Approaches for DNA-Encoded Library Screening of Transcription Factors

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DEL and Protein Sciences Group

Drug Discovery Chemistry – Encoded Libraries for Drug Discovery

April 2nd, 2024

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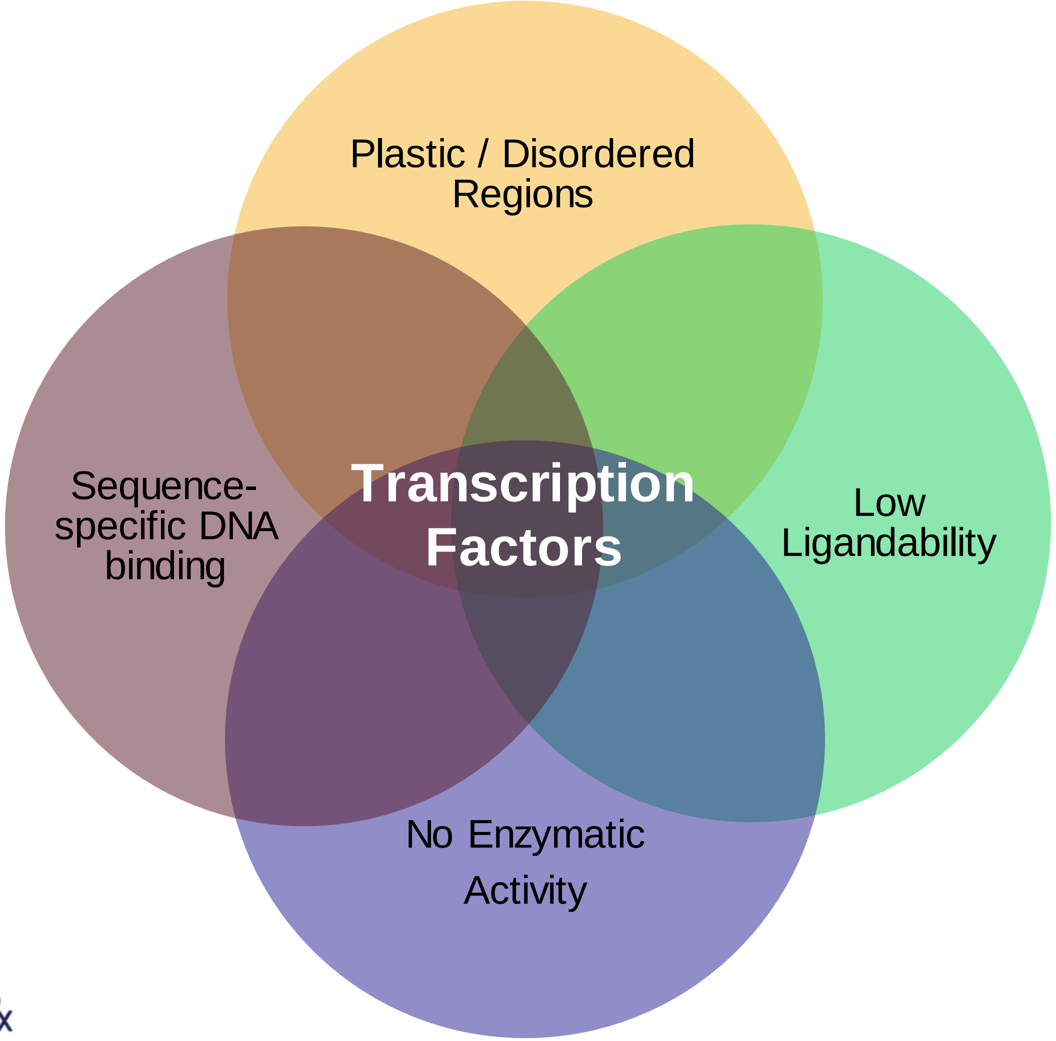
The Nurix DEL Screening and Analysis Process is Designed to Unlock Challenging Targets, including Transcription Factors

Challenges in ligand identification for transcription factors



- Nurix's DEL collection is designed to address targets with low ligandability
  - >5 billion molecule DEL
    - Including scaffold-based libraries
  - Diverse chemical space
- Approach to DEL affinity screening focused on capturing novel binding sites
- In DEL screening, the DNA tag may contain consensus sequence for transcription factor
  - A combination experimental and bioinformatic approaches can mitigate this issue

EWS-FLI1 Is a Fusion Protein and the Main Oncogenic Driver for Ewing Sarcoma

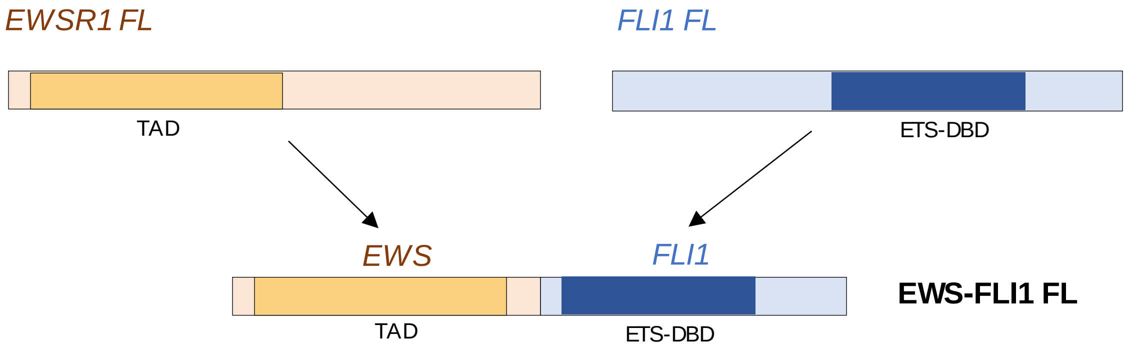
- **Ewing sarcoma (ES)** is a pediatric bone and soft tissue cancer with **no therapies available**.

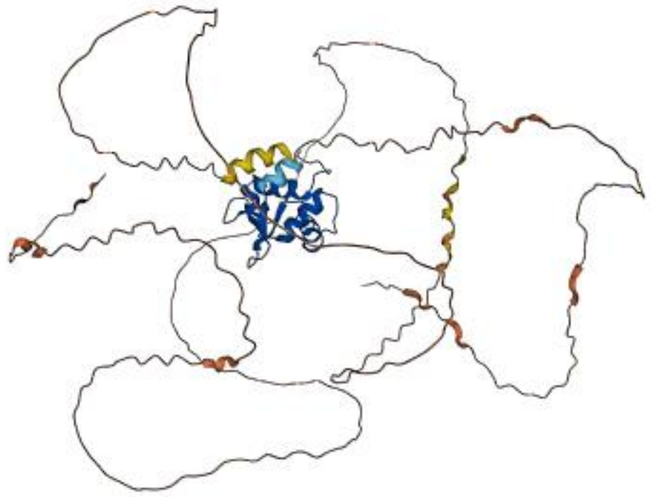
- Ewing sarcoma impacts children and young adults, constituting 10-15% of all bone sarcomas.

- ~200 patients are diagnosed with Ewing sarcoma each year in the United States.

- **EWS-FLI1** is a fusion protein caused by chromosomal translocation.
    - **EWSR1 -** strong transactivation domain (TAD)
    - **FLI1 –** ETS-DBD transcription factor
        - Binds to 5' GGAA 3' dsDNA sequences
    - This leads to **aberrant transcription of oncogenes in Ewing sarcoma.**

- >85% of Ewing sarcoma's have EWS-FLI1

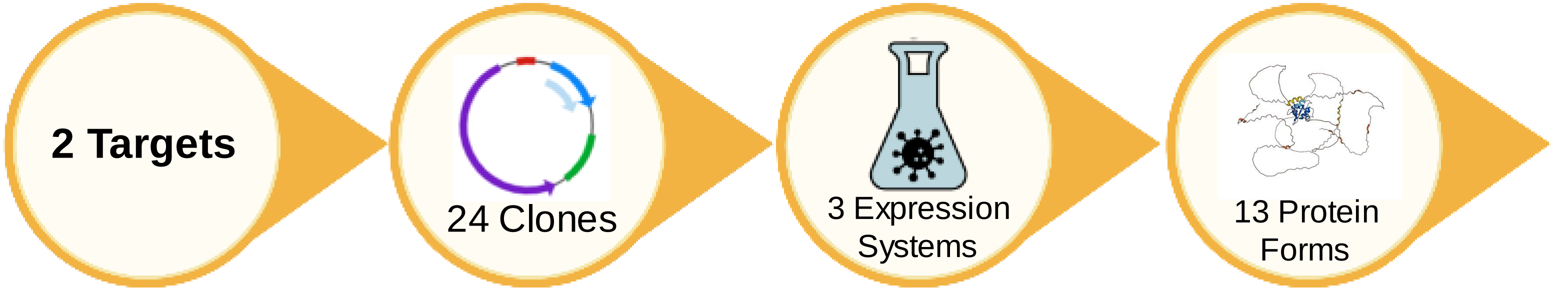




EWS-FLI1 Alphafold Model

Extensive Protein Screening was Required to Enable Productive DEL Discovery

High-Throughput Protein Expression



2 Targets

24 Clones

3 Expression Systems

13 Protein Forms

EWS-FLI1

EWSR1

24 Target Forms

5 Mutants

2 Fusion Partners

3 Affinity Tags

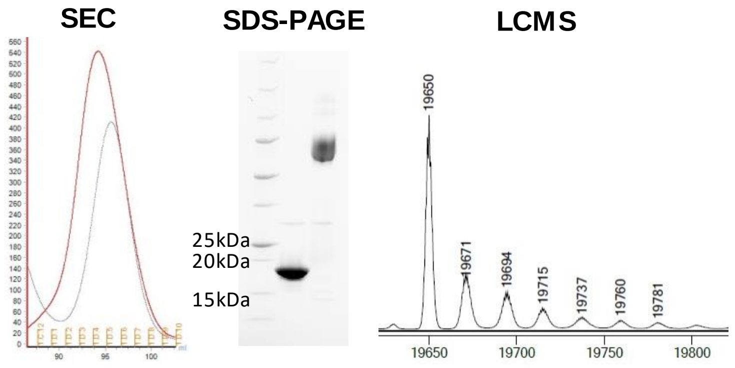

Insect


E.coli

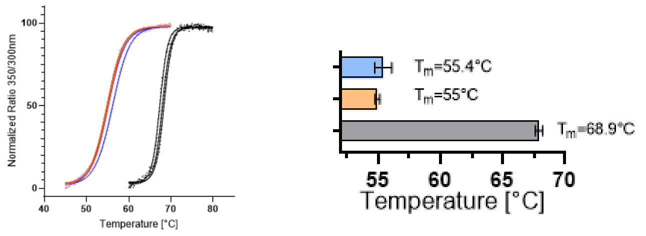

Mammalian

- ☑ EWS-FLI1 FL
- ☑ EWS-FLI1 FL Mutants
- ☑ FLI1 DBD
- ☑ FLI1 DBD Mutants
- ☑ EWSR1

