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Next Generation Technology for Identification of Genomic Alterations in MPNs

Presented by Terra Lasho

Mayo Clinic, Rochester, MN, USA

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Advanced Genomics Technology Center

Genotyping Shared Resource Vision

To become a **leader** in genomic analyses for Mayo investigators and medical **researchers** around the world providing the highest **quality** data in order to enable enhanced understanding of human disease, improve health, and promote **excellence** in patient care

Genotyping Shared Resource Mission

Providing **centralized** genomic analyses services to Mayo investigators and collaborators around the world. Offering individualized customer **service**, including study design consultation and result interpretation. We strive for the highest quality data in the most **efficient** manner.

- Whole-Genome
- Gene Association
- Copy Number Variation
- Focused and Custom Analyses
- Repeat and Single Nucleotide Polymorphism's
- Methylation Analyses
- Linkage Analyses
- Array CGH

Next Generation Technology represents a quantum advance in the ability to understand cancer genetics

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Use of Mate Pair Library Sequencing to Identify a Novel Recurrent Translocation in T-cell Lymphomas

Andrew L. Feldman, M.D.

Brief report

Discovery of recurrent t(6;7)(p25.3;q32.3) translocations in ALK-negative anaplastic large cell lymphomas by massively parallel genomic sequencing

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The genetics of peripheral T-cell lymphomas are poorly understood. The most well-characterized abnormalities are translocations involving *ALK*, occurring in approximately half of anaplastic large cell lymphomas (ALCLs). To gain insight into the genetics of ALCLs lacking *ALK* translocations, we combined mate-pair DNA library construction, massively parallel ("Next Generation") sequencing, and a novel bioinformatic algorithm. We identified a balanced translocation disrupting the *DUSP22* phosphatase gene on 6p25.3 and adjoining the *FRA7H* fragile site on 7q32.3 in a systemic ALK-negative ALCL. Using fluorescence in situ hybridization, we demonstrated that the t(6;7)(p25.3;q32.3) was recurrent in ALK-negative ALCLs. Furthermore, t(6;7)(p25.3;q32.3) was associated with down-regulation of *DUSP22* and up-regulation of *MIR29* microRNAs on 7q32.3. These findings represent the first recurrent translocation reported in ALK-negative ALCL and highlight the utility of massively parallel genomic sequencing to discover novel translocations in lymphoma and other cancers. (*Blood*. 2011;117(3):915-919)

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Mate Pairs

- Fragment DNA (5kb)
- End Repair/ Label
- Gel for size (5kb)
- Circularize
- Digest Linear DNA
- Fragment circularized DNA (500bp)
- Purify Biotinylated DNA
- End Repair/ligate adaptors
- Final Size selection (350-600bp)
- Bridge amplification

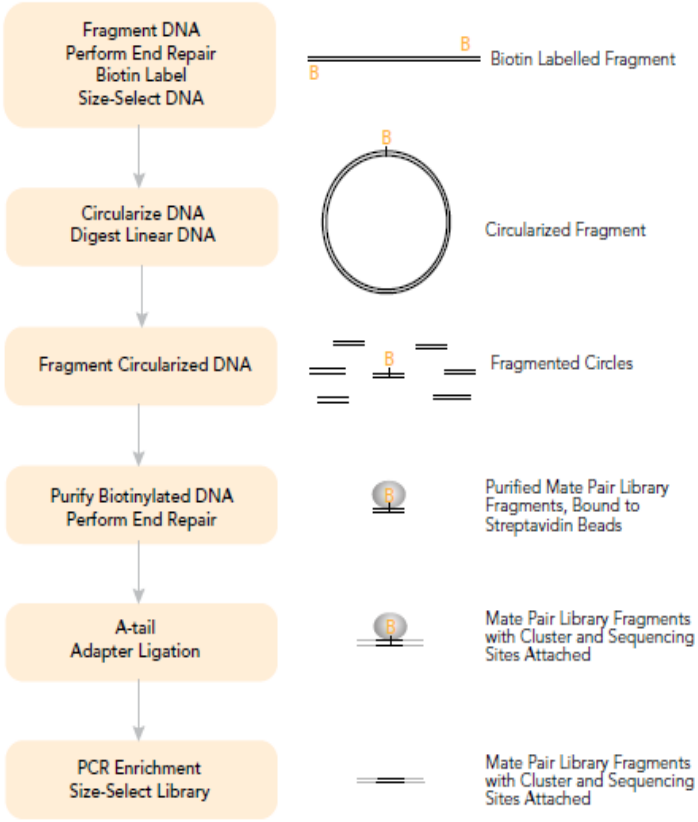
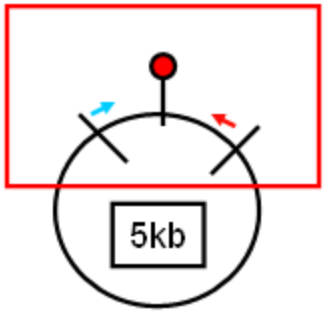
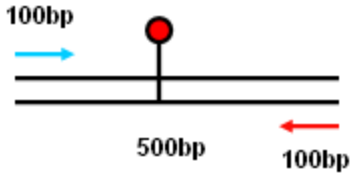


