Weill Cornell Medicine

DnD-Seq: A Versatile Platform for High-Resolution Protein-DNA Interaction Mapping in Single Cells

Lead Inventors:

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Background & Unmet Need

- Over 1,600 transcription factors orchestrate gene regulation through complex DNA interactions, which are often disrupted in disease and aging
- Current bulk methods like ChIP-seq and CUT&TAG require stringent conditions that disrupt weaker protein-DNA interactions and mask cellular heterogeneity by providing only population averages
- While techniques like DamID can map protein-DNA interactions genome-wide, they cannot capture the dynamic regulatory networks at single-cell resolution needed to understand cell-type specific responses and disease mechanisms
- **Unmet Need:** Methods that can directly identify and map non-histone protein binding events with single-cell resolution

Technology Overview

- **The Technology:** A sequencing method (DnD-seq) that combines nanobody-directed targeting with controlled DNA deamination to record protein-DNA binding events at single-cell resolution
- The technology utilizes an engineered split DddA enzyme with nanobody targeting enables controlled, protein-specific DNA modification that permanently marks binding sites
- The system remains inactive until deliberately activated, ensuring specific recording of protein-DNA interactions
- **PoC Data:** Demonstrated high specificity with clear identification of transcription factor footprints and concordance with ChIP-seq reference data
- Demonstrated CTCF profiling in primary human CD8 T cells, revealing how IDH2 mutations alter CTCF binding patterns and chromatin organization across different cell types, validated by genotype-specific analysis

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Ivan Raimondi
Wei-Yu Chi

Patents:
Provisional Filed

Publications:
Chi et al. *bioRxiv* [Preprint]. 2025

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Cornell Reference:
D-11042

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DddA: Double-stranded DNA deaminase A
CTCF: CCCTC-binding factor I
DH2: Isocitrate dehydrogenase 2

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Technology Applications

- Enhanced AI/ML cell modeling through high-resolution protein-DNA interaction data
- Optimization of chromatin-targeted therapeutic development
- Improved detection of off-target effects in genetic engineering
- Advanced cellular reprogramming through precise transcription factor mapping

Technology Advantages

- Integration with diverse platforms (PTA, DLP, 10X Multiome) for comprehensive analysis
- Enables simultaneous profiling of protein binding and chromatin accessibility at single-cell resolution
- Reveals cellular heterogeneity previously masked in bulk approaches
- Expandable platform allowing incorporation of additional protein-specific nanobodies

Supporting Data / Figures

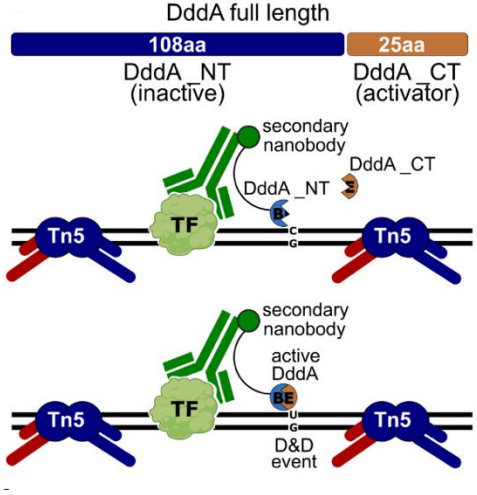

Figure 1: Schematic representation of the DnD-seq split enzyme system. The engineered construct comprises an inactive DddA N-terminal domain fused to a nanobody that recognizes target antibodies, and a separate C-terminal domain. When brought together at antibody-bound transcription factor (TF) sites, the enzyme becomes active.