Protein Quality Control



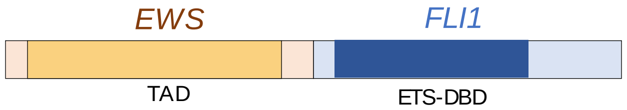
DSF with binder (if available)



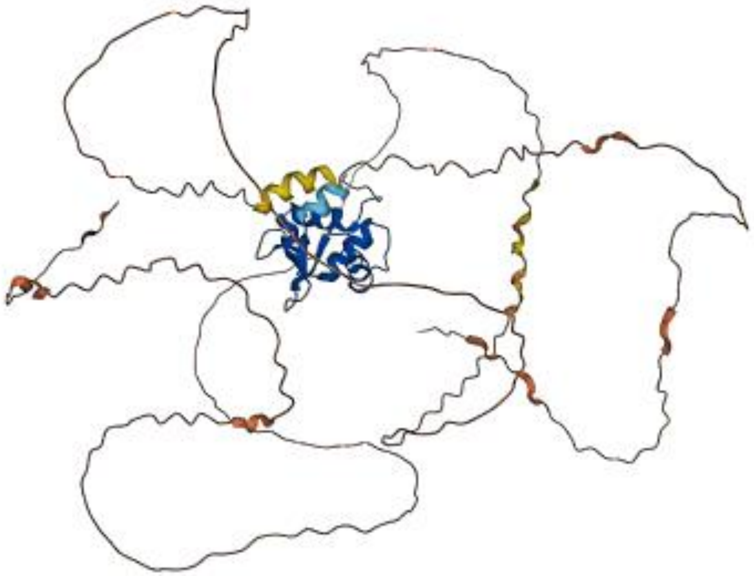
DEL Screen

Structured FLI1 DNA Binding Domain Prioritized for DEL Screening

EWS-FLI1 full length fusion phase separates, requiring denaturation and refolding

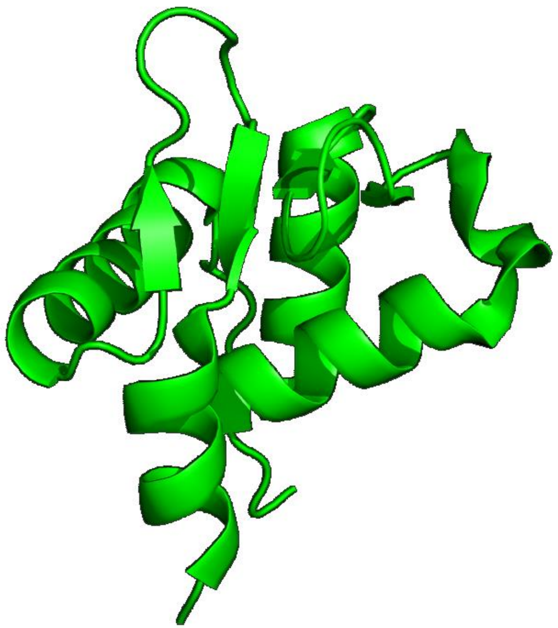


EWS-FLI1 FL

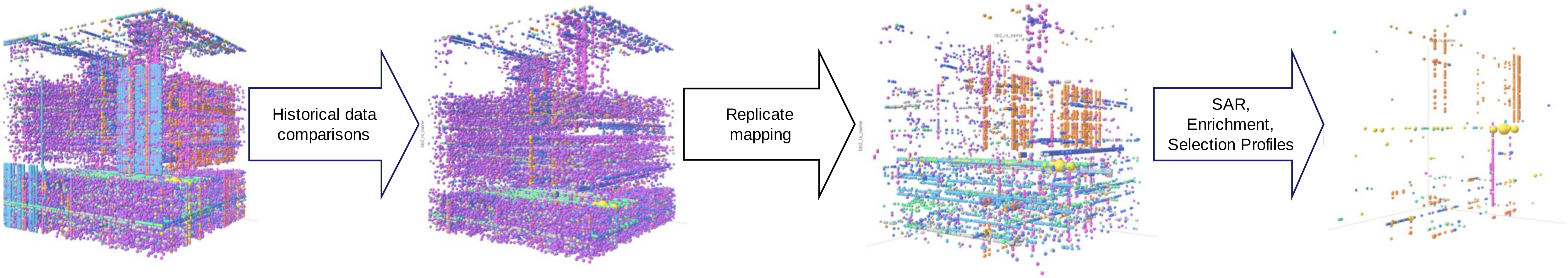


EWS-FLI1 Alphafold Model

WT FLI1 DBD



Nurix's DEL Analysis Platform Applies Multiple Rounds of Filtering To Identify Hits

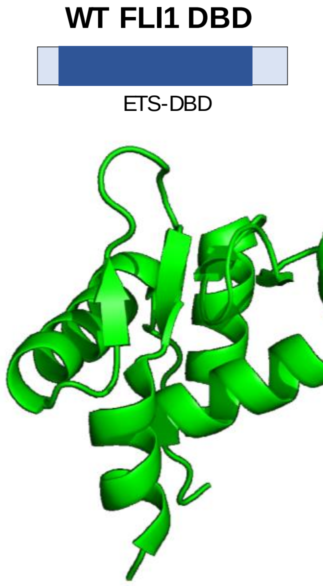


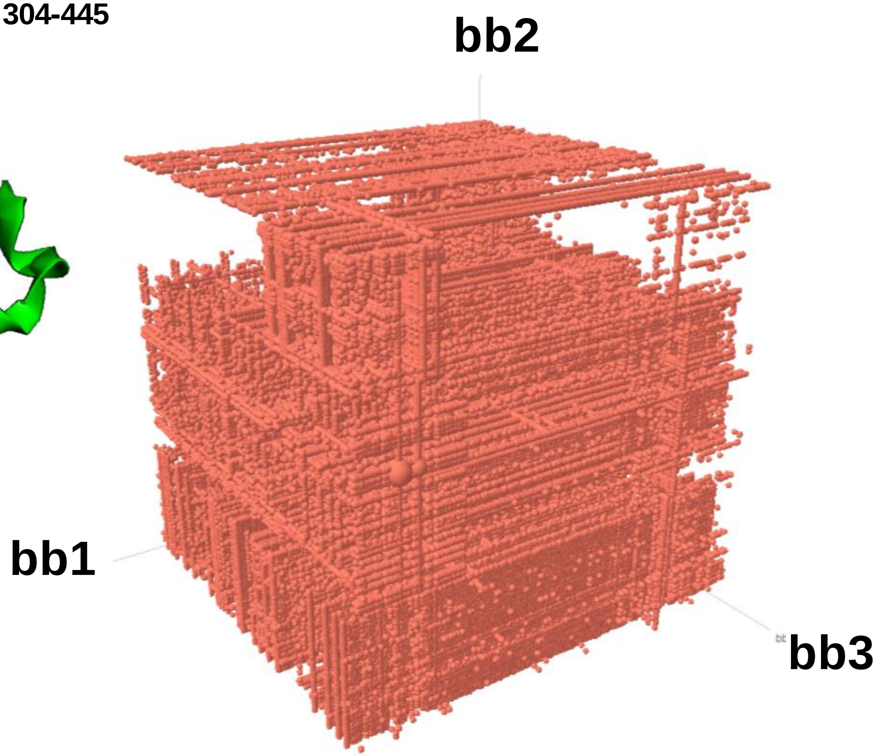
Historical data comparisons

Replicate mapping

SAR,
Enrichment,
Selection Profiles

FLI1 DNA Binding Domain Library Enrichment Driven by Affinity to DNA Tag





Informatic Flagging of Consensus Sequence

72% FASTA reads contain known consensus sequence

Strategies to mitigate DNA tag-driven enrichment of consensus sequence

DNA blockers

- Literature-reported DNA consensus sequence
- Computationally identify DNA consensus sequence by analyzing DEL sequence output