Figure 1 *Mate Pair Library Preparation Overview*

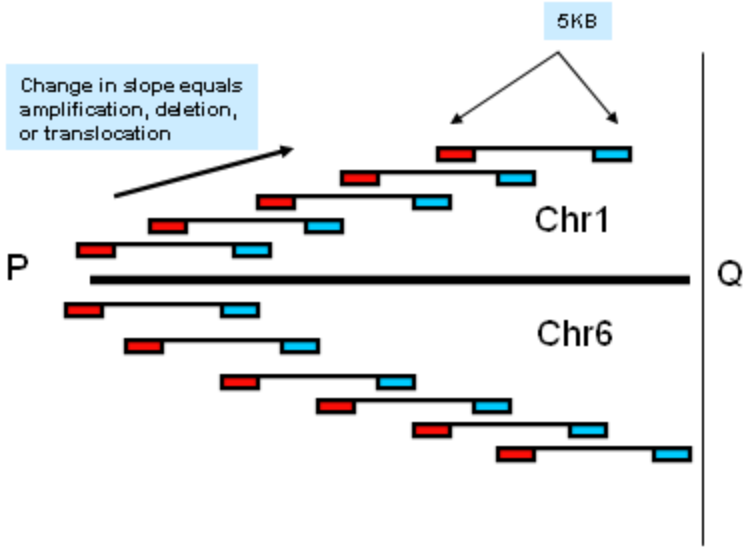


5kb fragment is circularized to capture 5kb in 300bp



300bp fragment contains two ends that are read in both directions (100bp)

Genomic DNA is cut into 5kb fragments and end labeled with biotin



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Algorithm

32bp fragments

Address

AAATTGTCGTAGTCGATGCTAGTCG…. → 00000101100101101010

→ Steps through Genome

AAAATTGTCGTAGTCGATGCTAGTCG…. → 00000101110111011010

A - 00
T – 01
C – 10
G - 11

Binary code:

Index table construction: AGTA to code (0010) to address in index (1718215) to genome position (1)

000000000000000	1
000000000000001	2
111111111111111	$3x10^9$

This is not a number index, but an address to a location per the reference Genome

George Vasmatzis, Ph.D.

Sarah H. Johnson

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Table of putative translocations

16	AATTTG	TCAAAC	8	11	53	-39	52730159	-38809361	8q11.23a	11p12c
20	GGAATC	TTAGCA	8	11	53	-39	52731338	-38809506	8q11.23a	11p12c
20	TATGTT	AAAATG	8	11	53	-39	52731096	-38809651	8q11.23a	11p12c
21	CATTCT	TTATCT	8	11	53	-39	52730285	-38809642	8q11.23a	11p12c
37	TATATC	ACAATA	8	11	53	-39	52731324	-38810513	8q11.23a	11p12c
17	CAGATC	ACTTTT	8	11	53	-39	52730533	-38808643	8q11.23a	11p12c

Human BLAT Results

BLAT Search Results

ACTIONS	QUERY	SCORE	START	END	QSIZE	IDENTITY	CHRO	STRAND	START	END	SPAN
browser details	YourSeq	100	1	100	200	100.0%	11	-	38809390	38809489	100
browser details	YourSeq	99	101	200	200	100.0%	8	+	52730224	52730323	100
browser details	YourSeq	29	133	173	200	96.8%	3	+	107608969	107609281	313
browser details	YourSeq	26	157	200	200	67.9%	X	-	50157571	50157601	31
browser details	YourSeq	26	132	173	200	78.6%	1	+	75234446	75234483	38
browser details	YourSeq	22	156	178	200	100.0%	7	-	71904460	71904486	27
browser details	YourSeq	21	151	172	200	100.0%	2	-	53132815	53132837	23
browser details	YourSeq	20	125	144	200	100.0%	4	+	31617668	31617687	20

