Plant Biotechnology

Bayer Russia Plant Biotechnology Conference

July 2023

Molecular Assays

Edit Detection

Gene Editing Detection and Analysis

Starting Materials:

1. Sequencing Data
2. Target sequences
3. Query sequences
4. Metadata

Desired Output:

1. A report of editing events
2. A report on quality of input data
3. Provenance

Corn Zm 7.1 target information

gRNA target highlighted

Gene-specific primer underlined

>Zm7.1_amplicon

CGAAGTACCATGTTTCAAATGCTGGCCGGCTACTGCCTAAGAGGGCATGCCATCATATTATACTAAATAGACTATCATAAAGAACAGATGGTATTATTTC
ACATCGAATGACTCATTGTCCTCGCTAGCACTGTCAACATAAACCAATGTTATTAGAGCCAATTTCCGCGCTGCTGAAGAATGAAGACTGAGCTTGCA
CATCCAGAAGCATCCATCACATGCTTTGTTTTCTTCTTCTTACATTTTCAAGTCCTCCCAACTCACTCCC

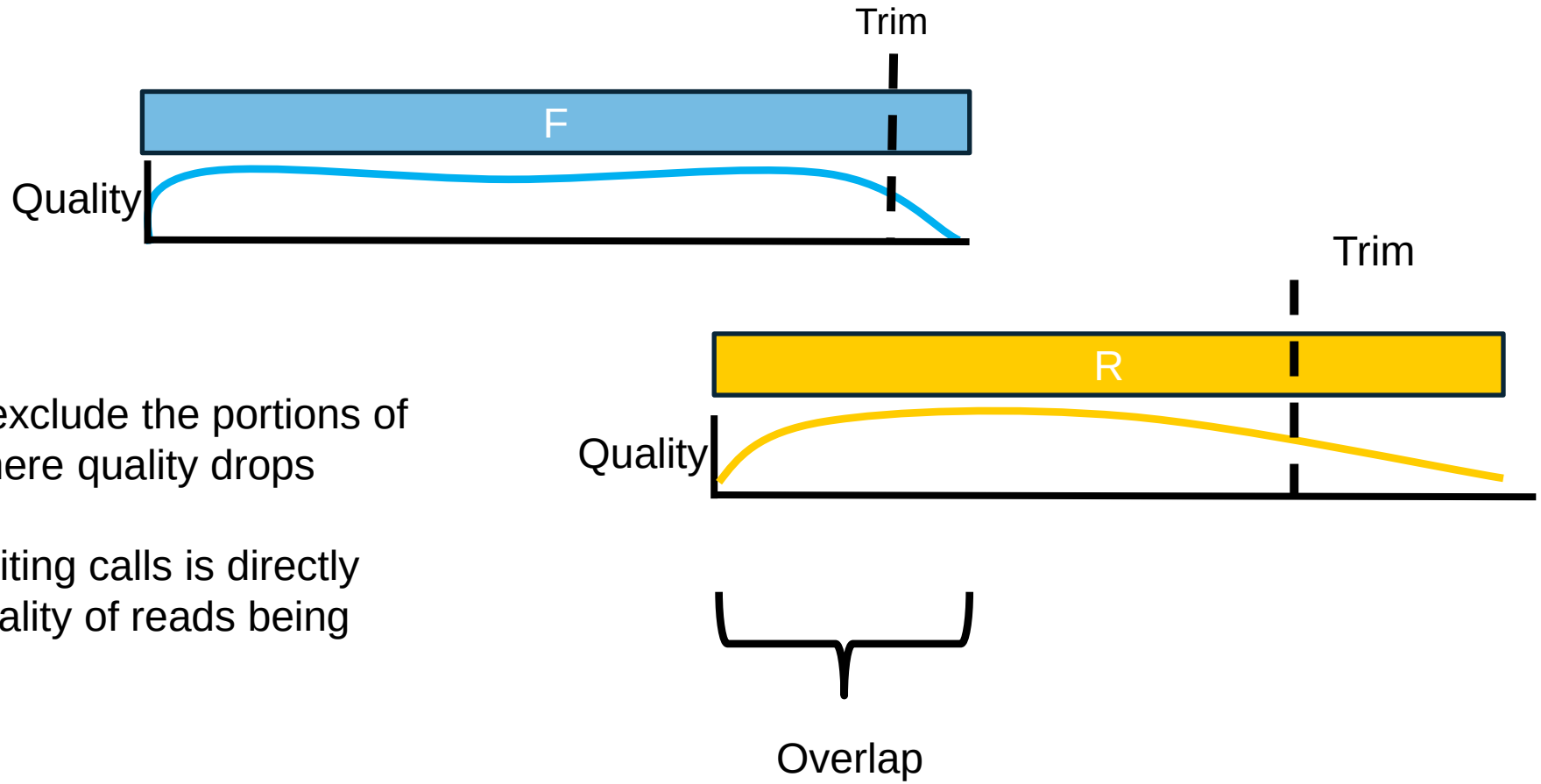
Soy Fad2-1A target information

gRNA target highlighted
Gene-specific primer underlined

>Expected Chromosome 10 Fad2-1A_amplicon sequence from gene-specific primers
