DNA Repair Factor Overexpression Screen Identifies Factors Required for Repair Pathway Choice

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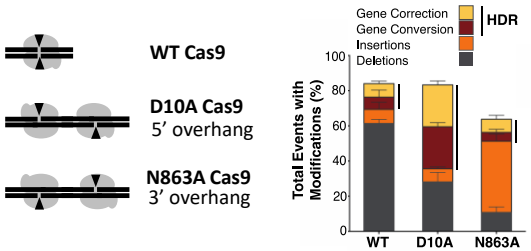
Introduction

Using Cas9 to Introduce DSBs

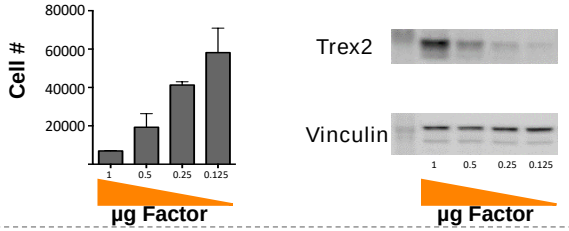
- Cas9 and its variants can be used to introduce a variety of different DNA cuts including blunt double-strand breaks (DSBs) or dual nicks leaving either 3' or 5' overhangs.
- The type of overhangs determines repair pathway choice and repair outcome:
  - **WT Cas9** -> **NHEJ** (small deletions and insertions)
  - **D10A Cas9** (5' overhang) -> **HDR** (gene correction through ssODN and gene conversion using endogenous HBD gene)
  - **N863A Cas9** (3' overhang) -> **A-NHEJ** (insertions)



Screen Workflow and Analysis

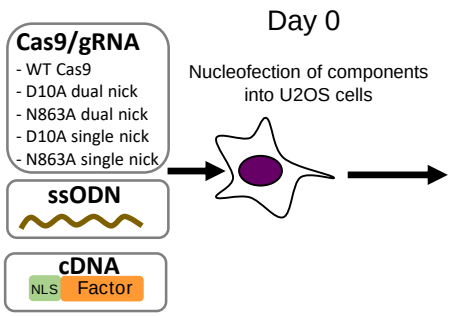
Viability and Expression Analysis

- All factors were screened for expression and cells were analyzed for viability
- Optimal factor concentration for screen was determined to maximize viability and factor expression



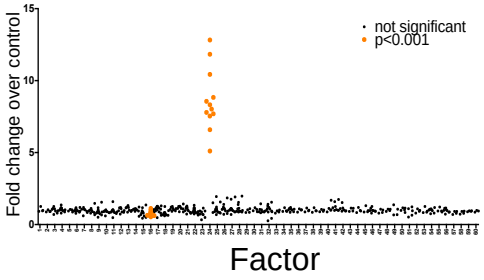
In this example, a concentration of 0.5 µg was chosen to obtain optimal protein expression with acceptable levels of factor induced cellular toxicity.

Setup



- Cell Lysis
- gDNA Extraction
- Locus PCR
- High throughput sequencing (MiSeq)
- Analysis
  - Representation is normalized to respective control
  - Divided by type of Cas9 lesions and repair event

Analysis Example:
WT Cas9 - INSERTIONS



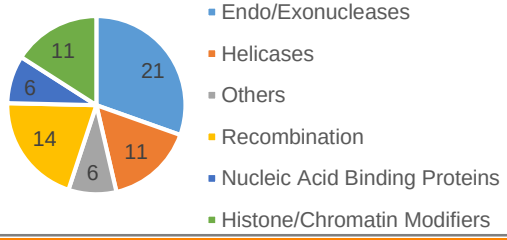
Focused Overexpression Screen

Goal

- Identify factors that modulate repair pathway choice and alter the frequency of repair outcomes:
  - Deletions
  - Insertions
  - Gene Conversion
  - Gene Correction
- Assess repair pathway modulation in the context of different Cas9-induced lesions

Factors

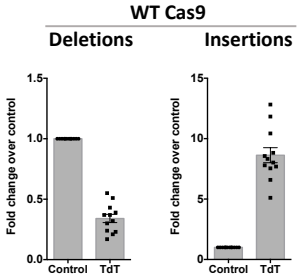
- Overexpressed cDNA library of 64 DNA-repair factors



Results and Conclusions

TdT

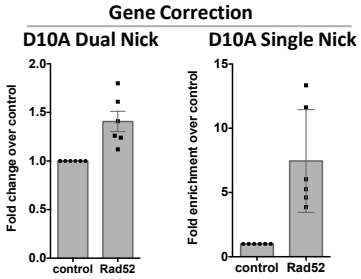
TdT is a polymerase physiologically expressed during V(D)J recombination



- Decrease in deletions
- Strong enhancement of insertions

Rad52

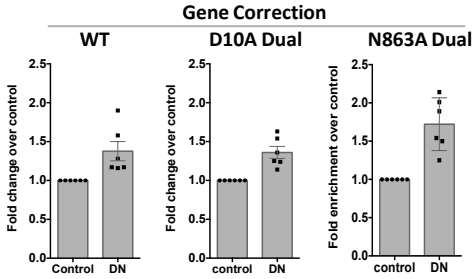
Rad52 is a single stranded DNA binding protein promoting strand annealing



- Increase in gene correction

53BP1 Dominant Negative

Dominant Negative version of 53BP1 (53BP1-DN) prevents inhibition of end resection



- Increase in gene correction