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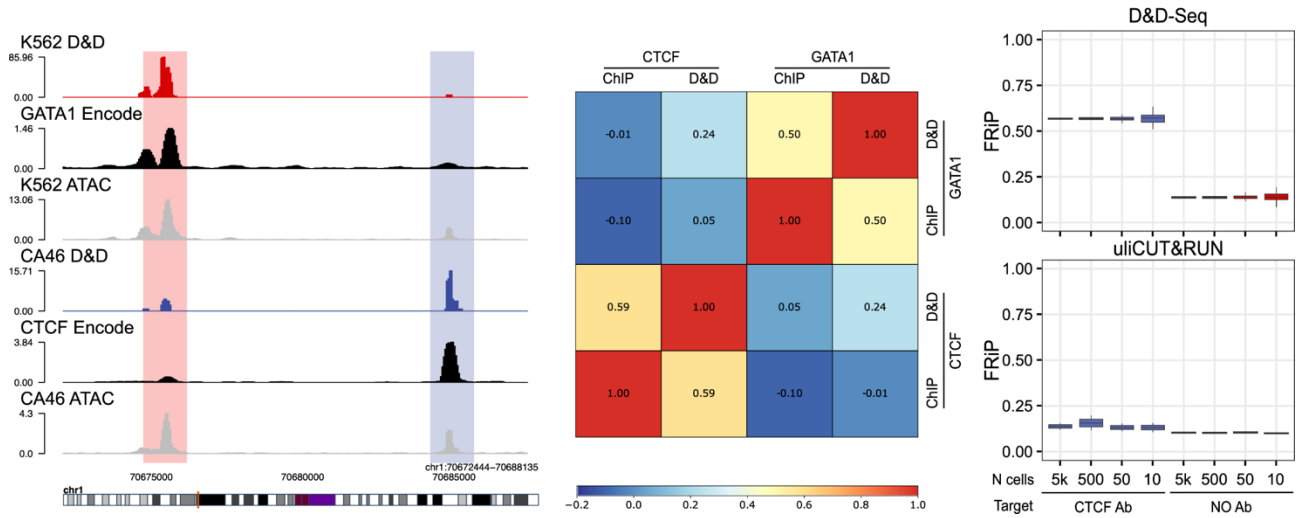

Figure 2: Left: DNA binding locations shown as peaks - red peaks show where GATA1 binds in K562 cells, blue peaks where CTCF binds in CA46 cells, with black lines showing reference data.
Middle: Correlation matrix demonstrating strong matches between D&D-seq and reference data for each protein.
Right: Comparison using Fraction of Reads in Peaks (FRiP) shows D&D-seq achieves 57% of DNA reads in known binding sites versus only 14% for current methods, maintaining this performance even with as few as 10 cells.

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DnD-Seq: Simultaneous Profiling of Transcription Factor Occupancy and Accessible Chromatin

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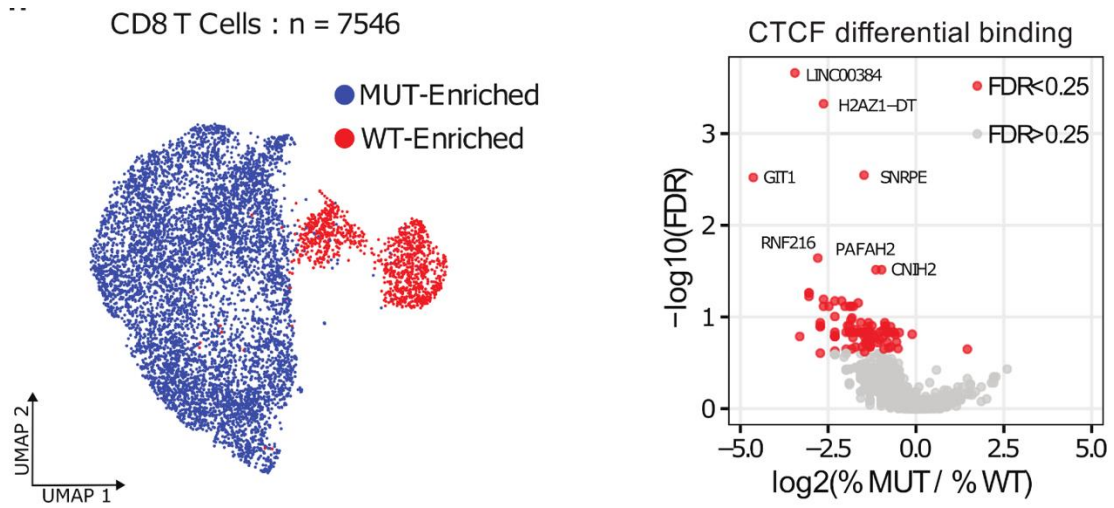

Figure 3: Left: Cell clustering analysis showing clear separation of CD8 T cells into two distinct populations- one enriched for IDH2 wild-type cells and another for IDH2 mutant cells.
Right: Analysis of CTCF binding differences between wild-type and mutant cells. Red dots highlight regions where CTCF binding is significantly different, with most showing decreased binding in mutant cells.

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