CCATGCCTTCAGCAAGTACC***A***ATGGGTTGATGATGTT***G***TGGGTTTGACC***C***TTCACTCA***A***CACTTTTAGTCCCTTATTTCTCATGGAAA
ATAAGCCATCGCCGCCA***T***CACTCCAACAC***A***GGTTCCCTTGACCGTGATGAAGTGTTTGTCCCAAAACCAAAATCCAAAGTTGCATG
GT***TTT***CCAAGTAC***T***T***A***AACAACCCTCTAGGAAGGGCTG***T***TTCTCTTCTC***G***TCACACTCACAATAGGGTGGCCT***A***TGTATTTAGCCTT
CAATGTCT
>Expected Chromosome 20 Fad2-1A_amplicon sequence from gene-specific primers
CCATGCCTTCAGCAAGTACC***C***ATGGGTTGATGATGTT***A***TGGGTTTGACC***G***TTCACTCA***G***CACTTTTAGTCCCTTATTTCTCATGGAAA
ATAAGCCATCGCCGCCA***C***CACTCCAACAC***G***GGTTCCCTTGACCGTGATGAAGTGTTTGTCCCAAAACCAAAATCCAAAGTTGCATG
GT***ACA***CCAAGTAC***C***T***G***AACAACCCTCTAGGAAGGGCTG***C***TTCTCTTCTC***A***TCACACTCACAATAGGGTGGCCT***T***TGTATTTAGCCTT
CAATGTCT

- There are 14 SNPs that can be used to differentiate edits between Chr10 and Chr20 (SNPs bold and italicized)

