Frequent KIT Mutations in Human Gastrointestinal Stromal Tumors

Zhi Xu[1#], Xinying Huo[1#], Chuanning Tang[2], Hua Ye[2], Vijayalakshmi Nandakumar[3], Feng Lou[2], Dandan Zhang[2], Shouwen Jiang[2], Hong Sun[2], Haichao Dong[2],Guangchun Zhang [2], Zhiyuan Liu[2], Zhishou Dong[2], Baishuai Guo[2], He Yan[2], Chaowei Yan[2], Lu Wang[2], Ziyi Su[2], Yangyang Li[2], Dongying Gu[1], Xiaojing Zhang[1], Xiaomin Wu[1], Xiaowei Wei[1], Lingzhi Hong[1], Yangmei Zhang[1], Jinsong Yang[1], Yonglin Gong[1], Cuiju Tang[1], Lindsey Jones[3], Xue F Huang[3], Si-Yi Chen[3]*, Jinfei Chen[1]*

[1]Department of Oncology, Nanjing First Hospital,
Nanjing Medical University, Nanjing 210006, China.

[2]San Valley Biotechnology Inc., Beijing 100044, China

[3]Norris Comprehensive Cancer Center,
Department of Molecular Microbiology and Immunology, Keck School of Medicine,
University of Southern California, Los Angeles, CA 90033, USA.

[#]These two authors contributed equally.

*Corresponding Authors:

Jinfei Chen, MD, Department of Oncology, Nanjing First Hospital, Nanjing Medical University, 68 Changle Road, Nanjing, 210006, P. R. China; Fax: +86 25 87726234; Tel: +86 25 87726242; Email: jinfeichen@sohu.com.

Dr. Si-Yi Chen, MD., Ph.D., Norris Comprehensive Cancer Center, Department of Molecular Microbiology and Immunology, Keck School of Medicine, University of Southern California, Los Angeles, CA 90033, USA. E-mail: siyichen@usc.edu; Tel: 323 442 7727; Fax: 323 442 7729.

Supplemental Figure Legend

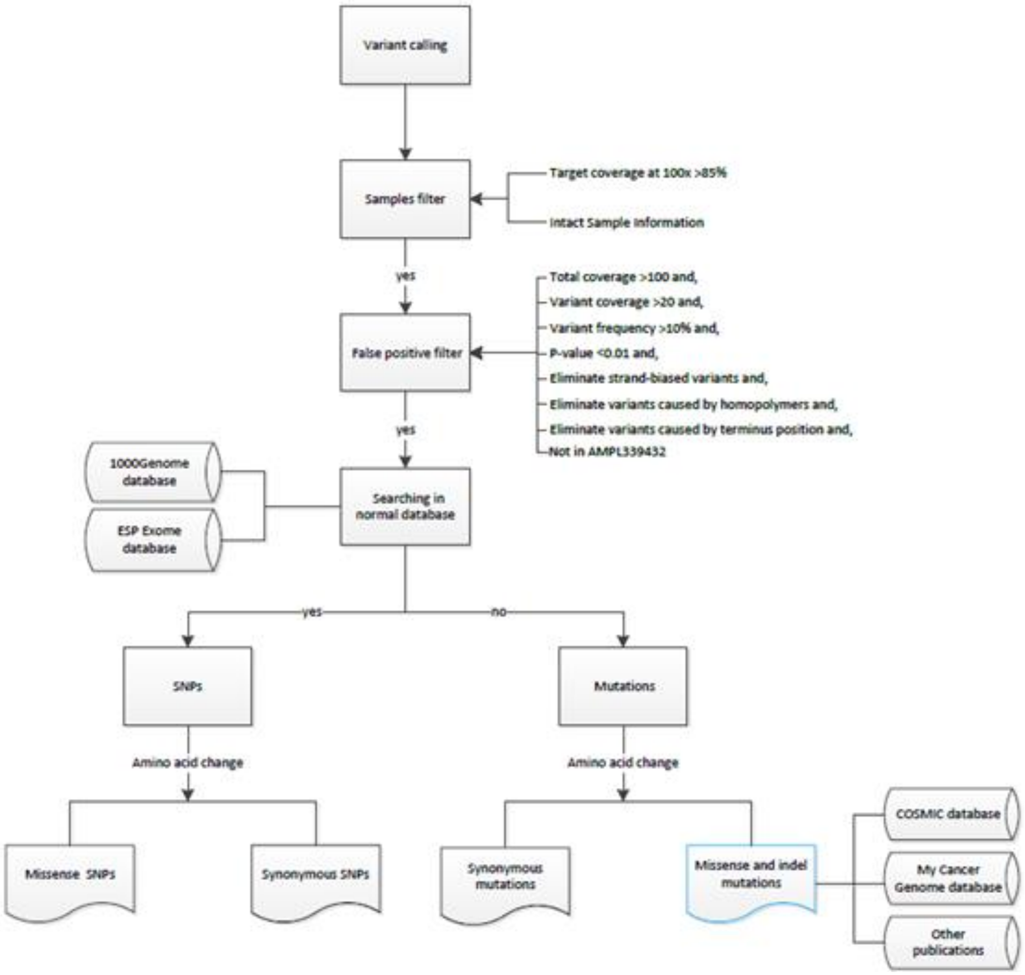
Supplementary Fig. 1. Filter process of variants. Note: (a) Strand-biased variants were eliminated using Integrative Genomics Viewer (IGV) software (http//www.broadinstitute.org/igv); (b) Variants in AMPL339432 should be eliminated, because this amplicon is not uniquely matched to PIK3CA in the human genome; (c) All of our statistical analysis was based on the data in blue box (Missense and in/del mutations).

