STILLA

EMBL European Molecular Biology Laboratory

Crystal digital PCR™ for genome editing and high multiplexing mutation detection

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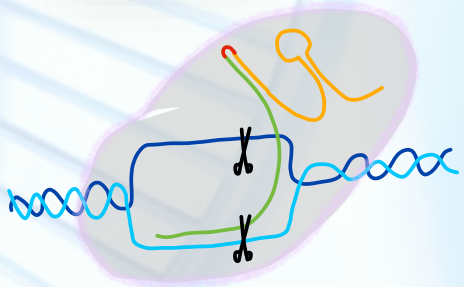
Stilla Technologies Inc

NextGen Omics Series
Boston, MA
April 7, 2020

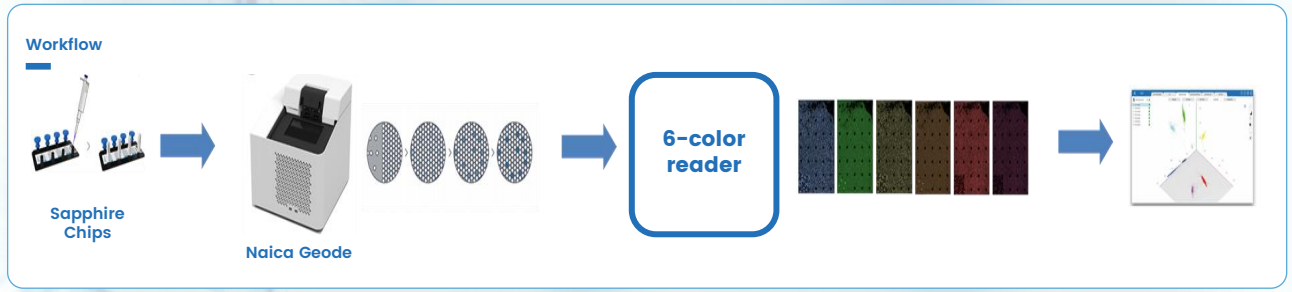
Presentation Outline

- Crystal Digital PCR workflow for multiplexing DNA assays

- Tag copy number assessment in CRISPR-edited cell lines

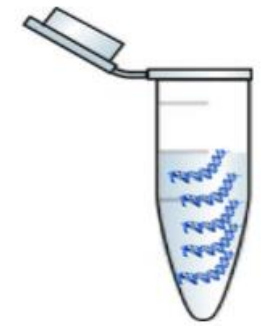


- 6-color Crystal Digital PCR for cancer monitoring in clinical trials



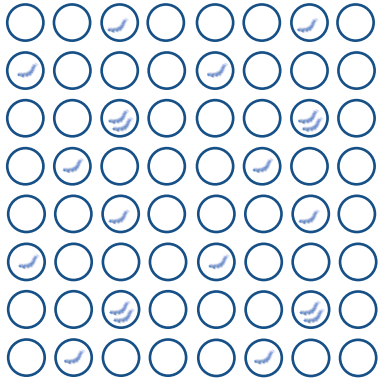
PRINCIPLE
OF DIGITAL PCR

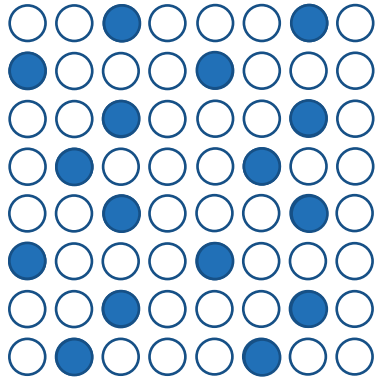
PARTITIONING



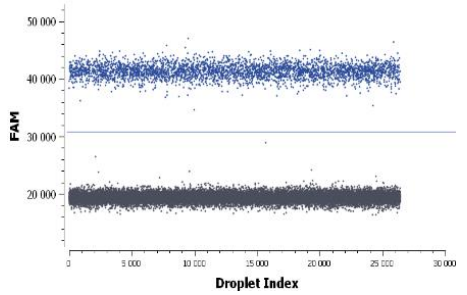
Wild-type DNA

PCR





READING & ANALYSIS



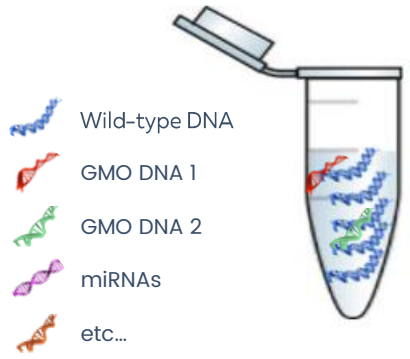
RESULTS
636 cp/µL with **2.2 %** uncertainty

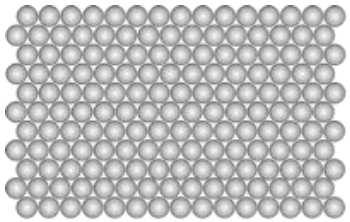
POISSON STATISTICS

$$\frac{N_{pos}}{N_{tot}}$$

PRINCIPLE
OF CRYSTAL DIGITAL PCR™

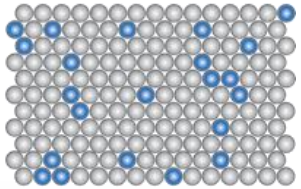
PARTITIONING

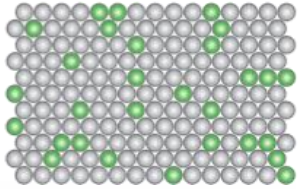


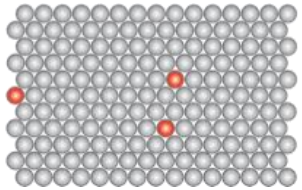


Droplet crystal:
Self-assembled
array of droplets

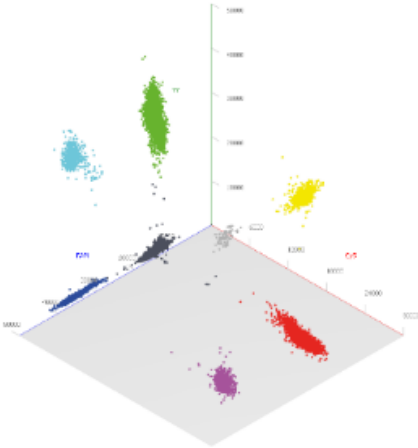
PCR







READING & ANALYSIS



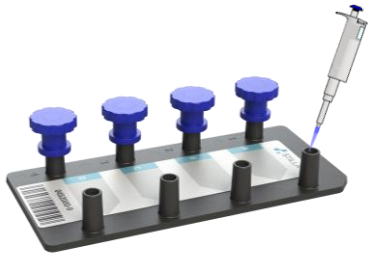
C = 102 cp/µL
C = 152 cp/µL
C = 8 cp/µL

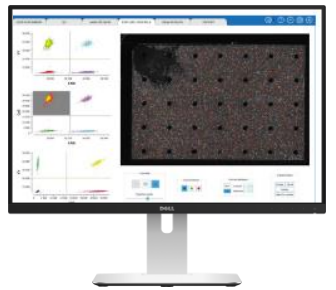
2 Parameters for good quantification In dPCR:

- Number of droplets
- Size of the droplets

PERFORM CRYSTAL DIGITAL PCR™

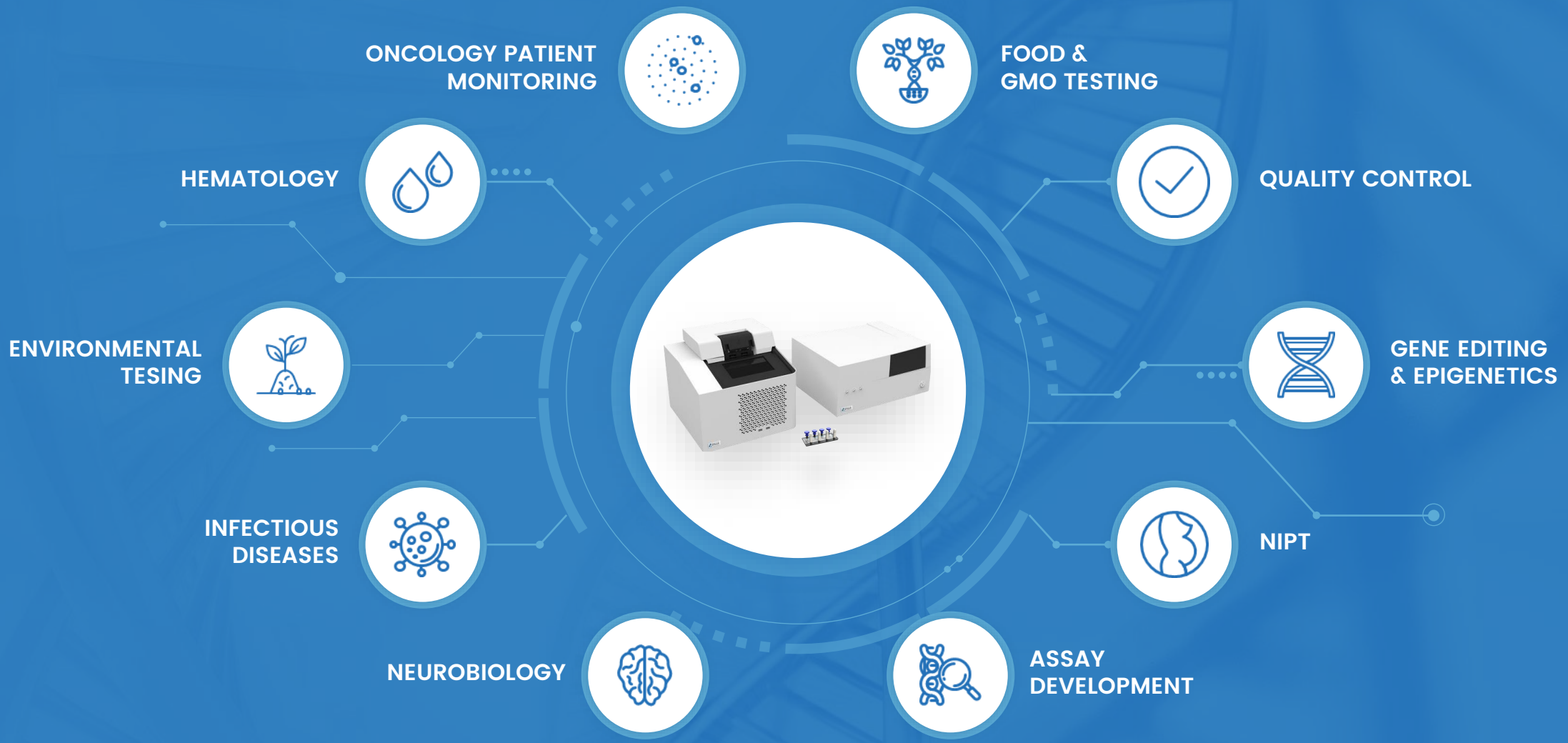
IN 2H30 WITH MINIMUM HANDS-ON TIME





DESCRIPTION	Pipette 25 µL of PCR mix into the Sapphire Chips and seal with cap	Place Sapphire chip into the Geode and launch the combined partitioning and thermocycling program	Image Chips using three fluorescent detection channels	Analyze results using our intuitive Crystal Miner software
PROCESS TIME 2H30	**5 min**	**2h10 min**	**10 min**	**5 min**
HANDS-ON TIME 5 min	**5 min**	**15 s**	**15 s**	

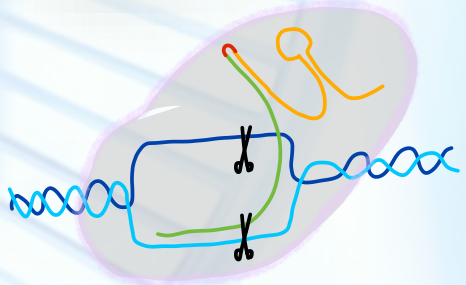
The Naica™ System Applications Across Life Sciences & Translational Research



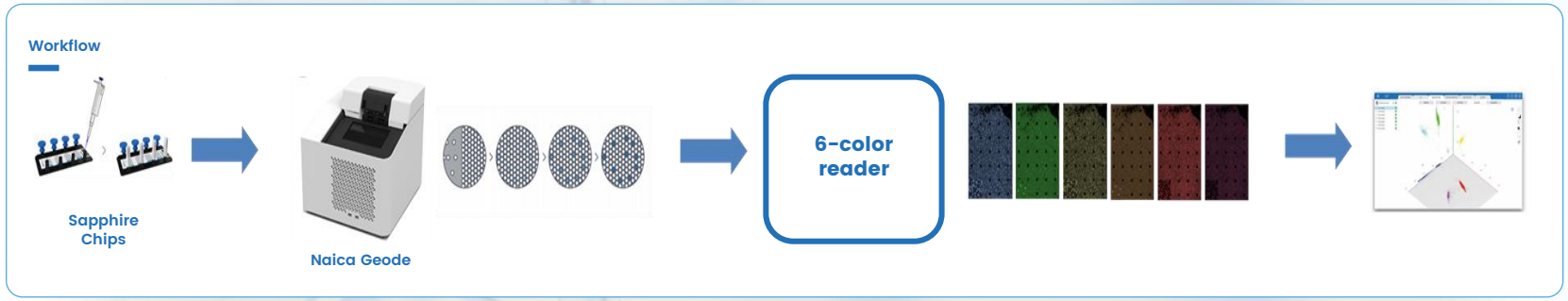
Presentation Outline

- Crystal Digital PCR workflow for multiplexing DNA assays

- Tag copy number assessment in CRISPR-edited cell lines



- 6-color Crystal Digital PCR for cancer monitoring in clinical trials



AIMS of the project

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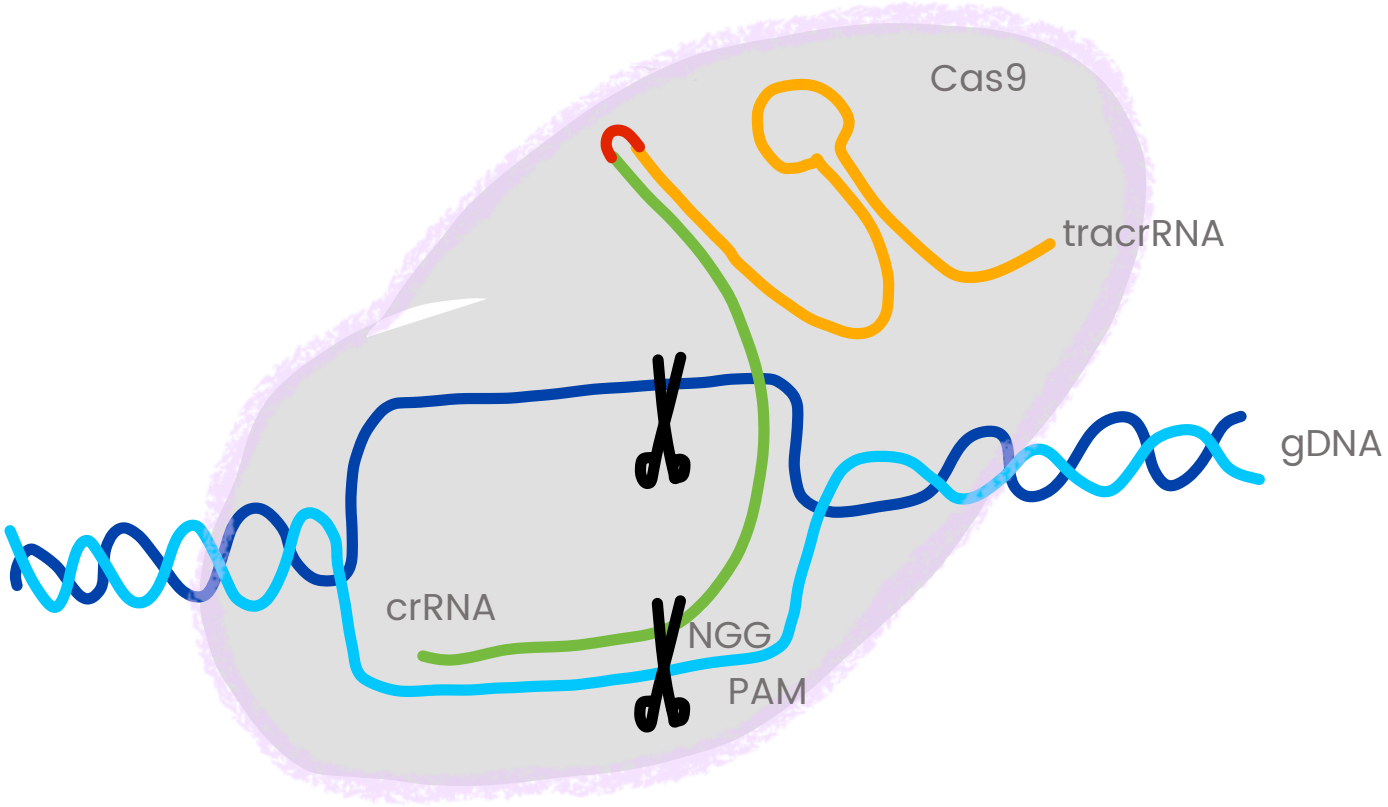
Generate homozygous knock-in clones from human cell lines using CRISPR