AATGCGATCGACCGGTGTGTACAGG----------GTACATGTACAGATAGACACAGTGGGG

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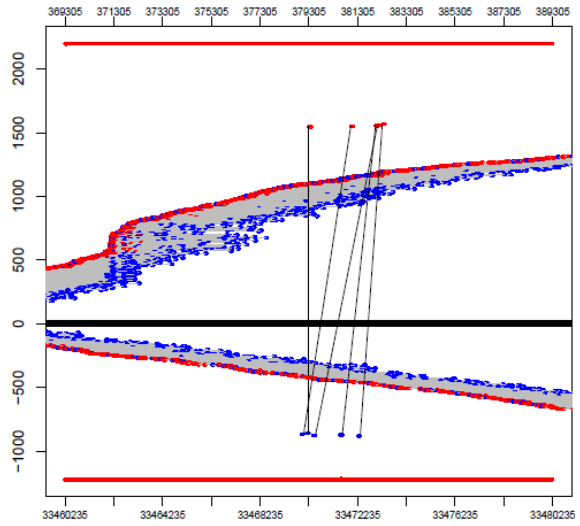
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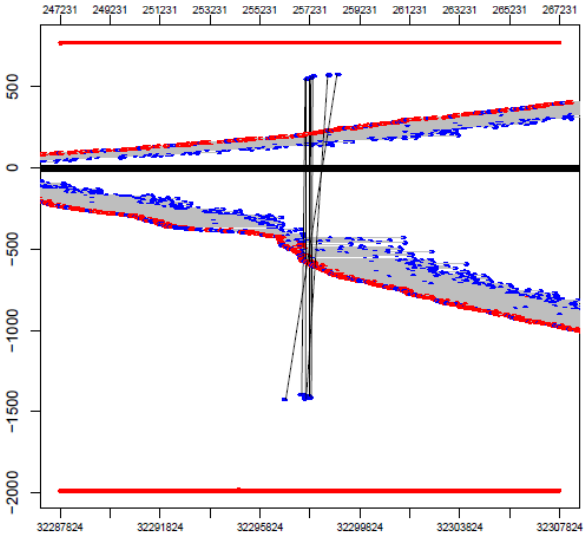
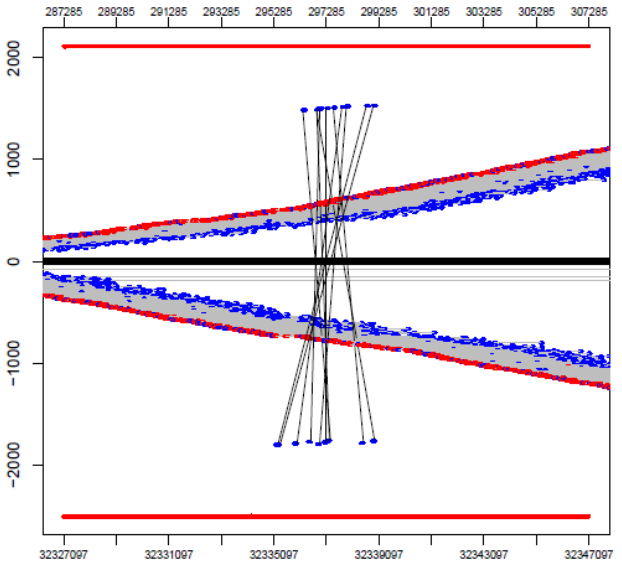
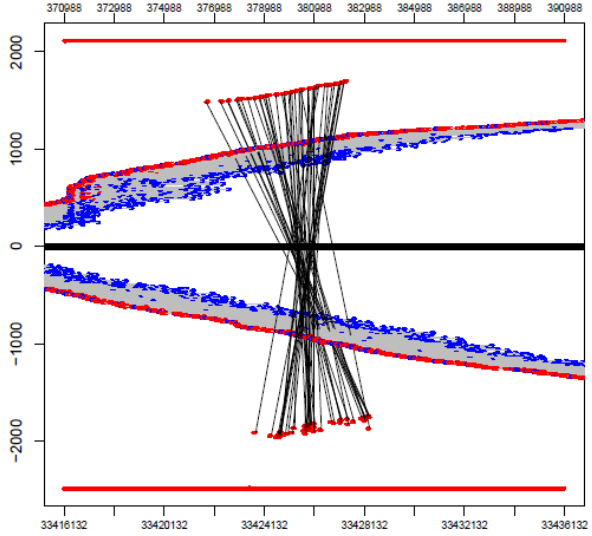
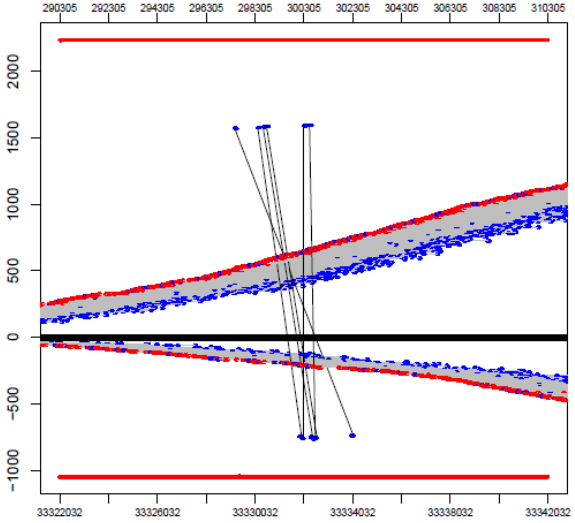
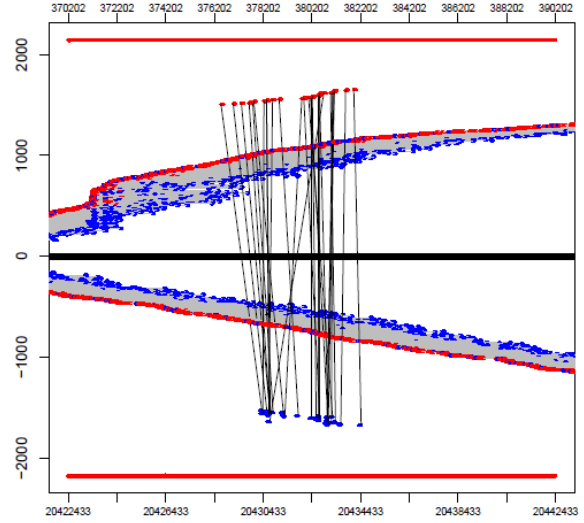
2000 1500 1000 500 0 −500 −1000
33460235 33464235 33468235 33472235 33476235 33480235


247231 249231 251231 253231 255231 257231 259231 261231 263231 265231 267231
500 0 −500 −1000 −1500 −2000
32287824 32291824 32295824 32299824 32303824 32307824


287285 289285 291285 293285 295285 297285 299285 301285 303285 305285 307285
2000 1000 0 −1000 −2000
32327097 32331097 32335097 32339097 32343097 32347097


370988 372988 374988 376988 378988 380988 382988 384988 386988 388988 390988
2000 1000 0 −1000 −2000
33416132 33420132 33424132 33428132 33432132 33436132


290305 292305 294305 296305 298305 300305 302305 304305 306305 308305 310305
2000 1500 1000 500 0 −500 −1000
33322032 33326032 33330032 33334032 33338032 33342032


370202 372202 374202 376202 378202 380202 382202 384202 386202 388202 390202
2000 1000 0 −1000 −2000
20422433 20426433 20430433 20434433 20438433 20442433

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Immediate Objectives

- **Interrogate cytogenetically apparent translocations in order to define precise breakpoints and involved genetic regions.**
- **Identify cytogenetically occult novel genetic translocations that are recurrent in MPN and characterize them further.**

