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Detection and Interpretation of Partial Tandem Duplication in the KMT2A/MLL gene

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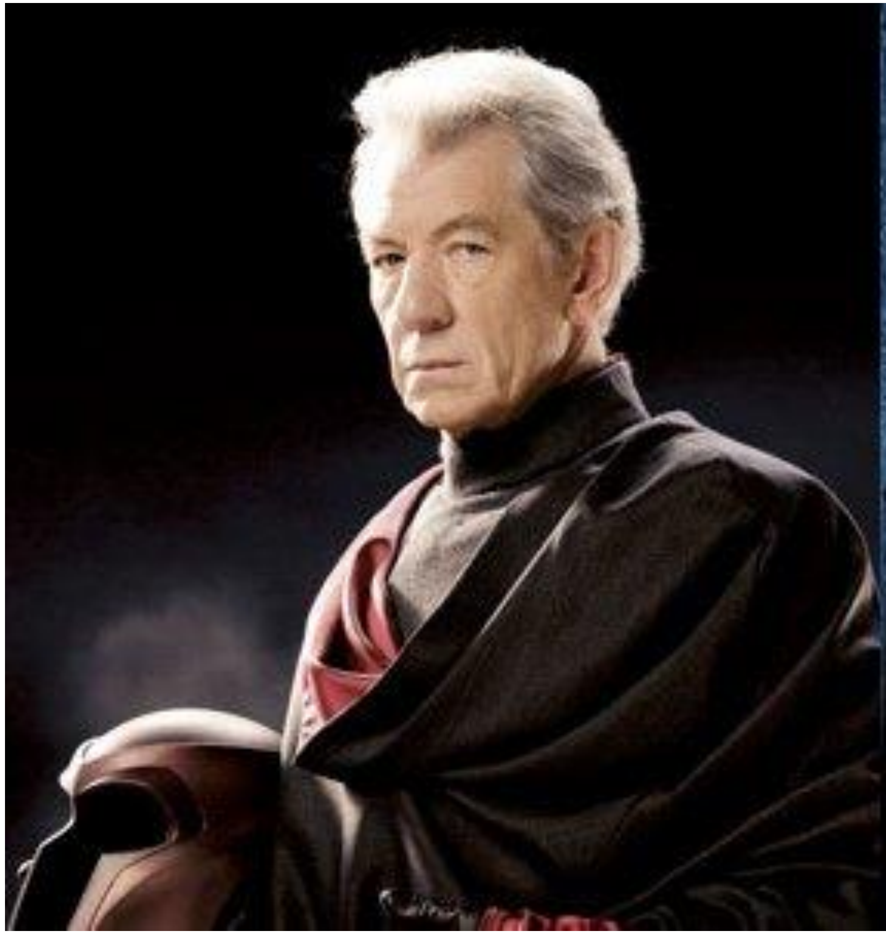
University Health Network

KMT2A (Lysine methyltransferase 2) / MLL (myeloid/lymphoid or mixed-lineage leukemia)

KMT2A-Partial Tandem Duplication

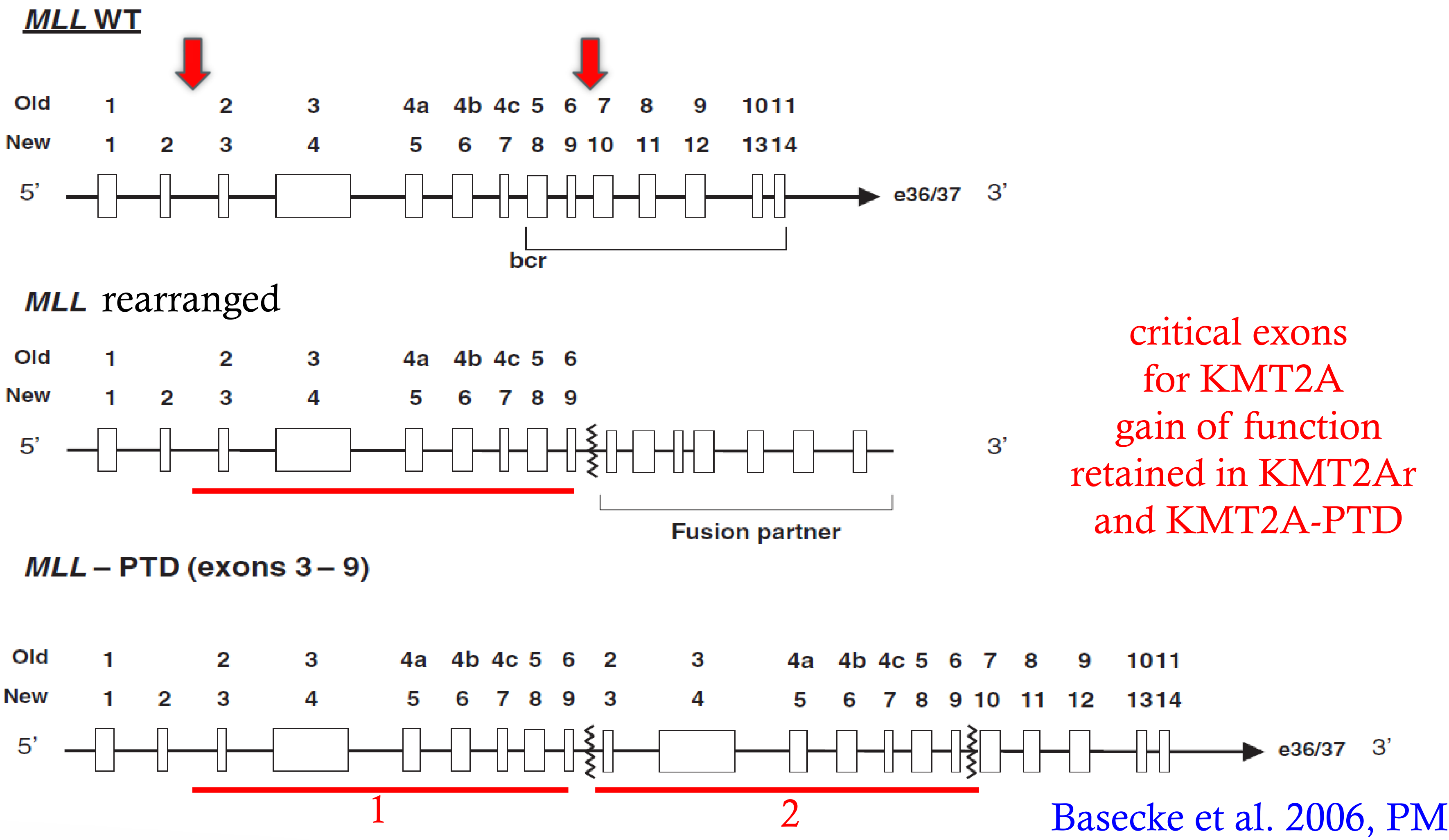


KMT2A fusion/rearrangements

KMT2A - Partial Tandem Duplications

- KMT2A partial tandem duplication (PTD) are cryptic KMT2A mutations that are not detectable by conventional cytogenetics
- KMT2A-PTD occur in 3-10% of AML/MDS patients
- 25% of AML cases +11, also have a KMT2A-PTD
- KMT2A-PTD is associated with a poor prognosis in AML and higher risk of MDS transformation to AML

KMT2A-PTD originates from recombination between Alu elements



critical exons
for KMT2A
gain of function
retained in KMT2Ar
and KMT2A-PTD

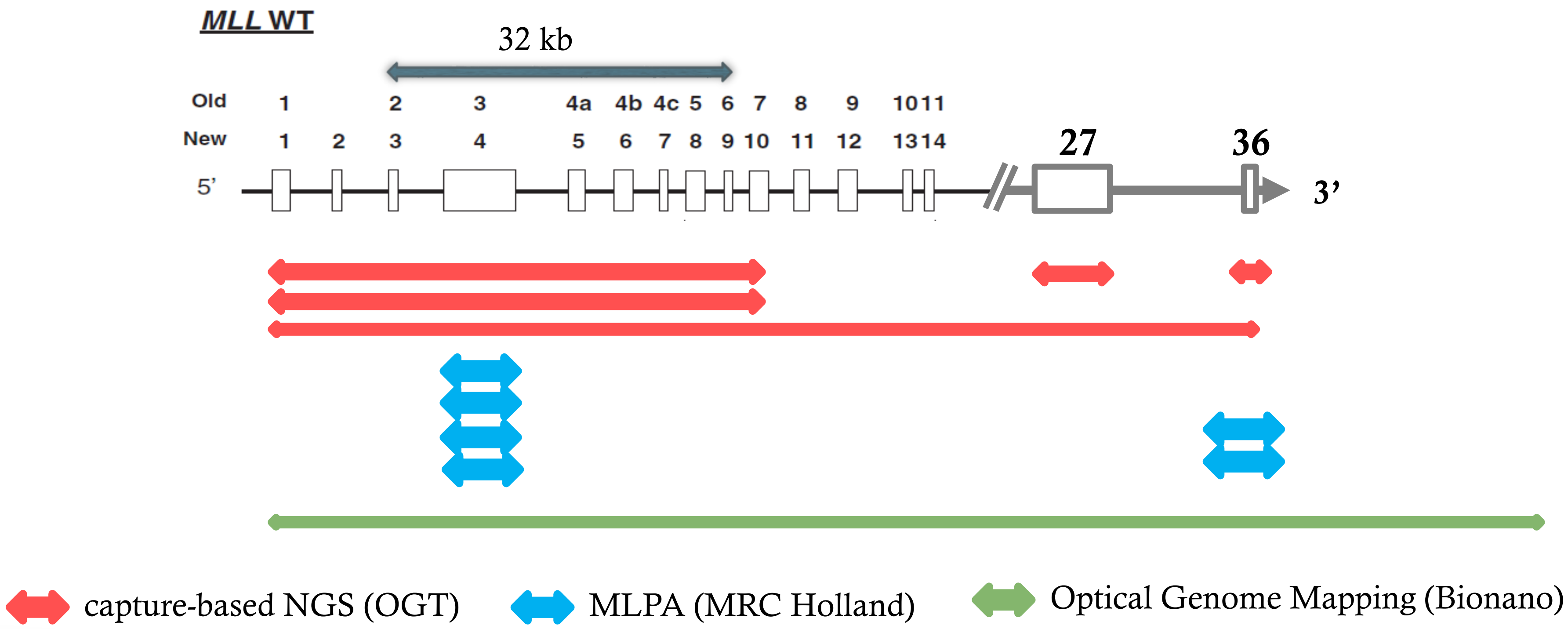
Basecke et al. 2006, PMID:16965385

- NGS: Relative overage ratio of commonly duplicated KMT2A exons (eg. exons 2 to 10) versus non duplicated ones (eg. exons 27, 36)

- MLPA : Similar to NGS
    - PTD probe (exon 4) control probe (exon 36)

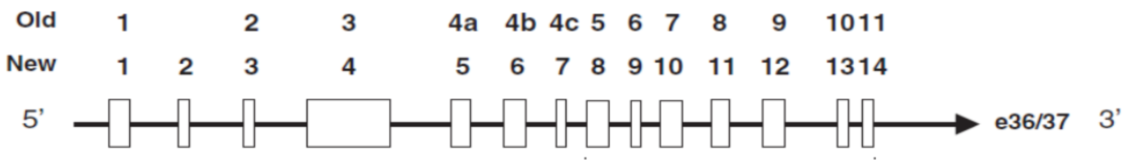
- OGM : High resolution structural variant analysis of genomic DNA molecule

Too large for amplicon NGS?



