NMIN Capacity-Building Webinar

rhAmpSeq™ CRISPR:

Multiplexed Amplicon Sequencing & Analysis

Adam Chernick

Field Application Manager

Integrated DNA Technologies (IDT)

rhAmpSeq™ CRISPR MULTIPLEXED AMPLICON SEQUENCING AND ANALYSIS

ADAM CHERNICK, PhD

November 16, 2020

INTEGRATED DNA TECHNOLOGIES

THE WORLD'S LARGEST SUPPLIER OF CUSTOM NUCLEIC ACIDS

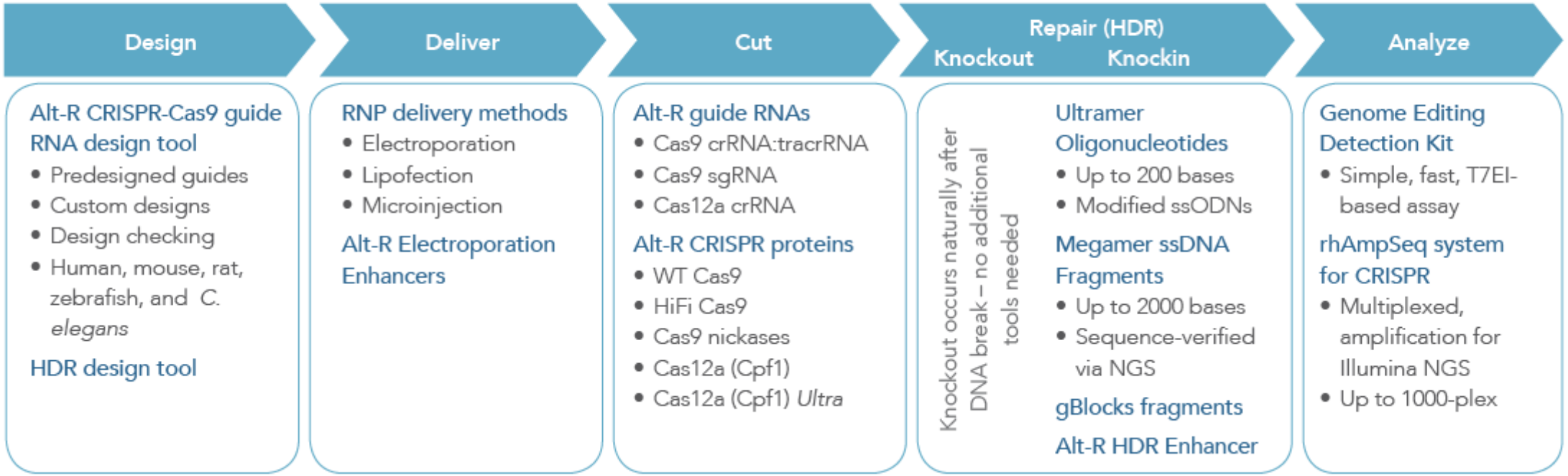
>64,000 oligos synthesized every day

- **Major R&D teams (~100 employees)**
- CRISPR
- NGS
- Genotyping
- qPCR
- Synthetic Biology
- RNAi
- Bioinformatics

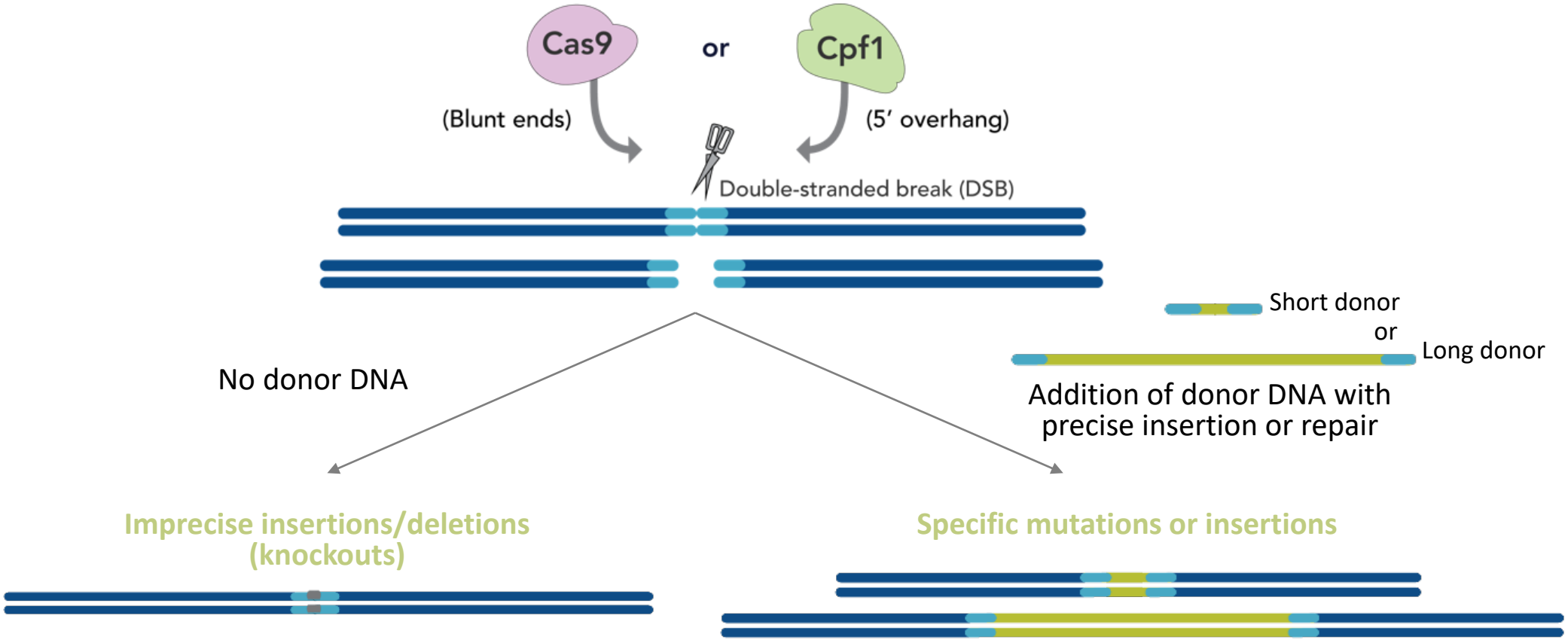
IDT™ INTEGRATED DNA TECHNOLOGIES

IDT SUPPORTS THE CRISPR WORKFLOW

Alt-R CRISPR System – a complete workflow



CRISPR GENOME EDITING OVERVIEW



Non-homologous end joining (NHEJ)

Knockouts

Genomic screens

Homology-directed repair (HDR)

Change allele (e.g., mimic disease)

Gene fusions (e.g., tagging)

ON-TARGET AND OFF-TARGET EDITING

- Genome editing is not perfect
- CRISPR can result in edits throughout the genome
- On-target and off-target edits must be identified and quantified

WHAT DOES rhAmpSeq DO?