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der(6)t(1;6)(q21–23;p21.3)

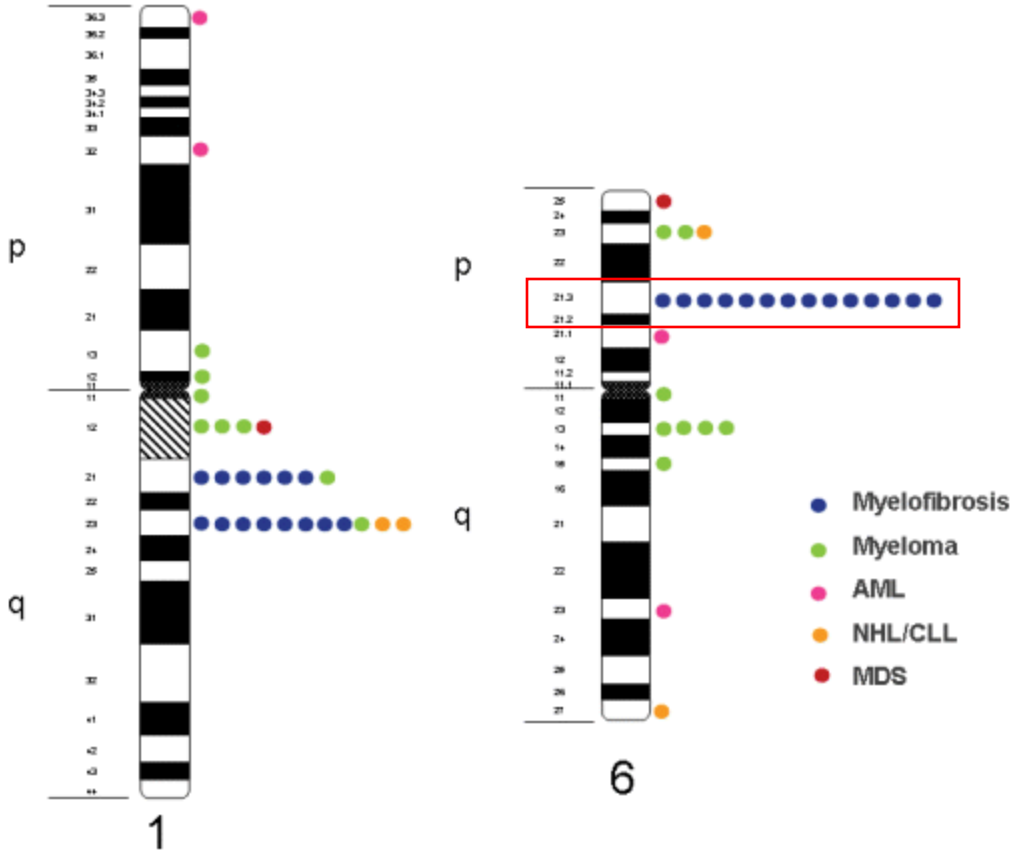

Figure 1. Ideogram showing breakpoints on both chromosomes matched with respective diagnosis. All patients with MMM had the same breakpoint on 6p21.3 and clustering of breakpoints on 1q(21–23). None of the remaining 13 patients with t(1;6) had the same breakpoints, which makes this chromosome anomaly specific for MMM. AML, acute myeloid leukaemia; NHL, non-Hodgkins lymphoma; CLL, chronic lymphocytic leukaemia; MDS, myelodysplastic syndrome.

17,791 pts tested (cyto reports)

-25 pts had translocations involving Chr 1 and Chr 6
-12 pts had same breakpoint and were all PMF (1 PPMM,1PTMM)

 British Journal of Haematology, **130**, 229–232

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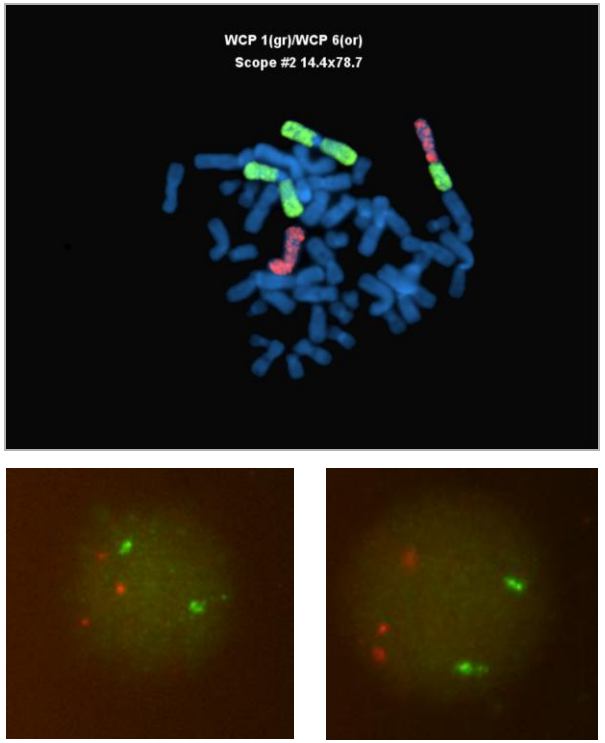
Der(6)t(1;6)(q21-23;p21.3): the most specific chromosomal translocation in myelofibrosis with myeloid metaplasia.

