Team 17

Improving CRISPR Off-Target Detection Using Transposases

DREXEL UNIVERSITY
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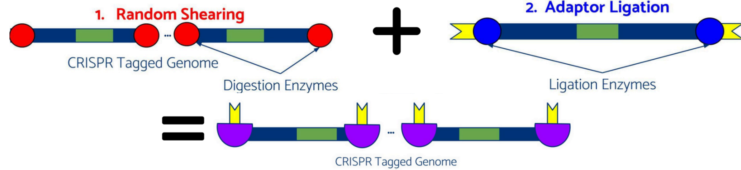
NEED:

Simplify DNA processing by combining separate Cut and Label steps into 1 Step



FUTURE:

Summary:
Combine 2 steps into 1, enhancing efficiency

Impact:
Simpler and quicker GUIDE-seq protocol.

Future Revisions:
Use an enzyme that can cut more TN5 reaction and directly test the sequencing (using comparable samples)

DESIGN INPUTS:

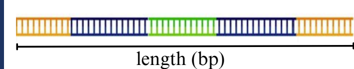
Constraints:

Compatible adapters: i5 and i7 ends needed for sequencing

Unique Molecular Index: needed to identify PCR duplicates

Requirements:

Fragment length: 100-2000 bp

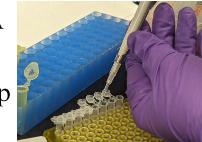


PCR Enrichment: higher DNA C. increase

TESTING RESULTS

DNA Fragmentation Test (R1) Run several TN5 Reaction

Results: Pass DNA fragments averaging ~1000 bp obtained



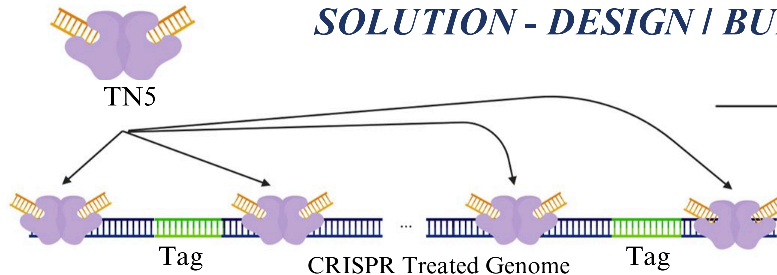
PCR Enrichment Test (R2) Run PCR, measure DNA concentration before and after

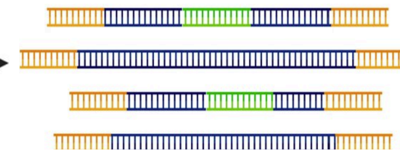
dna conc (ng/ul	A1	A2	B1	B2
Before PCR	6.55	6.39	4.29	5.96
After PCR	10.75	12.35	21.25	19.3

Results: Pass adapter B samples showed 3x adapter A's DNA C. Increase.

SOLUTION - DESIGN / BUILD

Utilizes TN5 enzymes to shear and ligate adapters to an in tact genome, produces same product as the GUIDE-seq shearing and ligation steps.





PCR Enrichment + DNA Sequencing