- Minimize the number of clones selected for in-depth analysis
  - Validate digital PCR (dPCR) with reference genomes as a high-predictive tool for genotype assessment
  - Quantitatively assess the number of on-target and off-target events using dPCR

CRISPR-Cas9 machinery

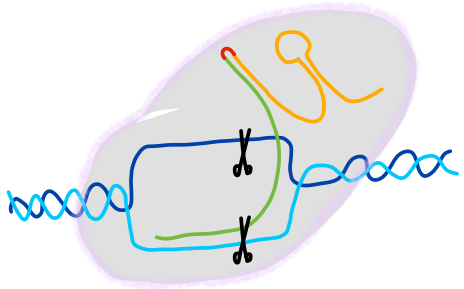
EMBL European Molecular Biology Laboratory

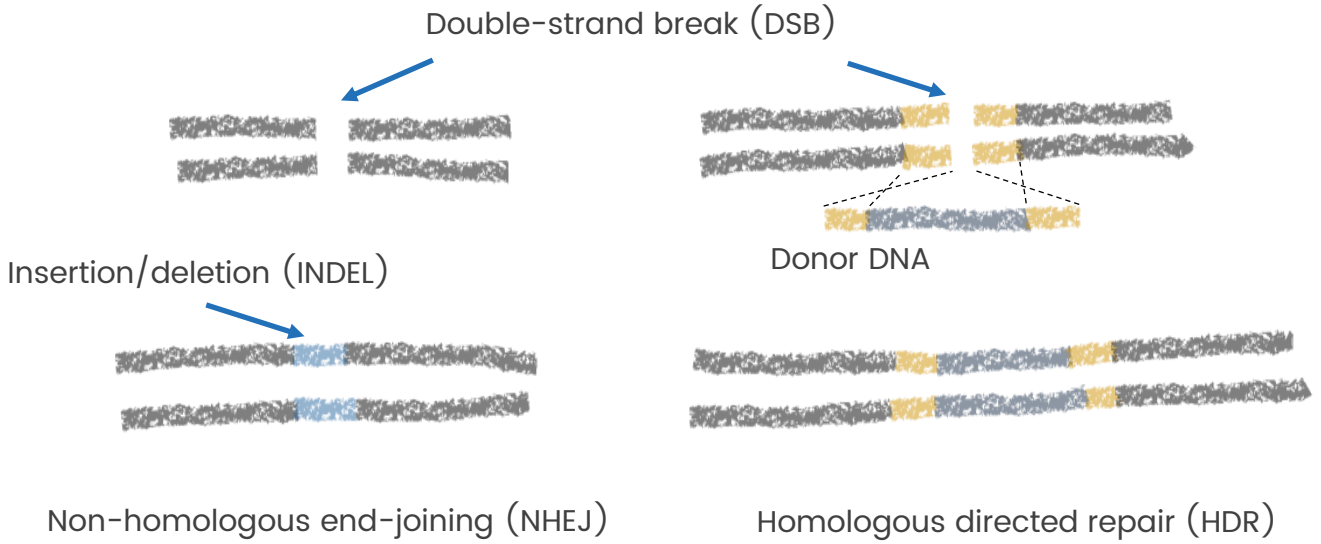
- CRISPR components to perform locus-specific genome breaks



- Jinek *et al.*, Science 2012
- Mali *et al.*, Science 2013
- Cong *et al.*, Science 2013
- CRISPR 101: A desktop resource, Addgene
- How To Use CRISPR: Your Guide to Successful Genome Engineering, Synthego

Repair mechanisms





- For knock-in generation, we exploit the HDR pathway

Ran *et al.*, Nat Protoc. 2013

CRISPR Workflow

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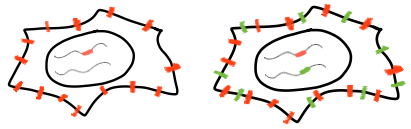
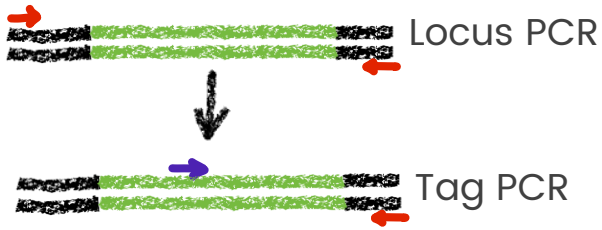
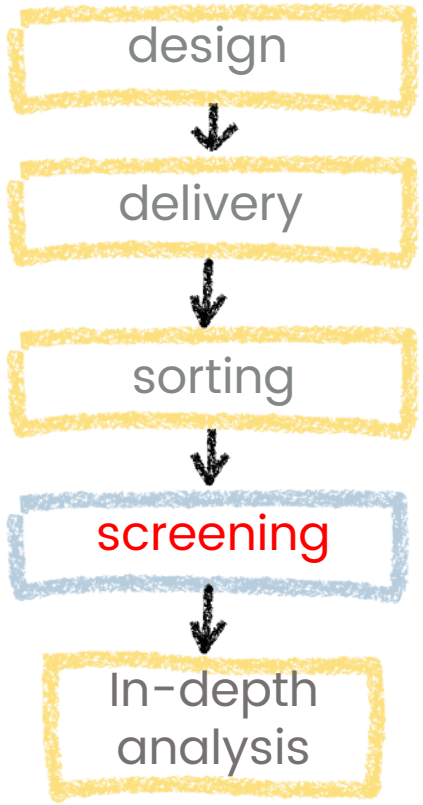
- Screening of CRISPR genome-edited knock-ins

Dual surface-exposed tags (1)

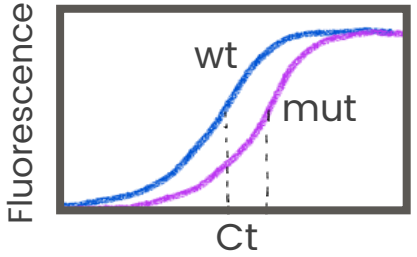


Tandem PCR (2)

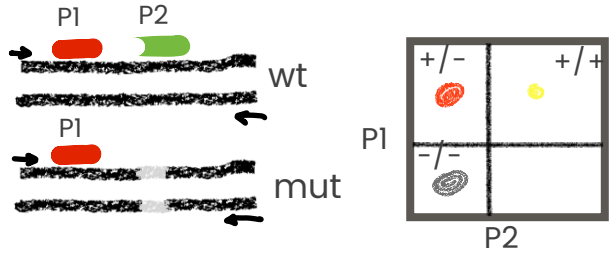




Mismatch qPCR (3)



Drop-off dPCR (4)



