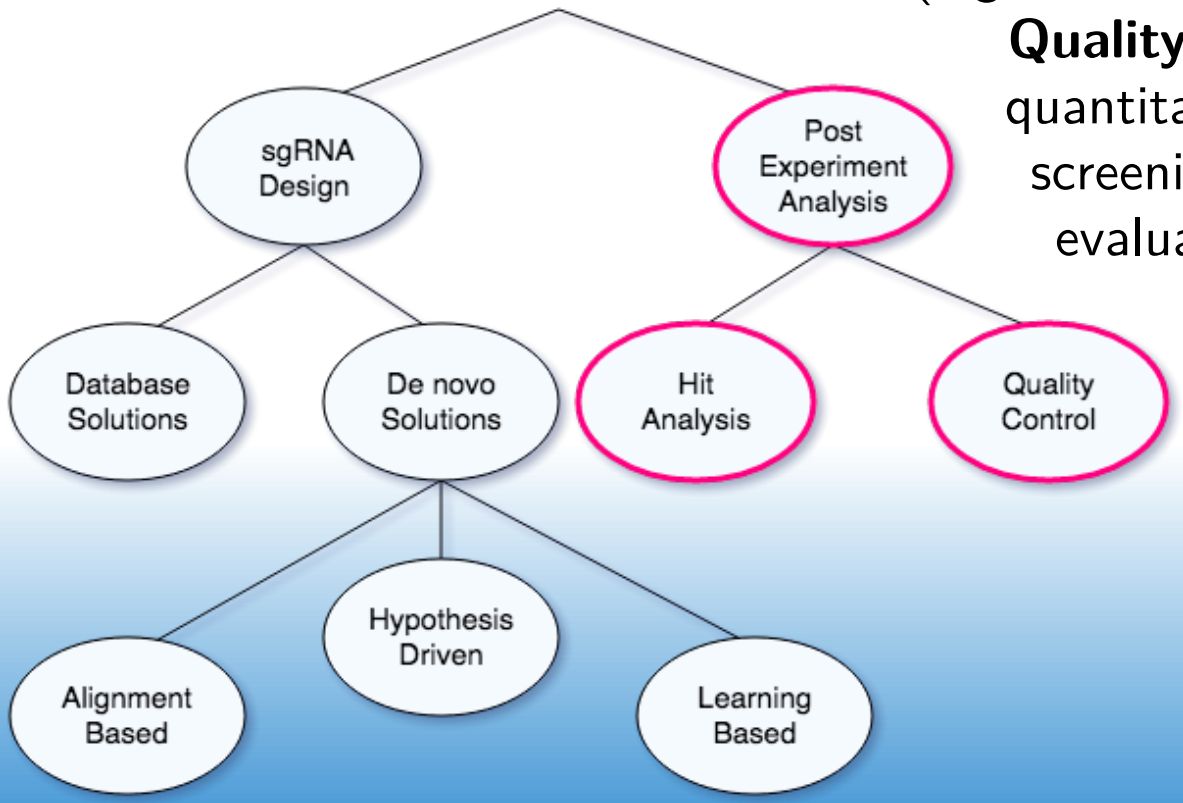
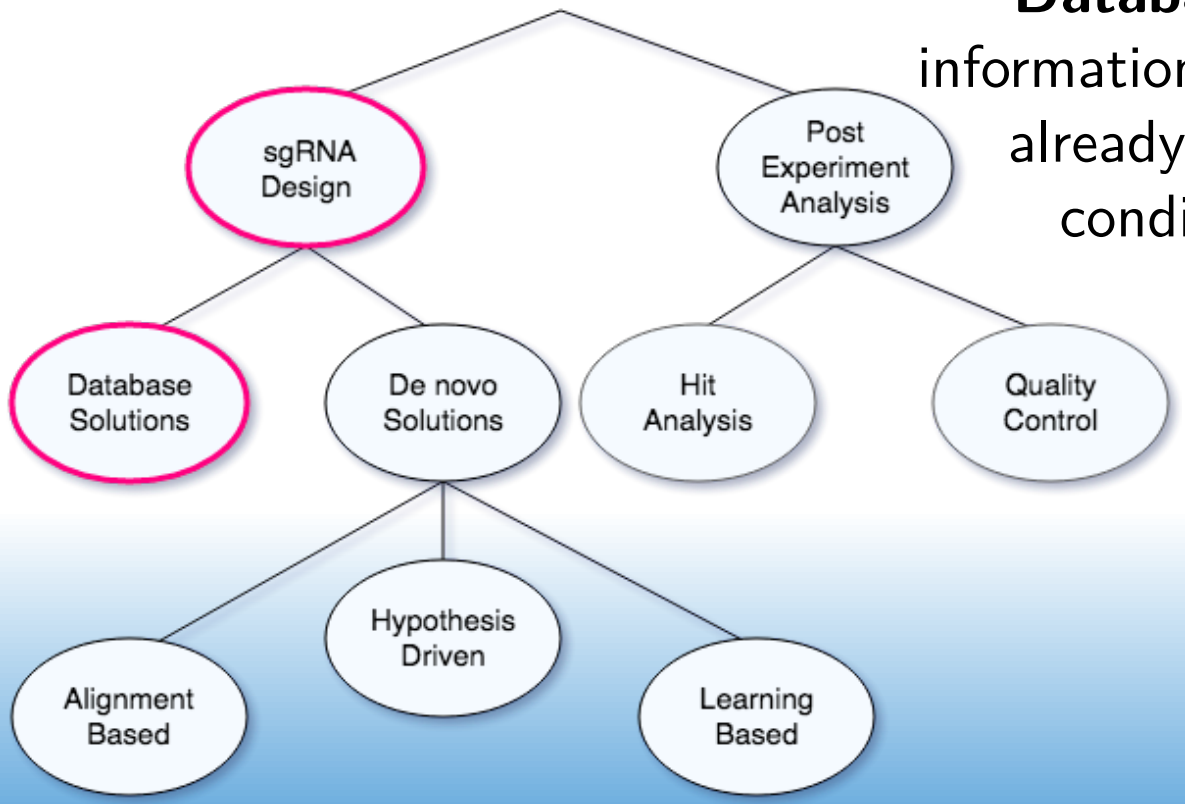
Post Experiment Analysis