DEL selections performed against mutant proteins

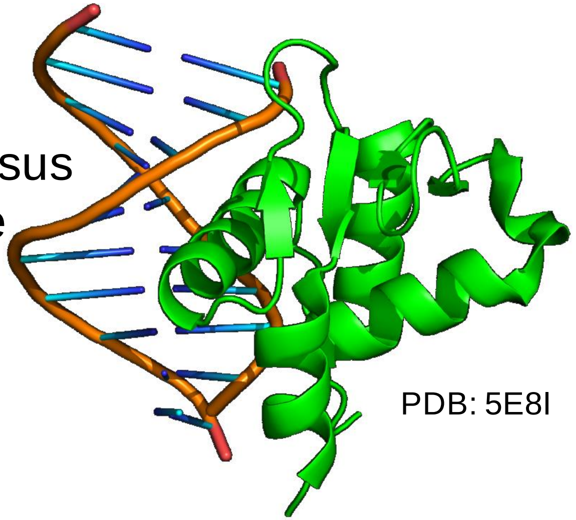
- Mutations that lack ability to bind DNA reported

Literature DNA Consensus Sequence Binds to FLI1 DNA Binding Domain (DBD)

WT FLI1 DBD

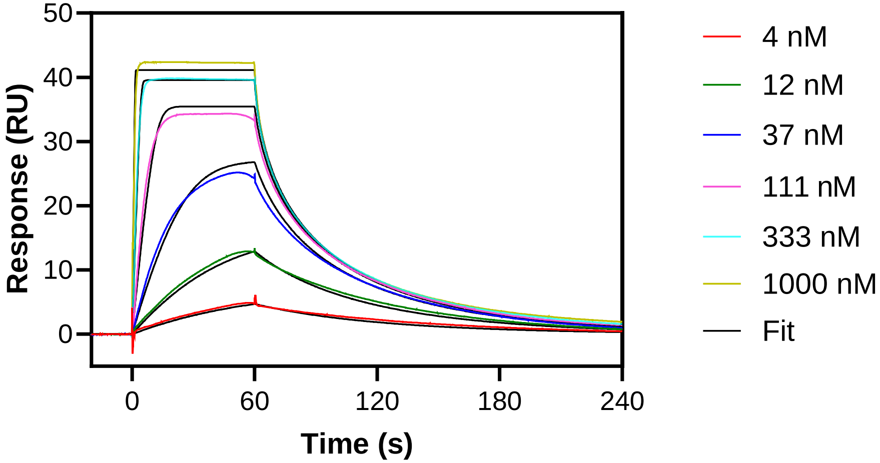
304-445

DNA consensus sequence



PDB: 5E8I

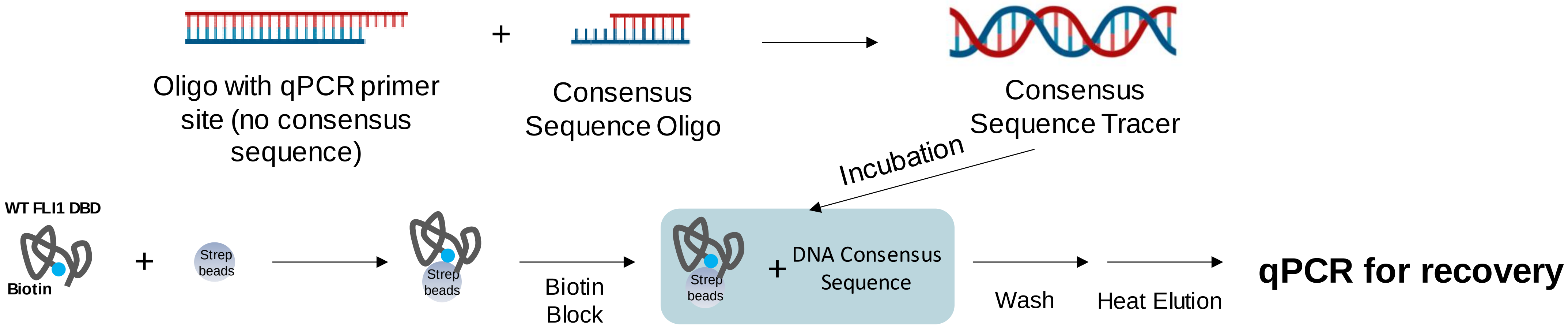
SPR confirms binding of DNA consensus sequence to FLI1 DBD



K_D = 20 nM

Hou and Tsodikov, *Biochemistry* (2015)

Addition of DNA Consensus Sequence Significantly Reduces DNA Driven Enrichment



Oligo with qPCR primer site (no consensus sequence)