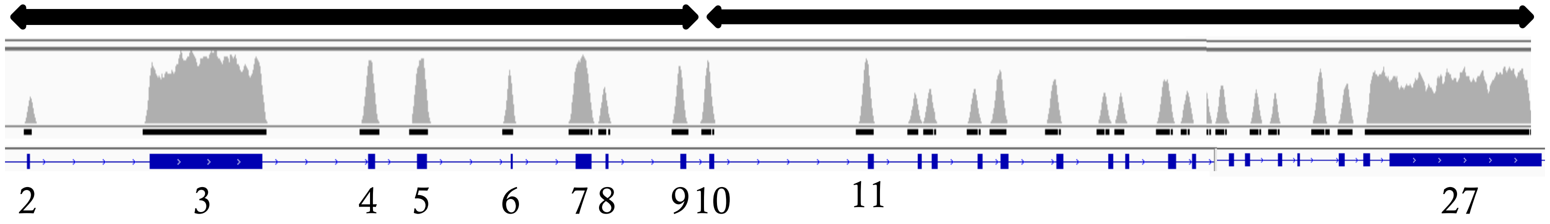
Visual inspection of KMT2A-PTD on NGS

***MLL* WT**



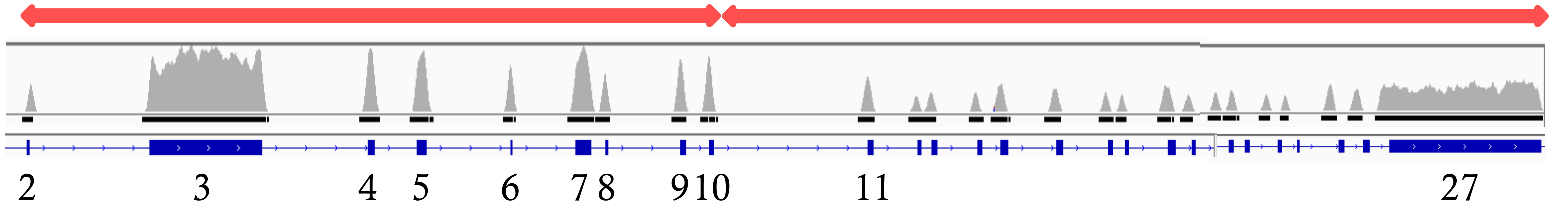
No KMT2A-PTD : Uniform coverage across CCDS of KMT2A

AML1

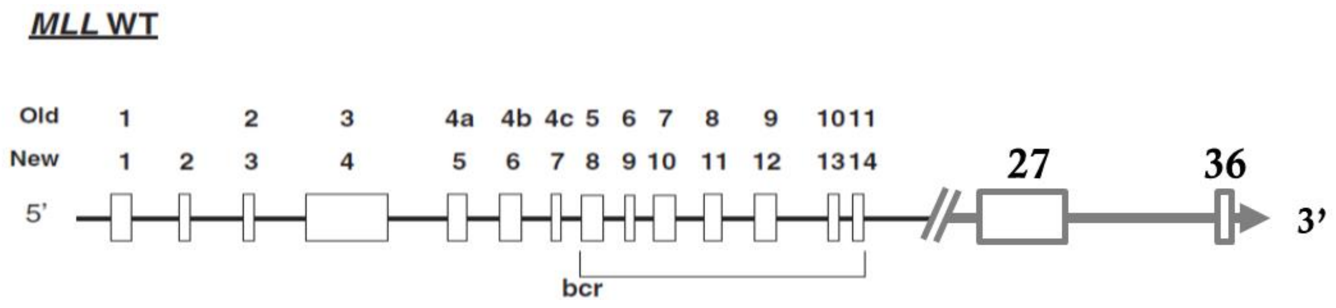


KMT2A-PTD exons 2 to 10 - Lower coverage of non duplicated regions (eg. exons 11-27)

AML2



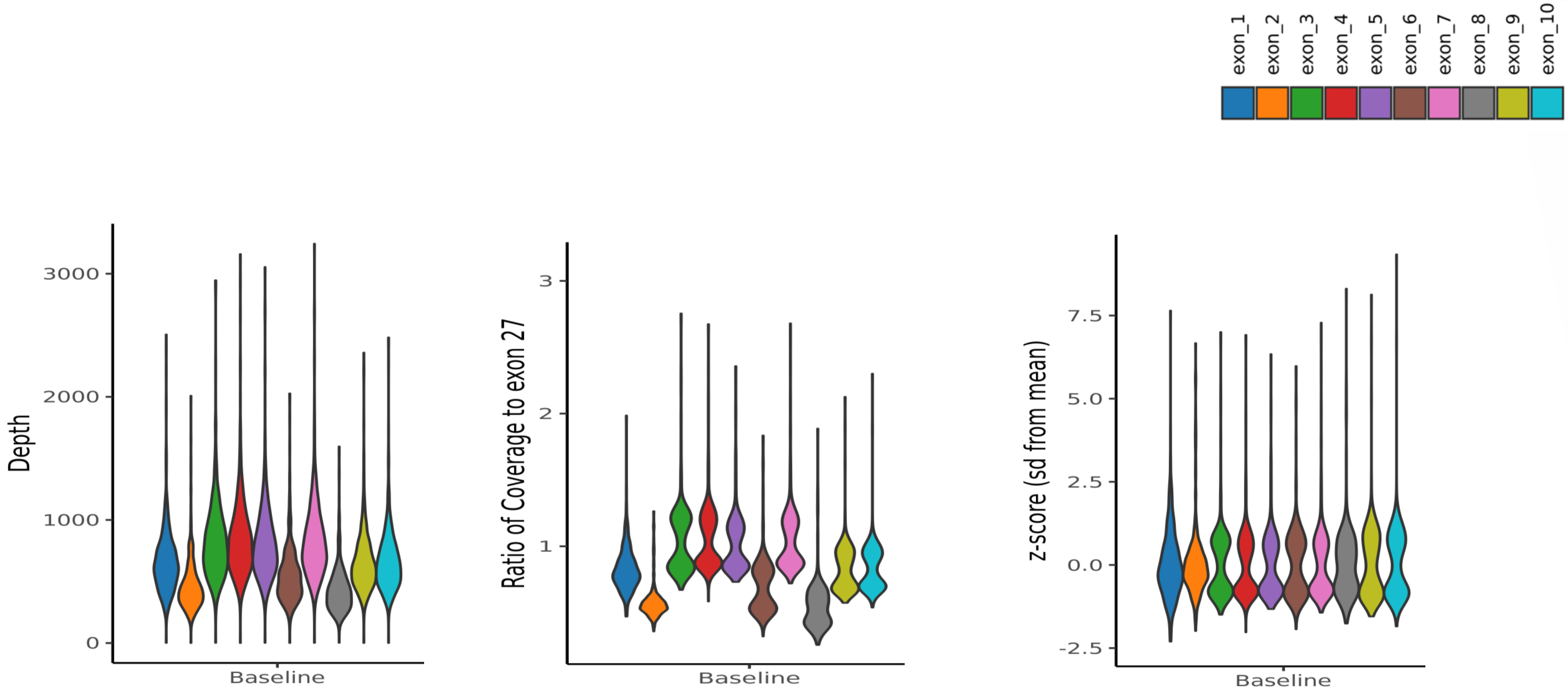
KMT2A-PTD analysis by NGS

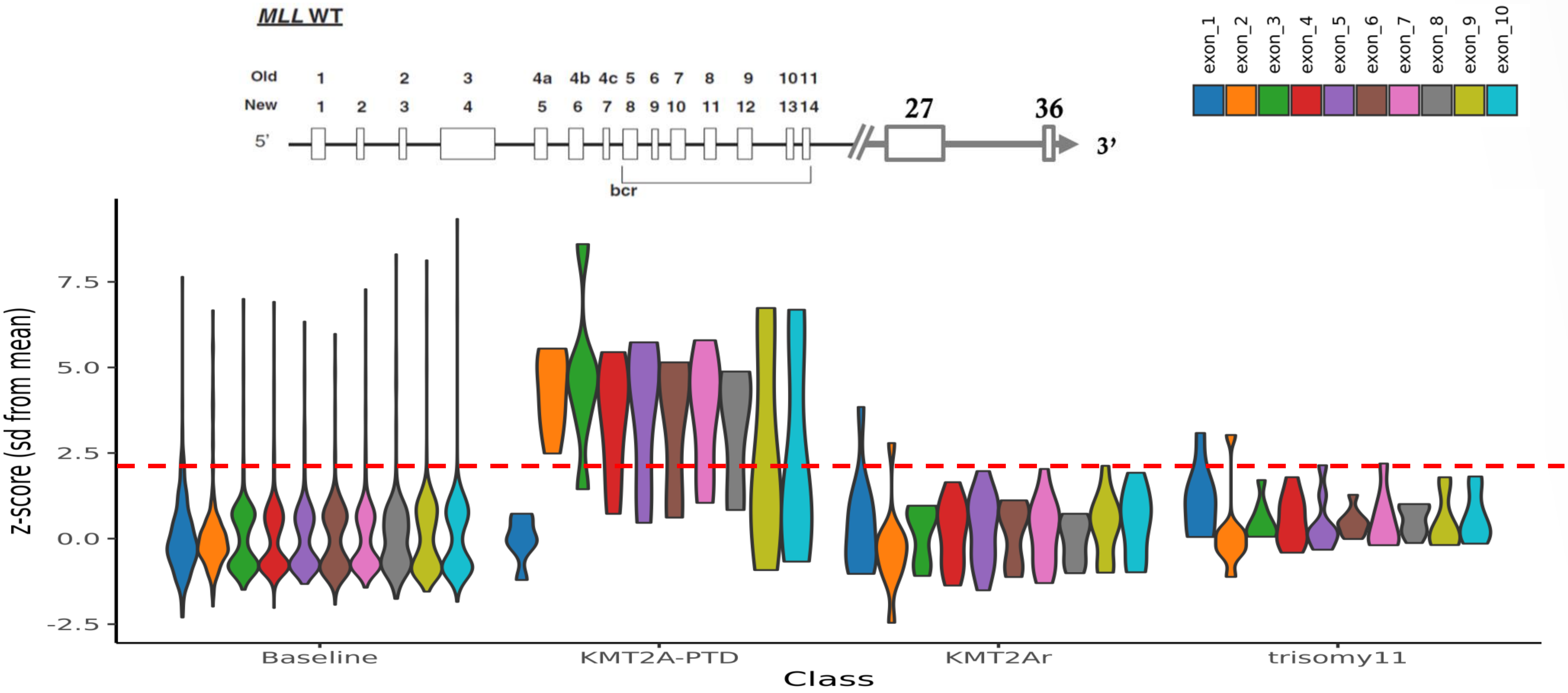


Baseline cohort :

- 193 cases
- >500x coverage of KMT2A exons:
  - N-ter exons (1-10)
  - C-ter exons (27, 36)
- no PTD
- no structural variation on chr11 (at cytogenetic resolution)
- no trisomy 11

KMT2A exon z-score = (depth of N-ter exon /depth exon 27 or 36) – ratio_mean) / ratio_sd


exon_1
exon_2
exon_3
exon_4
exon_5
exon_6
exon_7
exon_8
exon_9
exon_10
Depth
3000
2000
1000
0
Baseline
Ratio of Coverage to exon 27
3
2
1
Baseline
z-score (sd from mean)
7.5
5.0
2.5
0.0
-2.5
Baseline

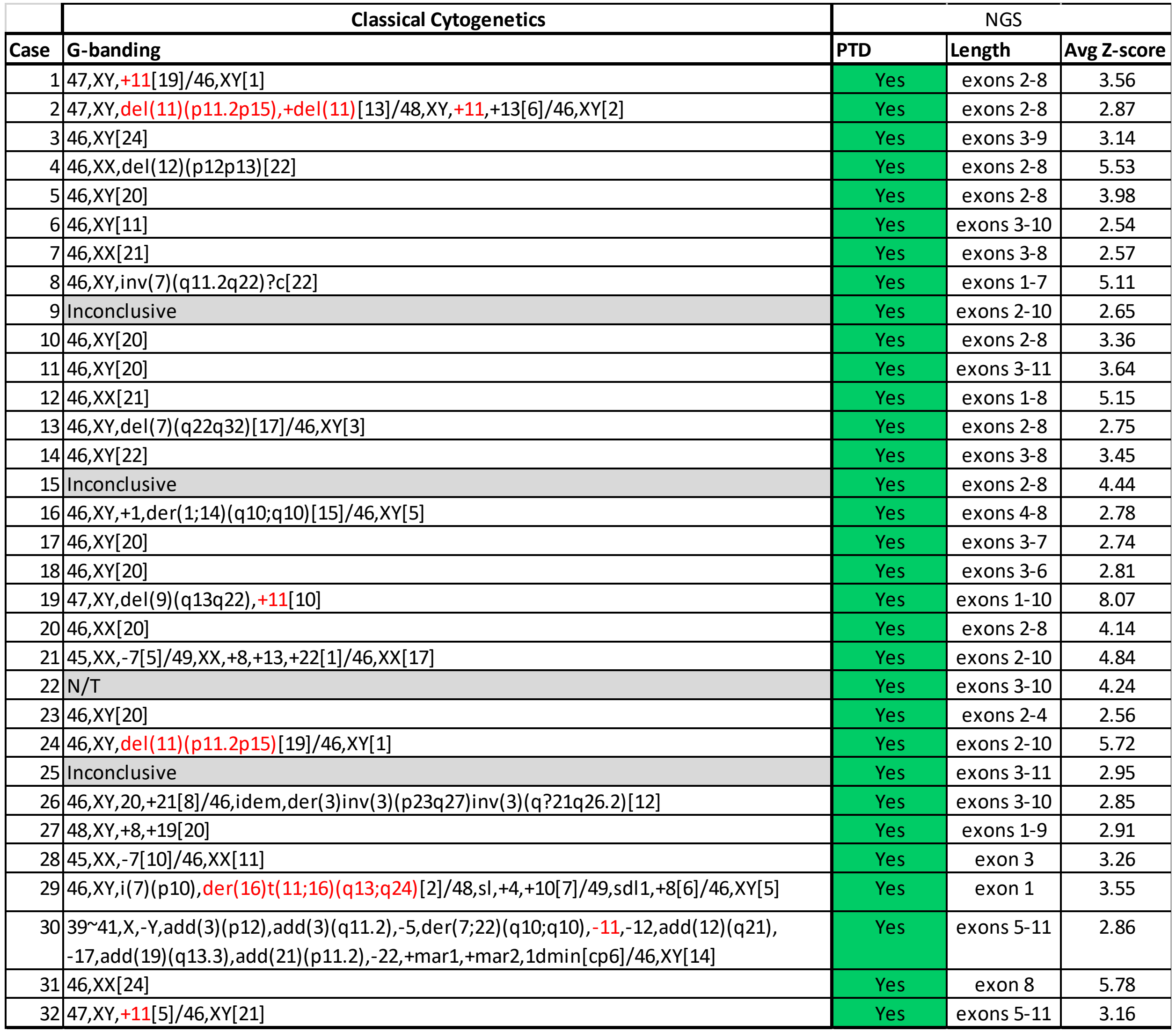

MLL WT
Old 1 2 3 4a 4b 4c 5 6 7 8 9 10 11
New 1 2 3 4 5 6 7 8 9 10 11 12 13 14
5'
27
36
3'
bcr
exon_1
exon_2
exon_3
exon_4
exon_5
exon_6
exon_7
exon_8
exon_9
exon_10
z-score (sd from mean)
7.5
5.0
2.5
0.0
-2.5
Baseline
KMT2A-PTD
KMT2Ar
trisomy11
Class

