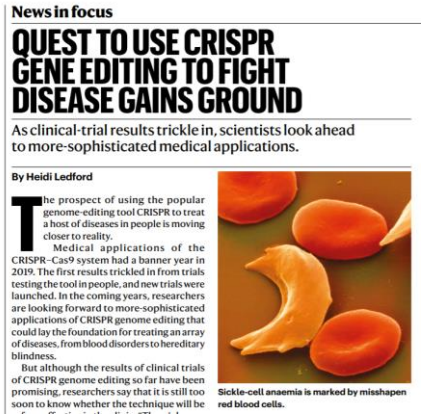
Advance Sequencing Technologies for Identification of Off-Target Mutations Associated with Genome Editing

Mick Fellows, AstraZeneca

Cell Therapy - TRAcking, Circulation, & Safety (CT-TRACS) committee

Introduction

- The promise of curative therapies from genome editing technologies such as CRISPR is becoming a reality
- Current trials are underway in five treatment areas: blood disorders, cancers, eye disease, chronic infections, and protein-folding disorders



News in focus

QUEST TO USE CRISPR GENE EDITING TO FIGHT DISEASE GAINS GROUND

As clinical-trial results trickle in, scientists look ahead to more-sophisticated medical applications.

By Heidi Ledford

The prospect of using the popular genome-editing tool CRISPR to treat a host of diseases in people is moving closer to reality.

Medical applications of the CRISPR–Cas9 system had a banner year in 2019. The first results trickled in from trials testing the tool in people, and new trials were launched. In the coming years, researchers are looking forward to more-sophisticated applications of CRISPR genome editing that could lay the foundation for treating an array of diseases, from blood disorders to hereditary blindness.

But although the results of clinical trials of CRISPR genome editing so far have been promising, researchers say that it is still too soon to know whether the technique will be

Sickle-cell anaemia is marked by misshapen red blood cells.

Patient Care · March 30, 2021

UC Consortium Launches First Clinical Trial Using CRISPR to Correct Gene Defect That Causes Sickle Cell Disease

Project Uses Nobel-Prize-Winning Technology to Directly Correct Sickle Mutation in Blood Stem Cells, Addressing Underlying Cause of Debilitating Disorder

By Lorna Fernandes

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How CRISPR Is Changing Cancer Research and Treatment

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July 27, 2020, by NCI Staff

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Introduction

- However, there are still safety gaps, including delineating the risk of off target editing[1,2]
  - Several commonly used technologies may not have the required sensitivity (e.g., indel freq of <0.5% not reproducibly detected[3])
  - There are 33 trillion cells in a human!
- We are investigating a new unbiased technology (Induce-Seq) invented by AZ/Cardiff Uni graduate student Felix Dobbs
- INDUCE-Seq can identify DNA double strand breaks without the need for DNA amplification, thus greatly increasing sensitivity

[1] Kenpton and Qi, 2019. https://science.sciencemag.org/content/364/6437/234.summary
[2] Han *et al. 2020.* https://link.springer.com/article/10.1007/s00109-020-01893-z
[3] Chaudhari *et al.* 2020. https://www.liebertpub.com/doi/full/10.1089/crispr.2020.0053

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Project Proposal

Primary proposal

- Utilise novel unbiased NGS technology (INDUCE-seq) to identify CRISPR off-target DNA strand breaks
- Follow-up with secondary analysis (e.g. DUPLEX-seq) to confirm if identified DNA break sites lead to mutation