Consensus Sequence Oligo

Consensus Sequence Tracer

Incubation

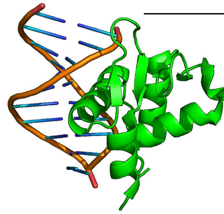
Biotin Block

DNA Consensus Sequence

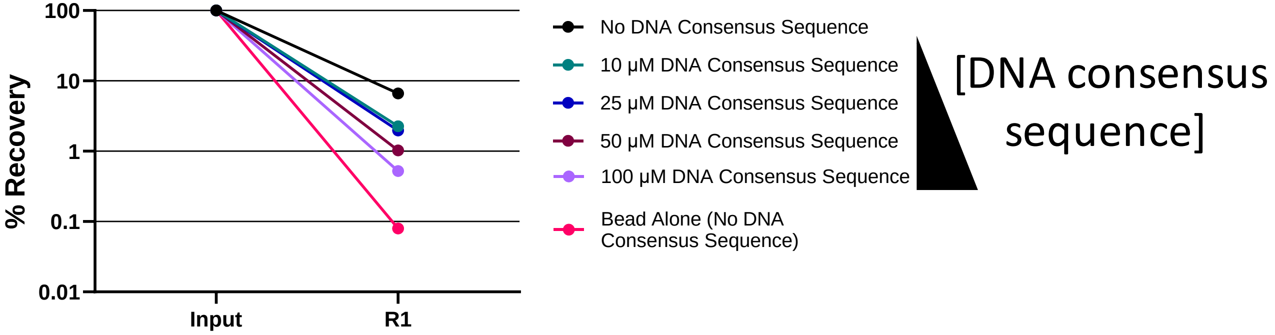
Wash

Heat Elution

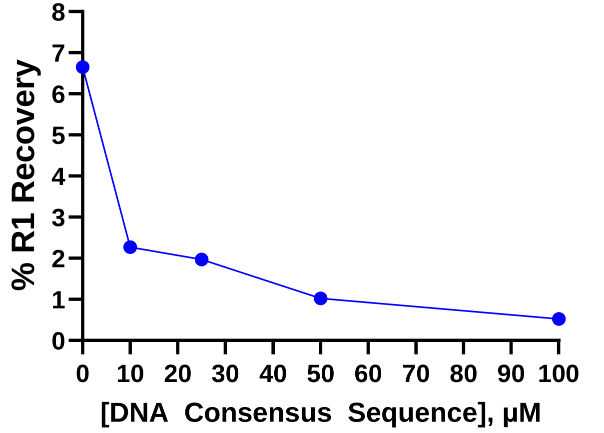
qPCR for recovery



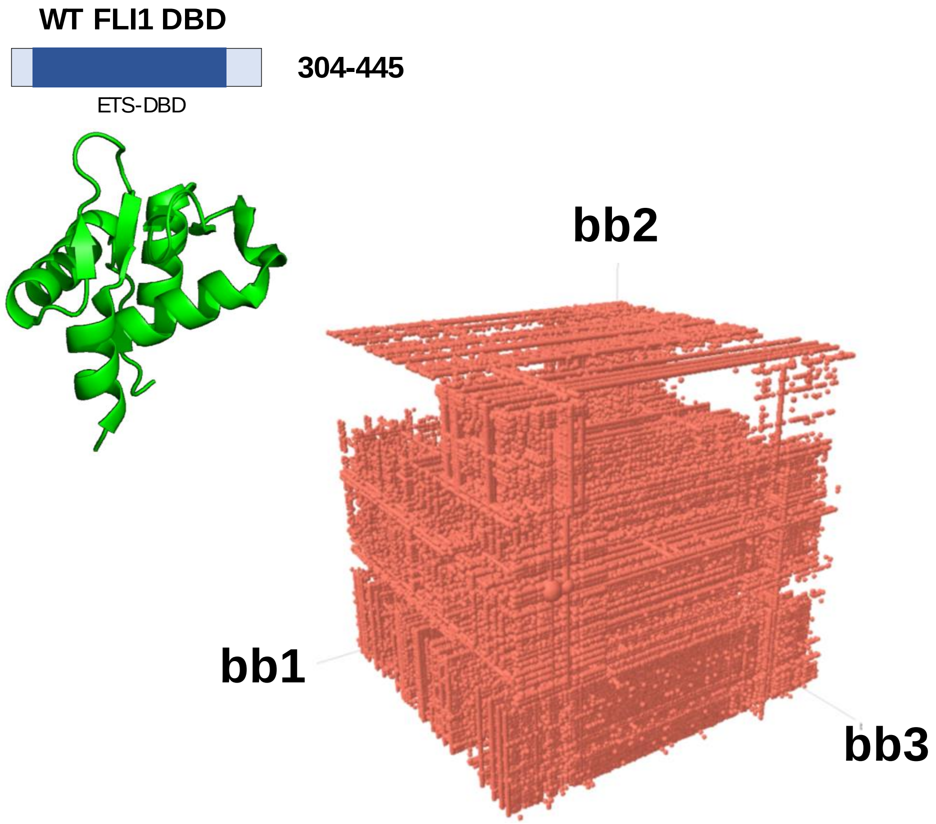
DNA consensus sequence

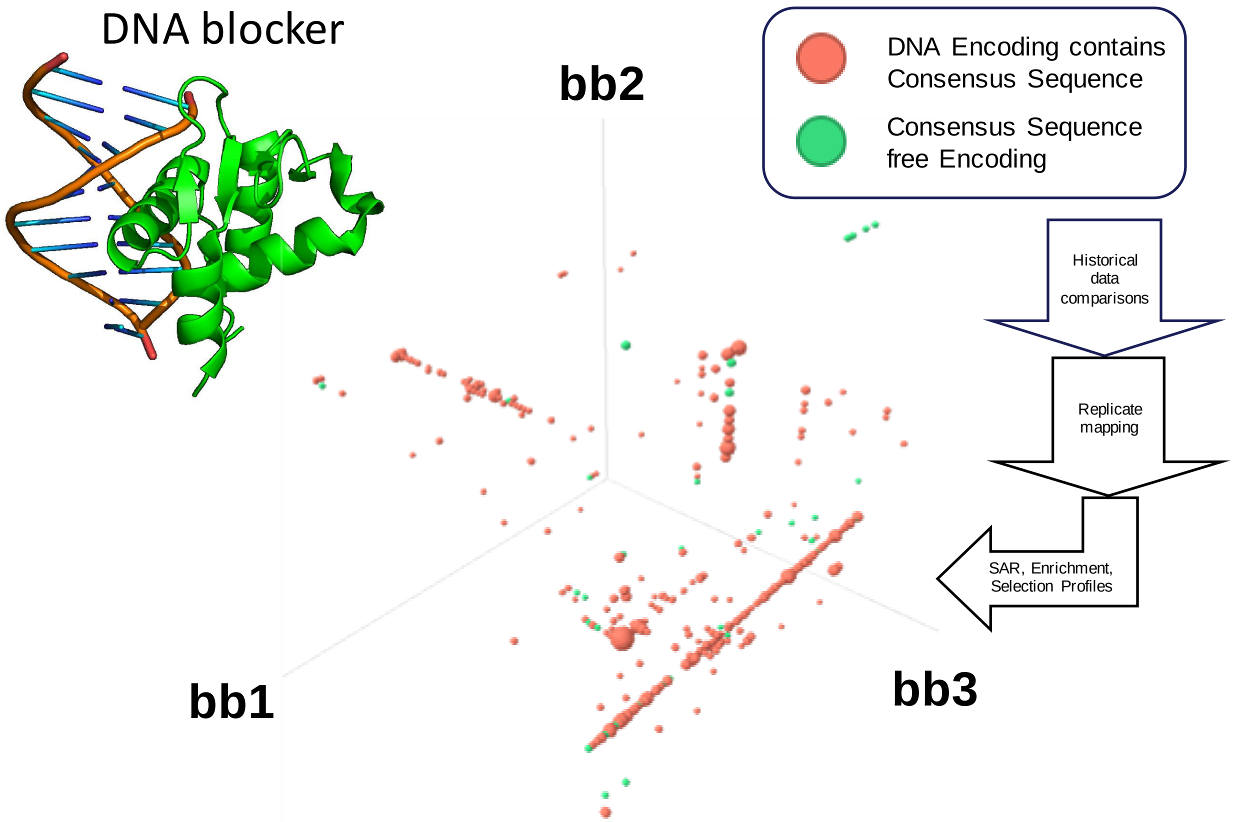


Recovery of consensus sequence tracer



DNA Blocker Reduces Recovery of Consensus Sequence Binders to Near Baseline



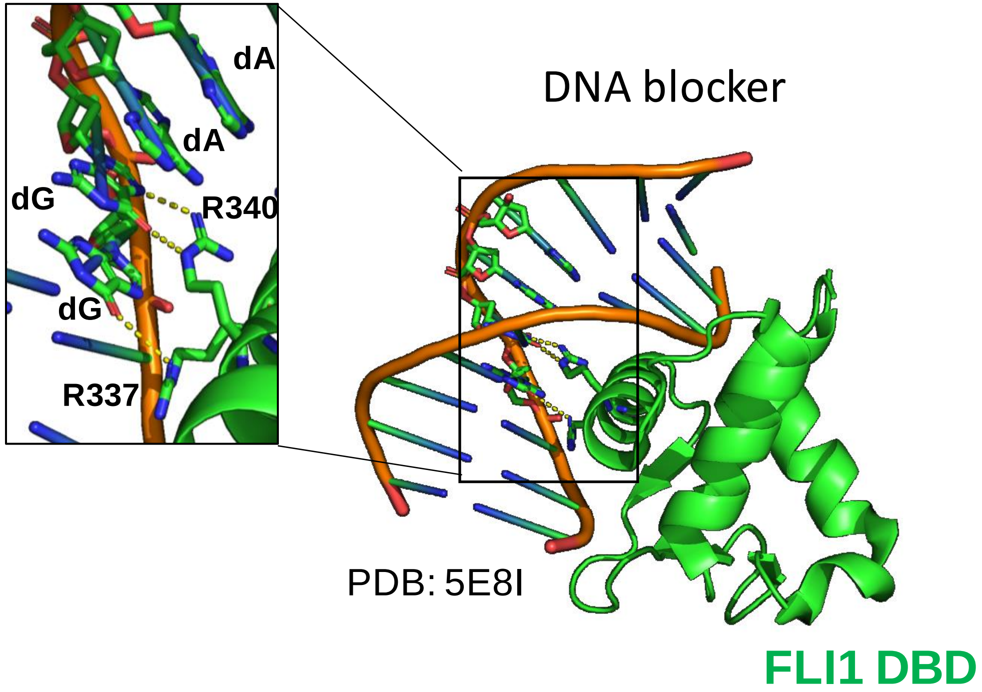


Informatic Flagging of Consensus Sequence

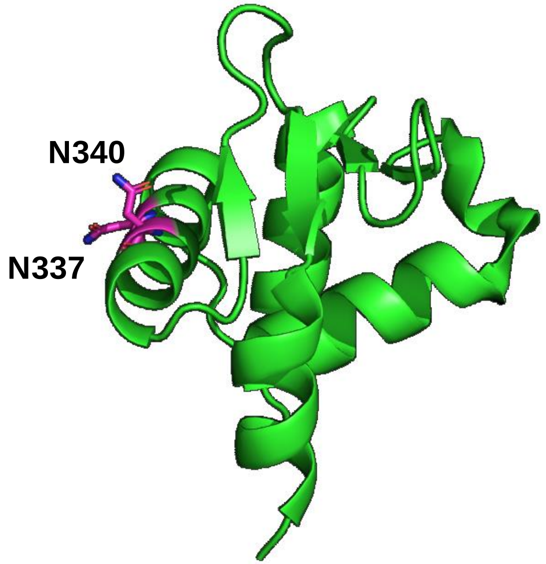
72% Consensus Sequence + DNA Blocker → **25% Consensus Sequence**

Bead Only = 23%
Naïve = 26%

Screening of FLI1 DBD Mutant Abrogates Consensus Sequence Driven Enrichment

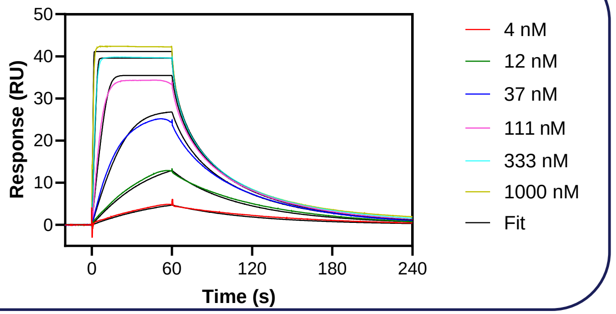


FLI1 DBD$^{R337N/R340N}$ mutant

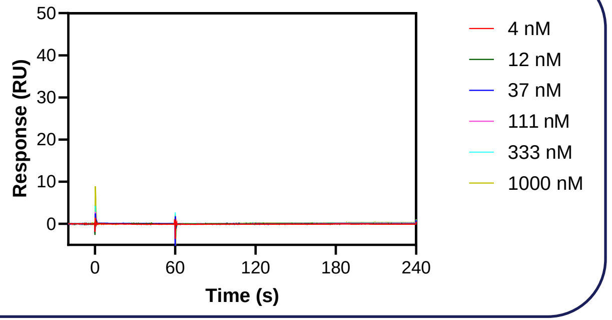


SPR confirms DNA blocker binds to WT FLI1 DBD

K_D = 20 nM

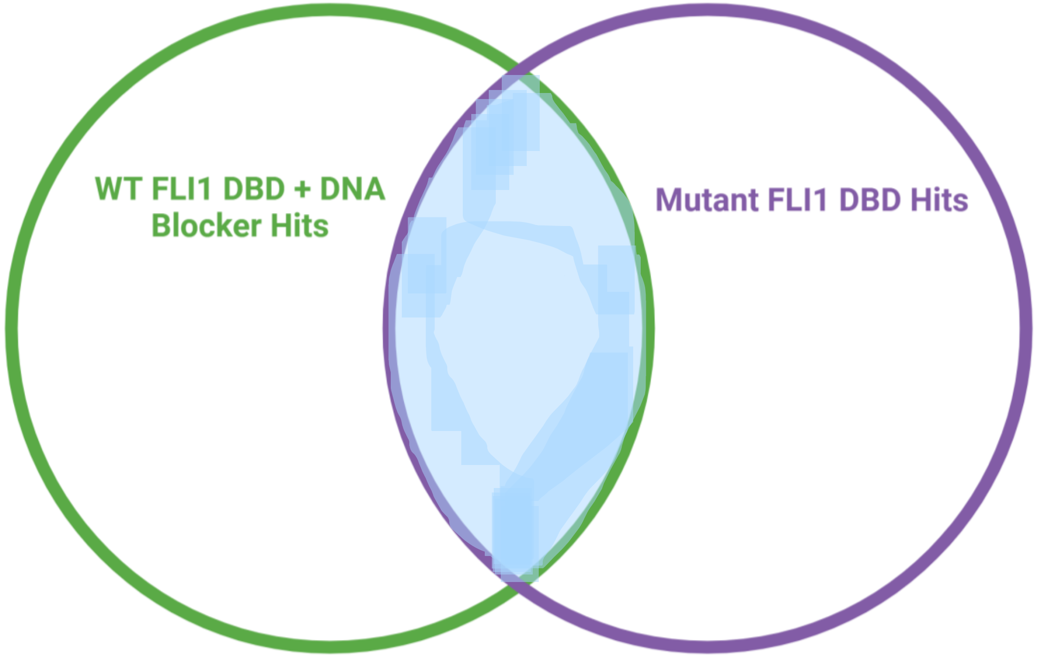


SPR confirms no DNA blocker binding to FLI1 DBD$^{R337N/R340N}$ mutant



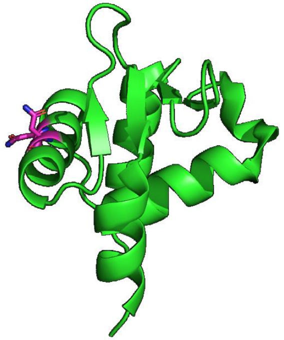
Hou and Tsodikov, *Biochemistry* (2015)