943 de novo
AMLs

myeloid-NGS

➡ KMT2A z-score ➡ KMT2A-PTD
(n=32) ➡

Confirmation by:
- MLPA
- OGM



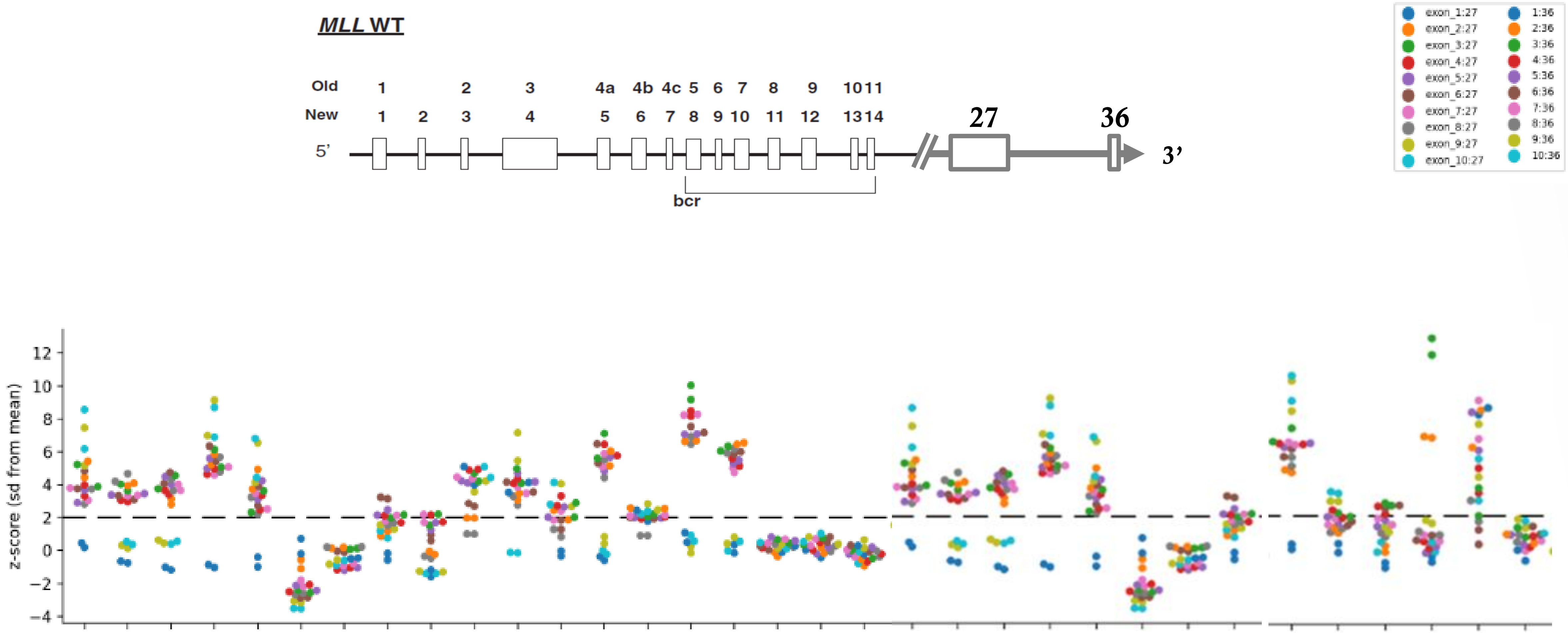
	Classical Cytogenetics	NGS		
Case	G-banding	PTD	Length	Avg Z-score
1	47,XY,+11[19]/46,XY[1]	Yes	exons 2-8	3.56
2	47,XY,del(11)(p11.2p15),+del(11)[13]/48,XY,+11,+13[6]/46,XY[2]	Yes	exons 2-8	2.87
3	46,XY[24]	Yes	exons 3-9	3.14
4	46,XX,del(12)(p12p13)[22]	Yes	exons 2-8	5.53
5	46,XY[20]	Yes	exons 2-8	3.98
6	46,XY[11]	Yes	exons 3-10	2.54
7	46,XX[21]	Yes	exons 3-8	2.57
8	46,XY,inv(7)(q11.2q22)?c[22]	Yes	exons 1-7	5.11
9	Inconclusive	Yes	exons 2-10	2.65
10	46,XY[20]	Yes	exons 2-8	3.36
11	46,XY[20]	Yes	exons 3-11	3.64
12	46,XX[21]	Yes	exons 1-8	5.15
13	46,XY,del(7)(q22q32)[17]/46,XY[3]	Yes	exons 2-8	2.75
14	46,XY[22]	Yes	exons 3-8	3.45
15	Inconclusive	Yes	exons 2-8	4.44
16	46,XY,+1,der(1;14)(q10;q10)[15]/46,XY[5]	Yes	exons 4-8	2.78
17	46,XY[20]	Yes	exons 3-7	2.74
18	46,XY[20]	Yes	exons 3-6	2.81
19	47,XY,del(9)(q13q22),+11[10]	Yes	exons 1-10	8.07
20	46,XX[20]	Yes	exons 2-8	4.14
21	45,XX,-7[5]/49,XX,+8,+13,+22[1]/46,XX[17]	Yes	exons 2-10	4.84
22	N/T	Yes	exons 3-10	4.24
23	46,XY[20]	Yes	exons 2-4	2.56
24	46,XY,del(11)(p11.2p15)[19]/46,XY[1]	Yes	exons 2-10	5.72
25	Inconclusive	Yes	exons 3-11	2.95
26	46,XY,20,+21[8]/46,idem,der(3)inv(3)(p23q27)inv(3)(q?21q26.2)[12]	Yes	exons 3-10	2.85
27	48,XY,+8,+19[20]	Yes	exons 1-9	2.91
28	45,XX,-7[10]/46,XX[11]	Yes	exon 3	3.26
29	46,XY,i(7)(p10),der(16)t(11;16)(q13;q24)[2]/48,sl,+4,+10[7]/49,sdl1,+8[6]/46,XY[5]	Yes	exon 1	3.55
30	39~41,X,-Y,add(3)(p12),add(3)(q11.2),-5,der(7;22)(q10;q10),-11,-12,add(12)(q21), -17,add(19)(q13.3),add(21)(p11.2),-22,+mar1,+mar2,1dmin[cp6]/46,XY[14]	Yes	exons 5-11	2.86
31	46,XX[24]	Yes	exon 8	5.78
32	47,XY,+11[5]/46,XY[21]	Yes	exons 5-11	3.16

N/T not tested

NGS:

- Single and multiple exons PTDs
- PTDs with higher z-scores: >2 copies?
- PTD size suggest at least duplication of some exons within PTD

MLL WT


Old
1
2
3
4a
4b
4c
5
6
7
8
9
10
11
New
1
2
3
4
5
6
7
8
9
10
11
12
13
14
27
36
5'
3'
bcr
z-score (sd from mean)
12
10
8
6
4
2
0
−2
−4
exon_1:27
exon_2:27
exon_3:27
exon_4:27
exon_5:27
exon_6:27
exon_7:27
exon_8:27
exon_9:27
exon_10:27
1:36
2:36
3:36
4:36
5:36
6:36
7:36
8:36
9:36
10:36

	Classical Cytogenetics	NGS			MLPA			
Case	G-banding	PTD	Length	Avg Z-score	PTD	exon4/exon 36	exon 4	exon 36
1	47,XY,+11[19]/46,XY[1]	Yes	exons 2-8	3.56	Yes	1.3	1.8	1.3
2	47,XY,del(11)(p11.2p15),+del(11)[13]/48,XY,+11,+13[6]/46,XY[2]	Yes	exons 2-8	2.87	Yes	1.5	2.0	1.4
3	46,XY[24]	Yes	exons 3-9	3.14	Yes	1.3	1.3	1.0
4	46,XX,del(12)(p12p13)[22]	Yes	exons 2-8	5.53	Yes	2.1	2.2	1.0
5	46,XY[20]	Yes	exons 2-8	3.98	Yes	1.6	1.6	1.0
6	46,XY[11]	Yes	exons 3-10	2.54	Yes	1.3	1.4	1.0
7	46,XX[21]	Yes	exons 3-8	2.57	Yes	1.4	1.5	1.0
8	46,XY,inv(7)(q11.2q22)?c[22]	Yes	exons 1-7	5.11	Yes	1.5	1.6	1.0
9	Inconclusive	Yes	exons 2-10	2.65	Yes	2.1	2.3	1.1
10	46,XY[20]	Yes	exons 2-8	3.36	Yes	1.6	1.6	1.0
11	46,XY[20]	Yes	exons 3-11	3.64	Yes	1.9	1.9	1.0
12	46,XX[21]	Yes	exons 1-8	5.15	Yes	1.8	1.9	1.0
13	46,XY,del(7)(q22q32)[17]/46,XY[3]	Yes	exons 2-8	2.75	Yes	1.7	1.9	1.1
14	46,XY[22]	Yes	exons 3-8	3.45	Yes	1.5	1.5	1.0
15	Inconclusive	Yes	exons 2-8	4.44	Yes	1.9	1.9	1.0
16	46,XY,+1,der(1;14)(q10;q10)[15]/46,XY[5]	Yes	exons 4-8	2.78	Yes	1.3	1.3	1.0
17	46,XY[20]	Yes	exons 3-7	2.74	Yes	1.6	1.5	1.0
18	46,XY[20]	Yes	exons 3-6	2.81	Yes	1.4	1.4	1.0
19	47,XY,del(9)(q13q22),+11[10]	Yes	exons 1-10	8.07	Yes	1.6	2.1	1.3
20	46,XX[20]	Yes	exons 2-8	4.14	Yes	2.7	2.0	0.7
21	45,XX,-7[5]/49,XX,+8,+13,+22[1]/46,XX[17]	Yes	exons 2-10	4.84	Yes	1.9	1.9	1.0
22	N/T	Yes	exons 3-10	4.24	Yes	1.1	1.0	0.9
23	46,XY[20]	Yes	exons 2-4	2.56	Yes	1.6	1.6	1.0
24	46,XY,del(11)(p11.2p15)[19]/46,XY[1]	Yes	exons 2-10	5.72	Yes	1.8	1.8	1.0
25	Inconclusive	Yes	exons 3-11	2.95	Inconclusive	1.7	1.1	0.6
26	46,XY,20,+21[8]/46,idem,der(3)inv(3)(p23q27)inv(3)(q?21q26.2)[12]	Yes	exons 3-10	2.85	Inconclusive	2.2	1.1	0.5
27	48,XY,+8,+19[20]	Yes	exons 1-9	2.91	Inconclusive	1.8	1.0	0.6
28	45,XX,-7[10]/46,XX[11]	Yes	exon 3	3.26	No	1.0	1.0	1.0
29	46,XY,i(7)(p10),der(16)t(11;16)(q13;q24)[2]/48,sl,+4,+10[7]/49,sdl1,+8[6]/46,XY[5]	Yes	exon 1	3.55	No	1.0	1.4	1.4
30	39~41,X,-Y,add(3)(p12),add(3)(q11.2),-5,der(7;22)(q10;q10),-11,-12,add(12)(q21), -17,add(19)(q13.3),add(21)(p11.2),-22,+mar1,+mar2,1dmin[cp6]/46,XY[14]	Yes	exons 5-11	2.86	No	0.9	0.9	1.0
31	46,XX[24]	Yes	exon 8	5.78	No	0.9	1.0	1.0
32	47,XY,+11[5]/46,XY[21]	Yes	exons 5-11	3.16	No	1.1	1.1	1.0