Hit Analysis : identification of significantly selected sgRNAs, genes and pathways involved in CRISPR screens (e.g. MAGeCK)

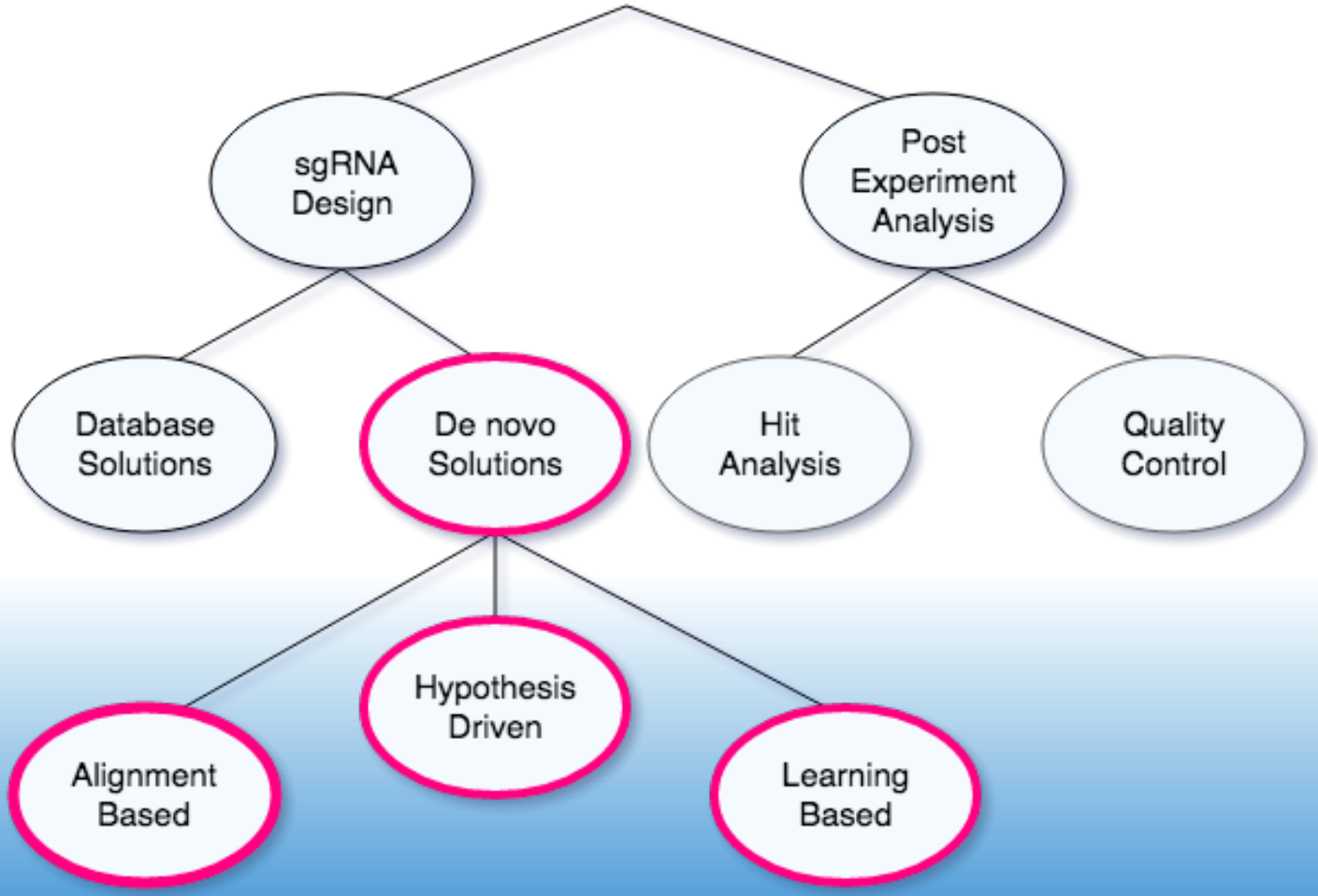
Quality Control : qualitative and quantitative evaluation of CRISPR screening experiments mostly by evaluating resulting mutations (e.g. CRISPResso)