- Enables the best-in-class insertion/deletion (indel) quantification of genome editing
- Sequences on-target and off-target sites simultaneously
- Yields great uniformity and high “map rate” as well as quantitative readout
  - GUIDE-seq or other unbiased methods result in significant noise (>80% unmapped or non-target reads) in library prep; the rhAmpSeq system does not generate these wasted reads
- Provides detailed info at on-target sites, as well as off-target sites, all in one reaction
  - The condensed workflow saves time and reagents during design and experiment

The rhAmpSeq system is NOT an off-target identification method; it is a tool used to evaluate up to 5000 *known* or *predicted* off-target sites genome-wide.

STEP 1: IDENTIFY OFF-TARGET SITES

- Unbiased detection methods
  - Cell-based—GUIDE-seq
  - *In vitro*—SITE-Seq
- *In silico* prediction methods
  - IDT “gRNA checker”
  - Cosmid, CRISTA, RGEN

GUIDE-seq results

Internal, improved protocols to share with customers

Tsai, *et al*., *Nat Biotech* (2015)

		Guide Coordinates				*Sequence Alignments*	
Mismatch	Assay	Chr	Start	Stop	Strand	G T T G G A G C A T C T G A G T C C A G	GS Reads
0	AR_iGS_1	chrX	67545904	67545924	+	G T T G G A G C A T C T G A G T C C A G	4030
4	AR_iGS_2	chr17	14626784	14626804	-	A A T G G G C A T C T G A G T C C A T	1671
3	AR_iMS_2	chr1	27592693	27592713	-	G A T G G A G C A A C C G A G T C C A G	1190
2	AR_iGS_3	chr7	22126335	22126355	-	A T T G G A G C C T C T G A G T C C A G	940
2	AR_iGS_4	chr20	46362541	46362561	-	G T T G G A G A A A C T G A G T C C A G	562
1	AR_iMS_3	chr8	70014903	70014923	-	G T T G G A G C C T C T G A G T C C A G	418
3	AR_iMS_4	chr12	122113358	122113378	-	G G A G G A G C A C C T G A G T C C A G	409
3	AR_iGS_5	chr10	75605948	75605968	-	G G A A G A G C A T C T G A G T C C A G	341