- cdPCR was chosen to screen CRISPR edited genomes by assessing the integrated tag copy-number and comparing it with the expected number

- Zotova *et al.*, Sci. Rep. 2019
- Guo *et al.*, Plant Meth. 2018
- Gehre *et al.*, preprint https://doi.org/10.1101/635151
- Bulletin 6872, Biorad

The Validation Principle

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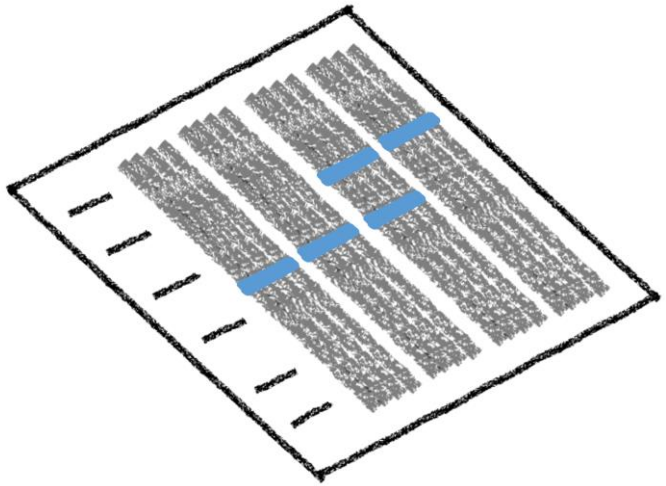
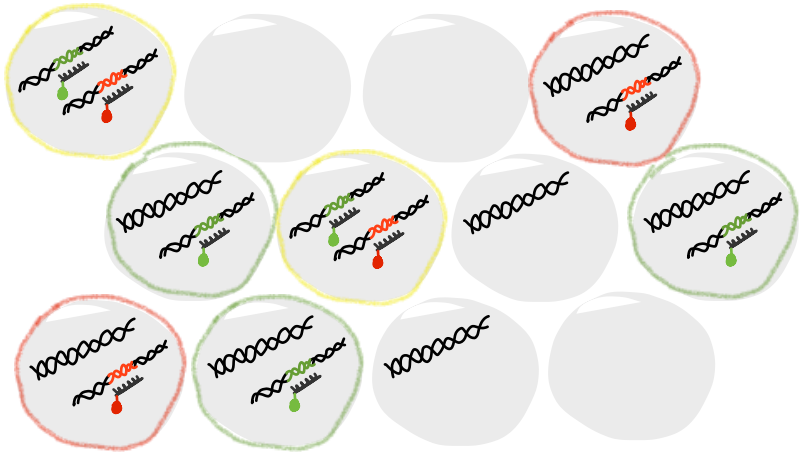
- Southern blot (SB) *vs.* cdPCR: interrogating the whole genome

Southern Blot



~2 weeks

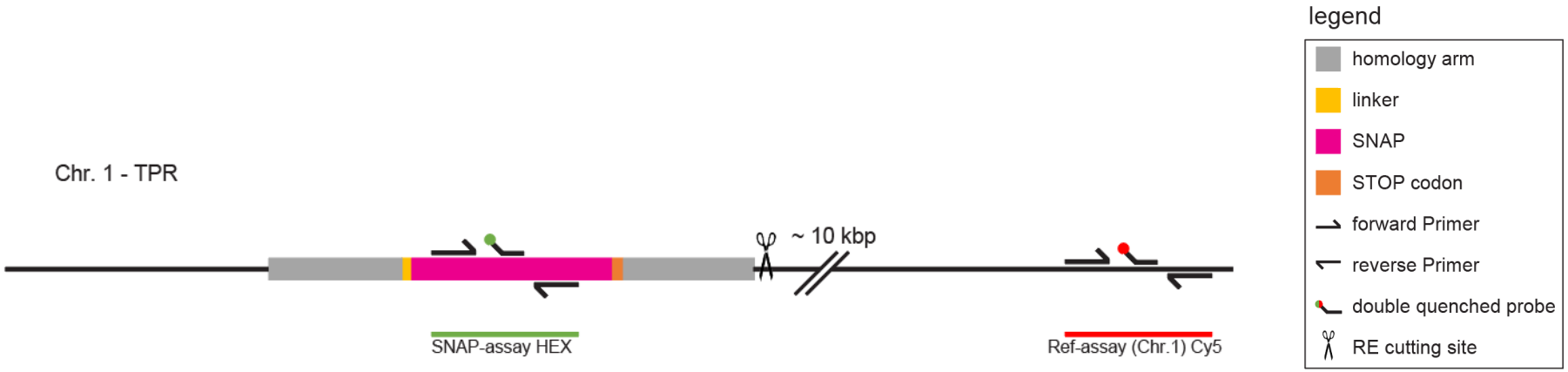
Crystal Digital PCR



~3 hours

dPCR validation – assay design

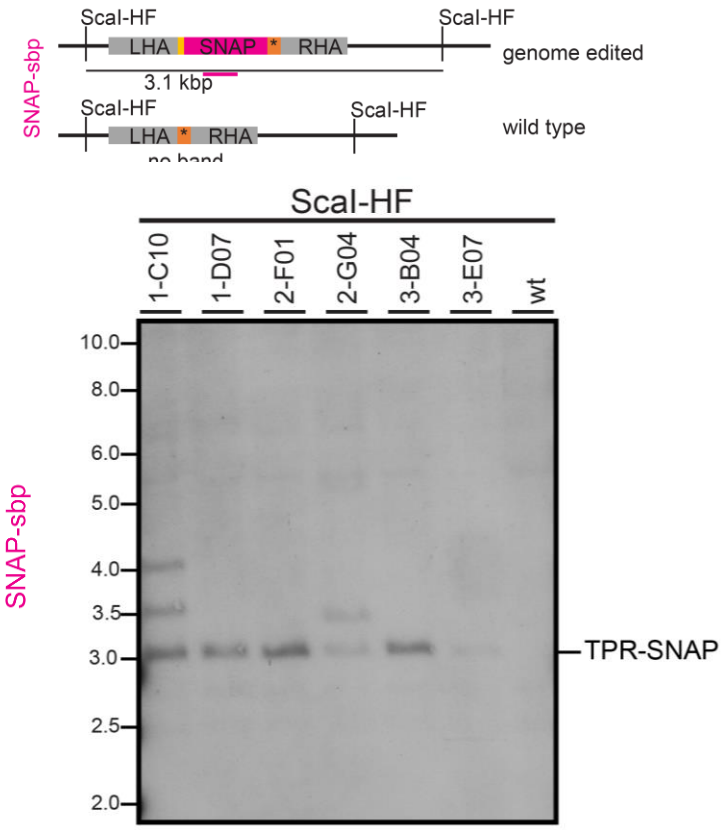
- dPCR assay to assess total SNAP-tag integrations

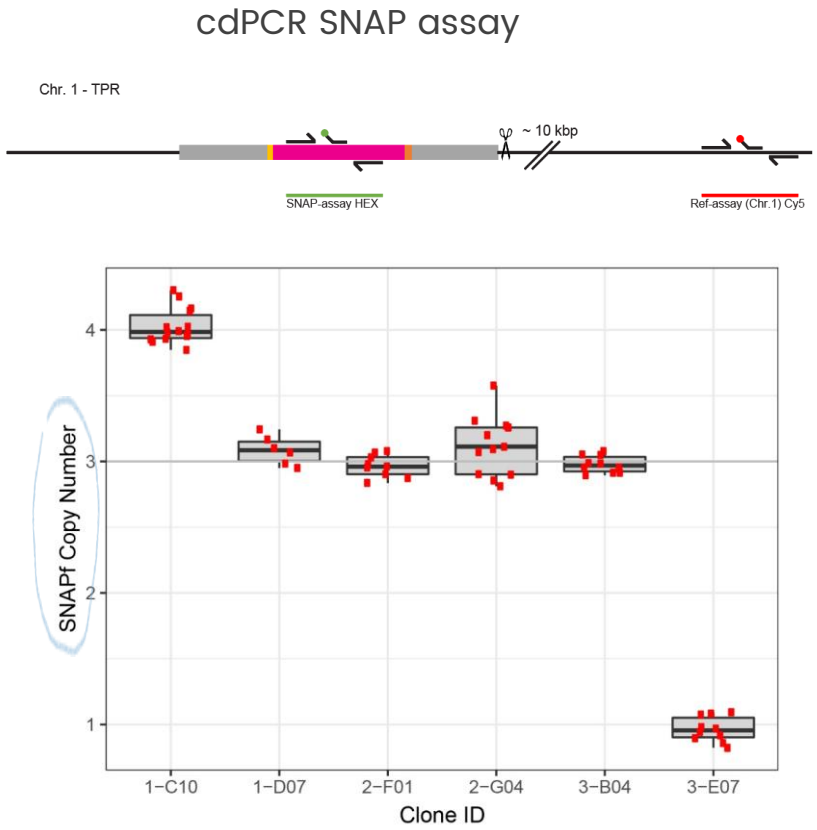


- Primers of the cdPCR SNAP-assay are within the SNAP tag
- Reference assay on the same chromosome ~10kbp away

dPCR Validation results

- Counting SNAP-tag copy number at TPR locus (triploid in U-2 OS cells)