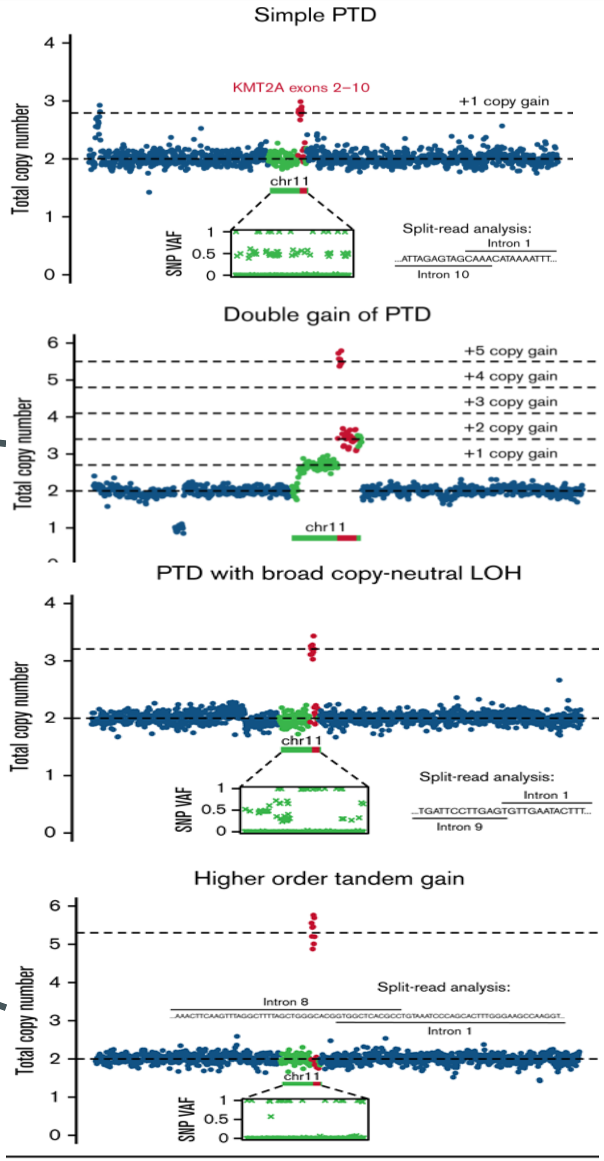
MLPA:

- 75% concordance with NGS results
- Discordant calls:
  - C-ter deletion of KMT2A
  - PTD not involving exon 4
- MLPA supports duplication or higher copy number within PTD
  (i.e triplication ratio: 1.75-2.15)

N/T not tested

MLPA inconclusive: copy number control probe (exon36) <0.7

Levels of magnitude of PTDs

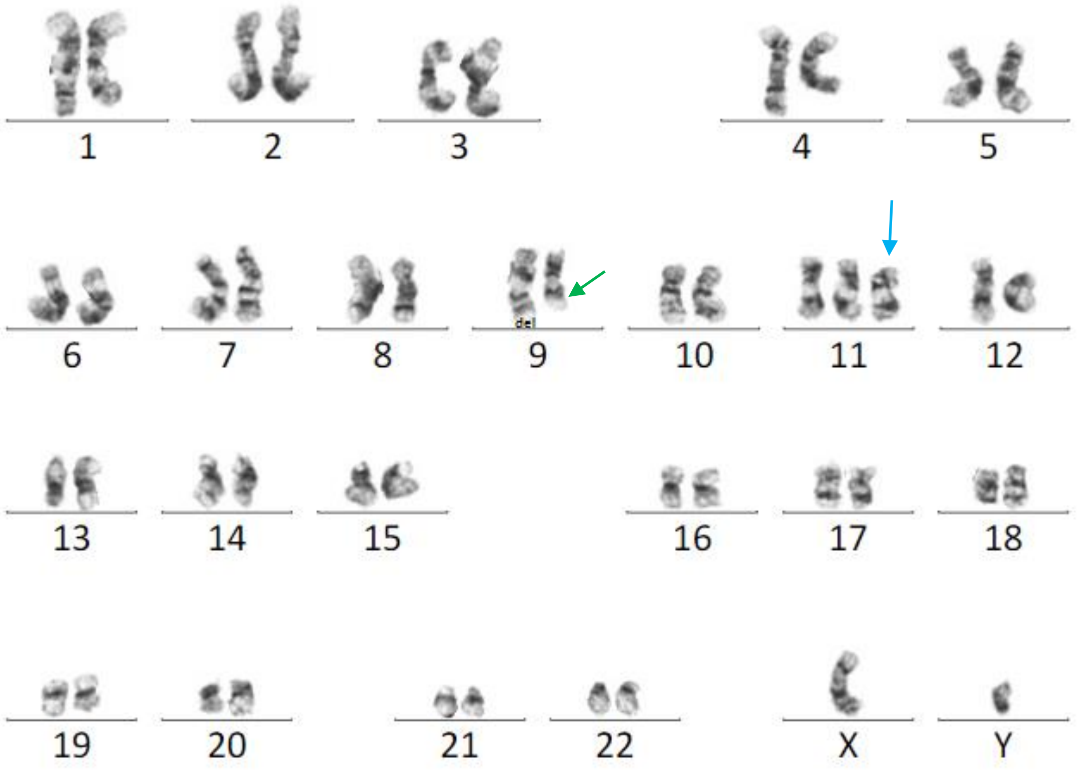


Tsai et al. 2022, PMID:35584376

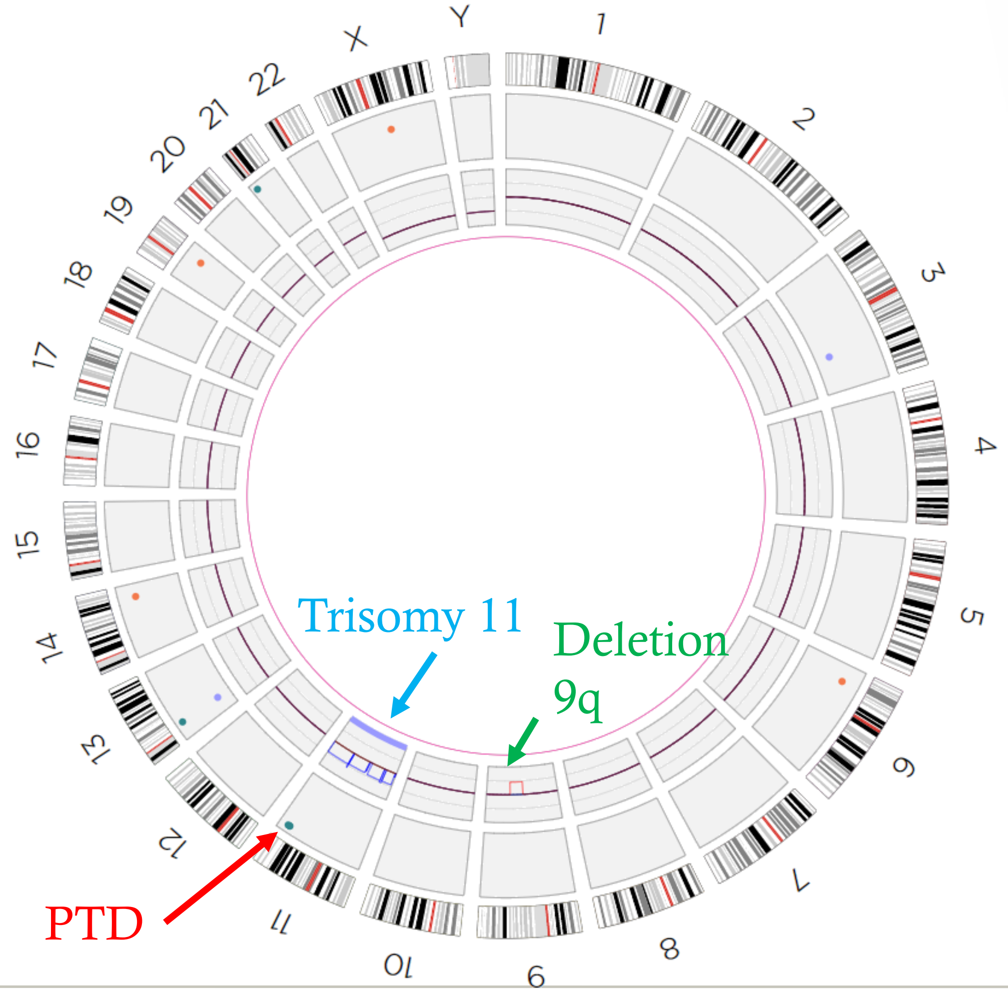
	Classical Cytogenetics		NGS			**MLPA**				OGM
Case	**G-banding**	**KMT2A FISH**	**PTD**	**Length**	**Avg Z-score**	PTD	**exon4/exon 36**	**exon 4**	**exon 36**	**KMT2A-PTD**
1	47,XY,+11[19]/46,XY[1]	N/T	Yes	exons 2-8	3.56	Yes	1.3	1.8	1.3	N/T
2	47,XY,del(11)(p11.2p15),+del(11)[13]/48,XY,+11,+13[6]/46,XY[2]	Negative	Yes	exons 2-8	2.87	Yes	1.5	2.0	1.4	N/T
3	46,XY[24]	Negative	Yes	exons 3-9	3.14	Yes	1.3	1.3	1.0	N/T
4	46,XX,del(12)(p12p13)[22]	Negative	Yes	exons 2-8	5.53	Yes	2.1	2.2	1.0	N/T
5	46,XY[20]	Negative	Yes	exons 2-8	3.98	Yes	1.6	1.6	1.0	N/T
6	46,XY[11]	N/T	Yes	exons 3-10	2.54	Yes	1.3	1.4	1.0	N/T
7	46,XX[21]	Negative	Yes	exons 3-8	2.57	Yes	1.4	1.5	1.0	N/T
8	46,XY,inv(7)(q11.2q22)?c[22]	Negative	Yes	exons 1-7	5.11	Yes	1.5	1.6	1.0	N/T
9	Inconclusive	Negative	Yes	exons 2-10	2.65	Yes	2.1	2.3	1.1	N/T
10	46,XY[20]	N/T	Yes	exons 2-8	3.36	Yes	1.6	1.6	1.0	N/T
11	46,XY[20]	N/T	Yes	exons 3-11	3.64	Yes	1.9	1.9	1.0	N/T
12	46,XX[21]	N/T	Yes	exons 1-8	5.15	Yes	1.8	1.9	1.0	N/T
13	46,XY,del(7)(q22q32)[17]/46,XY[3]	N/T	Yes	exons 2-8	2.75	Yes	1.7	1.9	1.1	N/T
14	46,XY[22]	Negative	Yes	exons 3-8	3.45	Yes	1.5	1.5	1.0	N/T
15	Inconclusive	Negative	Yes	exons 2-8	4.44	Yes	1.9	1.9	1.0	N/T
16	46,XY,+1,der(1;14)(q10;q10)[15]/46,XY[5]	N/T	Yes	exons 4-8	2.78	Yes	1.3	1.3	1.0	N/T
17	46,XY[20]	Negative	Yes	exons 3-7	2.74	Yes	1.6	1.5	1.0	N/T
18	46,XY[20]	Negative	Yes	exons 3-6	2.81	Yes	1.4	1.4	1.0	N/T
19	47,XY,del(9)(q13q22),+11[10]	N/T	Yes	exons 1-10	8.07	Yes	1.6	2.1	1.3	Yes
20	46,XX[20]	N/T	Yes	exons 2-8	4.14	Yes	2.7	2.0	0.7	Yes
21	45,XX,-7[5]/49,XX,+8,+13,+22[1]/46,XX[17]	N/T	Yes	exons 2-10	4.84	Yes	1.9	1.9	1.0	Yes
22	N/T	Negative	Yes	exons 3-10	4.24	Yes	1.1	1.0	0.9	Yes
23	46,XY[20]	N/T	Yes	exons 2-4	2.56	Yes	1.6	1.6	1.0	Yes
24	46,XY,del(11)(p11.2p15)[19]/46,XY[1]	Negative	Yes	exons 2-10	5.72	Yes	1.8	1.8	1.0	No
25	Inconclusive	Positive	Yes	exons 3-11	2.95	Inconclusive	1.7	1.1	0.6	No
26	46,XY,20,+21[8]/46,idem,der(3)inv(3)(p23q27)inv(3)(q?21q26.2)[12]	N/T	Yes	exons 3-10	2.85	Inconclusive	2.2	1.1	0.5	N/T
27	48,XY,+8,+19[20]	N/T	Yes	exons 1-9	2.91	Inconclusive	1.8	1.0	0.6	No
28	45,XX,-7[10]/46,XX[11]	N/T	Yes	exon 3	3.26	No	1.0	1.0	1.0	N/T
29	46,XY,i(7)(p10),der(16)t(11;16)(q13;q24)[2]/48,sl,+4,+10[7]/49,sdl1,+8[6]/46,XY[5]	Negative	Yes	exon 1	3.55	No	1.0	1.4	1.4	N/T
30	39~41,X,-Y,add(3)(p12),add(3)(q11.2),-5,der(7;22)(q10;q10),-11,-12,add(12)(q21),-17,add(19)(q13.3),add(21)(p11.2),-22,+mar1,+mar2,1dmin[cp6]/46,XY[14]	N/T	Yes	exons 5-11	2.86	No	0.9	0.9	1.0	N/T
31	46,XX[24]	N/T	Yes	exon 8	5.78	No	0.9	1.0	1.0	N/T
32	47,XY,+11[5]/46,XY[21]	N/T	Yes	exons 5-11	3.16	No	1.1	1.1	1.0	N/T

- Structural variations on chromosome 11 interfere with ability to detect PTD by NGS

What does a KMT2A-PTD Look Like on OGM?

