# Characterization Of Multiple Myeloma Genomes With LinkPrep™ Assay Enables Detection Of Somatic Variation And SV-driven Interactions Of The 3D Genome

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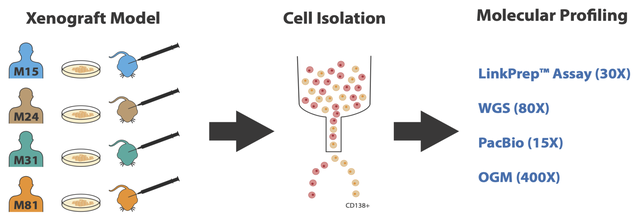
## Introduction

Multiple myeloma (MM) is characterized by its genomic complexity that includes recurrent driver single nucleotide variation (SNVs) alongside a high burden of structural variation (SV). Complete characterization of somatic variation in a MM genome is dependent on a technology that can accurately and sensitively detect both small and large variants. Here, we demonstrate performance of a novel linked-read approach, called LinkPrep™ technology, for high accuracy detection of both small and large somatic variants in four separate relapse MM genomes.

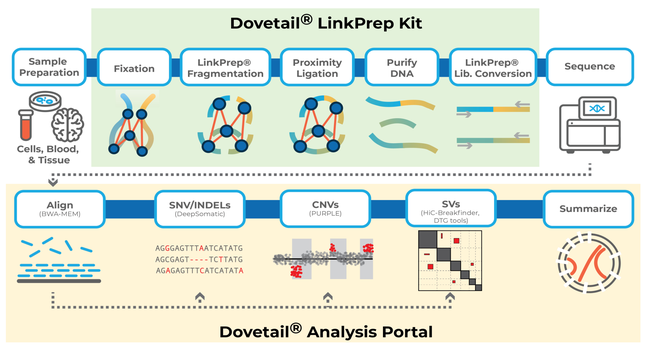
## Methods

Four relapse MM (rMM) patient-derived xenografts were profiled with Dovetail® LinkPrep™ kit[1] and sequenced to ~30X on an Illumina platform. Data was analyzed through the Dovetail Analysis Portal[2] to detect SNVs/Indels (DeepSomatic), CNVs (Purple), and SVs (HiC-breakfinder and proprietary tools). Call sets were compared against other genomics technologies including WGS (80X tumor/ 30X normal), PacBio (15X), and OGM (400X).

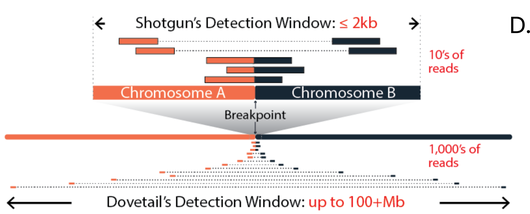
A.




B.




C.

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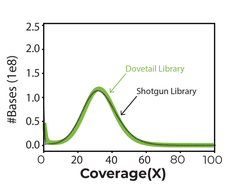
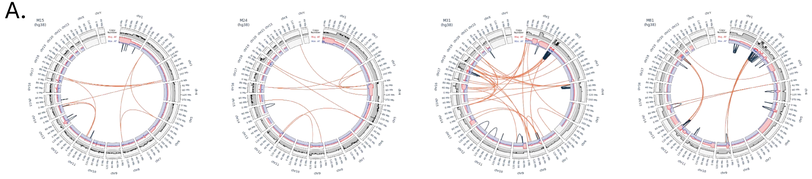



**Figure 1. A. Experiment overview.** 4 patient-derived xenograft rMM patient samples (M15, M24, M31, M81) were profiled with 4 molecular technologies. **B. Dovetail LinkPrep and the Dovetail Variant Analysis workflow.** The Dovetail LinkPrep kit supports fresh frozen samples and generates linked-read libraries compatible with short read sequencers. LinkPrep data analysis was supported by the Dovetail Analysis Portal, variant workflow to generate SNV/indel, CNV, and SV call sets. **C. Dovetail linked-reads expand the SV detection window to enable high sensitivity SV detection.** Shotgun's detection window is limited to reads aligning within 2 kb of the breakpoint resulting in low sensitivity SV detection. Dovetail's linked-reads utilize both breakpoint spanning and breakpoint flanking reads to expand the SV detection window over 100 Mb. **D. Dovetail linked-reads have shotgun-like, uniform coverage distribution.** Uniform coverage enables high quality SNV/Indel and CNV detection with standard short-read compatible tools.

## Results

### LinkPrep Key Findings - Cohort Overview

A.