STEP 2: STUDY THEM IN DETAIL WITH rhAmpSeq

STEP 1: IDENT TES

- Unbiased detection n rediction methods
  - Cell-based—GUI gRNA checker”
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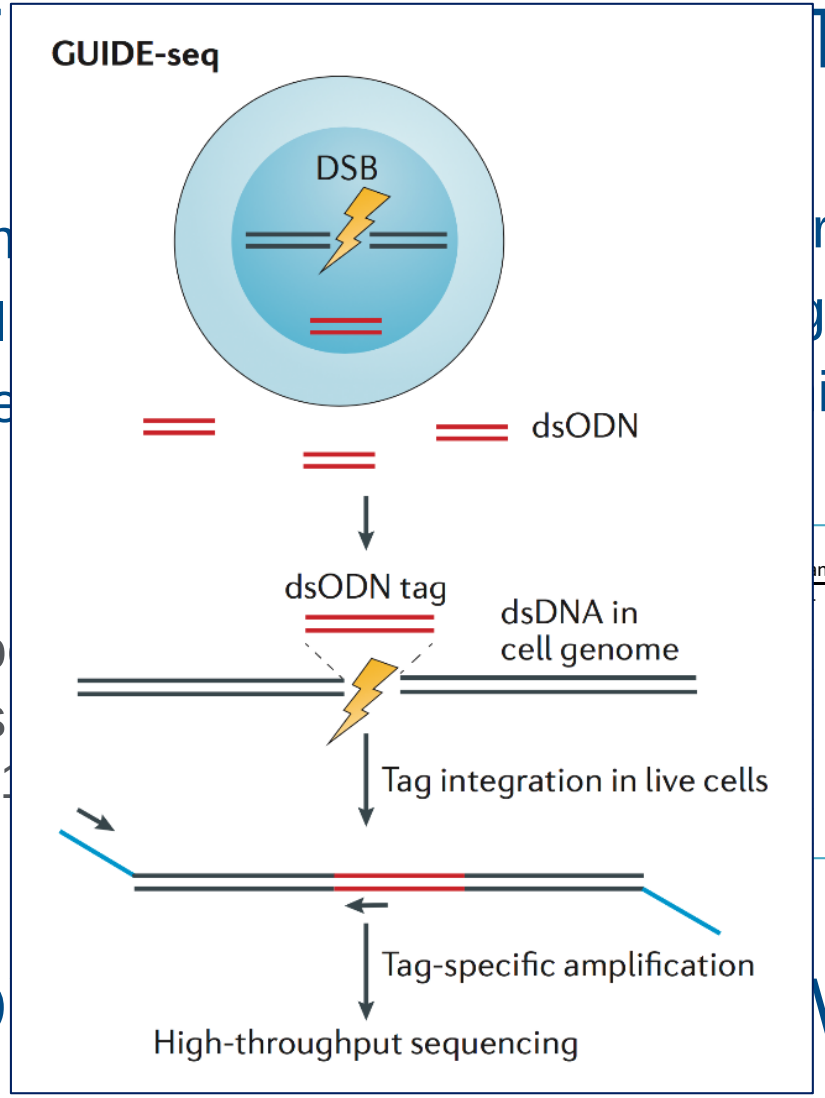
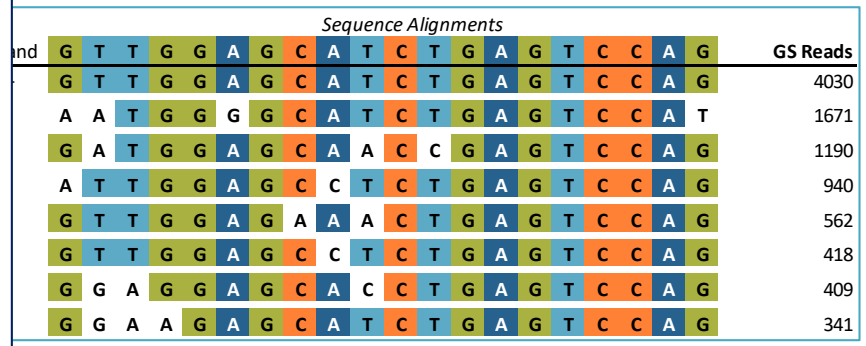
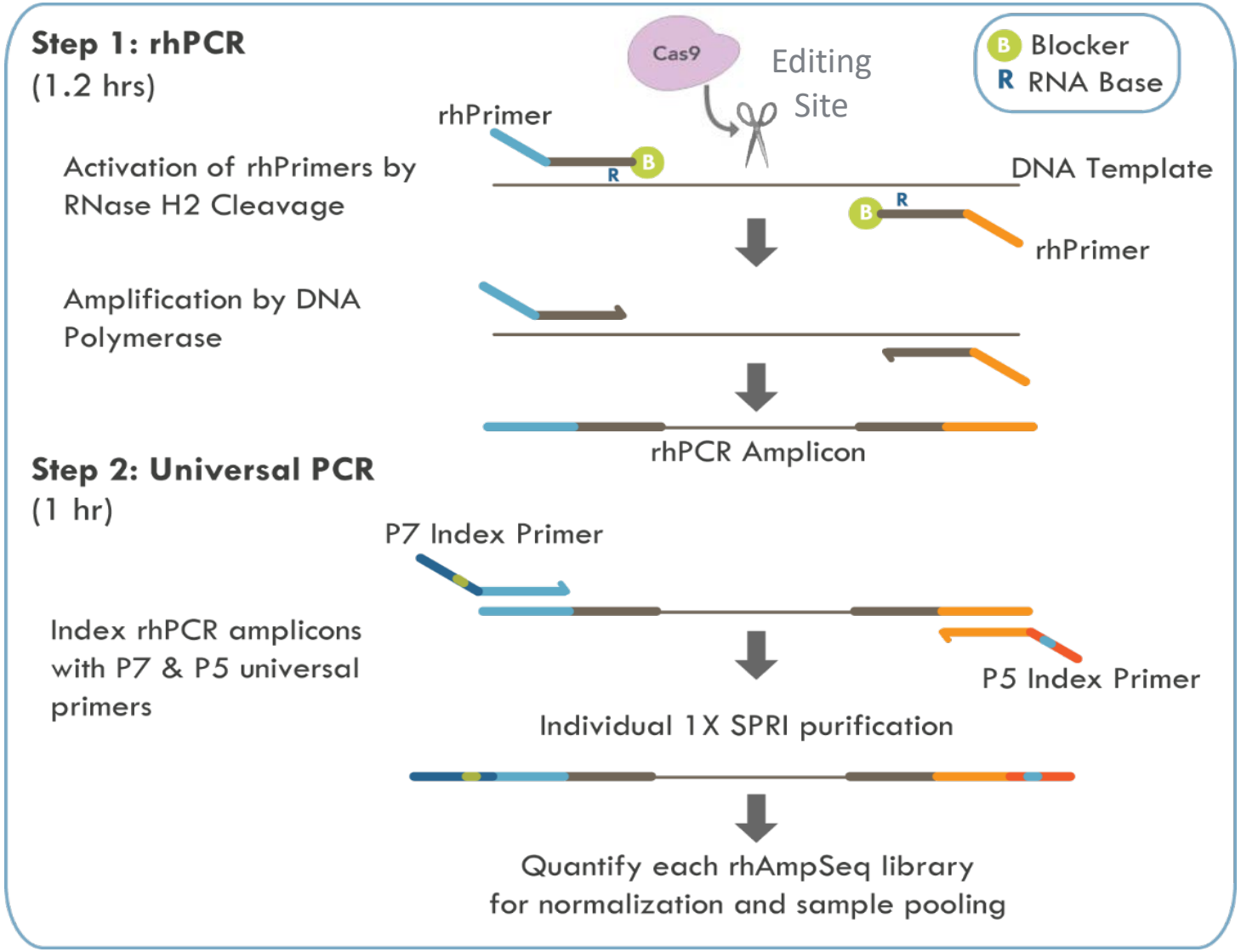


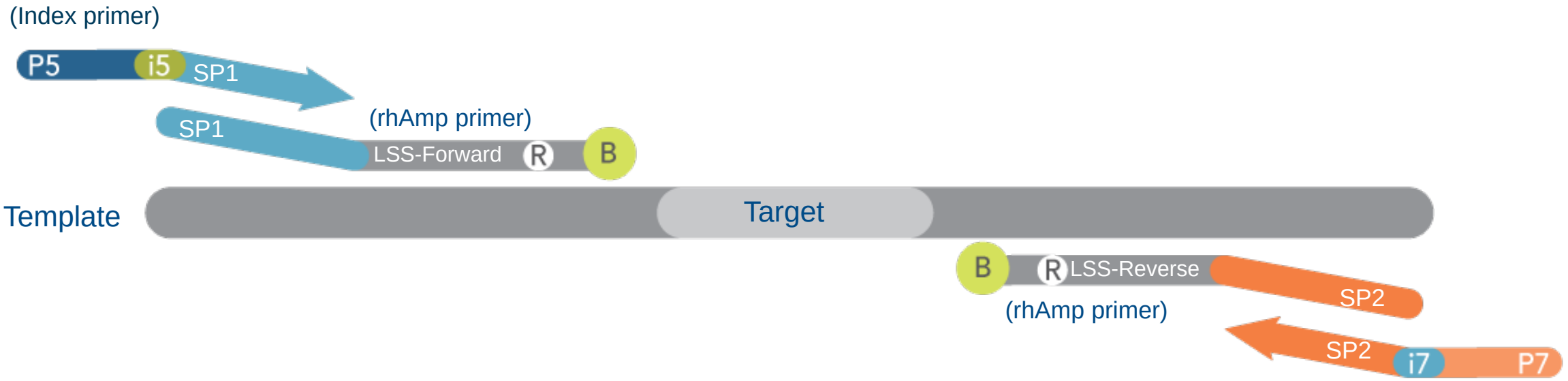


STEP 2: STUD VITH rhAmpSeq

rhAmpSeq METHODOLOGY?



