**UNRAVELLNG RECQ HELICASE FUNCTION IN GENOME**

**STABILITY USING STRAND-SEQ**

by

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# Abstract

Helicases are a highly conserved motor protein responsible for interacting with and unwinding canonical and non-canonical DNA structures. The RecQ class of helicases, known to suppress illegitimate recombination, are implicated in aging and cancer with three of the five human RecQ helicases directly linked to premature aging syndromes characterized by strong cancer predisposition. While no human disease has been associated with the RECQL5 helicase, loss of this gene in cells is known to result in elevated double strand breaks (DSBs) and sister chromatid exchange events (SCEs), a phenotype of genome instability similar to what is observed in RecQ helicase-linked diseases of strong cancer predisposition. Until recently, studying SCEs has been limited to cytogenetic assays that map at megabase resolution. We used single cell template strand sequencing (Strand-seq) to map SCEs as changes in template strand orientation before and after loss of RECQL5 at kilobase resolution. We generated over 20 single and double knockout models for RECQL5 as well as BLM, WRN and RECQL1 helicases using CRISPR-Cas9 in the human haploid cell line, KBM7, and mapped SCEs to the genome using custom bioinformatic approaches to improve resolution and accuracy of SCE detection. We performed enrichment analysis to show SCEs are frequently occurring near actively transcribed genes with guanine quadruplexes and common fragile sites further supporting the role of these genes in suppressing inappropriate recombination at specific genomic elements. We also developed novel bioinformatic approaches to generate genotype-specific call sets for CNAs, inversions and translocations. Uncovering the role of DNA helicases in DNA repair and replication pathways is critical for understanding their significance in cancer and aging. Stand-seq offers a unique method to study helicases by mapping the location of SCEs arising in their absence.

# Lay Summary

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* Helpful fellow students, lab mates, etc.
* Family support

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I thank Dr. O. Meyer for enlarging my vision of science and providing coherent answers to my endless questions.

Special thanks are owed to my parents, whose have supported me throughout my years of education, both morally and financially.

# Dedication

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## Introduction

### Overview

#### The human genome

What is the human genome?

How big is it? How it fits in the central dogma of biology

#### Genetic disease

What are genetic diseases? Mutations in the human genome

Hereditary vs de novo mutations

Cancer and aging both stem from the accumulation of mutation rates

##### Cancer

What is cancer? Hallmarks

Genome stability and accumulation of mutation rate

Hereditary vs sporadic

##### Aging

What is aging? Hallmarks

Genome stability and accumulation of mutation rate

##### Other genetic diseases

Monogenic vs polygenic

#### DNA transactions

How do mutations occur?

##### DNA damage and repair

Exogenous vs endogenous

Different repair pathways and mutation types

##### DNA replication

##### DNA transcription

##### The cell-cycle

#### Mechanisms of genome instability

##### Replication-associated genomic instability

###### Replication fork stalling and collapse

###### Replication stress and fragile sites

###### Incomplete DNA replication and ultrafine bridges

##### Transcription-coupled genome instability

###### Collision between transcription and replication machinery

###### Co-transcriptional R-loops

##### Genome instability syndromes

#### Mechanisms of DNA repair

##### Repair of small DNA lesions

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#### Methods of detection

##### Cytogenetics/FISH

##### Challenges and limitations

#### High resolution SCE mapping using Strand-seq

##### Technology

##### Bioinformatic tools

### Thesis rationale, objective and research scope

#### Chapter 2: New OP-Strand-seq pipeline for studying DNA repair

#### Chapter 3: Bioinformatic tools for uncovering multiple SV classes

#### Chapter 4: Role of RecQ helicases and SCEs and genome stability

#### Chapter 5: Conclusions and future directions

## New OP-Strand-seq pipeline for studying DNA repair

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Table 2.1 A sample of a table in Chapter 2

### Introduction

#### Original Strand-seq protocol

#### Applications of original Strand-seq method for studying DNA repair

#### Limitations of original Strand-seq method for studying DNA repair

##### Background limits breakpoint resolution

##### Constraints on throughput of manual library quality selection

##### Need for automated library classification system

### Methods

#### Knockout model generation in haploid cell line using CRISPR-Cas9

We used CRISPR-Cas9 to generate knockout clones for RECQL1, BLM, WRN, RECQL5, and both BLM and RECQL5 and WRN and RECQL5 together in the haploid KBM7 cell line. Two guides were designed per gene with maximized predicted on-target efficiency and minimized predicted off-target efficiency [1]. Assembled ribonucleoprotein CRISPR-Cas9 complexes were electroporated into cells and after 24 hours single cells, positive for gRNA fluorescence, were sorted by FACS into individual wells of 96 well plates. Colonies were grown up and DNA isolated from expanded cultures was used for PCR amplification of segments flanking the gRNA sequences and subsequent Sanger sequencing to characterize frameshifting mutations. This procedure was used to isolate 1-4 distinct clones for RECQL1, BLM, WRN, RECQL5 single KO cells and WRN/RECQL5 and BLM/RECQL5 double KO cells.