Hits Enriched in Both the Wild Type and Mutant FLI1 Selections Considered Higher Confidence Hits

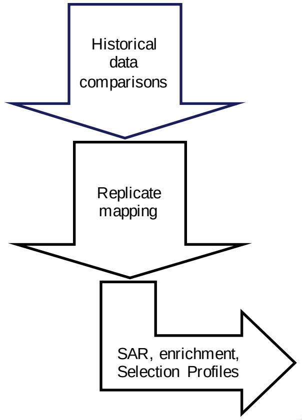


🟦 = Higher confidence hits for binding FLI1 DBD

FLI1 DBD$^{R337N/R340N}$ Mutant DEL Output Overlaps with WT FLI1 DBD + DNA Blocker Selections



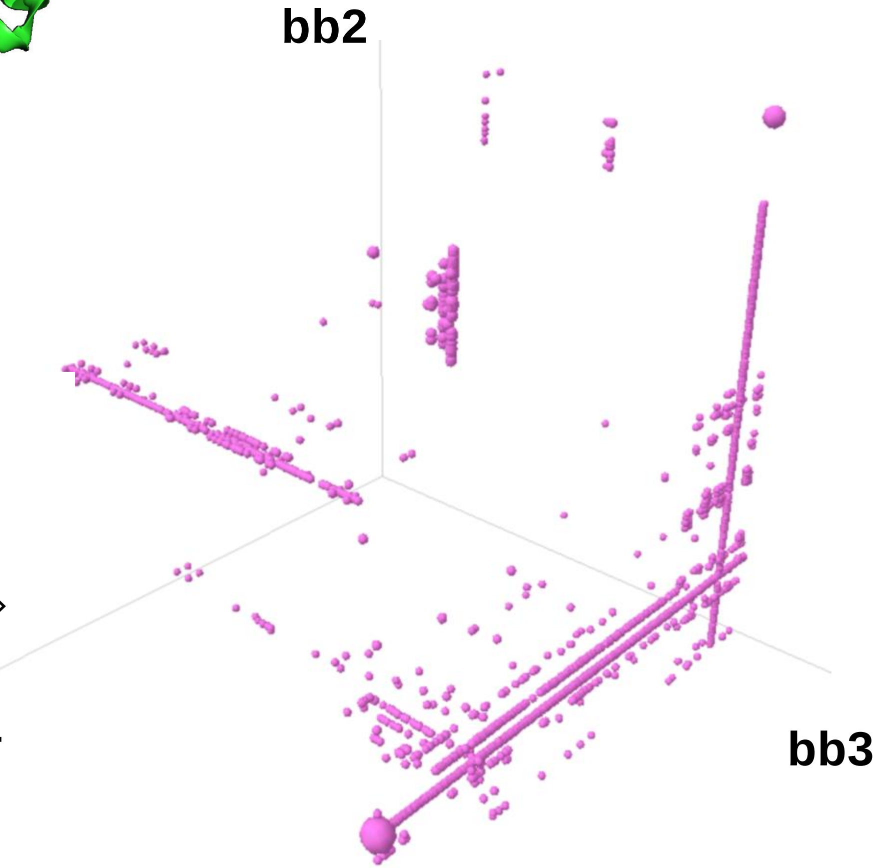
FLI1 DBD$^{R337N/R340N}$ Mutant Selection