rhAmpSeq LIBRARY FORMATION



LSS: Locus-specific sequence

Assay: A pair of forward and reverse rhAmp primers designed to amplify a specific region
Panel: Collection of assays (forward panel and reverse panel)

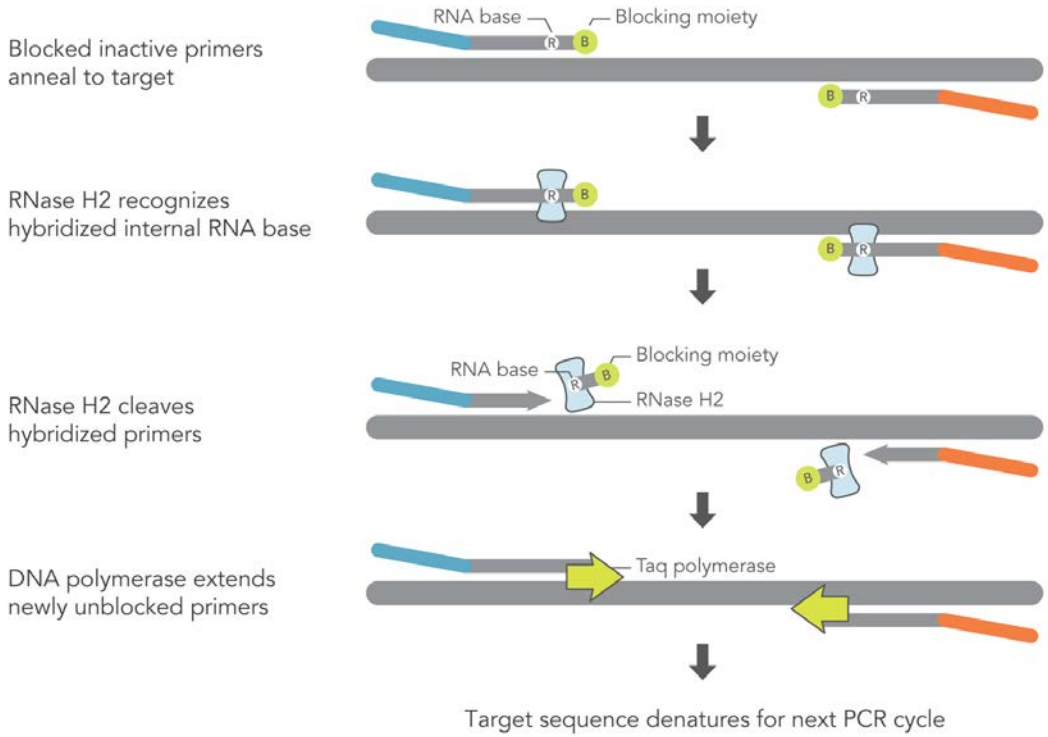
rhAmp PCR TECHNOLOGY INNOVATED AT IDT

BMC Biotechnology (2011) 11:80

METHODOLOGY ARTICLE **Open Access**

RNase H-dependent PCR (rhPCR): improved specificity and single nucleotide polymorphism detection using blocked cleavable primers

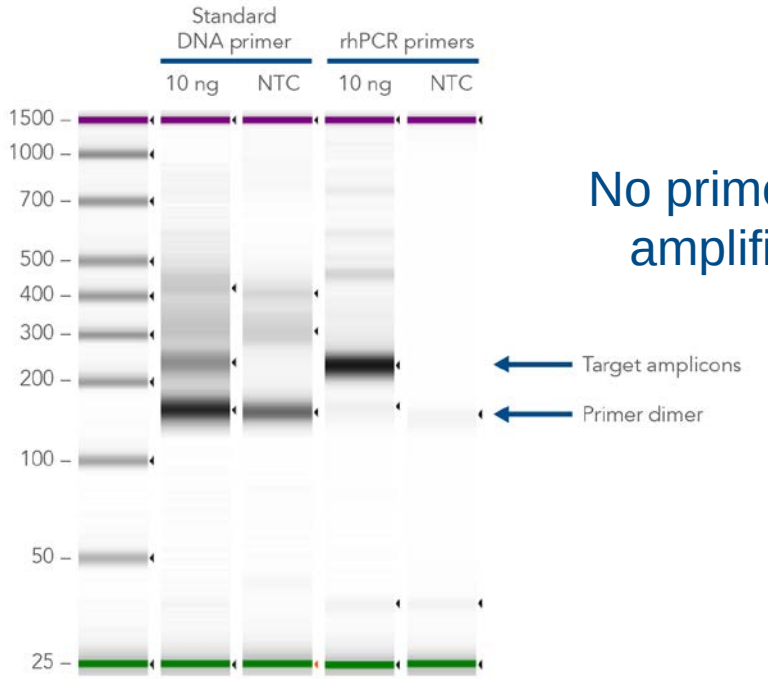
Joseph R Dobosy, Scott D Rose, Kristin R Beltz, Susan M Rupp, Kristy M Powers, Mark A Behlke* and Joseph A Walder