B.

| M15 | M24 | M31 | M81 |
|---|---|---|---|
| •t(11;14) *IGH/CCND1*<br>•t(6;8) *MYC* re-arr<br>•*TP53* biallelic disruption<br>•*NRAS* p.G12A (96%)<br>•del(13q) - *RB1* loss<br>•del(6q) *PRDM1, BLIMP1* loss | • inv(14)(q24;32) *IGH/RAD51B*<br>• t(6;12)(p25;p13) *IRF4* dysregulation<br>• *NRAS* p.G12D (100%)<br>• *TP53* p.A138P (100%)<br>• del(13q) - *RB1* loss<br>• del(17) - *TP53* loss<br>• *ELL* biallelic inactivation (t(6;19) and p.E105*) | • t(4;14) *IGH/NSD2*<br>• *KRAS* p.G12V (26%)<br>• *KRAS* p.G12F (18%)<br>• *NRAS* p.G13V (50%)<br>• del(13q) - *RB1* loss<br>• del(17) - *TP53* loss<br>• Chr2 chromothripsis | •t(4;14) *IGH/NSD2; IGH/FGFR3*<br>•t(12;20) *MAFB* re-arr<br>•*FGFR3* p.N262I (87%), p.S348F (30%)<br>•del(13q) - *RB1*, *DIS3* loss<br>•Chr2 chromothripsis |

**Figure 2. A. Circos plot overview of 4 MM samples (derived from 30X LinkPrep data only).** Copy number track (grey), B-allele frequency track (blue/pink), inter-chromosomal SVs (orange inner circle) and intra-chromosomal SVs (black inner circle) are shown for each of the 4 samples. **B. Key genomic alterations detected in 30X LinkPrep.** Distinct IGH rearrangements are detected in each sample along with copy number and SNV alterations consistent with myeloma genomes.

### LinkPrep Detects Structural Rearrangements With High Sensitivity

| Name | Break pt 1 (Strand) | Break pt 2 (Strand) | SV Type | SV-VAF | 30X T-only LinkPrep Support | 80X T/N Shotgun Support | 15X PacBio Support | 400X OGM |
|---|---|---|---|---|---|---|---|---|
| inv(14)(q24;q32) | chr14:68,792,091 (-) | chr14:105,863,768 (-) | inversion | 64% | 617 | 10 | - | Match |
| inv(14)(q24;q32)* | chr14:68,791,968 (+) | chr14:105,863,768 (+) | inversion | 51% | 410 | 8 | - | - |
| t(6;19)(p24;p13) | chr6:7,746,966 (+) | chr19:18,494,763 (-) | tloc unbalanced | 26% | 774 | 15 | - | Match |
| t(6;12)(p25;p13)** | chr6:217,321 (-) | chr12:4,188,673 (-) | tloc unbalanced | 87% | 2,993 | 25 | 7 | Match |
| t(2;4)(q36;q28) | *chr2:226,850,000 (+)* | *chr4:131,870,000 (+)* | tloc unbalanced | 10% | 73 | 8 | 11 | Match |
| t(3;16)(p21;q22) | chr3:48,330,320 (-) | chr16:67,881,818 (-) | tloc unbalanced | 30% | 648 | 11 | - | Match |
| t(3;4)(p21;p15) | chr3:48,431,204 (+) | chr4:12,635,126 (-) | tloc unbalanced | 43% | 736 | 20 | - | Match |
| t(9;15)(p23;q12) | chr9:11,349,359 (-) | chr15:25,704,525 (-) | tloc balanced | 38% | 388 | 20 | - | Match |
| t(9;15)(p23;q12) | chr9:11,349,314 (+) | chr15:25,704,491 (+) | tloc balanced | 37% | 373 | 11 | 9 | Match |
| t(9;12)(q33;p12) | chr9:117,382,596 (-) | chr12:16,720,009 (-) | tloc unbalanced | 35% | 380 | 14 | 8 | Match |
| t(4;16)(q22;p15) | chr4:12,788,908 (+) | chr16:68,086,162 (+) | tloc unbalanced | 29% | 439 | 15 | 4 | Match |
| t(4;19)(p15;q13) | chr4:14,455,422 (+) | chr19:39,919,241 (+) | tloc unbalanced | 37% | 529 | 3 | 8 | Match |
| t(4;X)(q12;q25) | *chr4:58,000,000 (+)* | *chrX:125,900,000 (-)* | tloc unbalanced | 13% | 101 | 5 | - | Match |
| t(6;21)(q25;q22) | *chr6: 157,000,000 (+)* | *chr21:37,000,000 (-)* | tloc unbalanced | 1% | 16 | - | - | - |

* IGH enhancer hijacking ** IRF4 dysregulation

**Table 1. Comparison of LinkPrep SV callset to other technologies (M24).** Large SVs detected with 30X tumor-only LinkPrep data were compared against 80X tumor/normal shotgun (Manta), 15X tumor-only PacBio (pbsv), and 400X OGM call sets. Breakpoints were identified using LinkPrep + Dovetail Analysis Portal, achieving base-pair resolution except for the three low-VAF calls (italicized), which are reported at lower resolution. LinkPrep call support is 10x-100x higher than orthogonal technologies.