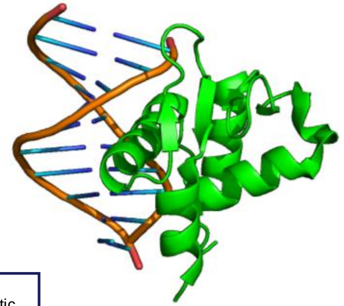


Historical data comparisons

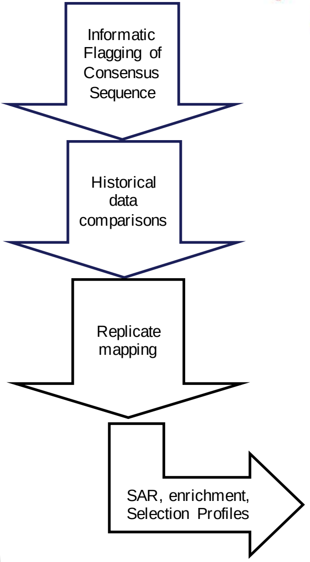
Replicate mapping

SAR, enrichment, Selection Profiles





WT FLI1 DBD + DNA Blocker Selection

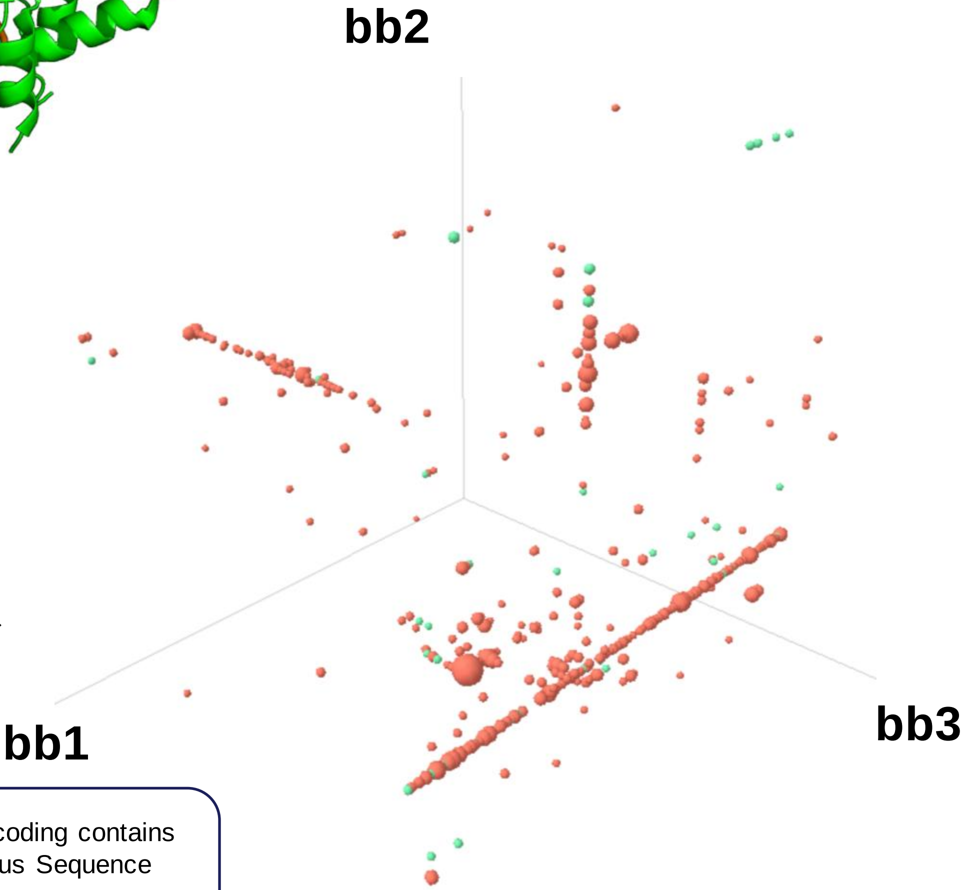


Informatic Flagging of Consensus Sequence

Historical data comparisons

Replicate mapping

SAR, enrichment, Selection Profiles

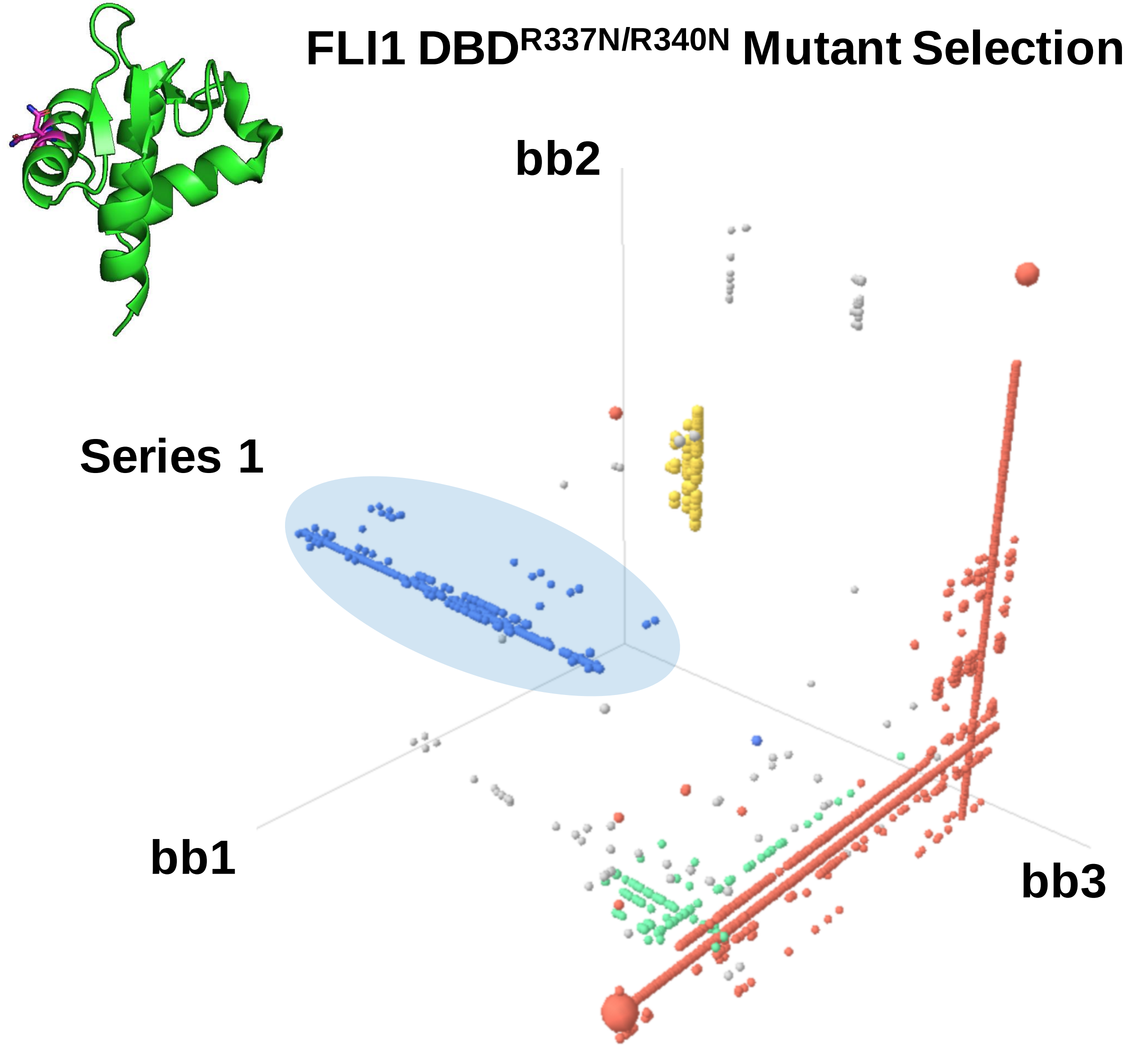


🔴 DNA Encoding contains Consensus Sequence

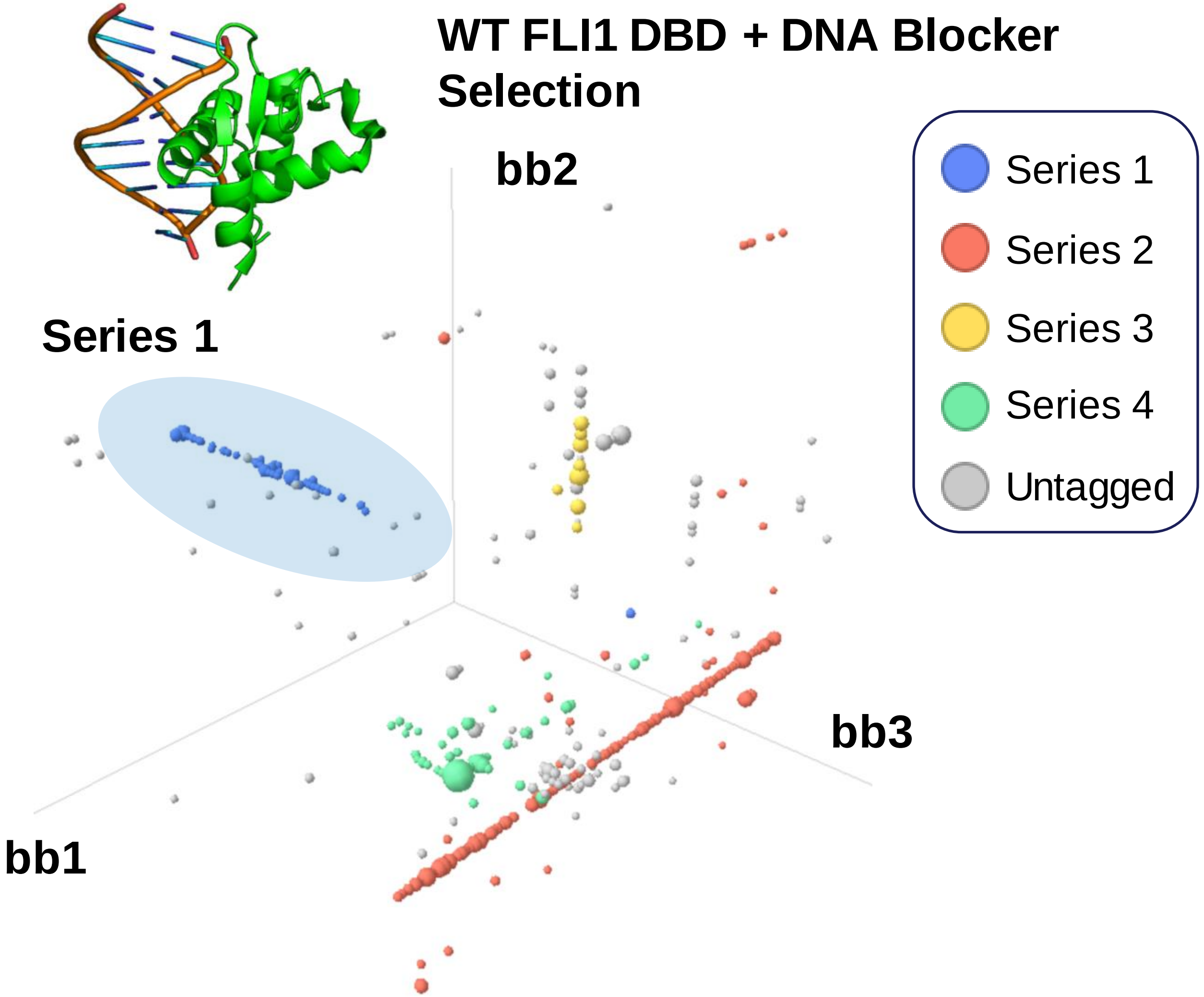
🟢 Consensus Sequence free Encoding

Four Overlapping Series Identified From FLI1 DBD$^{R337N/R340N}$ Mutant and WT FLI1 DBD + DNA Blocker Selections

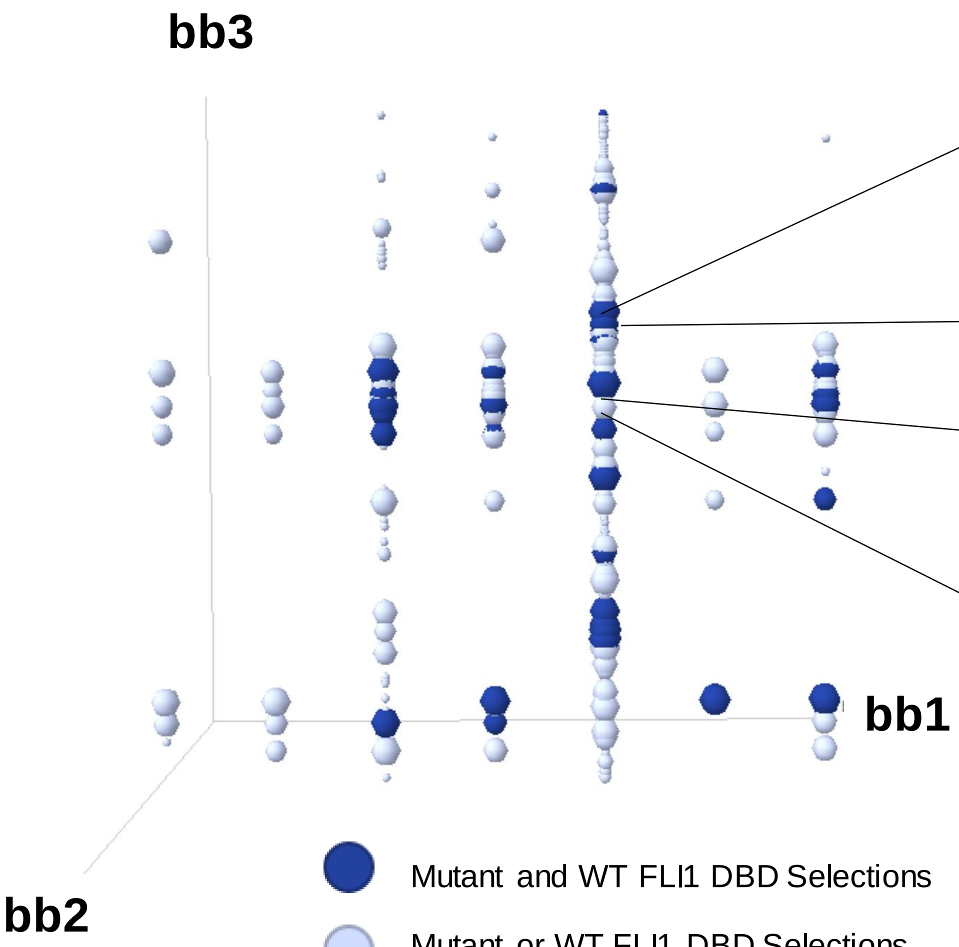
FLI1 DBD$^{R337N/R340N}$ Mutant Selection