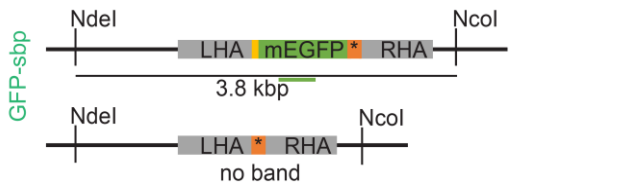


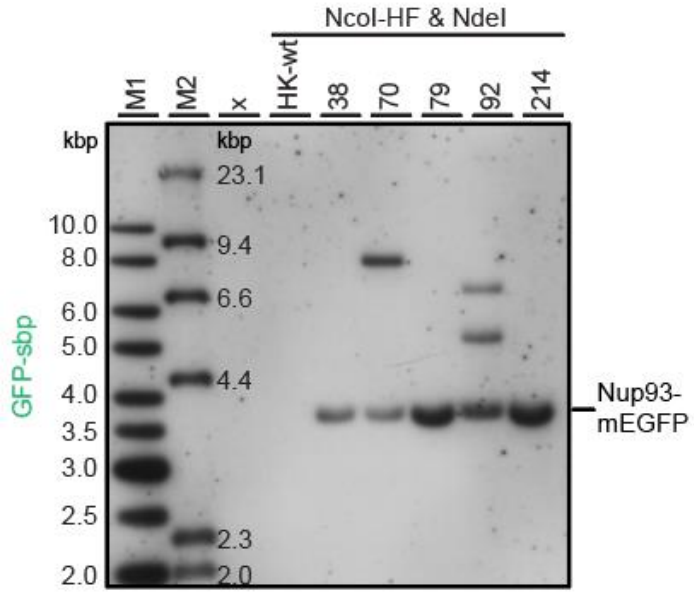


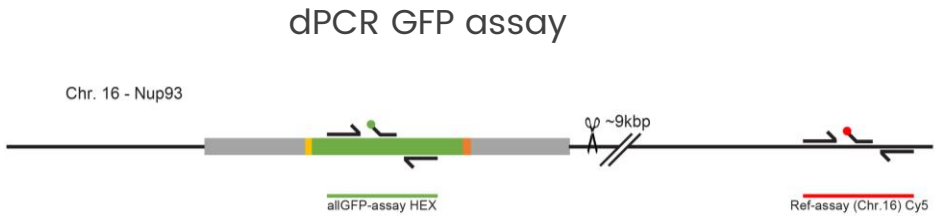
- Clone 1-C10 resulted in one extra integration
- Clone 3-E07 resulted in ~1 integration – speculatively heterozygous
- Clone 2-G04 resulted in expected copy-number but major rearrangements in SB
- cdPCR SNAP copy-number mostly matches genotype predictions out of SB

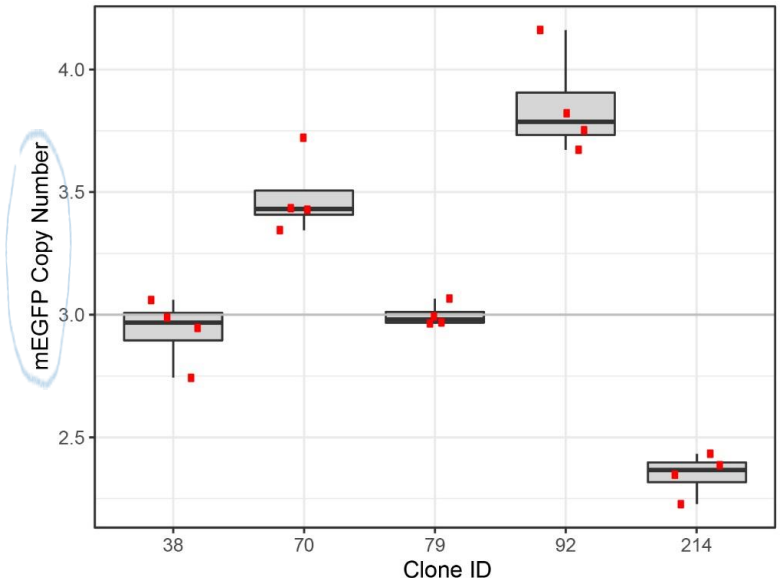
dPCR Validation results

- Counting mEGFP copies at the Nup93 locus (triploid in HeLa Kyoto cells)





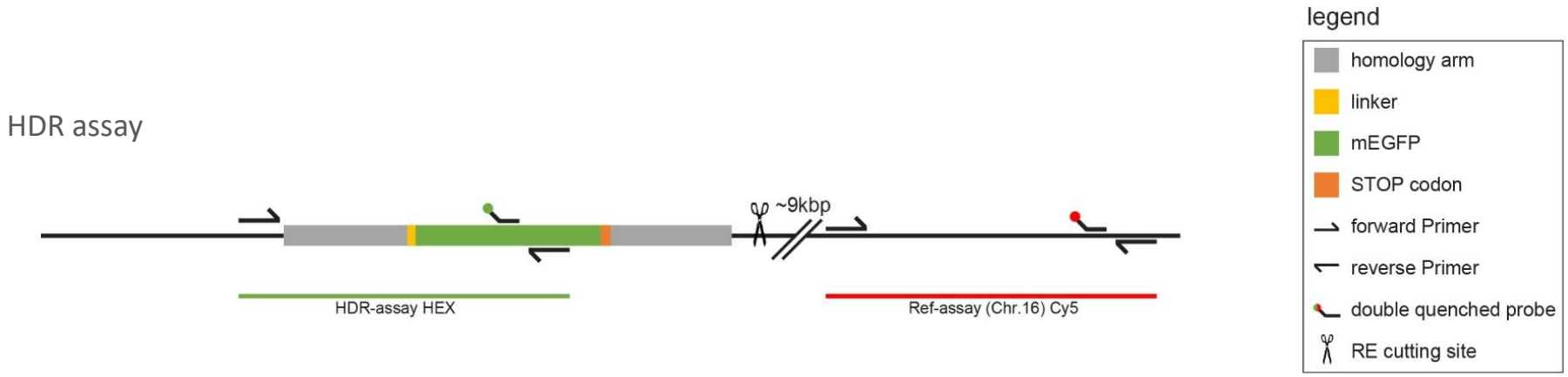




- Clone 214 resulted in ~2 integrations - not detectable with GFP-sbp
- mEGFP copy-number mostly matches genotype predictions out of SB