Supplementary Fig. 2. Sequence read distribution across 189 amplicons generated from 121 FFPE specimens, normalized to 300,000 reads per sample.
A. Distribution of average coverage of each amplicon. Data are showed as mean ±SD.
B. Number of amplicons with a given read depth, sorted in bins of 100 reads. (blue bars represent number of target amplicons within read depth, red line represents % of target amplicons ≥ read depth).
Supplementary Table 1. Mutations (including Missense point mutations/deletion/insertion) frequencies in 45 genes (737 loci) in gastric and intestinal GISTs.

Supplementary Table 2. Frequencies of point mutations, insertion, and deletion mutations in 737 loci of 45 genes in 121 GISTs.

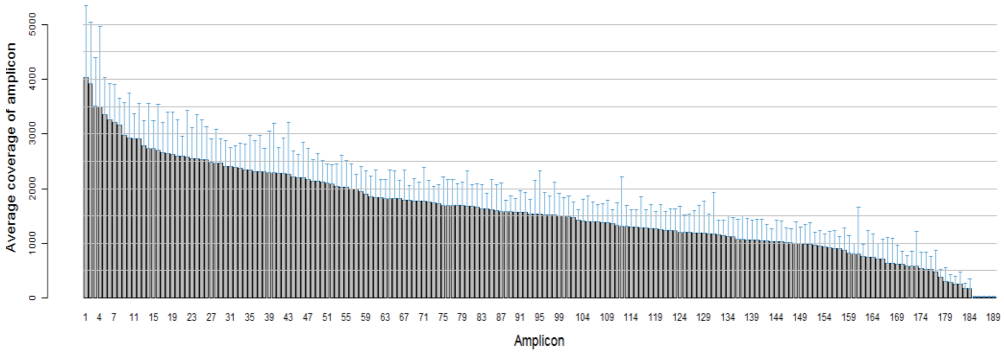
Supplementary Table 3. Validation by Sanger sequencing
Supplementary Table 4. Patients' characteristics for 121 GIST samples