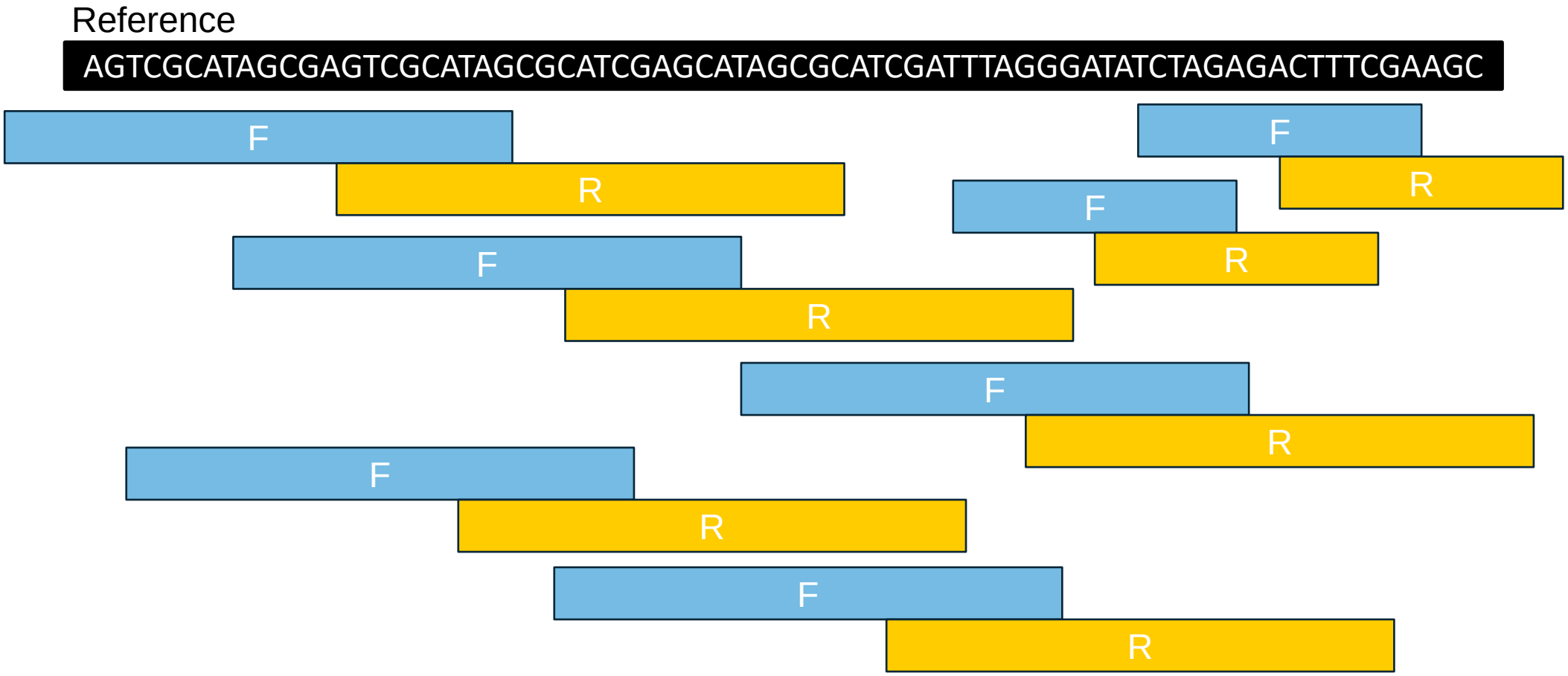
1. Perform quality control on sequencing data



- We want to exclude the portions of the reads where quality drops
- Quality of editing calls is directly related to quality of reads being used