dPCR validation – HDR assay design

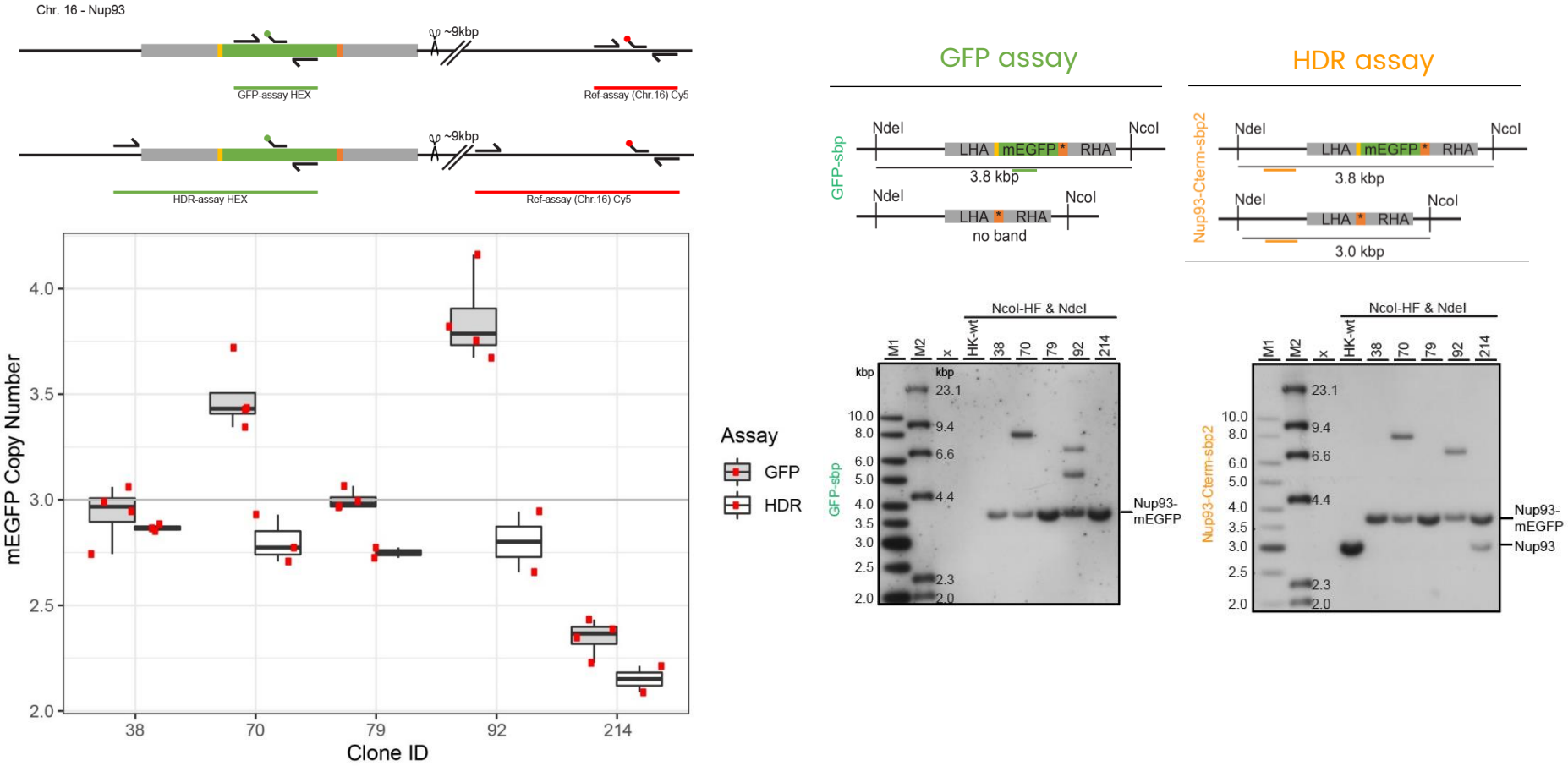
- Additional dPCR assay to check locus specificity of mEGFP integration



- Forward primer of the dPCR HDR-assay is moved outside of the left homology arm
- Reference assay was adapted to match the new amplicon size (~1.4 kbp)

dPCR Validation results

- Extending dPCR-based quantification of tag insertions at the target locus



- Clone 214 resulted to be heterozygous as predicted by dPCR
- HDR assay adds information about the number of specific integrations

Conclusions

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- dPCR robust tool to screen CRISPR clones with high predictivity of generated genotypes
- dPCR is a quantitative tool to assess CRISPR strategy efficiency
- HDR-based dPCR assays can be optimized by shortening homology arms of the donor DNA

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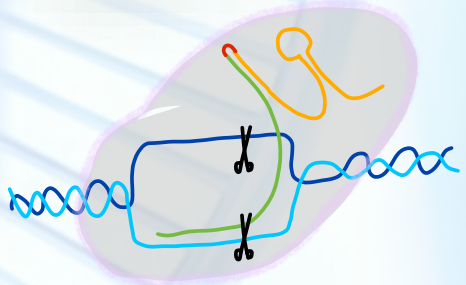
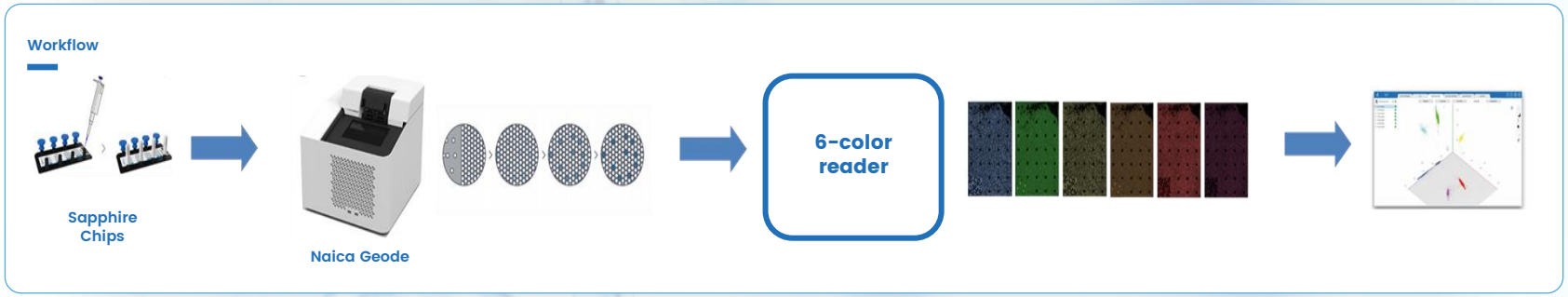


- 6-color Crystal Digital PCR for cancer monitoring in clinical trials

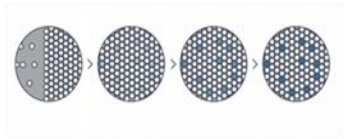


Announcing 6-color Crystal Digital PCR™

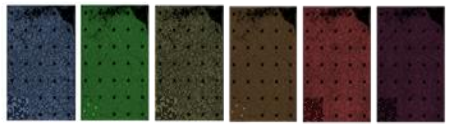
Workflow

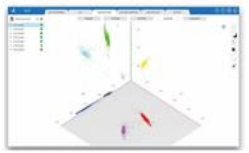
Sapphire Chips

Naica™ Geode



6-Color Reader





Introducing
6-color Crystal Digital PCR™

Examples of compatible fluorophores:

Channel	Fluorophores
1	FAM
2	YY®
3	Atto 550
4	ROX
5	Cy®5
6	Atto 700

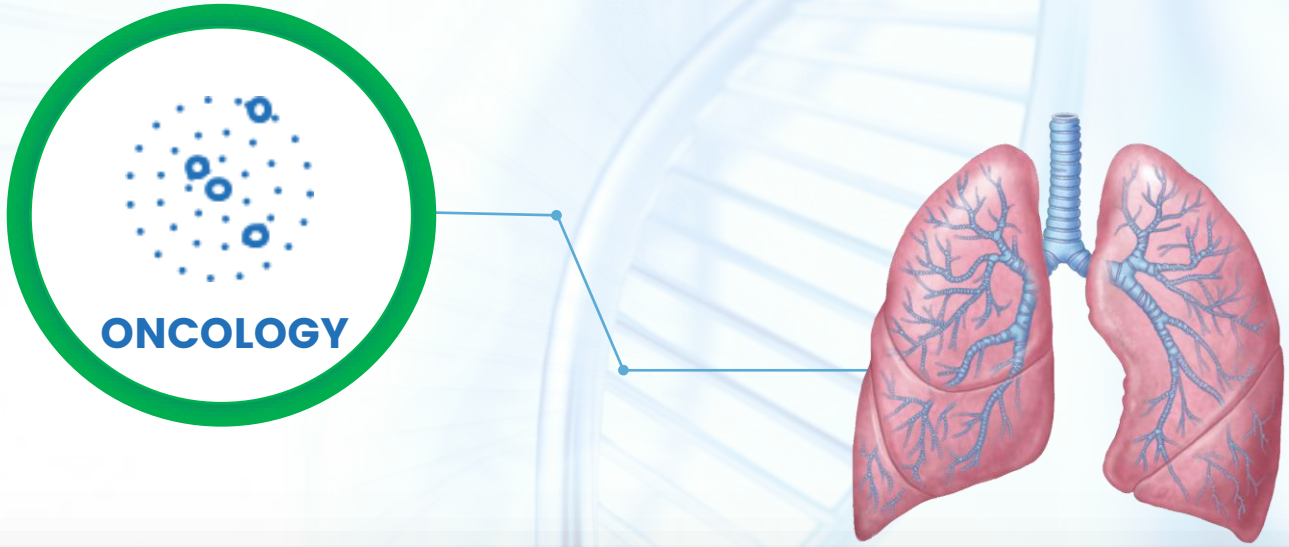
6-Color Reader

Chip Compatibility

- Sapphire chips (36 samples per day per 8h shift)
- 3 chips per run
- Time-to-result: ≤ 3 hours, for 6 channels

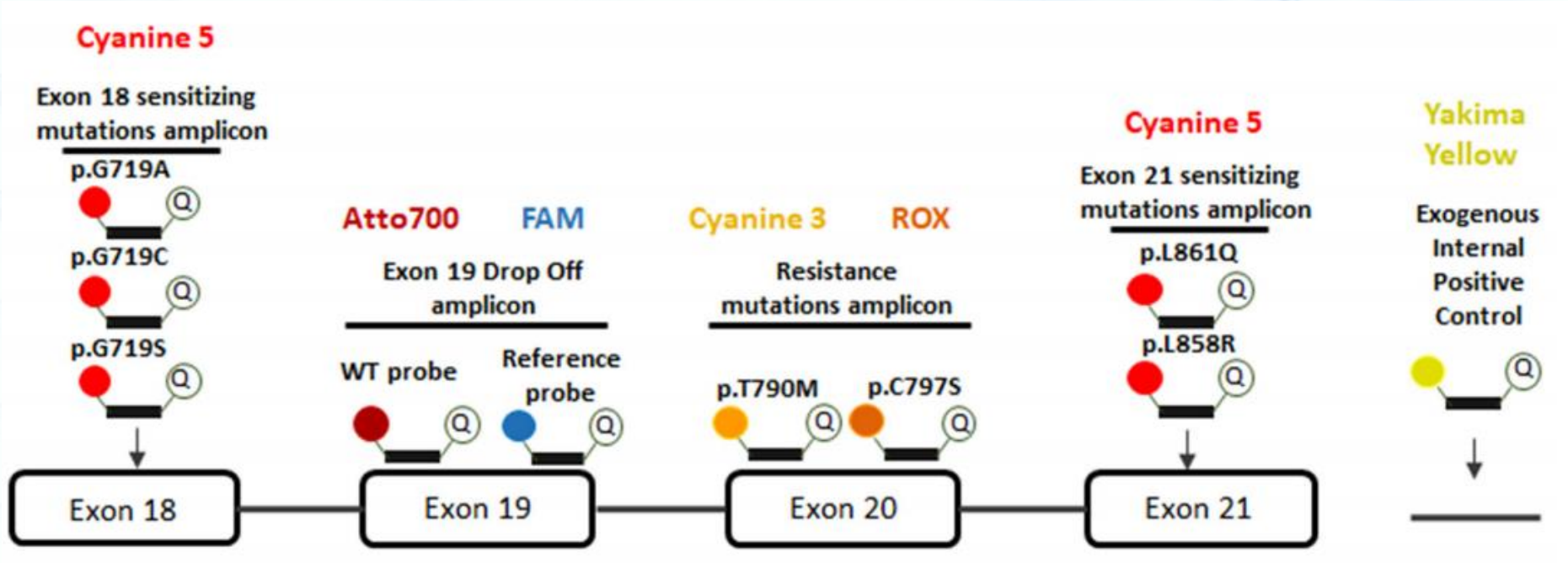
6-color Detection Channels : Proof of concept

6-color Lung Cancer Panel for *EGFR* mutation quantification



Workflow

6-color Crystal Digital PCR™ quantifies > 90% of known *EGFR* mutations in a single assay



- **Quantifies 19** of the most prevalent TKI sensitizing and resistance *EGFR* mutations
- **Maximize** the use of your precious sample
- **Minimize** time to results

Madic et Jovelet et al., 2018 Oncotarget

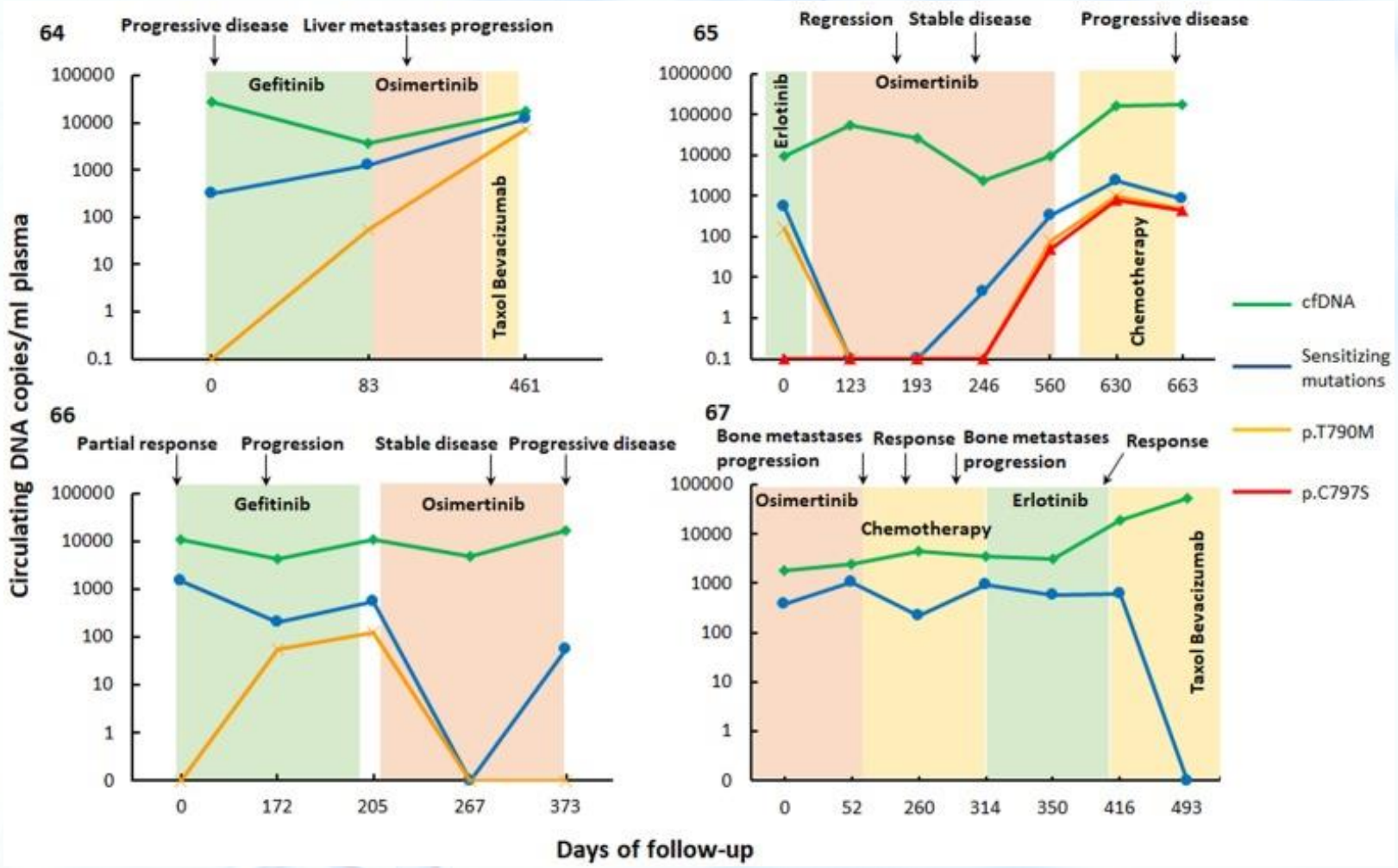
6-color Detection of the most prevalent sensitizing and resistance *EGFR* mutations in NSCLC