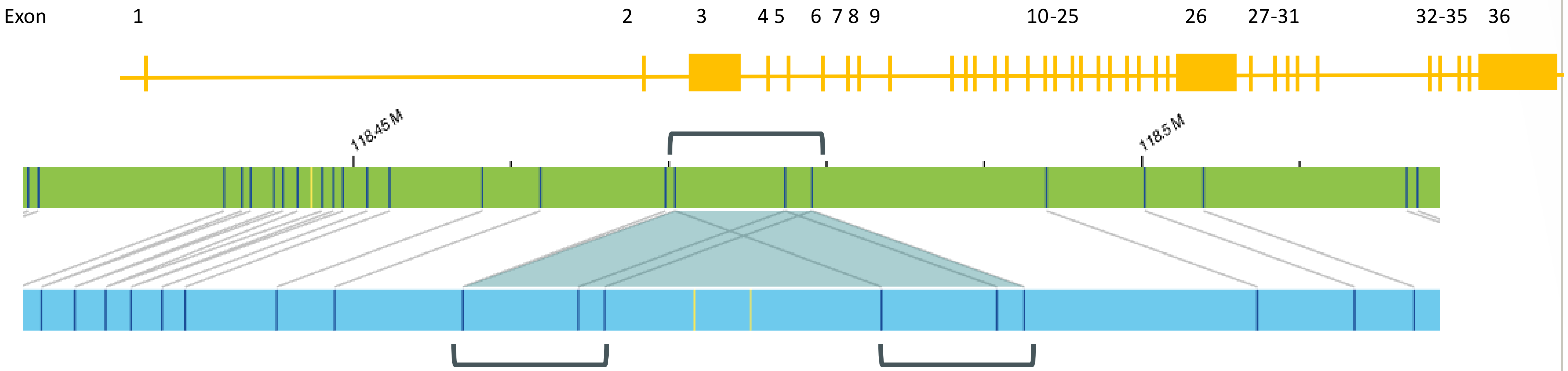


47,XY,del(9)(q13q22),+11[10]



Genome View – 11q23

KMT2A Exons

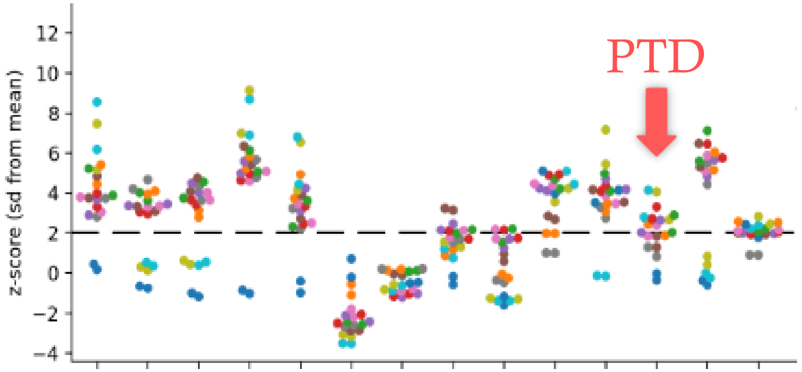


ogm ins(11;?)(q23.3;?)

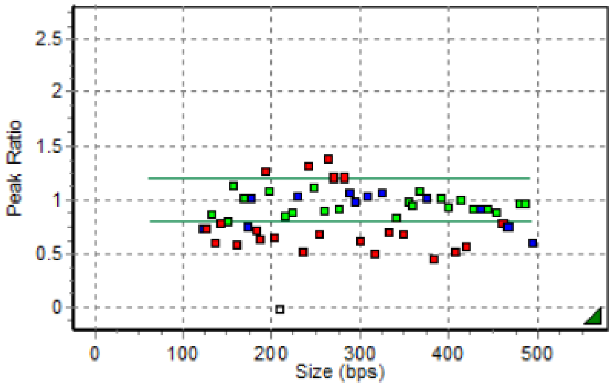
KMT2A-PTDs range in size from approximately 20kb to 50kb (in our experience). They are detected by the SV pipeline in OGM, not the copy number pipeline. SV pipeline 5kb or greater (unbal SV), CNV >500kb

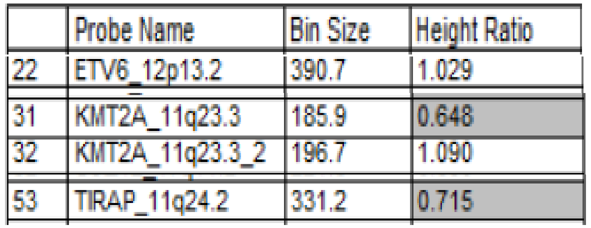
False positive by NGS: unbalanced t(11;19) *KMT2A::ELL*

- PTD positive exons 3-11

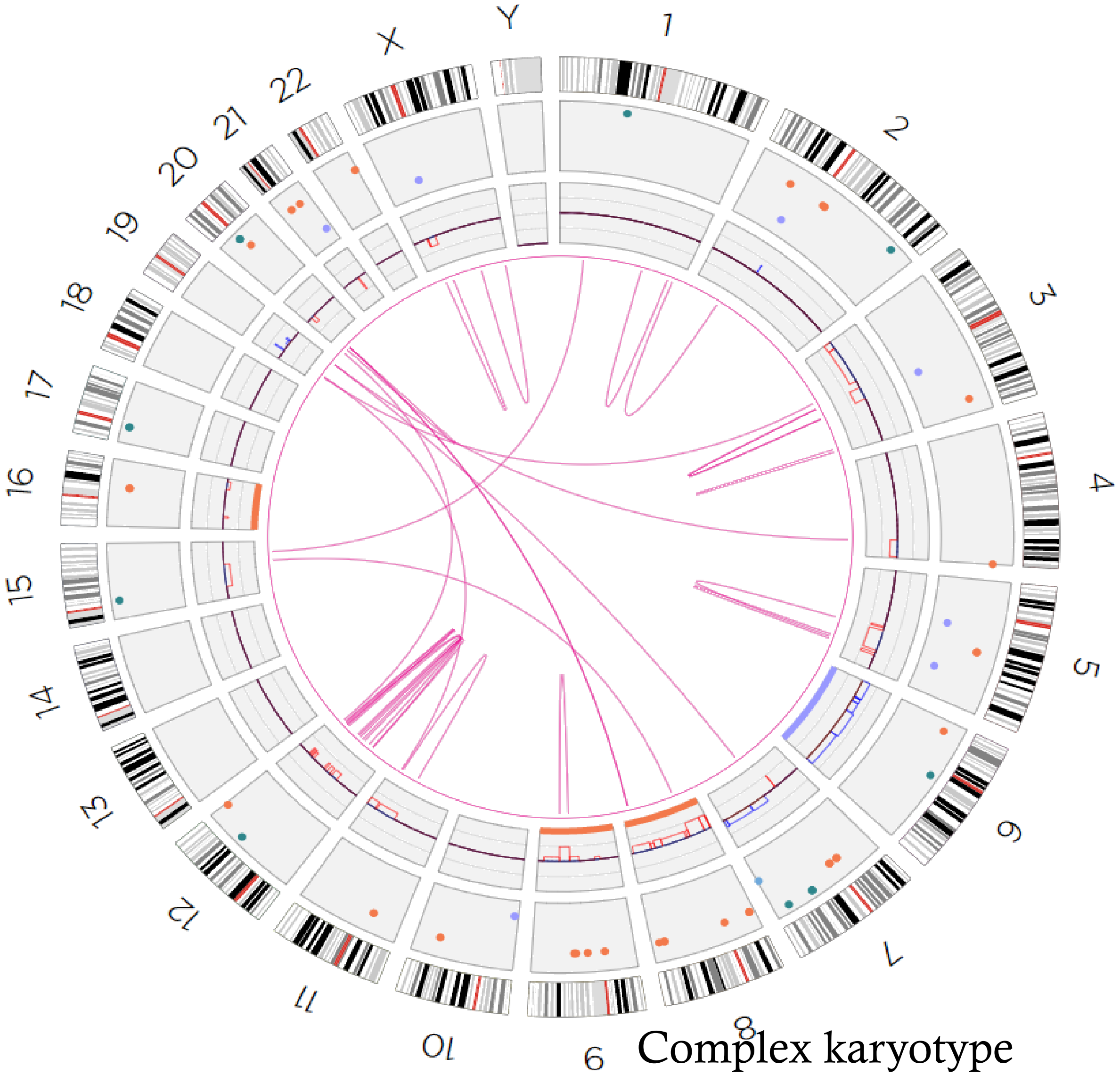


PTD





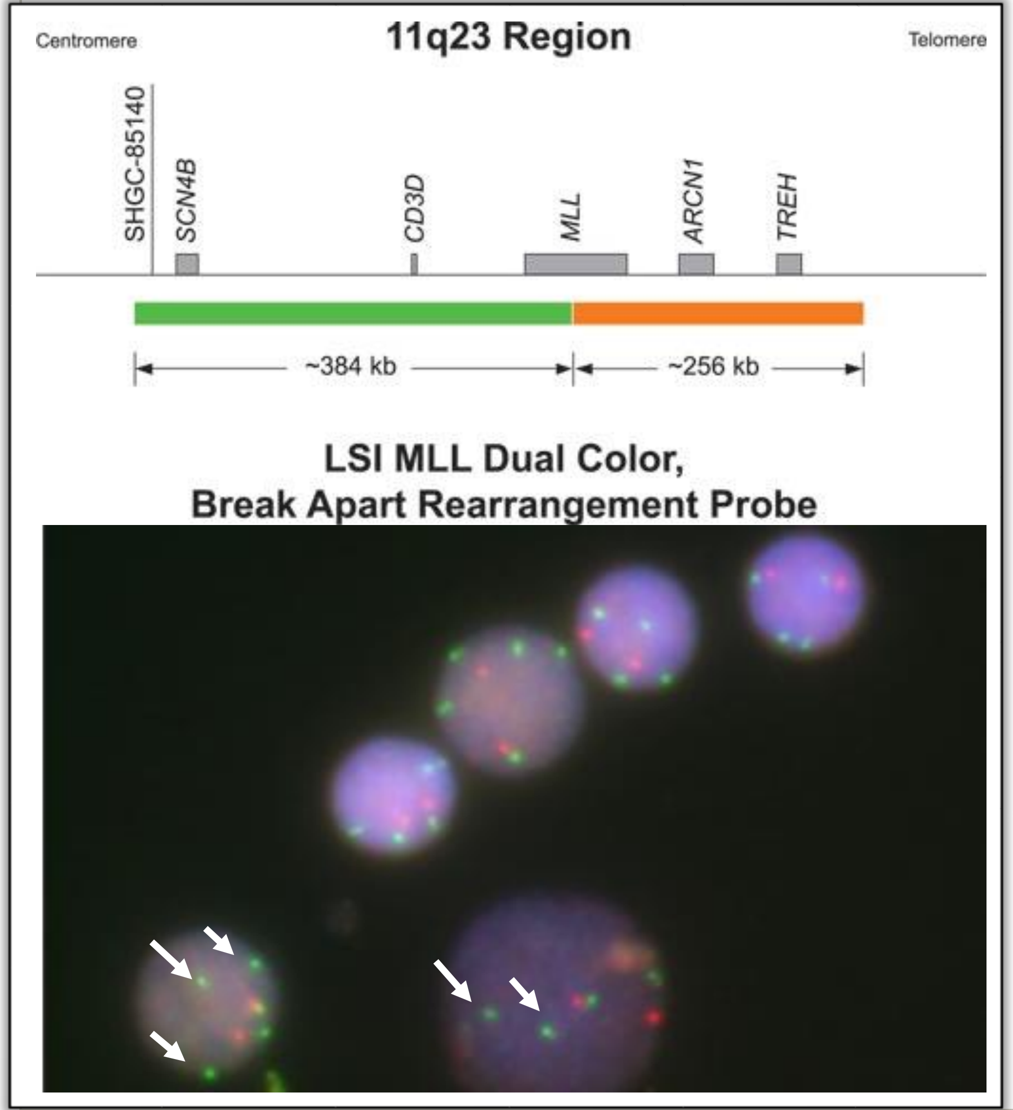
	Probe Name	Bin Size	Height Ratio
22	ETV6_12p13.2	390.7	1.029
31	KMT2A_11q23.3	185.9	0.648
32	KMT2A_11q23.3_2	196.7	1.090
53	TIRAP_11q24.2	331.2	0.715