BENEFITS OF rhAmp PCR

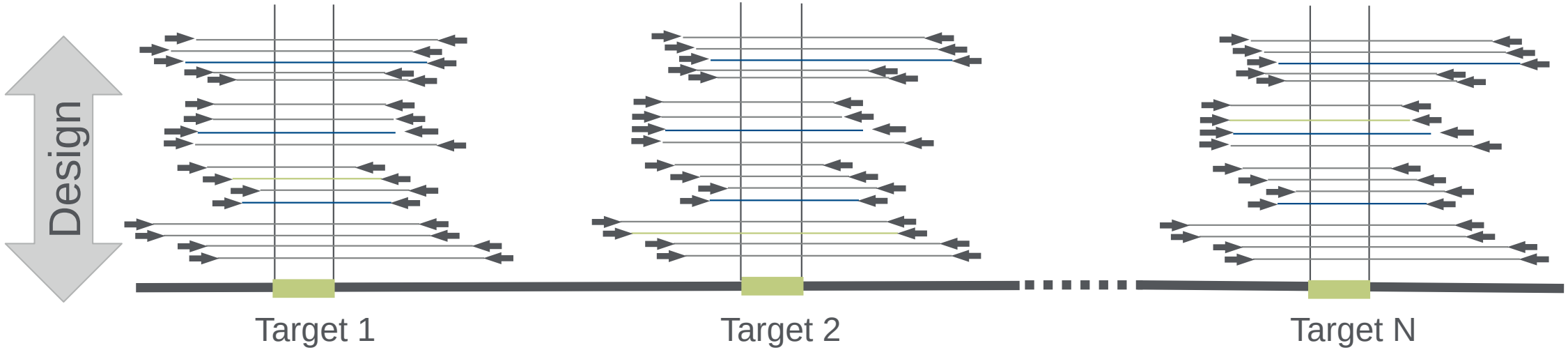
RNase H2 is sensitive to mismatches around the RNA base cleavage site, thus enabling:

- Higher specificity
- Lower potential for primer-dimers
- Higher multiplexing capability
- Modular panel designs
- Flexible amplicon sizes



No primer-dimer amplification

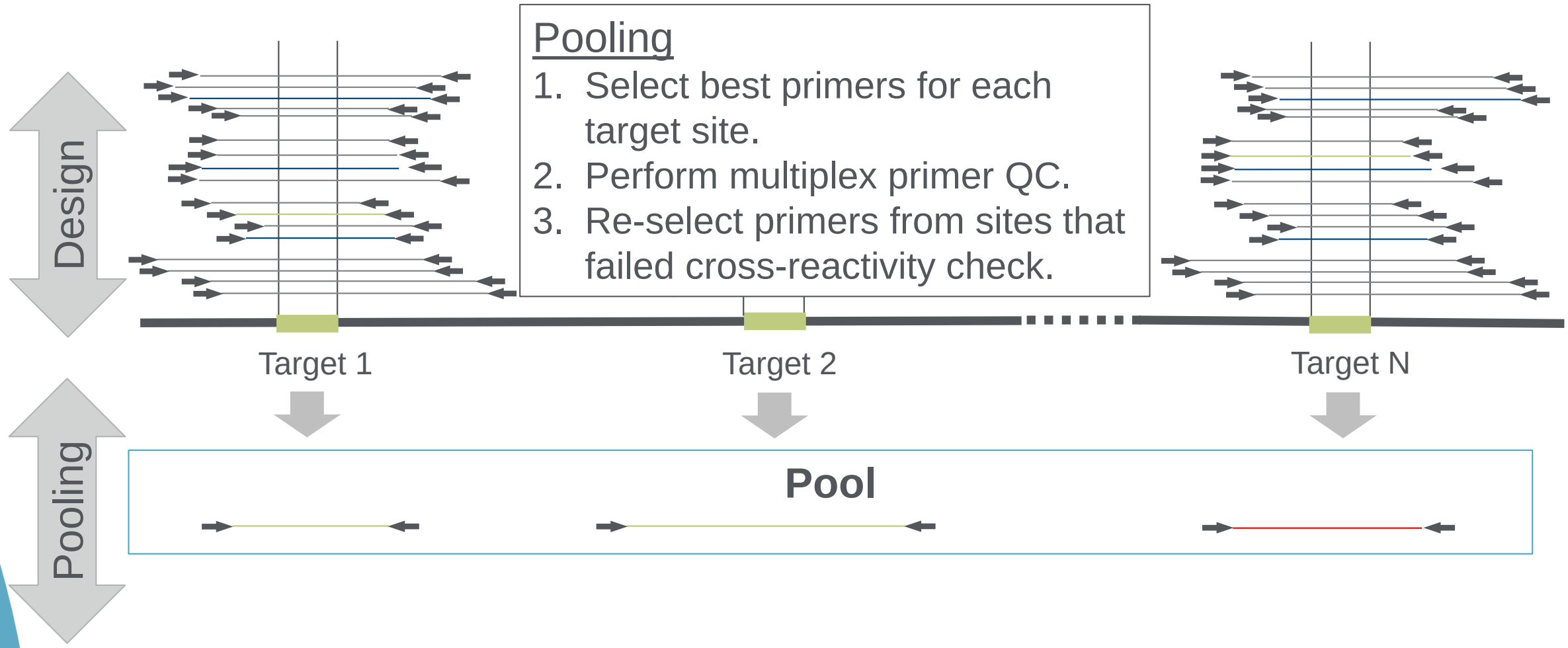
rhAmpSeq DESIGN APPROACH



Design

1. Flexible amplicon size
2. Exhaustive primer design around each target site
3. Comprehensive QC

rhAmpSeq DESIGN APPROACH



PUBLICATIONS: ON/OFF-TARGET WORKFLOW

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Original Article

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[1]Institute of Nanotechnology and Advanced Materials, The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 5290002, Israel; [2]Integrated DNA Technologies, Coralville, IA 52241, USA; [3]Department of Computer Science, Interdisciplinary Center, Herzliya 4610101, Israel; [4]Department of Computer Science, Technion–Israel Institute of Technology, Haifa 3200003, Israel

Cold Spring Harbor Laboratory
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CIS checkpoint deletion enhances the fitness of cord blood derived natural killer cells transduced with a chimeric antigen receptor