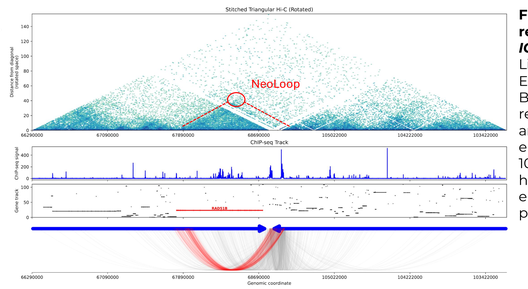
### LinkPrep Variant Calls Are Concordant With 80X T/N Shotgun

| Alteration | Truth VAF (Alt / Total) 80X T/N Shotgun | Test VAF (Alt / Total) 30X T-only LinkPrep |
|---|---|---|
| *NRAS*:p.Gly12Asp | **100.0%** (56 / 56) | **100.0%** (35 / 35) |
| *TP53*:p.Ala138Pro | **100.0%** (37 / 37) | **93.3%** (14 / 15) |

**Table 2. Comparison of LinkPrep variant calls to high coverage, T/N shotgun (M24).** 30X tumor-only LinkPrep SNV/Indels calls (DeepSomatic) were compared against 80X tumor/normal shotgun (Strelka) call set, defined as "truth". Overall, 30X tumor-only LinkPrep data recalled 94.2% of the shotgun truth set variant calls. Further, key pathogenic variants *NRAS* and *TP53* were detected with concordant VAF between the two datasets.

### LinkPrep Elucidates Enhancer Hijacking Targets

A.

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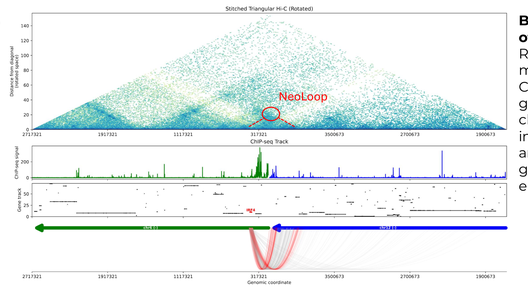


**Figure 3. A. Neo-chromosome reconstruction of inv(14) involving *IGH*.** Top panel: Reconstructed LinkPrep data Hi-C map. Middle panel, ENCODE H3K27ac ChIP-seq data[3]. Bottom panel: hg38 gene track of reconstructed chromosomes. Grey arcs indicate all interactions from IGH enhancer elements "bait" (chr14: 105,557,659-105,705,000). Red arcs highlight interactions from IGH enhancer elements "bait" to RAD51B promoter target (chr14q24).

**B. Neo-chromosome reconstruction of t(6;12) involving *IRF4*.** Top panel: Reconstructed LinkPrep data Hi-C map. Middle panel, ENCODE H3K27ac ChIP-seq data[3]. Bottom panel: hg38 gene track of reconstructed chromosomes. Grey arcs indicate all interactions from IRF4 gene "bait". Red arcs highlight interactions from IRF4 gene "bait" to chr12 regulatory enhancer targets.

## Summary

Dovetail® LinkPrep™ technology is a linked-read NGS assay with comprehensive genome coverage. LinkPrep + Dovetail Analysis Portal deliver comprehensive genomic variant characterization with a single sequencing approach.

LinkPrep linked-reads enable high sensitivity SV detection by expanding the detection window over standard short reads.

LinkPrep detected key SV, SNV and CNV drivers with high concordance to orthogonal methods in 4 patient-derived xenograft relapse multiple myeloma samples. Importantly, even with a 30X tumor-only dataset, the LinkPrep SV calls had 10-100x more read support than orthogonal technologies.

LinkPrep chemistry is performed *in situ* (rather than extracted DNA) allowing it to additionally capture Hi-C-like information about 3D genome folding and enhancer-promoter regulatory interactions.

Neo-chromosome reconstructions of SVs involving IGH enhancer and IRF4 help elucidate regulatory target interactions.

## References

1. Dovetail LinkPrep technology. https://cantatabio.com/linkprep
2. Dovetail Analysis Portal. https://portal.cantatabio.com
3. The ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. Nature 489, 57–74 (2012).

Learn more about Dovetail Genomics' solutions.