#### High throughput “one-pot” protocol

We developed a modified version of the original version of the Strand-seq protocol known as one-pot (OP)-Strand-seq to improve the quality and throughput of single cell sequencing libraries. This approach differs from the original method described in Sanders et al., 2017 in three ways. Firstly, the reagent volume was reduced 500- to 1,000-fold to improve the efficiency of the enzymatic steps involved in library preparation such as digestion and ligation. The reduction in individual reaction volumes also permitted the increase in the relative concentration of DNA fragments which further supports enzymatic efficiency. For example, the ligation of adapter to each other to form adaptor dimers rather than to DNA fragments is a common limitation of enzymatic efficiency and by increasing the relative concentration of DNA fragments in smaller reaction volumes, adaptor dimers are less likely to form [2], [3]. Second, genomic DNA was fragmented in bulk using micrococcal nuclease (MNase) to reduce the overall variability and GC-bias in standard library digestion and unevenness of sequencing coverage and a higher proportion of non-directional reads (herein referred to as background). Lastly bead clean-up steps are replaced with thermolabile protease treatments due to the loss of DNA reads associated with bead clean-up purification steps.

A dividing diploid cell replicates both Watson (W) and Crick (C) strands of each homologous chromosome. The two daughter cells can inherit opposing template strand for each homolog (WC, WC) or the same template strand for each homolog (WW, CC), generating three possible patterns of inheritance for each chromosome in a given daughter cell (WW, CC, WC). In a haploid cell this is simpler as there is only one homolog and only one of two possible DNA template strand inheritance patterns for each chromosome in a daughter cell (W, C).

##### Library preparation

For Strand-seq the thymidine analog, Bromodeoxyuridine (BrdU), is incorporated into nascent strand synthesis to allow selective nicking of nascent DNA with BrdU by UV irradiation in library fragments before PCR amplification [3]. This approach allows the selective sequencing of the parental DNA template strands. Illumina whole-genome sequencing (WGS) is used to generate directional libraries with reads mapping to the reference genome in the orientation of the native parental DNA template strands. Strand-seq libraries thus harbor unique signatures of intra-chromosomal template strand changes that represent orientation-dependent SVs.

{Strand-seq libraries were prepared as described using the one pot (OP-Strand-seq) library preparation protocol [4]}

Pools of Strand-seq libraries were size-selected and loaded onto a MiSeq or NextSeq instrument for paired end 75 bp sequencing [3], [4].

##### Illumina whole genome sequencing

##### Bioinformatic pre-processing

#### Bioinformatic classifier for sorting Strand-seq libraries

### Results

#### Comparison of breakpoint resolution between haploid and diploid cells

Diagram

Description automatically generated

Figure 2.1 SCE breakpoint resolution relative to sequencing effort for haploid and diploid cells

#### Comparison library classification accuracy to existing methods

### Discussion

#### Applications of novel methods for DNA repair studies

#### Conclusions

## Structural variant callers

### Introduction

#### Rationale for improving SV callers in the field to study intra-tumour heterogeneity

### Methods

#### SCEs calling

##### Bioinformatic approaches to SCE detection

##### Genotype-specific differences in SCE frequency

#### CNAs calling

##### Bioinformatic approaches to CNAs detection

##### Genotype-specific differences in CNAs frequency

#### Translocation calling

##### Bioinformatic approaches to translocations detection

##### Genotype-specific differences in translocations frequency

### Results

#### Compare accuracy (precision/recall) of different callers to existing tools

### Discussion

#### Applications for future studies in uncovering complete mutational landscape

## Role of BLM and RECQL5 in DNA repair using mainly SCE data

### Introduction

#### Role of DNA helicases in DNA repair

#### Non-redundant roles of RecQ helicases

### Methods

#### Generation of comprehensive SV call sets

#### Bioinformatic tools for running enrichment tests between genetic elements

#### Genetic element datasets

##### Collection of essential/non-essential genes for KBM7 cell line

##### Collection of actively/non-actively transcribed genes for KBM7 cell line

##### Collection of experimentally reported guanine quadruplexes (G4)

### Results

#### RecQ helicase KO genotype-specific differences in SCE frequency

#### Enrichment analysis of SCEs

### Discussion

##### Role of RecQ helicases in SCE and G4 biology

##### Conclusions

## Conclusion

### Discussion of results

#### Novel methods

##### Strand-seq library generation for DNA repair studies

##### Generation of complete SV call sets

##### Enrichment analysis of SV coordinates

#### Novel findings of RecQ helicases and implications on field of DNA repair

### Strengths and limitations of the research

#### Strengths

##### Novel method allows for improved resolution of studying DNA repair events

##### New callers exceed accuracy performance of existing/published methods

#### Weaknesses

##### No molecular work in protein or RNA to deduce functional consequences of different SVs

##### Limitations on overall accuracy of callers

### Discussion of any potential applications of the findings

#### Investigating RecQ helicases in the context of DNA repair

#### Using bioinformatic tools to uncover SVs in single cells

### A description of possible future research directions

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