May Daher, Rafet Basar, Elif Gokdemir, Natalia Baran, Nadima Uprety, Ana Karen Nunez Cortes, Mayela Mendt, Lucila Nassif Kerbauy, Pinaki P. Banerjee, Mayra Hernandez Sanabria, Nobuhiko Imahashi, Li Li, Francesca Lorraine Wei Inng Lim, Mohsen Fathi, Ali Rezvan, Vakul Mohanty, Yifei Shen, Hila Shaim, Junjun Lu, Gonca Ozcan, Emily Ensley, Mecit Kaplan, Vandana Nandivada, Mustafa Bdaiwi, Sunil Acharya, Yuanxin Xi, Xinhai Wan, Duncan Mak, Enli Liu, Sonny Ang, Luis Muniz-Feliciano, Ye Li, Jing Wang, Shahram Kordasti, Nedyalko Petrov, Navin Varadarajan, David Marin, Lorenzo Brunetti, Richard J. Skinner, Shangrong Lyu, Leiser Silva, Rolf Turk, Mollie S. Schubert, Garrett R. Rettig, Matthew S. McNeill, Gavin Kurgan, Mark A. Behlke, Heng Li, Natalie W. Fowlkes, Ken Chen, Marina Konopleva, Richard Champlin, Elizabeth J. Shpall, Katayoun Rezvani

doi: https://doi.org/10.1101/2020.03.29.014472

REGULAR ARTICLE
blood advances

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Nucleic Acids Research, 2019, Vol. 47, No. 15 7955–7972
doi: 10.1093/nar/gkz475

Highly efficient editing of the β-globin gene in patient-derived hematopoietic stem and progenitor cells to treat sickle cell disease

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nature medicine
ARTICLES
https://doi.org/10.1038/s41591-018-0137-0

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nature biotechnology
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Check for updates

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This article is a preprint and has not been certified by peer review [what does this mean?].

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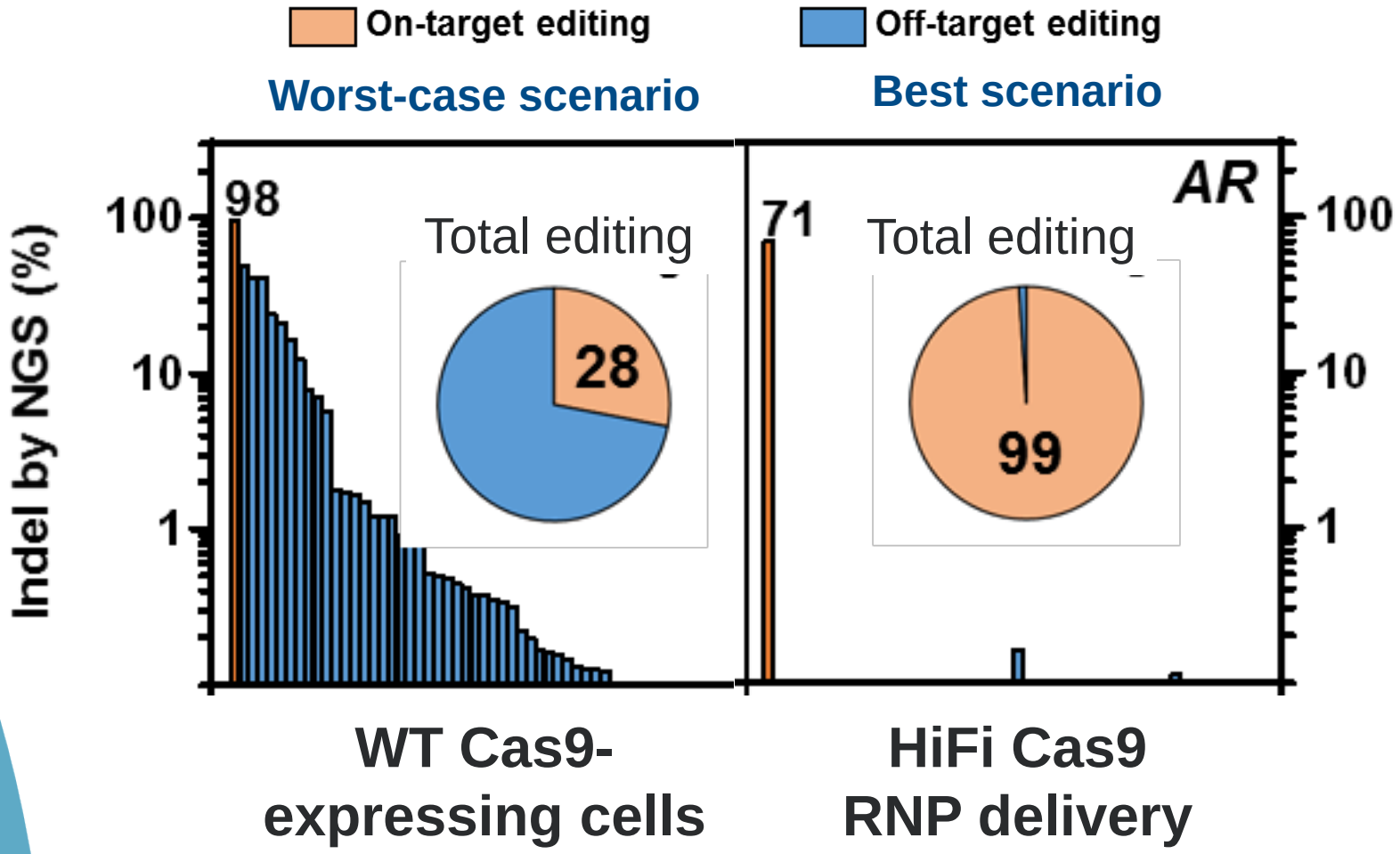
rhAmpSeq TECHNOLOGY AND Alt-R HiFi Cas9