p arm of 6 is replaced with q arm of chromosome 1



In situ fluorescence showing different Chromosome 6 breakpoints

http://www.cydas.org/OnlineAnalysis/

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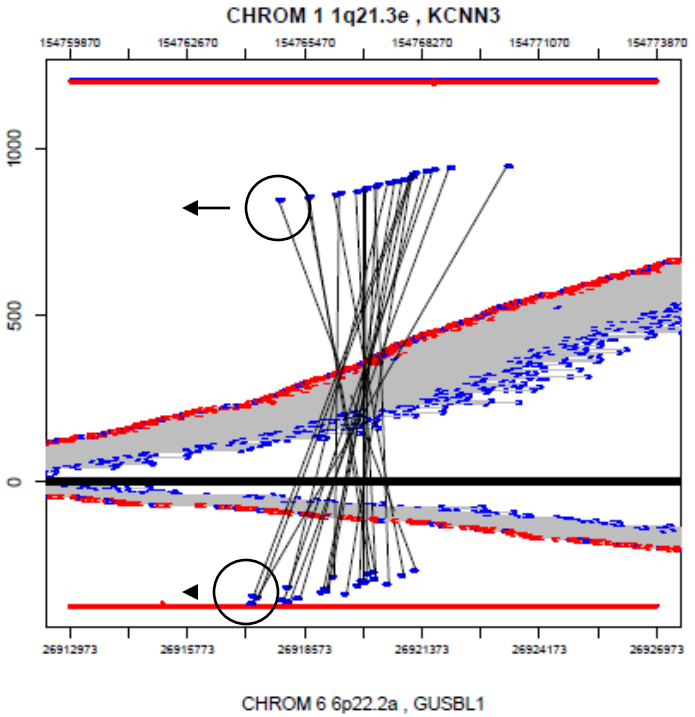
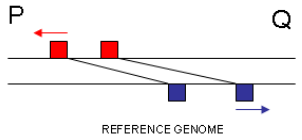
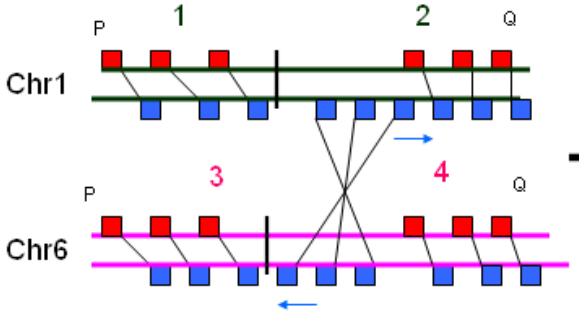
Case #1: 46,XY,der(6)t(1;6)(q21;p21.3),del(20)(q11.2)

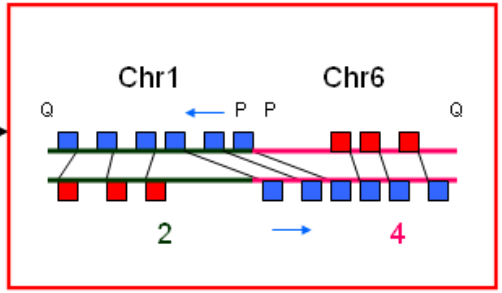


t(1;6) associates

	read1	read2	chrm 1 position	chrm 6 position
1	GAGCAGTGATGTAAACAGAGGAATATATTG	AGCCATGCCACTGGGTATTAGGCTAGCCACTG	154768021	26917362
2	ACACAAGAATTACTCATACATAAAAATCCTG	TGAGGCTCCGTGGGGGCACTGAAAGTTGGTGG	154768086	26917482
3	CTGGGTGGGAGAGCATCCGTCATTCCATCA	ATTCTAACATTGACCATATGCTTGGTCATAAAG	154766292	26919285
4	CAGCAAACCCAGCTAGGTACTATAAAAGGT	CTATACTTAGTTACTGAGACAGGCATAAATTTA	154767450	26918208
5	CTATTCCAGAAAAGTTGAAGAGAACGTACT	AAATCATGTGCCAAGTGAGGTGTCAGACATGCA	154765561	26920144
6	CAGGTCAATCAGAAAGAACGGGCTATTGAA	AGGTGACCACTGTTGATGAACATAGACTCAAAA	154765521	26920251
7	AGAAGATGTTTACAGGACTTTCTGCAACCA	CGGCTTGAGGCCAGGAGCTCAAGCCTGCAGTG	154764839	26921232
8	ATCTACGACTCATTGTCATTCCTAGAAGAG	TGTTGGGGGATGAGTGGAGGTGGCGAATACTG	**154768530**	**26918077**
9	TGGCTAACCAAAAACTGAAGGTCTGGGATT	ATTGTAGGAGACTTCAACACCCCCGCTGGCAG	154767618	26919109
10	GGAGTCTAGACTGTATTCTAAGGACAAGGA	TAAACTAAACTTCCTAAGTGAAGAAGAAACAAA	154768348	26918462
11	ATCTTCCAAGATTGAATCAGGAAGATACTTA	TGCAAGATCAGTCTTGCTTGTGAAGAACTGAGT	154766870	26919973
12	GCACTGCATCATCAAAGCAGACAACTAAGA	CACCTCTGCAGTGGTAGTGTCGAGTCCCCCACC	154767794	26919140
13	TTGAGGACAGAGACCATGTCTGTTTCTATG	GACTCAAAAATTCTCAACAAAGTACTAGCAAAC	154766689	26920275
14	TACTTAAGTACCCTAAATATAAATGCACCCA	TGCTGTATAAGGCATGGGGACTCAAAGAGGCA	154767955	26919010
15	TACCAAGCCAAAAAGCCCTGGACTATATGG	TCAGGGACCCTCTGAGAGGAGAAAAAACTTGC	154766915	26920057
16	TGAGCCCATCTAGAAAGAATGCTGGGATCG	CAGACATATAAAATAATCCTCAGAGAATACTAG	154767193	26919876
17	GTTAACGTAGTTGACTAAGCTGACTTAGTC	AAGAACACAATCATATTTACAACACCTAGGATG	154766168	26920941
18	AACCCAGCTAGGTACTATAAAAGGTGACTA	TAGATATCATTTTTTATTGGGTAAAAAAAAAATT	154768916	26918213
19	TAAATGCCTTTATCATAAAGTTAGAAATATT	GTAAATGAGCAGTGATGTAAACAGAGGAATATA	154768015	26919574
20	TCTAGGCCTCAGTTTCCTCATCTGTTCAATA	AGGAAGTATAAAACAGCCATGCCACTGGGTATT	154770292	26917348