WT FLI1 DBD + DNA Blocker Selection



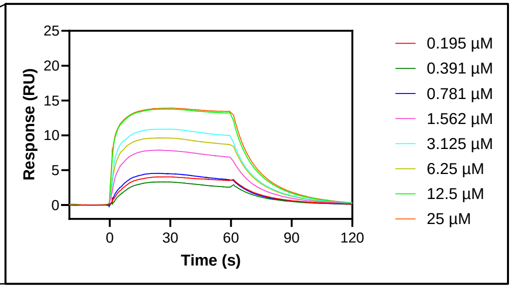
Series 1 Hits Enriched in WT and Mutant FLI1 Selections Validated by SPR To Bind to WT-FLI1 DBD



● Mutant and WT FLI1 DBD Selections

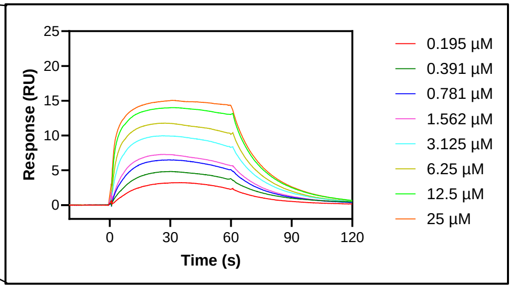
○ Mutant or WT FLI1 DBD Selections

Hit in WT and Mutant Selections



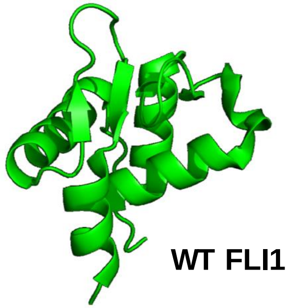
DEL Hit 1
K_D = 1.4 µM

Hit in Mutant Selection



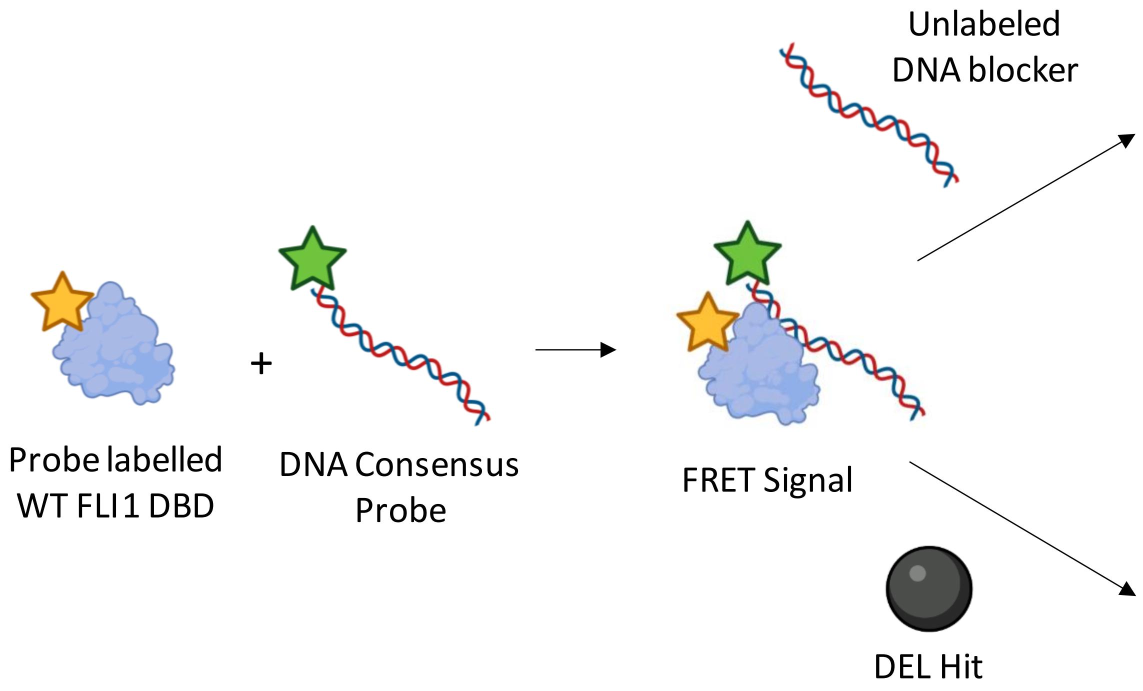
DEL Hit 2
K_D = 1.8 µM

Both ligands have FASTA reads containing consensus sequence



WT FLI1 DBD

FRET Displacement Assay Reveals Binding Mode of Series 1 off-DNA DEL Hits



Unlabeled DNA blocker

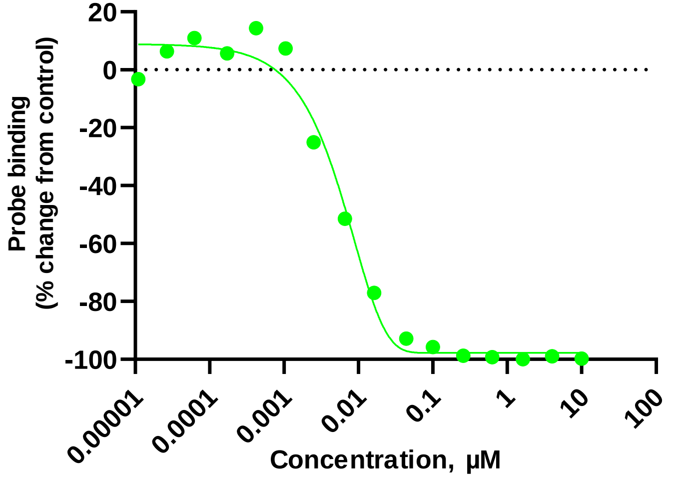
Probe labelled WT FLI1 DBD

+

DNA Consensus Probe

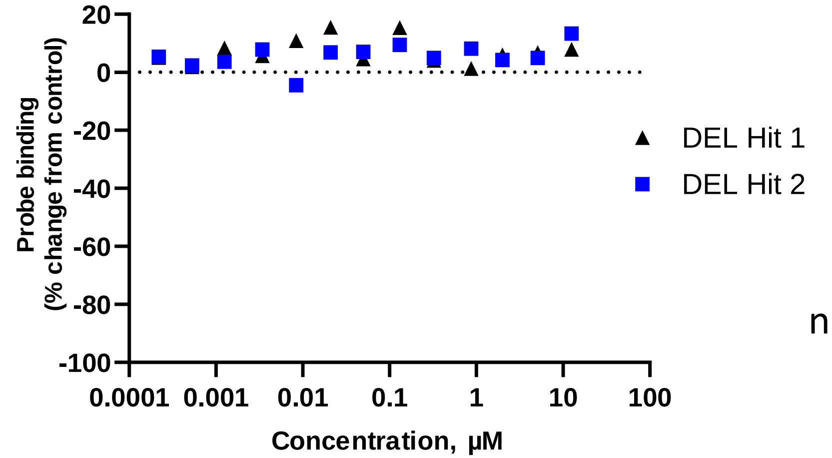
FRET Signal

DEL Hit



DNA blocker competes with DNA consensus probe

IC_{50} = 6.0 nM

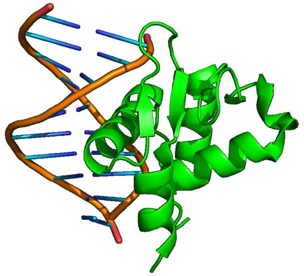


Series 1 DEL hits do not compete with DNA consensus probe

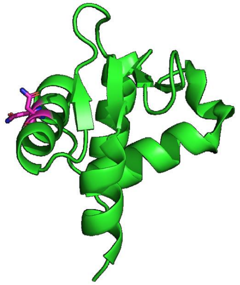
nurix

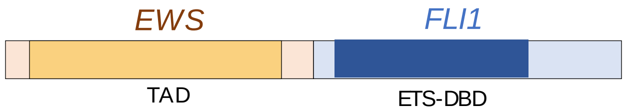
Validation of Ligand Binding to Oncogenic Fusion