Vakulskas, *et al.* (2018) *Nat Med* 24:1216

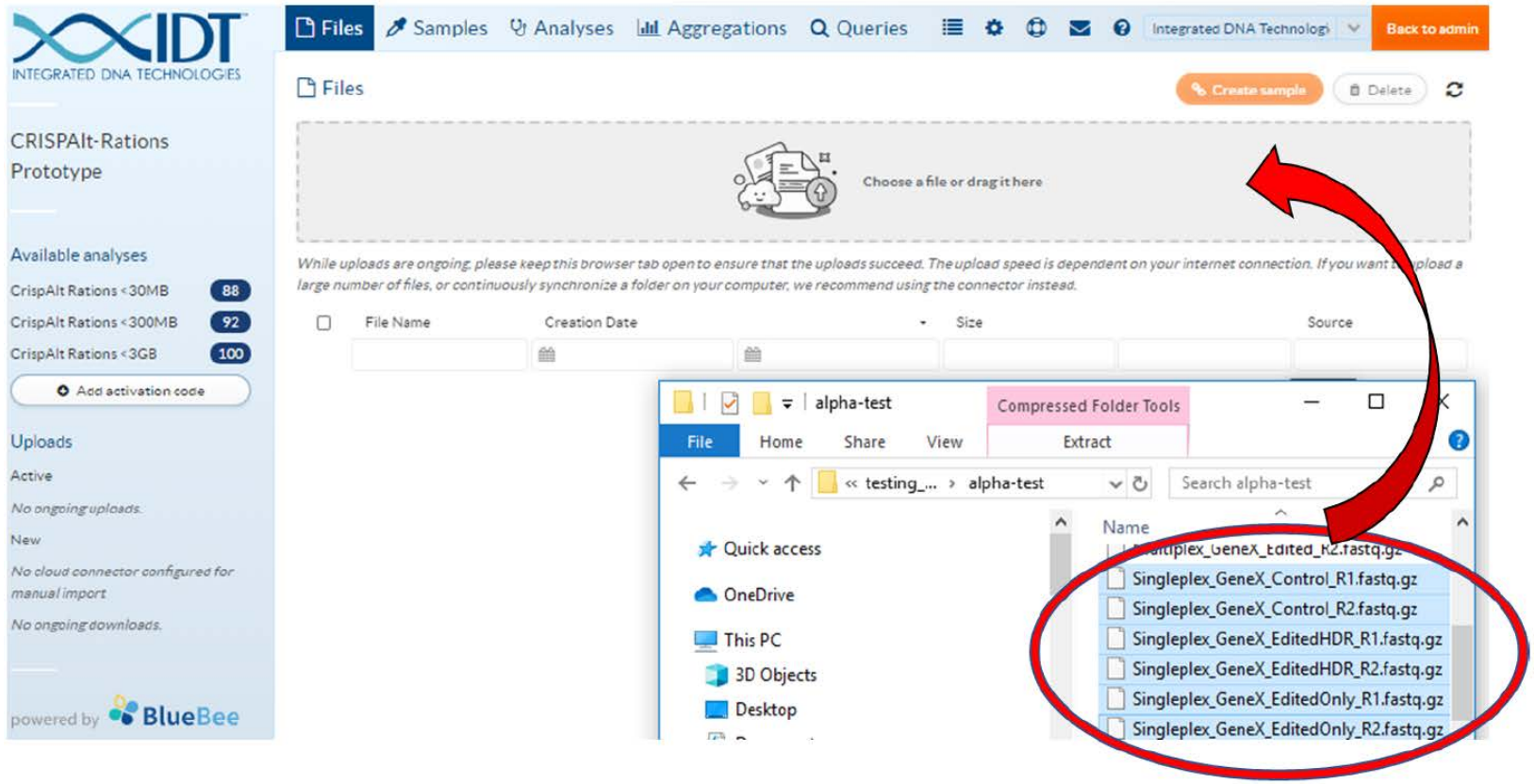
rhAmpSeq system savings:

- 8 x 40 assay designs
- ~1500 individual PCRs reduced to <96
  - Master mix
  - gDNA
- Library quantification
- Full-time equivalent hours
  - Months to days

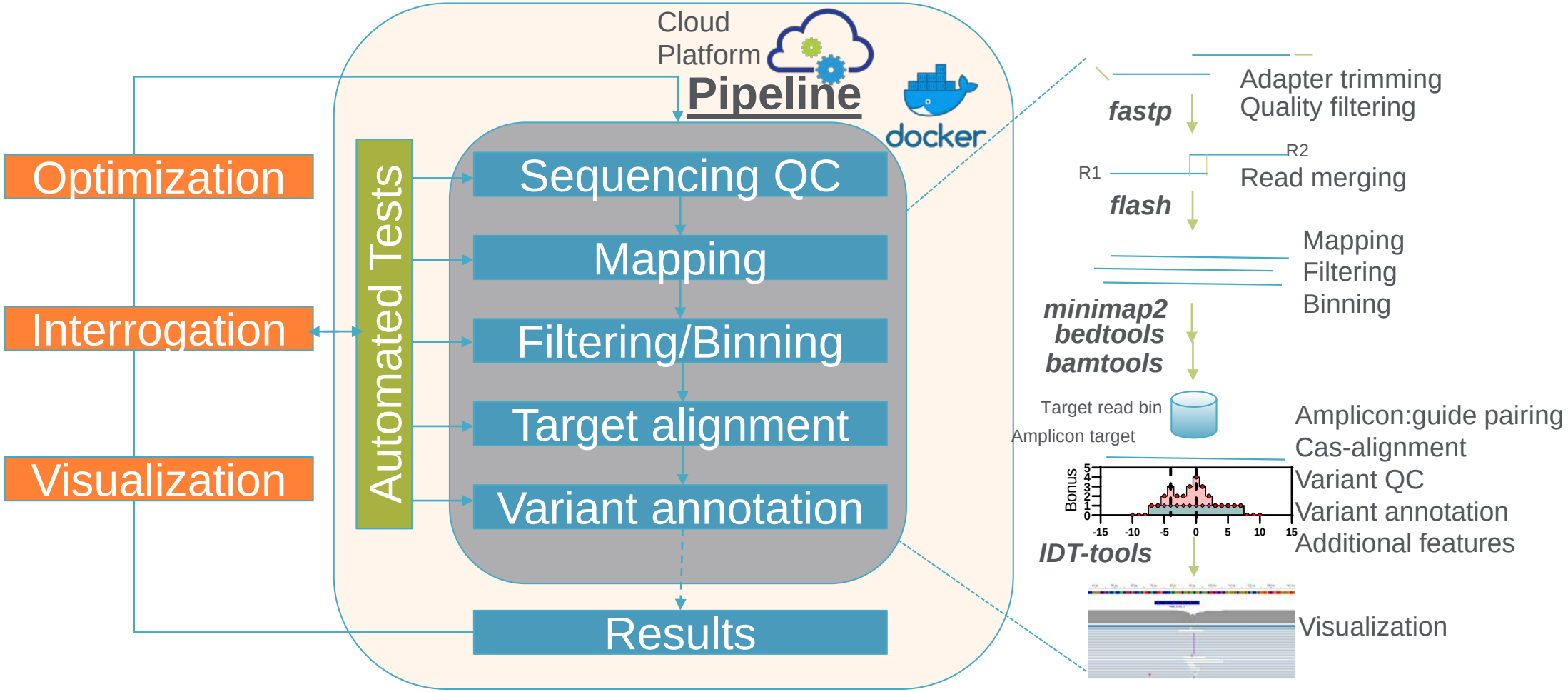
DATA ANALYSIS PIPELINE

Off-target identification
(stable Cas9, modified gRNAs)