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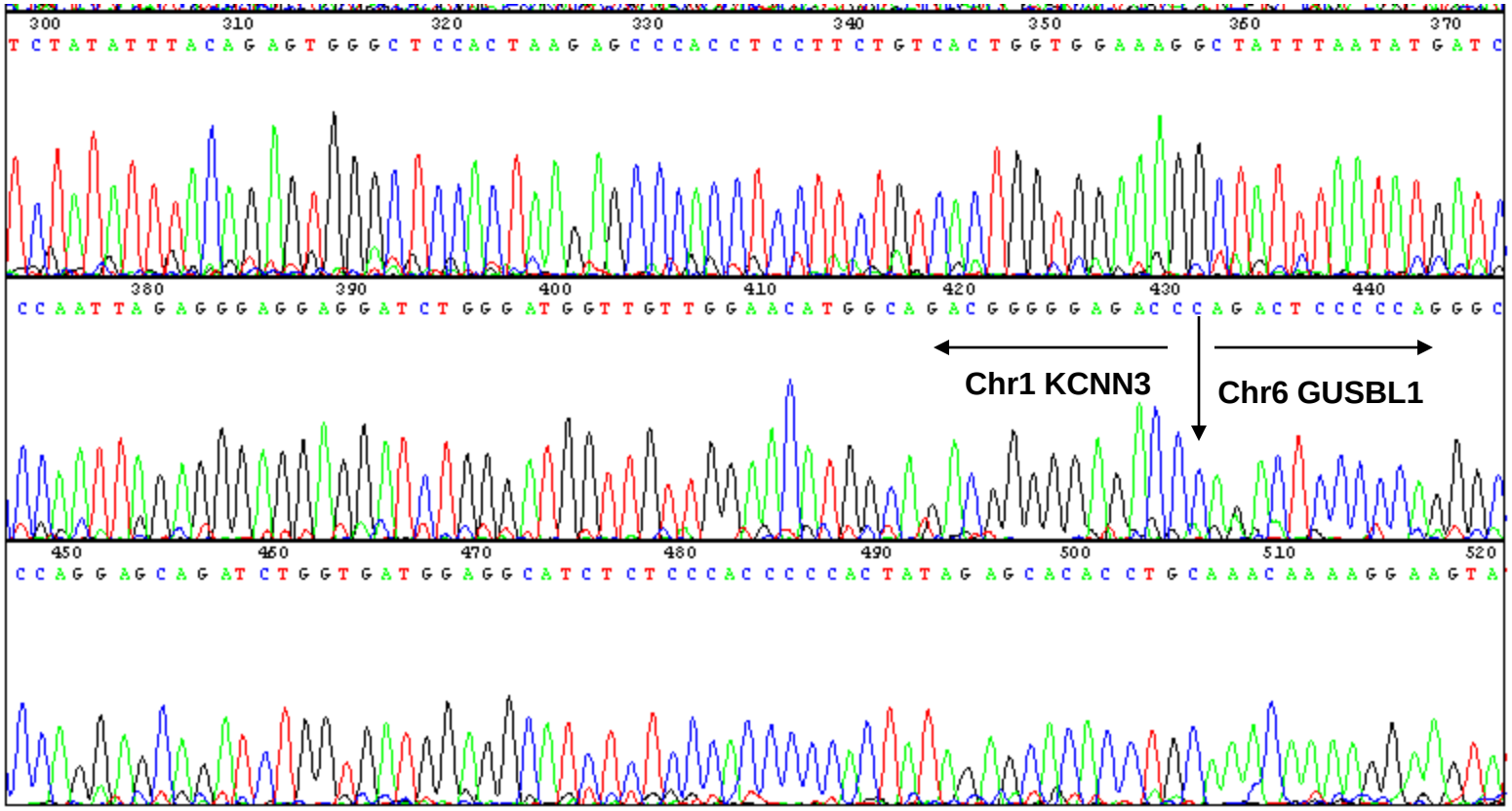
Case #1: 46,XY,der(6)t(1;6)(q21;p21.3),del(20)(q11.2)



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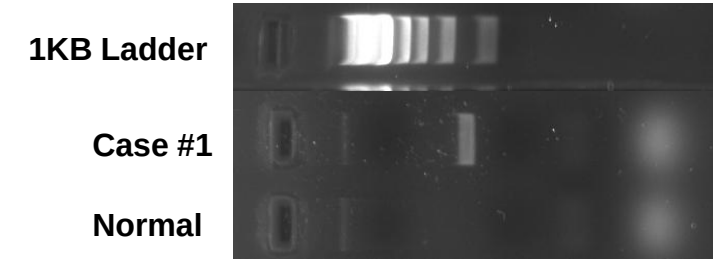