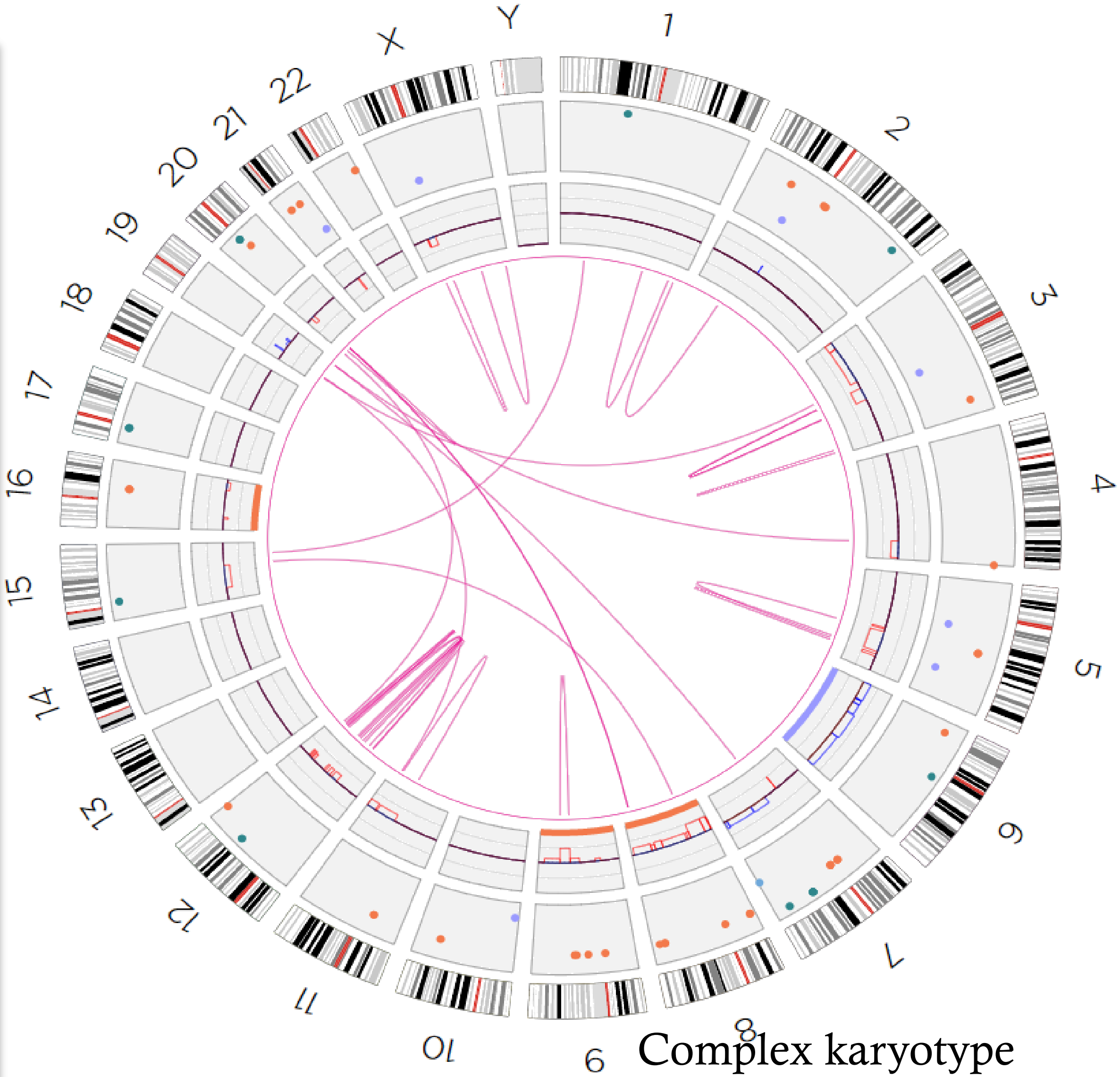


Complex karyotype
with KMT2A translocation

False positive by NGS: unbalanced t(11;19) *KMT2A::ELL*

- PTD positive exons 3-11

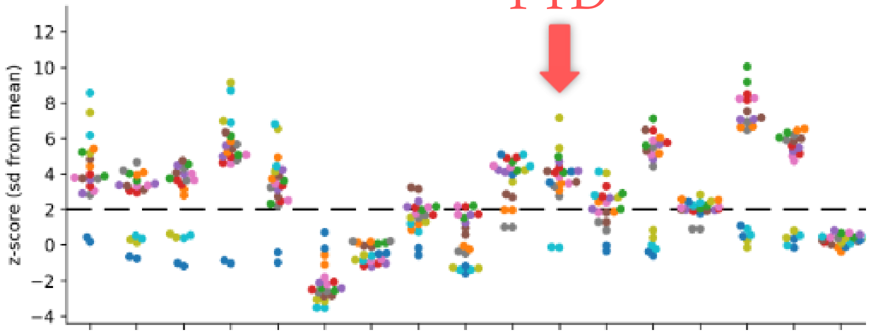




Complex karyotype
with KMT2A translocation

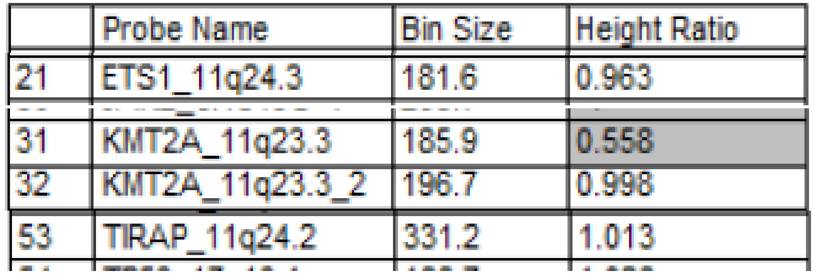
False positive by NGS: **deletion of 5'-KMT2A**

CG: 48,XY,+8,+19[20]



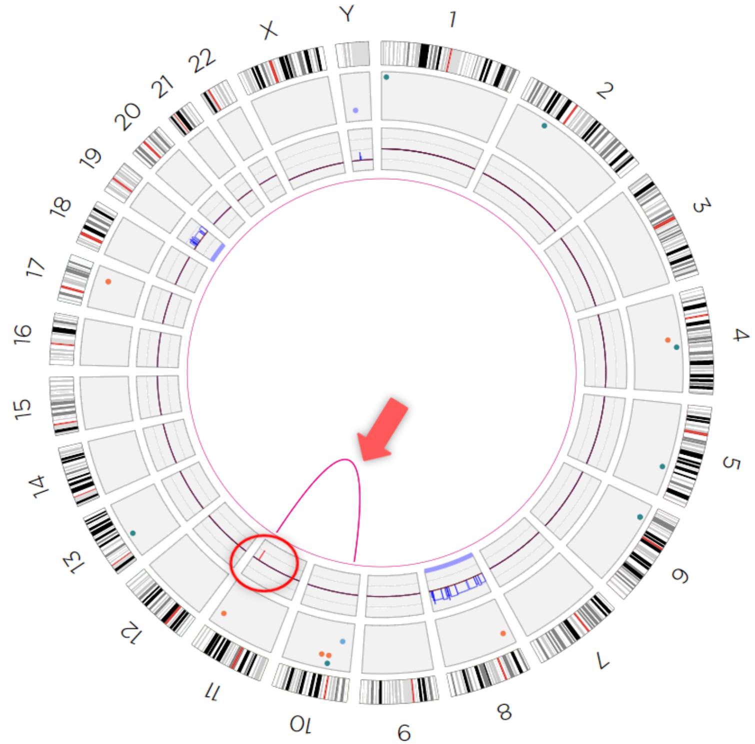
PTD

- PTD positive exons 1-9

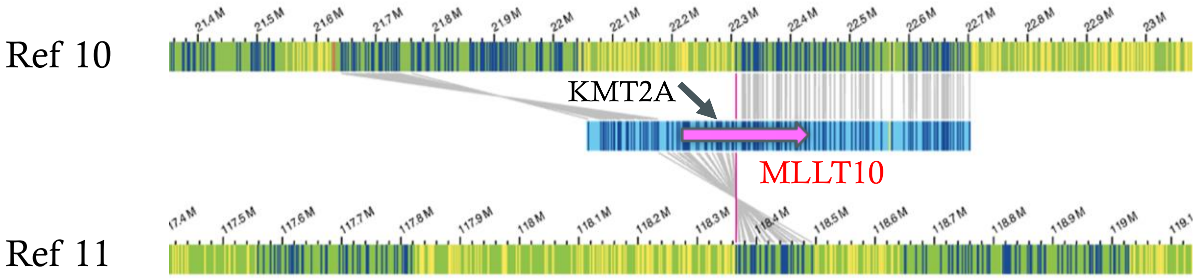


	Probe Name	Bin Size	Height Ratio
21	ETS1_11q24.3	181.6	0.963
31	KMT2A_11q23.3	185.9	0.558
32	KMT2A_11q23.3_2	196.7	0.998
53	TIRAP_11q24.2	331.2	1.013

- No PTD
  - 1 copy exon 36
  - 2 copies exon 4

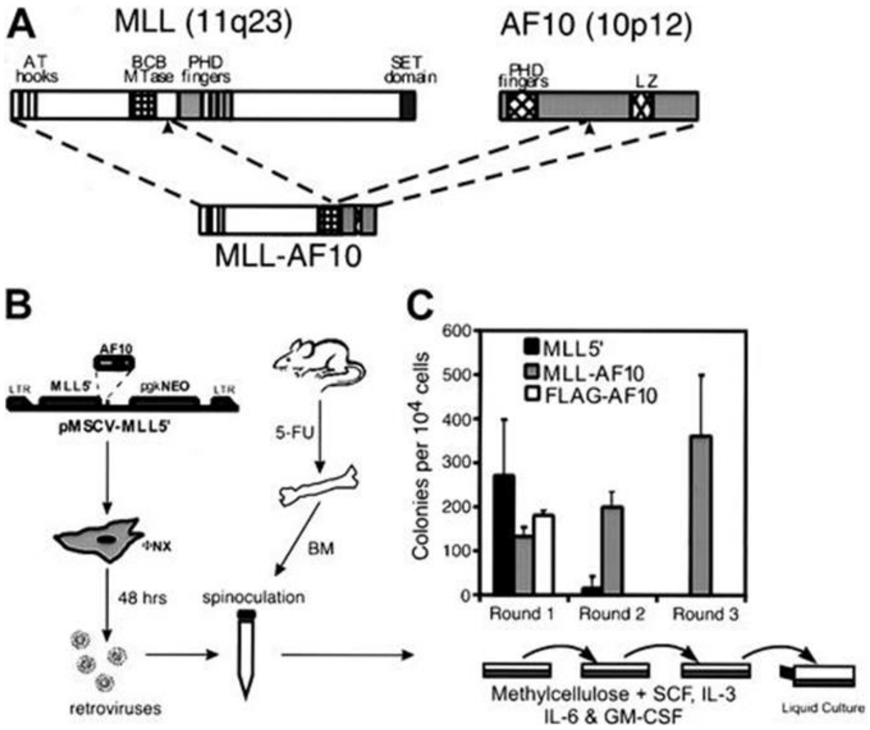


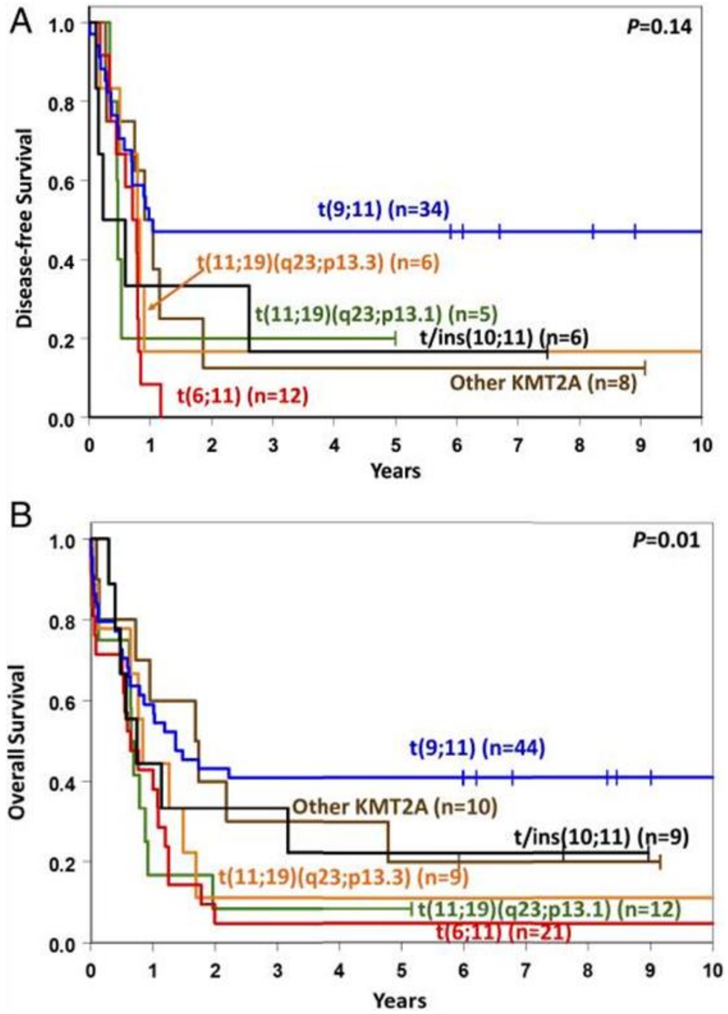
ogm (8)x3,t(10;11)(KMT2A::MLLT10),ins(11;?)(q23.3;?),(19)x3