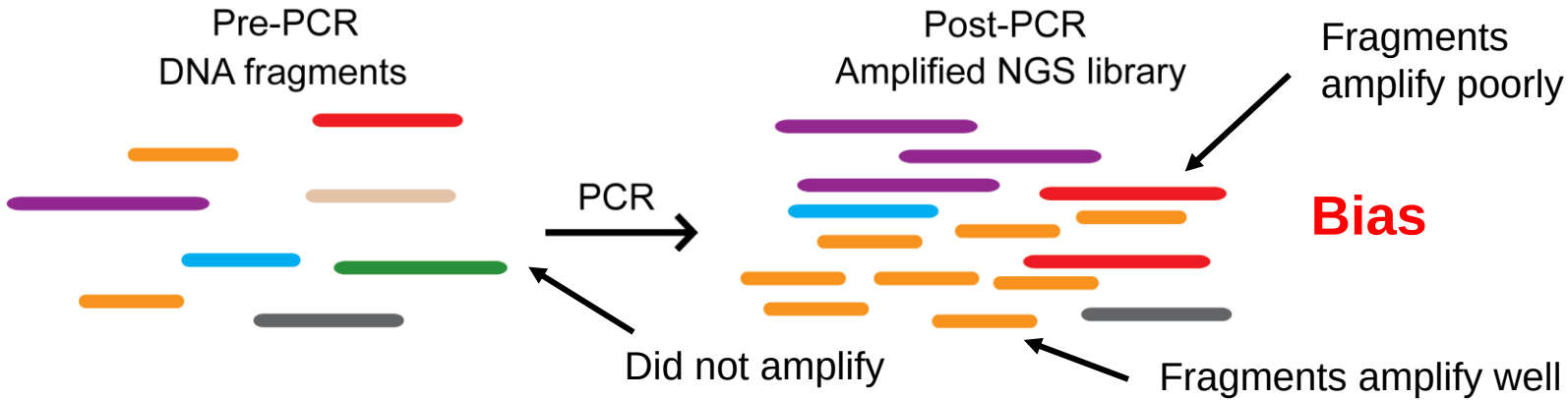
Secondary proposal

- *To utilise the technologies to identify mutational changes under different culture conditions for hPSC*

Accurate analysis of off-target CRISPR is essential

Numerous methods to detect genomic DSB events in cells
Direct break labelling: e.g. BLESS, DSBCapture, END-seq, BLISS

- Key limitation to PCR-based library preparation for NGS



- Break detection by this method is **biased** and **not** quantitative
- This introduces a **high background** reducing the signal:noise ratio for break detection

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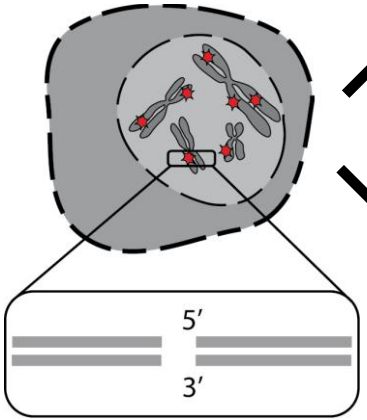
INDUCE-seq: a novel PCR-free NGS library preparation enables flow cell enrichment of DSBs*

DSBs in cells

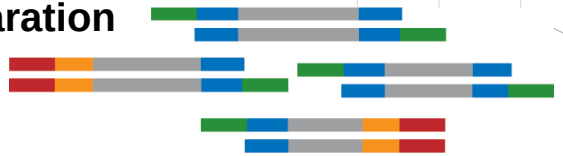


***In situ* break labelling**

INDUCE-seq

- DNA extraction
- Fragmentation

PCR-free library preparation



DSBCapture

BLISS

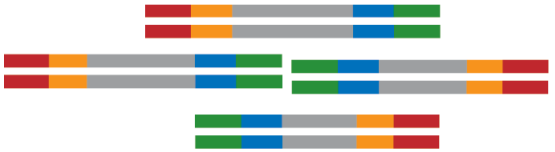
END-seq

Break-end enrichment

- Streptavidin-biotin
- *In vitro* transcription

- DNA extraction
- Fragmentation

Standard PCR based library preparation



*doi: https://doi.org/10.1101/2020.08.25.266239

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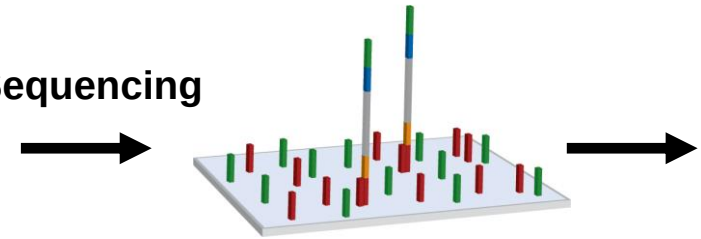
INDUCE-seq