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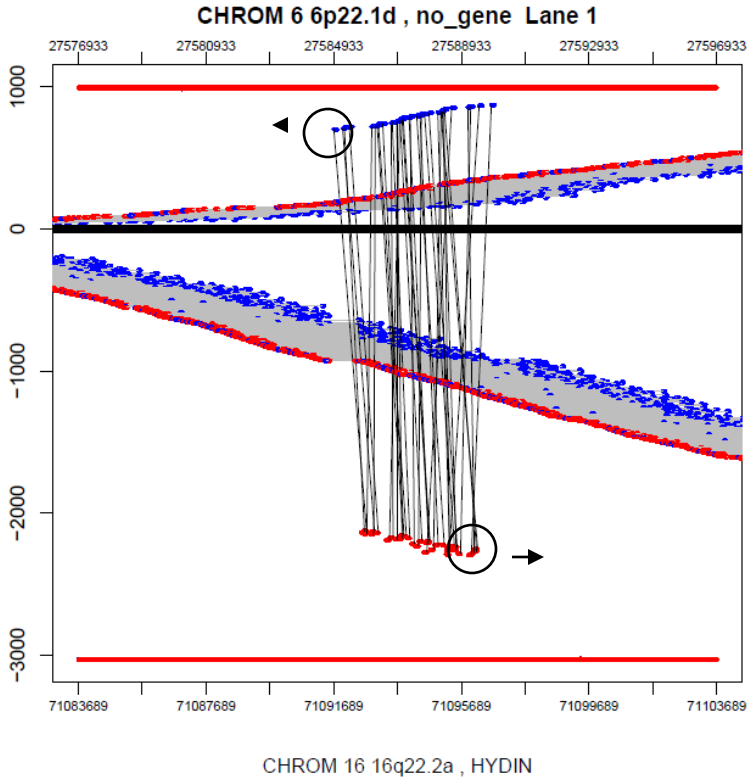
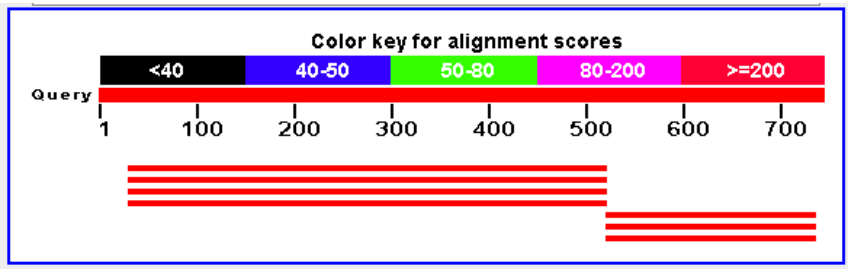
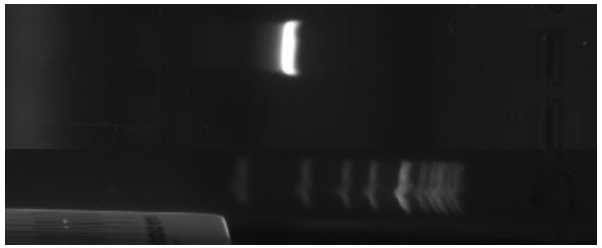
Case #2: 46,XX,der(6)t(1;6)(q23;p21.3)





Accession	Description
NT_167207.1	Homo sapiens chromosome 1 unlocalized genomic contig, GRCh37.p2
NW_927421.1	Homo sapiens unplaced genomic contig, alternate assembly Hs_Celer
NT_010498.15	Homo sapiens chromosome 16 genomic contig, GRCh37.p2 reference
NW_001838320.2	Homo sapiens chromosome 16 genomic contig, alternate assembly Hu
NT_007592.15	Homo sapiens chromosome 6 genomic contig, GRCh37.p2 reference p
NW_001838980.1	Homo sapiens chromosome 6 genomic contig, alternate assembly Hur
NW_923073.1	Homo sapiens chromosome 6 genomic contig, alternate assembly Hs_