sgRNA Design



Database Solutions : provide information about which sgRNAs have already worked and under what conditions. (e.g. CrisprGE)

sgRNA Design



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sgRNA Design - De novo Solutions

- **Alignement Based :**
  sgRNAs aligned from the given genome purely by locating the PAM. (e.g. Cas-OFFinder)
- **Hypothesis Driven :**
  sgRNAs aligned and scored heuristically, according to specific factors (e.g., GC content, exon position, etc.) to sgRNA on-target efficacy (e.g. Chopchop)
- **Learning Based :**
  sgRNAs scored from a training model & off-targets predicted (e.g. sgRNA Designer - Rule Set 2)

sgRNA Design - De novo Solutions

- First tools (Hsu et al., Doench et al. 2014) :
  empirically derived rules,
  prior knowledge &
  approximate or sensible rules (some rules **here**)
- Latest tool (Doench et al. 2016) :
  new regression models,
  larger datasets
  –> resulted in Rule Set 2

Our focus : on Learning Based Solutions for CRISPR

- **Motivation :**
  - Better performance is expected by Learning Based approaches (Doench et al. 2016, Chuai et al. 2016)
  - Certain features (e.g. chromatin features) not yet explored
  - Heterogenous results
- **What we've been doing :**
  - Studying different methods
  - Collecting available datasets
  - Applying new techniques
- **Future Work :**
  - Reveal new features
  - Improved new tool(s)

Duchenne Muscular Dystrophy (DMD)

- Affects $\sim$ 1 in 5000 males
- Caused by frameshift mutations in dystrophin, a gene that regulates muscle function
- Largest human gene (79 exons)
- Mutational hotspots : primarily exons 45-55
- No good treatment to date