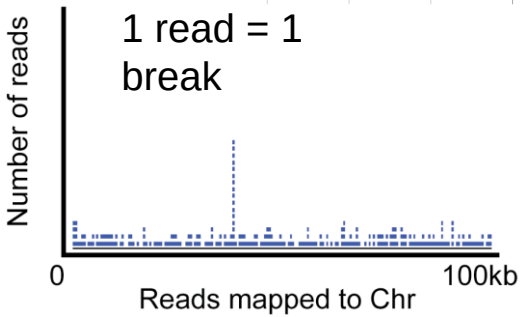
PCR-free library preparation

Sequencing



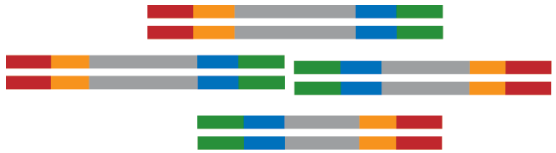
Mapped DSBs

1 read = 1 break

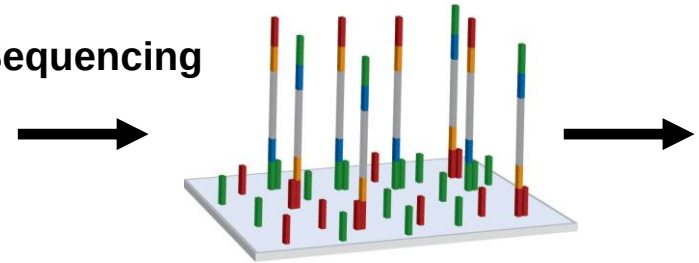


Break-end enrichment

Standard PCR based library preparation

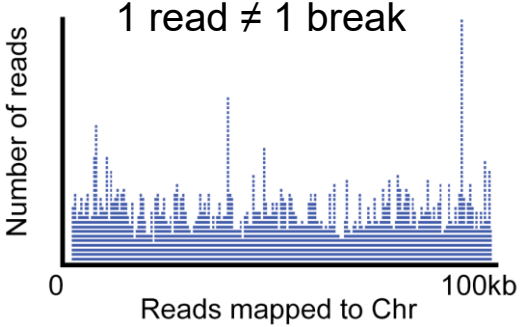


Sequencing



Mapped DSBs

1 read ≠ 1 break



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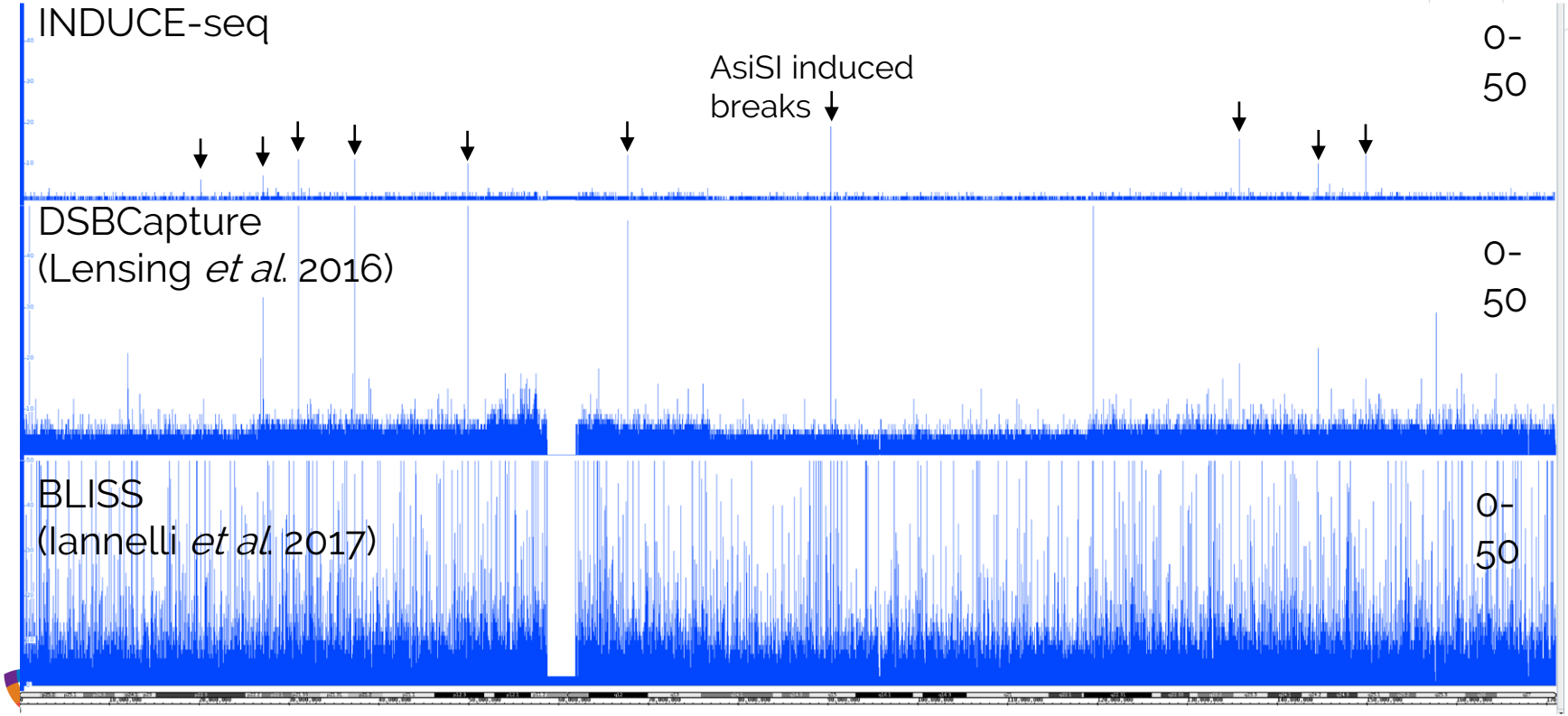
Genome browser comparison of AsiSI-inducible U2OS cell break capture by INDUCE-seq and alternative PCR-based approaches



Advance sequencing technologies for identification of off-target mutations associated with genome editing. *FIND*

Objectives

1. Understand the frequency and location of CRISPR Cas9 off target editing
2. Understand the mechanisms that underpin the formation of mutations caused by DSBs.
3. "Validate" (assess applicability) the assays in a multisite study and establish their value.

Starting materials

- CRISPR Cas9 edited cells (to a known site)
- Promiscuous and specific guide
- 2 cell lines
- Test in multiple labs

Step-1: Induce-Seq

- Samples to Broken String:
- Genome-wide, unbiased sequencing for DSB

Current plan is to use INDUCE-seq to 'FIND' double strand breaks

Anticipated outcomes

1. Joint publication with all collaborators; joint presentations at conferences.
2. Development of a method to detect unwanted genomic events with increased predictive ability and sensitivity >> de-risk CRISPR Cas9 edited cells for therapeutic use >> contribute to translation of CRISPR Cas9 cell therapies.