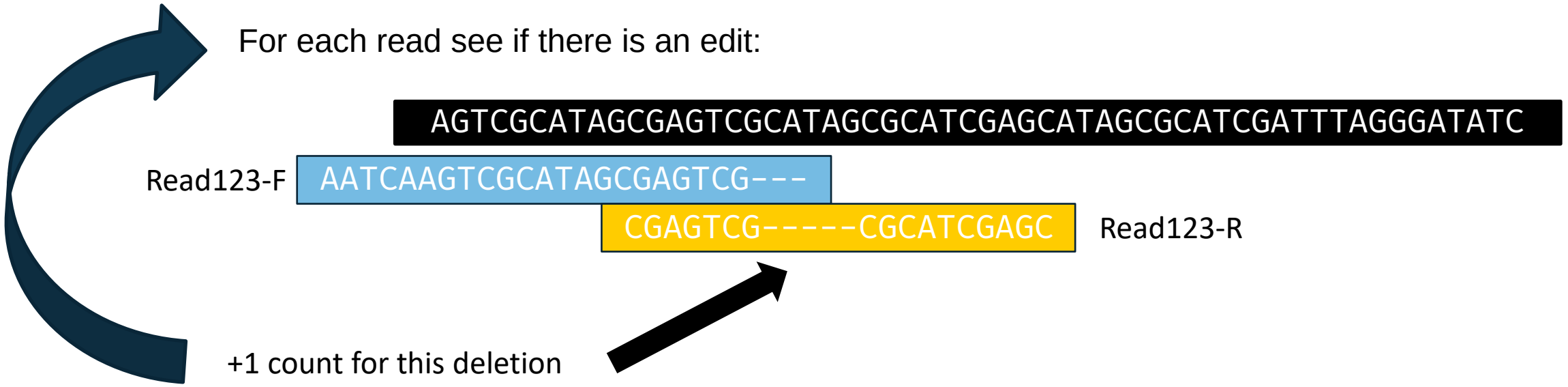
F = forward
R = reverse

2. Align sequencing data to target



F = forward
R = reverse

3. Find and count edits



F = forward
R = reverse

4. Report results

Sample	Target	Query	Type	Edit	%	Sequence
Sample001	Zeamays_chr3	Target1	Wt	-	99%	ACGTACGTA ACGTACGTA
Sample001	Zeamays_chr3	Target1	Edit	3M5D1M	15%	ACGTACGTA ACG-----A
Sample002	Zeamays_chr4	Target2	Edit	4M1D4M	40%	GTAGTCTTT GTAG-CTTT

A string annotation for the edit where M is “match” and D is “deletion”

Alignment to reference

5. Continue with promising edits

- At this point you can decide which edits are promising and move them forward in the pipeline
- Determine which editing technology is working well or not for specific targets
- Add this data to previous experiments for continued provenance tracking of an edit through generations

Available Applications

CRISPResso2

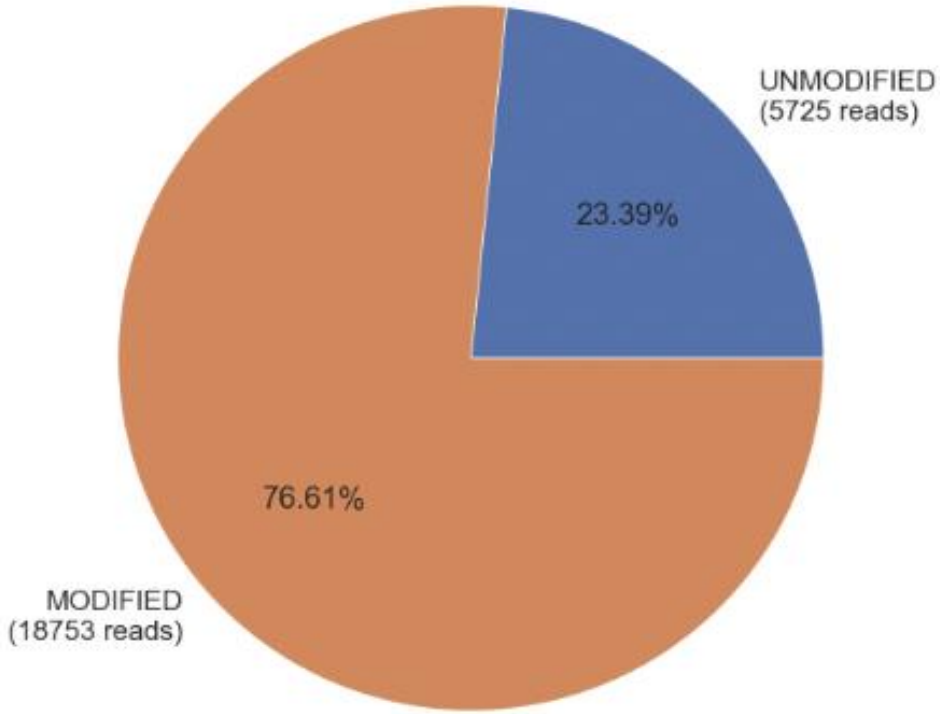
Analysis of genome editing outcomes from deep sequencing data

- http://crispresso2.pinellolab.org/submission

Cas-Analyzer
- http://www.rgenome.net/cas-analyzer

CRISPRmatch
- https://github.com/zhangtaolab/CRISPRMatch

Hi-TOM 2.0
- http://hi-tom.net/#/mutation/mutation_detection



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Thank you!

Any questions?