Deletion 5'-KMT2A and cryptic insertion of MLLT10

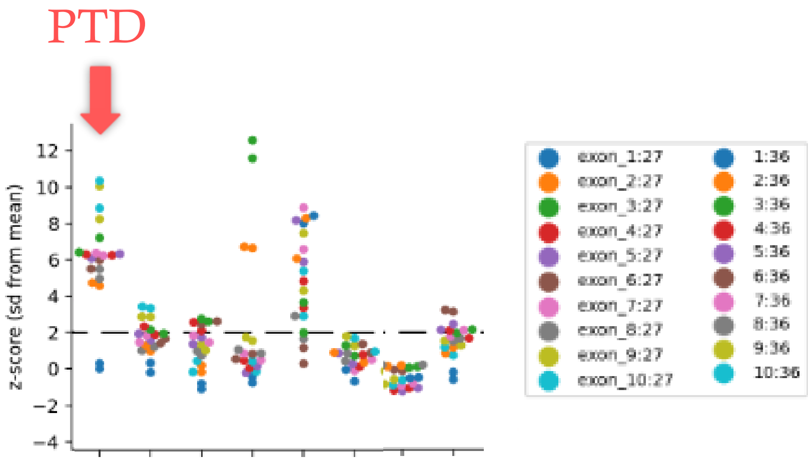
KMT2A-MLLT10/AFT10 fusion



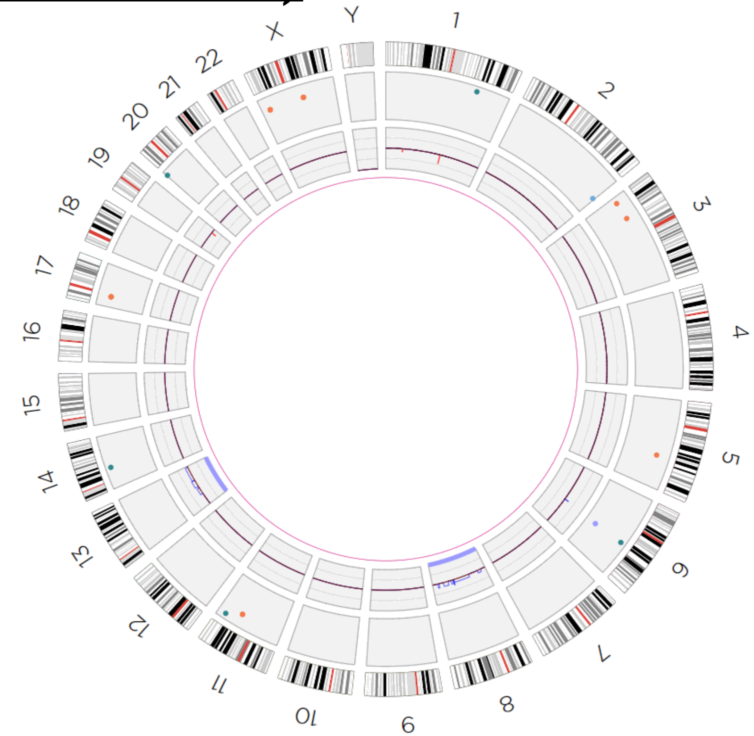


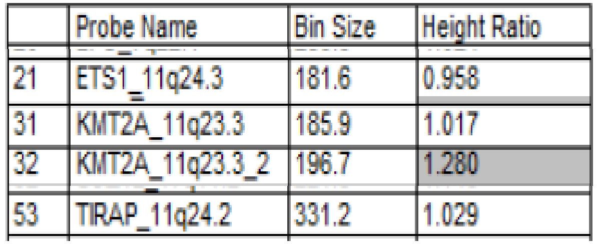
DiMartino et al. 2002, PMID:11986236
Bill et al. 2020, PMID: 33020282

KMT2A-PTD (TRIPLICATION?)



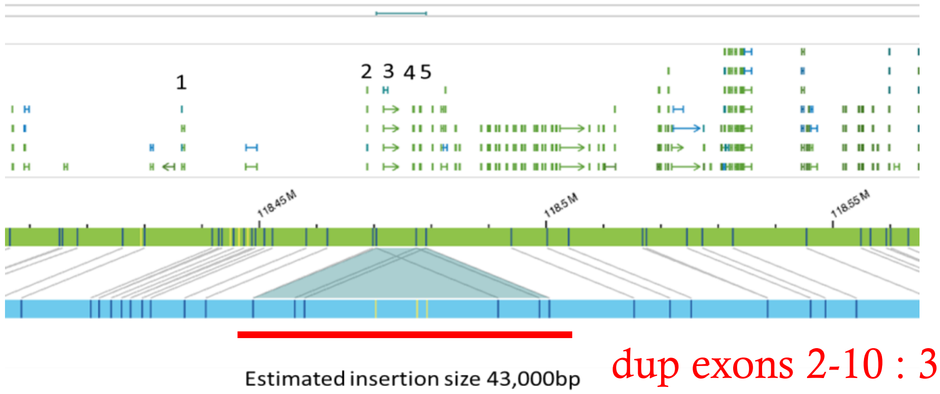
- PTD positive exons 2-10





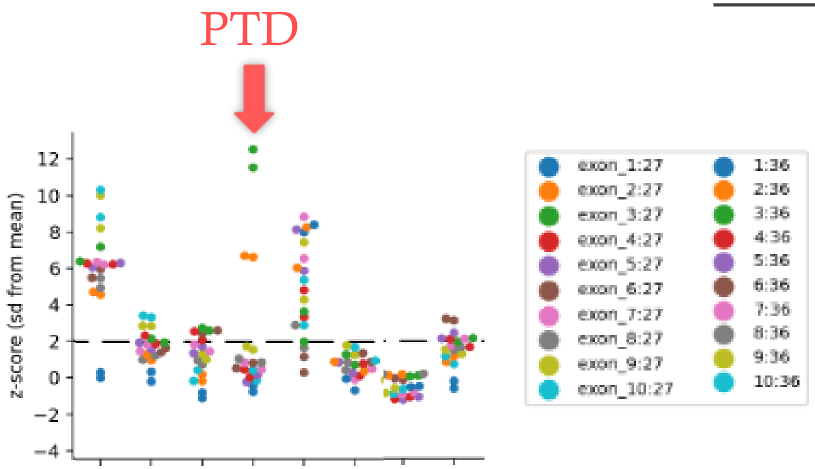
	Probe Name	Bin Size	Height Ratio
21	ETS1_11q24.3	181.6	0.958
31	KMT2A_11q23.3	185.9	1.017
32	KMT2A_11q23.3_2	196.7	1.280
53	TIRAP_11q24.2	331.2	1.029

- PTD:
  - 2 copies exon 36
  - 3 copies exon 4

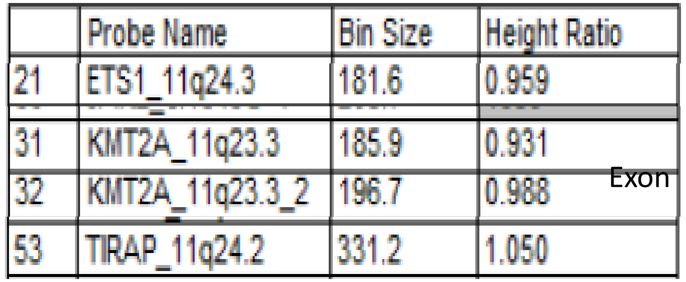


dup exons 2-10 : 32kb ?

PTD of Uncertain Significance?

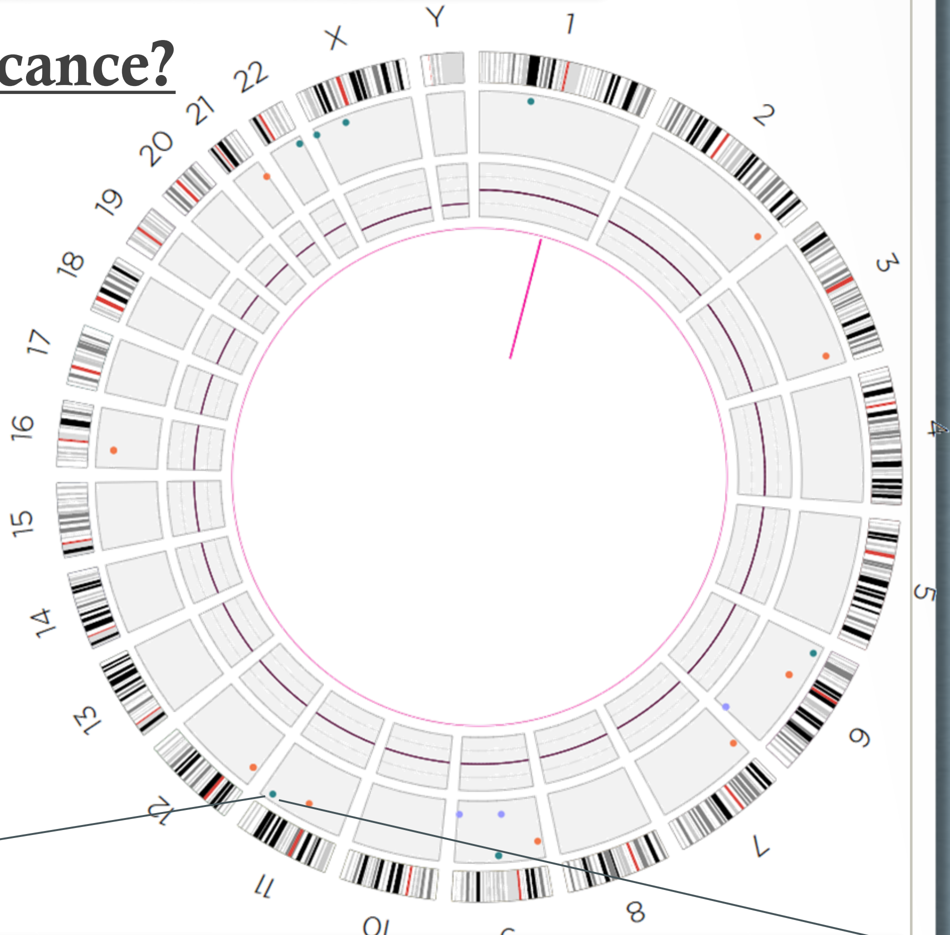

PTD

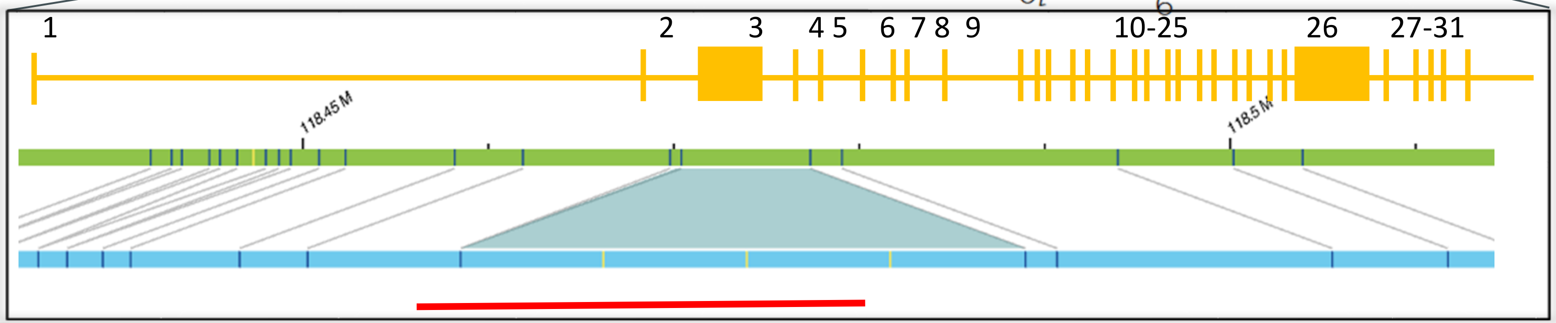
- PTD positive exons 2-3



	Probe Name	Bin Size	Height Ratio
21	ETS1_11q24.3	181.6	0.959
31	KMT2A_11q23.3	185.9	0.931
32	KMT2A_11q23.3_2	196.7	0.988
53	TIRAP_11q24.2	331.2	1.050

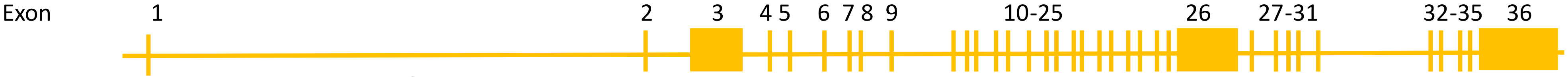
- No PTD:
  - Normal copy exons 4 & 36
  - No trisomy 11