WT FLI1 DBD

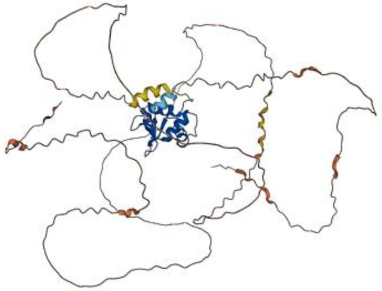


Mutant FLI1 DBD





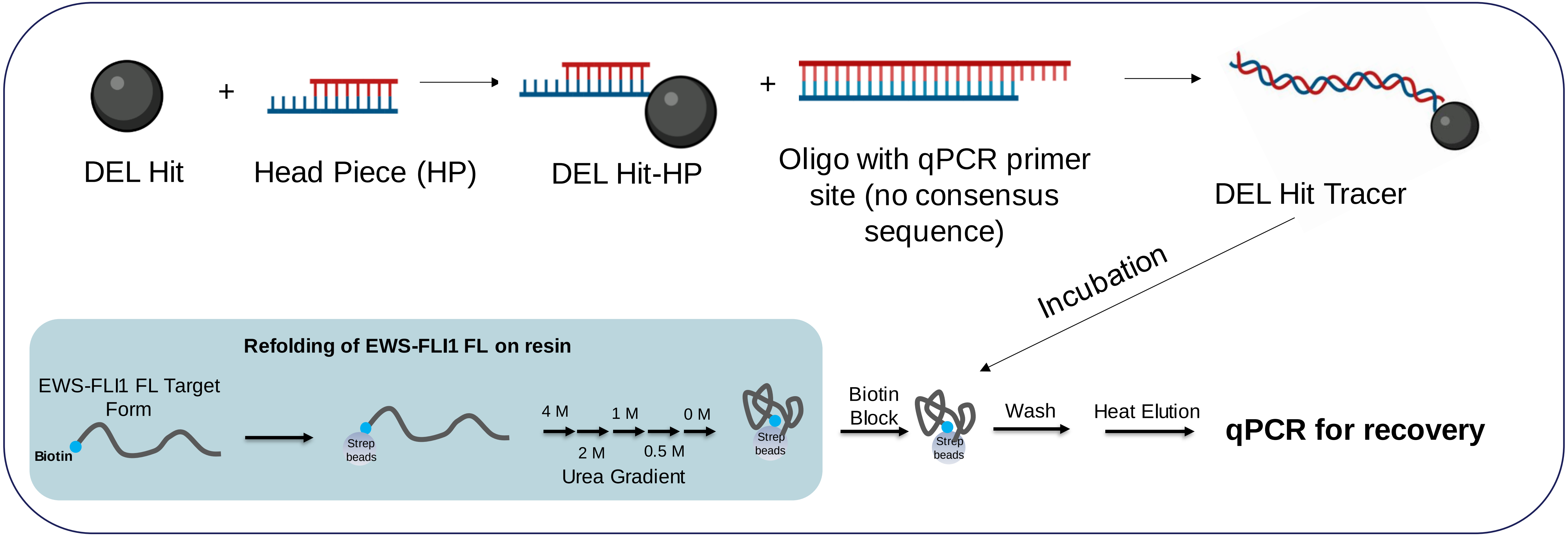
EWS-FLI1 FL



EWS-FLI1 Alphafold Model

→ **Refolding, followed by tracer screen test**

EWS-FLI1 Full Length Fusion Tracer Screen Test Set-Up



DEL Hit

\+

Head Piece (HP)

DEL Hit-HP

\+

Oligo with qPCR primer site (no consensus sequence)

DEL Hit Tracer

Incubation

Refolding of EWS-FLI1 FL on resin

EWS-FLI1 FL Target Form

Biotin

Strep beads

4 M

2 M

1 M

0.5 M

0 M

Urea Gradient

Strep beads

Biotin Block

Strep beads

Wash

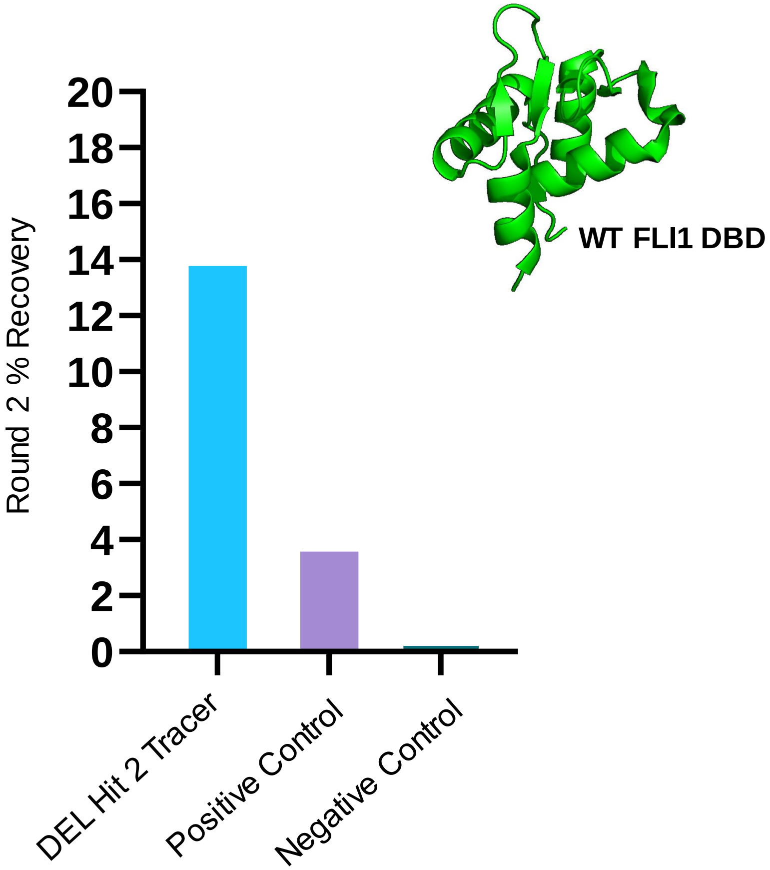
Heat Elution

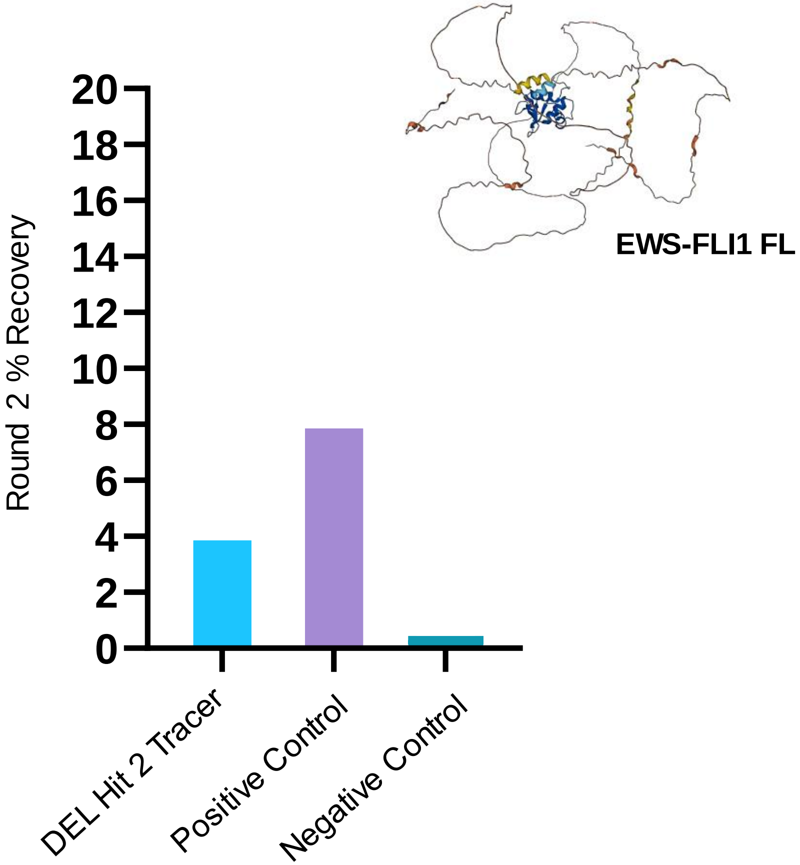
qPCR for recovery

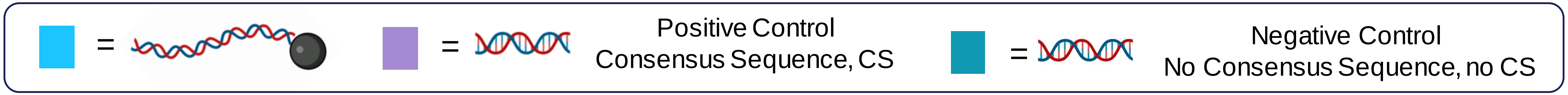
DEL Hit 2 Tracer Binds to EWS-FLI1 Fusion After On-Bead Refolding

Binder recovery
~10% per round

Background recovery
~1% per round







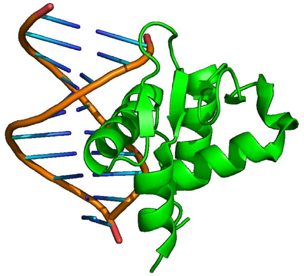
🟦 =

🟪 = Positive Control
Consensus Sequence, CS

🟦 = Negative Control
No Consensus Sequence, no CS

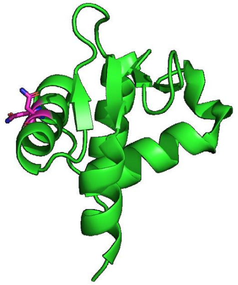
Conclusion : Experimental and Bioinformatic Strategies Enable Identification of Ligands for Transcription Factors

WT FLI1 DBD



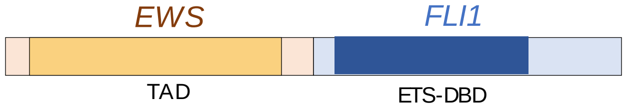
WT FLI1 DBD + DNA blocker screening decreased consensus sequence containing DEL binders

Mutant FLI1 DBD

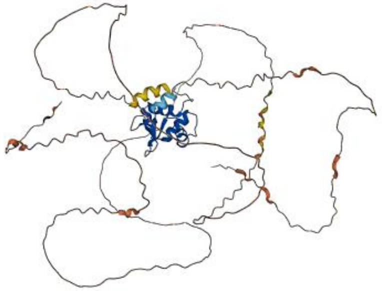


Mutant FLI1-DBD screening provided increased confidence of WT FLI1 DBD hits/features

Screening mutant FLI1-DBD can aid in identifying hits to WT-FLI1 that don’t enrich in WT FLI1 selections



EWS-FLI1 FL



Tracer screen test validated DEL ligand binding to refolded full length EWS-FLI1

Refolded oncogenic fusion protein can be screened using DELs

Thank You