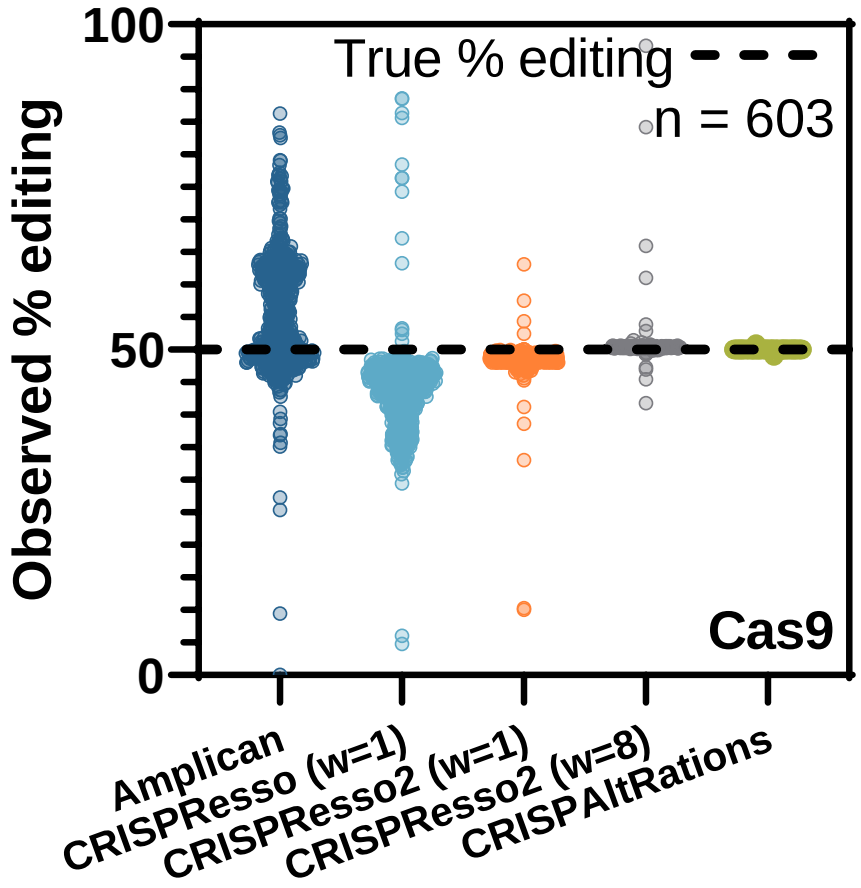
rhAmpSeq design, amplification, and sequencing
(multiple conditions, high-throughput)

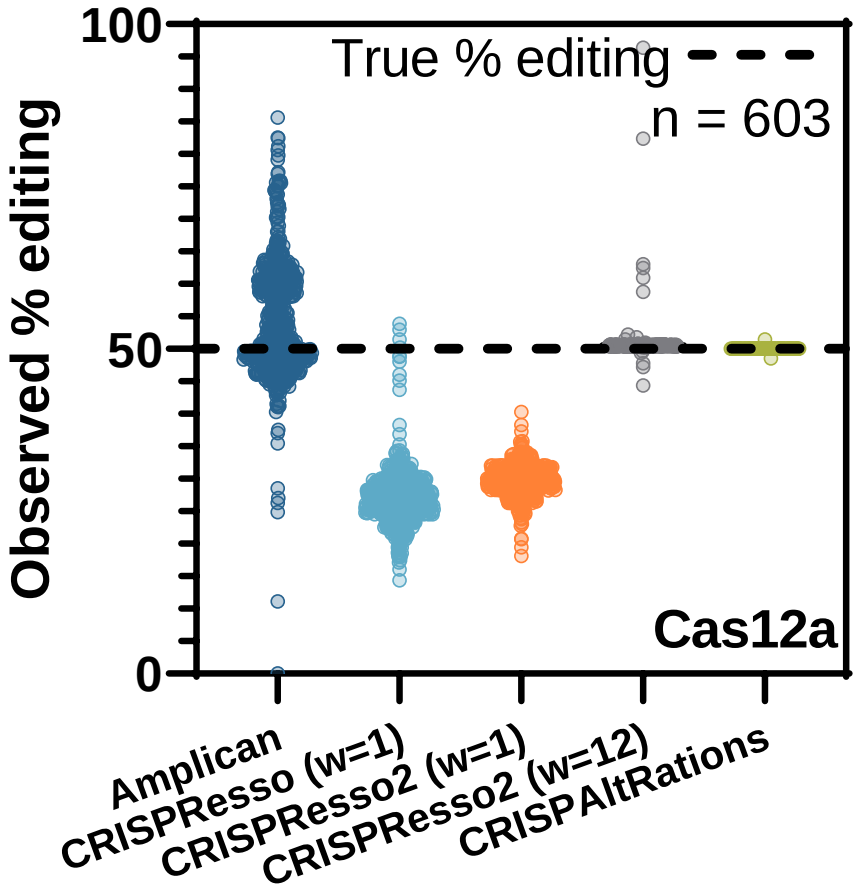


CRISPAltRations FOR THE ANALYSIS OF rhAmpSeq DATA



COMPARATIVE ANALYSIS OF CRISPR-NGS TOOLS





CONCLUSIONS

- rhAmp PCR enables high multiplexing levels.
- Up to 5000 targets are designed for a single-pool reaction.
- Simultaneously quantify editing for on- and off-target loci for CRISPR experiments.
- Streamlined, simple, 2-step PCR protocol generates NGS-ready libraries in approximately 4 hours.
- High on-target rate and coverage uniformity from as little as 10 ng of DNA input.
  - Increased % on-target reads
  - Higher degree of multiplexing
  - Better uniformity in coverage across the panel

THANK YOU

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NMIN Capacity-Building Webinar

Q&A

Adam Chernick

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