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Advance sequencing technologies for identification of off-target mutations associated with genome editing. *CONFIRM*

Objectives

1. Understand the frequency and location of CRISPR Cas9 off target editing
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Step-1: Induce-Seq

- Samples to Broken String:
- Genome-wide, unbiased sequencing for DSB

(Looking at this portion only right now)

Secondary plan is to use to CONFIRM mutations

Step-2: Duplex-Seq

- Targeted assessment of mutation at the sites identified in step-1

Step 3: Functional Assays

- Understanding function consequences of the mutations and translatability risk
- Methods: SACF, clonality, others?.

Anticipated outcomes

1. Joint publication with all collaborators; joint presentations at conferences.
2. Development of a method to detect unwanted genomic events with increased predictive ability and sensitivity >> de-risk CRISPR Cas9 edited cells for therapeutic use >> contribute to translation of CRISPR Cas9 cell therapies.

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Secondary objectives: Confirm

- DSB does not equal mutation following repair
- Mutation does not equal toxicological risk

Step-2: Duplex-Seq
- Targeted assessment of mutation at the sites identified in step-1

Step 3: Functional Assays
- Methods: SACF, clonality, others?.

Step 2: FIND DSB
- INDUCE-Seq identifies DSB

CONFIRM MUTATION
- DUPLEX-Seq
  - Random tagging of double-stranded DNA detect mutations with high accuracy
  - DUPLEX-Seq to confirm if INDUCE-Seq identified DSB generate stable mutations

Step 3: CONFIRM CONSEQUENCE
- In vitro
  - Soft Agar Colony Forming Assay (SACF)
  - Identifying potentially cariogenic cells
  - Links to other CT-TRACS project proposal: *'SACF assay for tumourigenicity assessment of genome edited adherent cells'*
  - Participants welcome?

Project Launch

Opportunity for HESI/Broken String collaboration (accelerator program) at reduced cost if enacted quickly (timeline: May-Sept. 2021).

- 2 company members ready to enroll immediately: AZ & Novartis
  - PoC study instead of larger roll-out in the immediate term
- PoC will generate data to inform next steps;
- "Smaller" format enables funding via CT-TRACS' budget *(in full)*.

✓ **Unanimous approval for project launch at May 2021 CT-TRACS Annual Meeting**

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Project Participants & Study plan

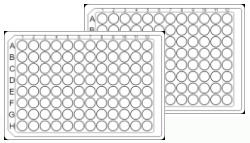
AstraZeneca
NOVARTIS

Steering team
- Project scoping
- Study design & protocols

2 sites
- Samples generation

(same cells; same edits with same methods)

Broken String
- INDUCE-Seq
- Bioinformatics Analysis



Steering team
- Results analysis
- Discussion & interpretation
- Scoping step-2 (Duplex-Seq)

June/July 2021

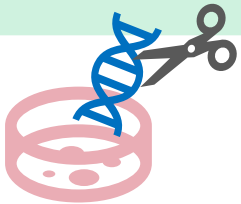
August/Sept. 2021

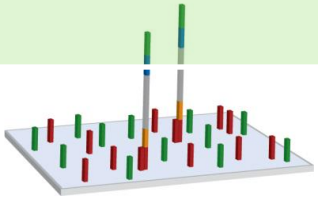
Q4 2021

CT/gene editing
- AstraZeneca
- Janssen
- NIST
- Novartis

Regulatory
- FDA
- MEB
- NIHS

Adv. sequencing
- Broken String/Cardiff Univ.
- NIST
- U. Sheffield





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Call for participants

- Follow-up work with Duplex-seq (Twin-Strand now joined CT-TRACS, eSTAR, GTTC)
- Interest in secondary proposal for identification of mutational changes under different culture conditions for hPSC?

https://hesiglobal.org/cell-therapy-tracking-circulation-safety-ct-tracs/

HESI Convene Translate Protect

https://hesiglobal.org/cell-therapy-tracking-circulation-safety-ct-tracs/

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