33 Tumor samples (21 Frozen, 12 FFPE)

- 24 *EGFR* sensitizing anomalies (73%)
- 13 T790M and 5 C797S resistance mutations (54%)
- 9 WT

49 cfDNA samples

- 35 *EGFR* sensitizing anomalies (71%)
- 14 T790M and 3 C797S resistance mutations (35%)
- 14 WT



Madic et Jovelet et al., 2018 Oncotarget

6-color Crystal Digital PCR™ Breast and Rectal cancer assays

6-color Monitoring of Breast & Rectal Cancer Mutations in two Clinical Studies

Motivation

- **4 year** EU-funded LIMA project led by Philips
- **Goal:** Combine liquid biopsy monitoring & MRI scans to predict and monitor cancer therapy response

Challenge

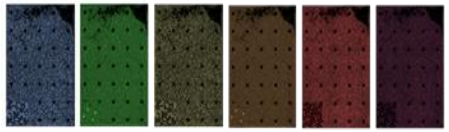
- **100 patients per trial**
  - Breast: 10 samples per patient - *1000 samples*
  - Rectal: 4 samples per patient - *400 samples*
- **Reliable, rapid and cost-effective quantification** of at least 6 targets from a single blood sample

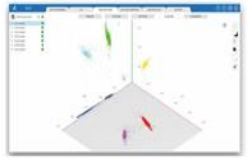
Workflow

Sapphire Chips

Naica™ Geode

6-Color Reader





Clinical Trial 6-color Crystal Digital PCR™ Panels

		RECTAL Cancer Panel patient coverage: 10% - 30%	**BREAST Cancer Panel** patient coverage: 25% - 35%
Channel	Fluorophores	Target	Target
1	FAM	***PIK3CA*** **H1047R**	***ERBB2*** **(*HER2* amp.)**
2	YY	***PIK3CA*** **H1047 WT**	***PIK3CA*** **H1047 WT**
3	Atto 550	***PIK3CA*** **E542K**	***MRM1*** **(Polysomy 17 Ref.)**
4	ROX	***PIK3CA*** **E545K**	***PIK3CA*** **Mut (H1047R / E542K / E545K)**
5	Cy®5	***PIK3CA*** **H1047L**	***TSN*** **(Amplification Ref.)**
6	Atto 700	**PhiX (Int. Ctrl.)**	**PhiX (Int. Ctrl.)**

Naica™ 6-color Crystal Digital PCR™: Conclusions

Workflow

- Multiplex oncology panels display high clinical utility :
  - Lung (>90% of known *EGFR* mutations in NSCLC)
  - Breast (25-35% patient coverage)
  - Rectal (10-30% patient coverage)

- Maximize information output of your precious samples while minimizing time to results

Acknowledgements

EMBL European Molecular Biology Laboratory

- Ellenberg Lab
  - Jan Ellenberg
  - Nathalie Daigle
- Cell Biology and Biophysics Computational Support
  - Beatriz Serrano Solano
- Genomics Core Facility
  - Vladimir Benes

STILLA

- 6-color development team at Stilla

Baden-Württemberg Stiftung

NIH National Institutes of Health

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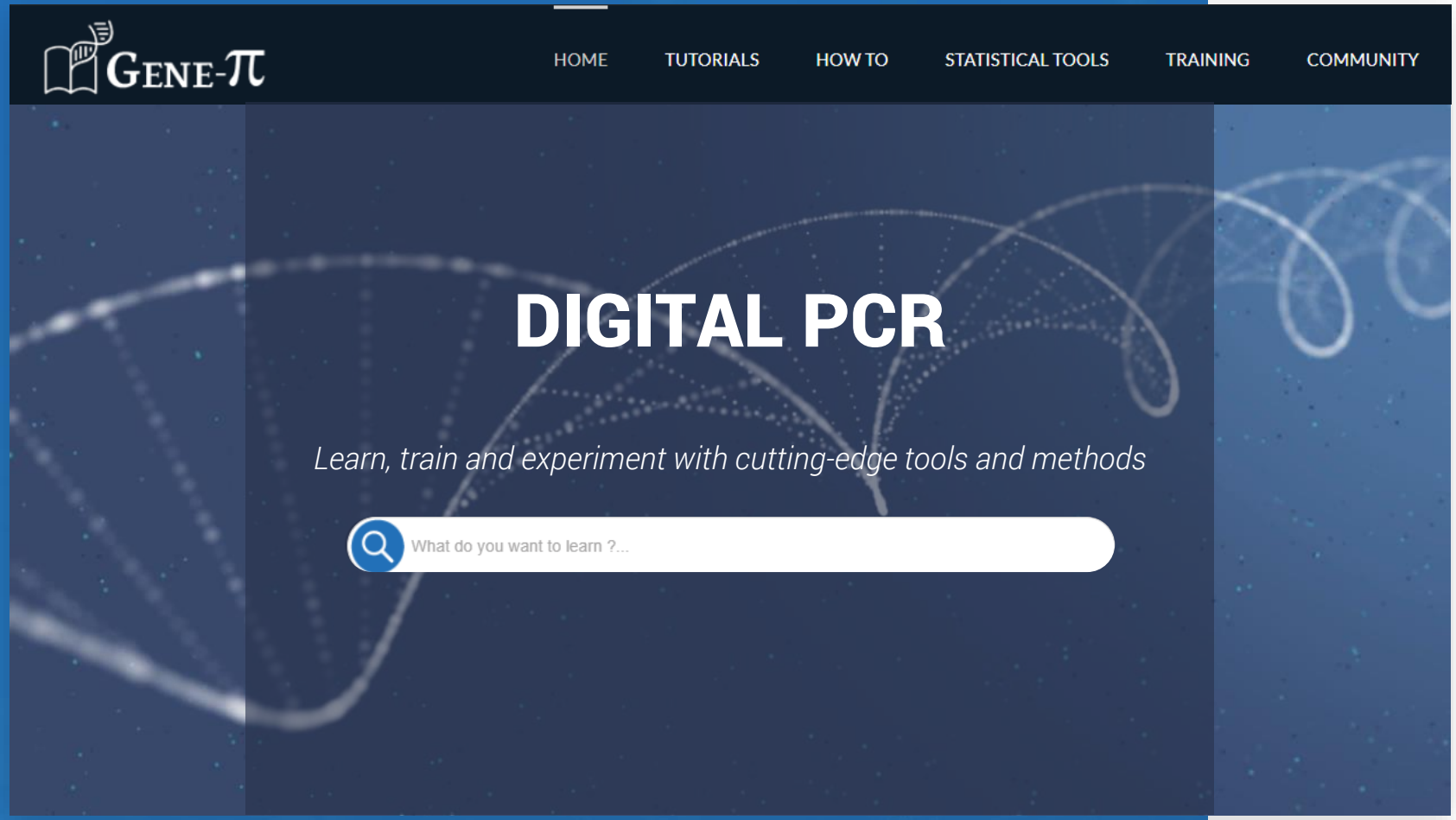

LAUNCH IN MARCH 2019:

- **2 tutorials**
  - Rare Mutation Detection
  - CNV
- **1 video**
- **20 items**
- **3 memos**
- **2 online statistical tools**
  - Poisson Law
  - CNV

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THANK YOU FOR YOUR ATTENTION!

ANY QUESTIONS?

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For more information on product and workflow, visit our website at

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