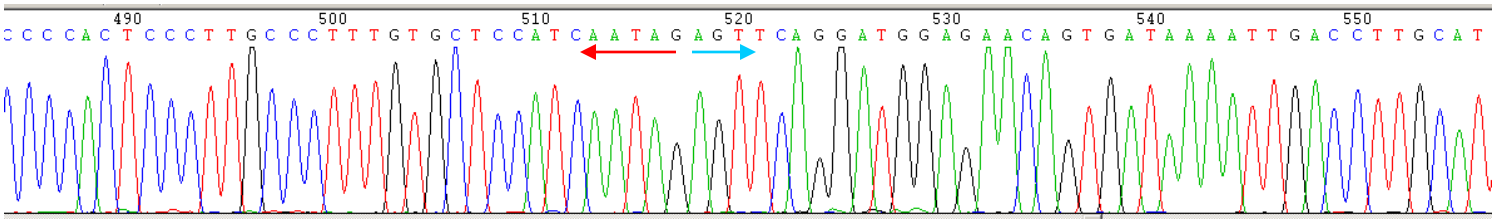


Case #2

Normal

1KB Ladder

unplaced **Chr1** **Chr6** 27,585,000



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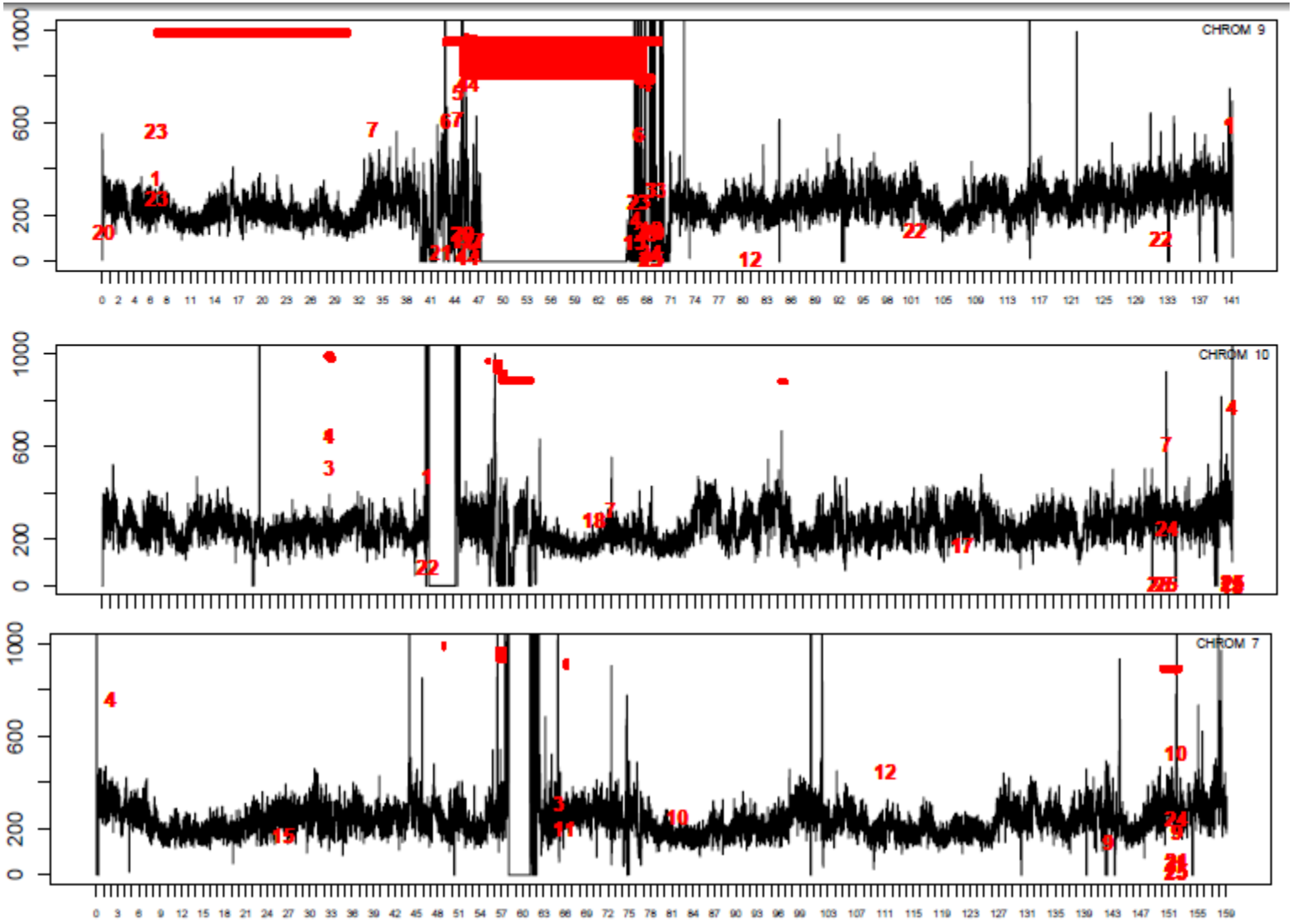
2nd Objective….



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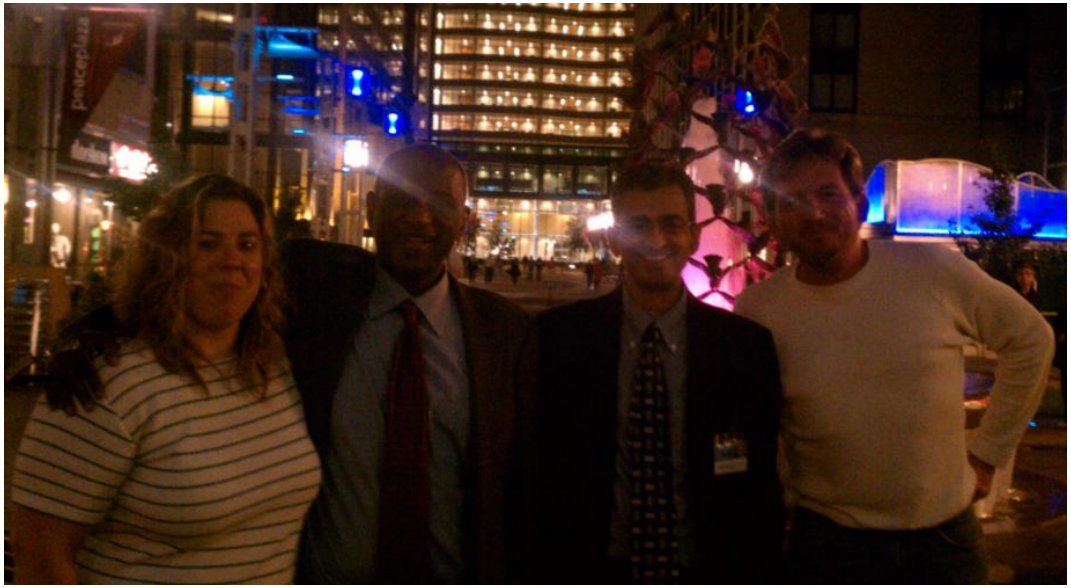
Tefferi Laboratory

- Ayalew Tefferi
- Animesh Pardanani
- Terra Lasho
- Christy Finke
- Steven Zincke



Bioinformatics

- George Vasmatzsis
- Sarah Johnson