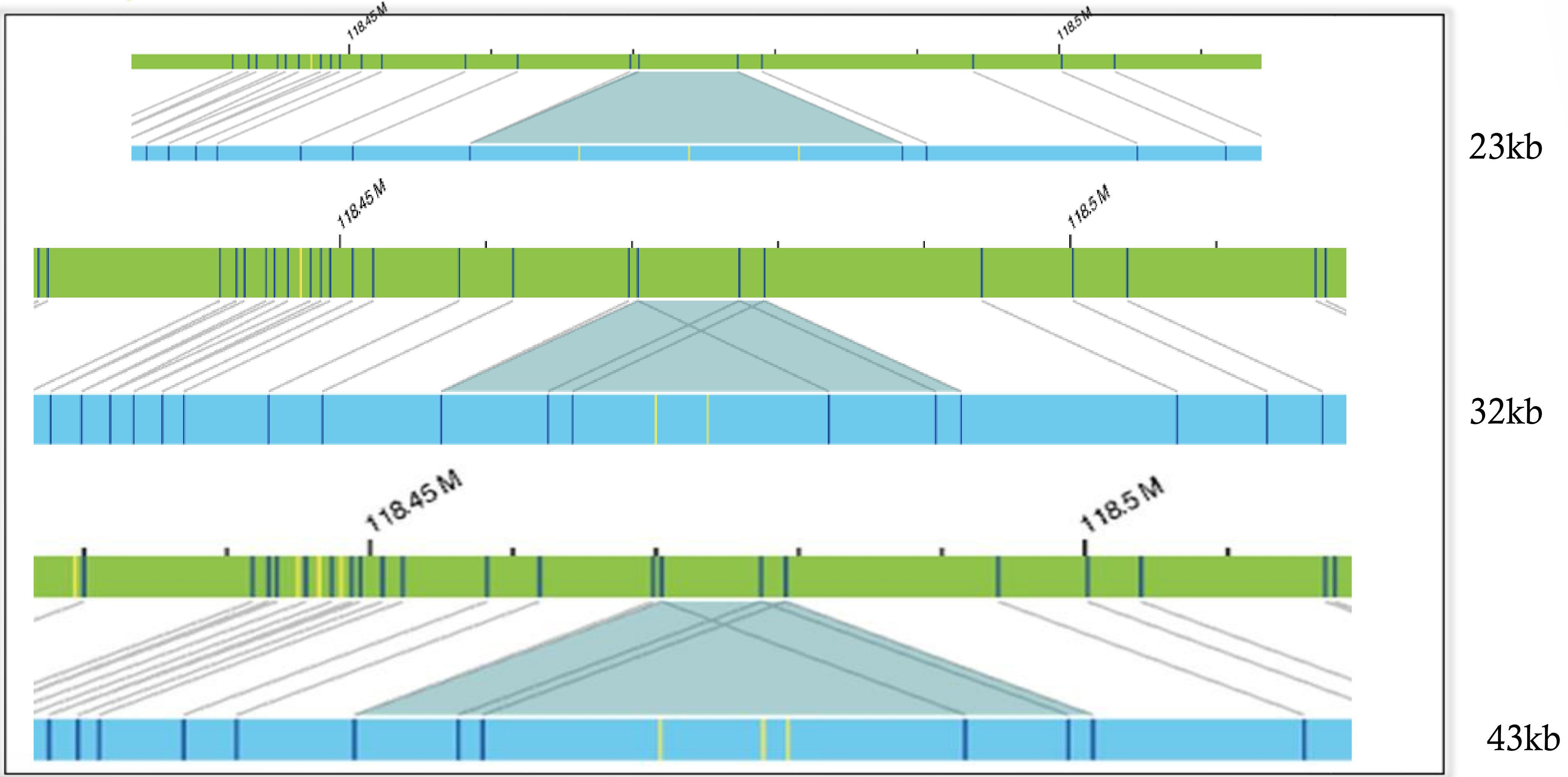




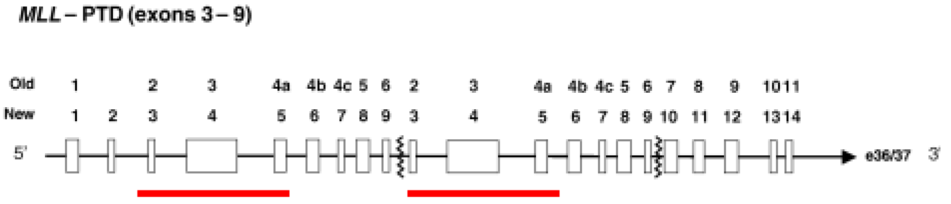
OGM PTD Patterns Observed

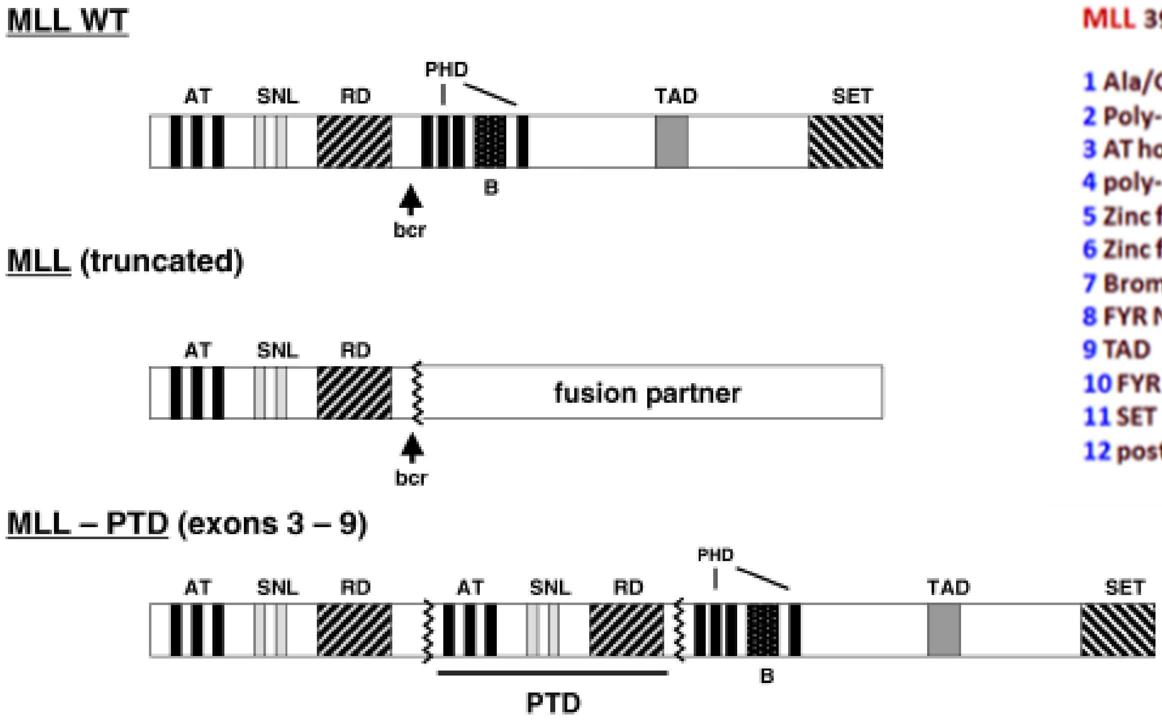
KMT2A Exons

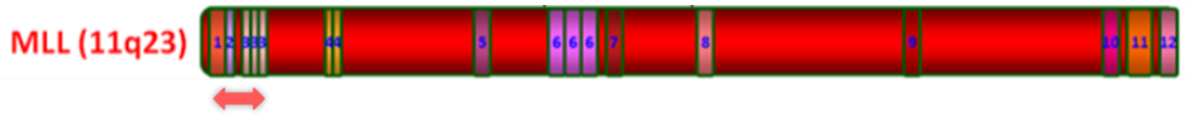




Exons 3 to 5 preserved in KMT2A-PTD ?

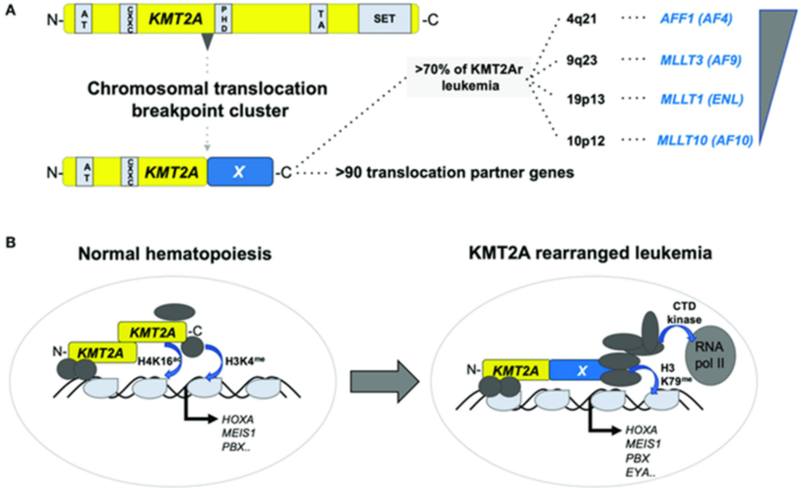




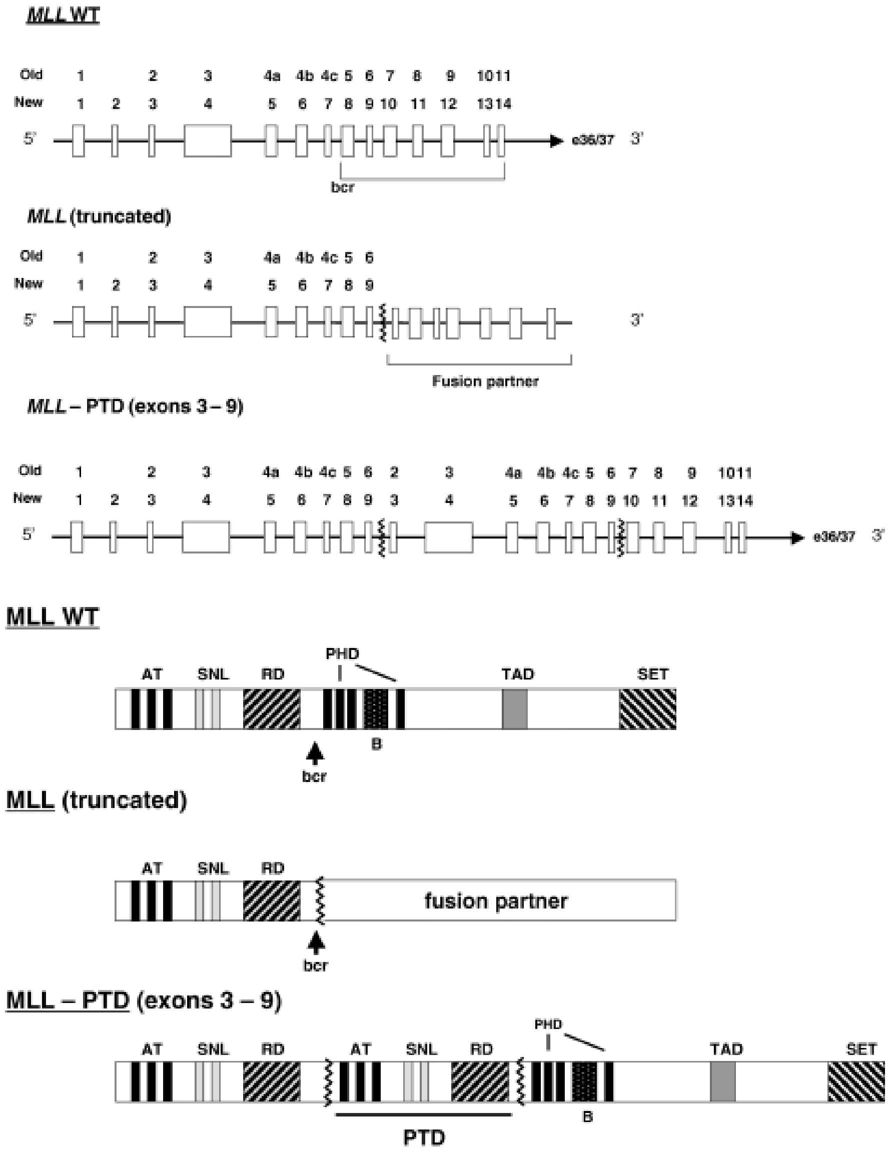


MLL 3969 amino acids

1 Ala/Gly/Ser-rich
2 Poly-Gly
3 AT hooks DNA binding
4 poly-Pro
5 Zinc finger CXXC-type
6 Zinc fingers PHD-type
7 Bromo domain
8 FYR N-term
9 TAD
10 FYR C-term
11 SET
12 post-SET



Basecke et al. 2006, PMID:16965385
Mercher et al. 2019, PMID: 31681706



- How to report the following PTD?
  - PTD exon 1
  - PTD exon 3
  - PTD exon 5-11
  - PTD exon 8


MERCI
THANK YOU