Exon Skipping Approach for DMD

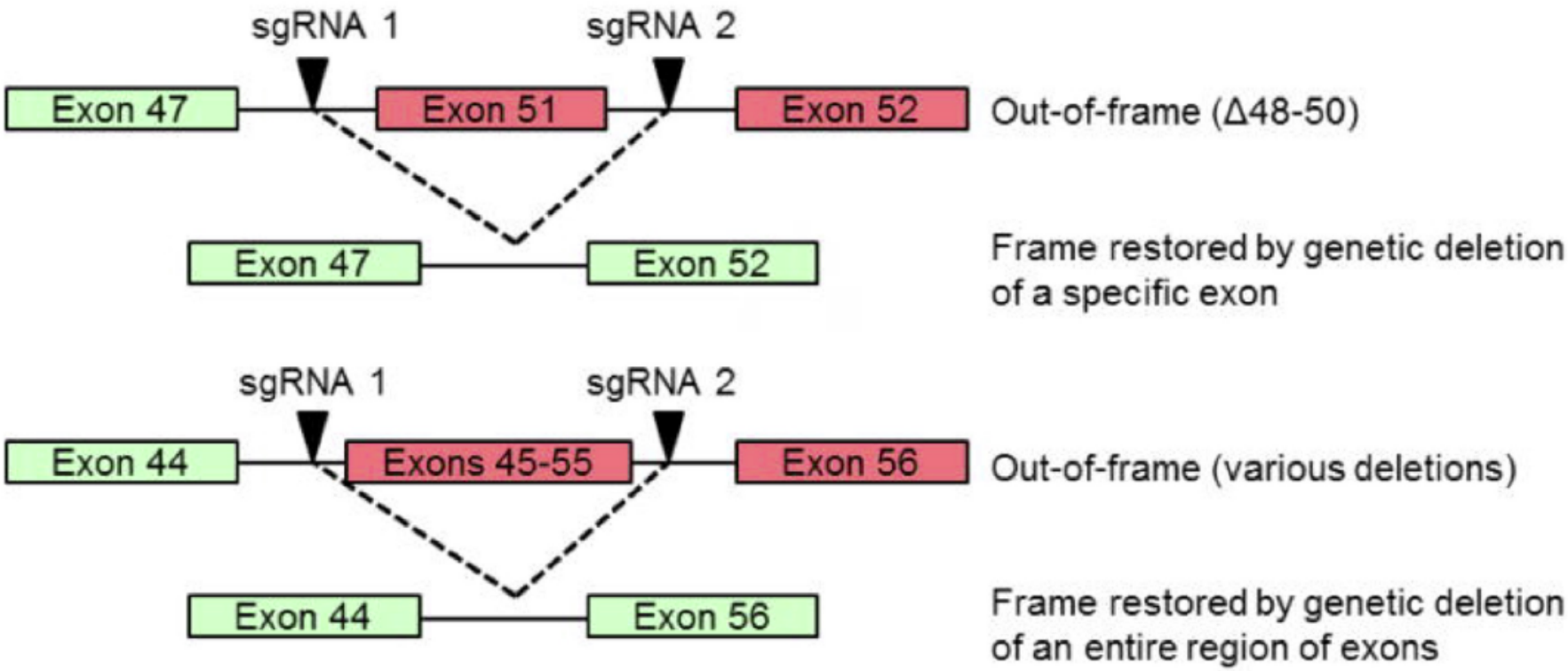
- Certain deletions maintain partially functional dystrophins (Monaco et al., 1988) which result in a milder condition known as **Becker muscular dystrophy (BMD)**.
- **Oligonucleotide-mediated exon skipping** has been used to restore the dystrophin reading frame during mRNA processing and convert DMD to a Becker-like phenotype.(Qi-Long Lu et al., 2011)
- **Disadvantages** : the oligonucleotides only modestly improve muscle function, and they must be injected regularly.

CRISPR-mediated Exon Skipping in DMD mice

Multiplex CRISPR system **(2 sgRNAS) to eliminate exon 23** in mouse model (Long et al., Nelson et al. & Tabebordbar et al.)

- **Targets :** Introns 22 & 23
  - By targeting intronic regions, indels generated by NHEJ should not affect trancript production
- **Delivery Method :** Viral vector (Adeno-Associated Virus (AAV))
- **Results :**
  - Positive phenotypes in cardiac and skeletal muscle
  - Very low to no off-target activity

CRISPR-mediated Exon Skipping in DMD myoblasts



Adapted from Osterout et al. 2015.

Issues to be adressed before human trials

- Immunogenicity of the AAV vector
- Off-targets
- Reach higher percentage of muscle cells

Numerous Exciting Possibilities

Perspectives

Kurzgesagt, 2016.

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Questions?

Thank you!
Contact for further information :
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