Supplementary Fig.1. Filter process of variants

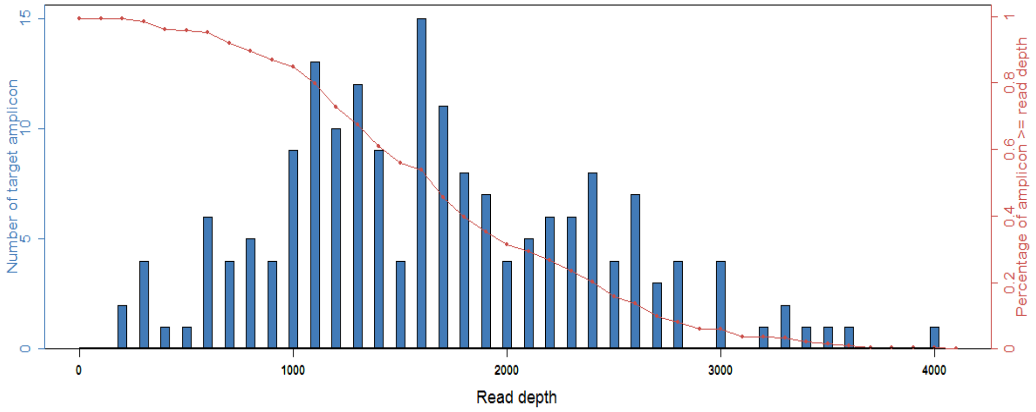


Supplementary Figure2

A



B



Supplementary Table 1. Mutations (including Missense point mutations/deletion/insertion) frequencies in 45 genes (737 loci) in gastric and intestinal GISTs.

Genes	Number of samples with mutations (Mutation frequency in 121 samples)	Number of gastric samples with mutations (Mutation frequency in 76 gastric samples)	Number of intestinal samples with mutations (Mutation frequency in 45 intestinal samples)
ABL1	0(0.0%)	0(0.0%)	0(0.0%)
AKT1	0(0.0%)	0(0.0%)	0(0.0%)
ALK	0(0.0%)	0(0.0%)	0(0.0%)
APC	0(0.0%)	0(0.0%)	0(0.0%)
ATM	0(0.0%)	0(0.0%)	0(0.0%)
BRAF	1(0.8%)	1(1.3%)	0(0.0%)
CDH1	1(0.8%)	0(0.0%)	1(2.2%)
CDKN2A	0(0.0%)	0(0.0%)	0(0.0%)
CSF1R	0(0.0%)	0(0.0%)	0(0.0%)
CTNNB1	0(0.0%)	0(0.0%)	0(0.0%)
EGFR	1(0.8%)	0(0.0%)	1(2.2%)
ERBB2	0(0.0%)	0(0.0%)	0(0.0%)
ERBB4	1(0.8%)	1(1.3%)	0(0.0%)
FBXW7	0(0.0%)	0(0.0%)	0(0.0%)
FGFR1	0(0.0%)	0(0.0%)	0(0.0%)
FGFR2	0(0.0%)	0(0.0%)	0(0.0%)
FGFR3	0(0.0%)	0(0.0%)	0(0.0%)
FLT3	3(2.5%)	3(3.9%)	0(0.0%)
GNAS	0(0.0%)	0(0.0%)	0(0.0%)
HNF1A	0(0.0%)	0(0.0%)	0(0.0%)
HRAS	0(0.0%)	0(0.0%)	0(0.0%)
IDH1	0(0.0%)	0(0.0%)	0(0.0%)
JAK3	0(0.0%)	0(0.0%)	0(0.0%)
KDR	0(0.0%)	0(0.0%)	0(0.0%)
KIT	60(49.6%)	41(53.9%)	19(42.2%)
KRAS	1(0.8%)	1(1.3%)	0(0.0%)
MET	0(0.0%)	0(0.0%)	0(0.0%)
MLH1	0(0.0%)	0(0.0%)	0(0.0%)
MPL	0(0.0%)	0(0.0%)	0(0.0%)
NOTCH1	0(0.0%)	0(0.0%)	0(0.0%)
NPM1	0(0.0%)	0(0.0%)	0(0.0%)
NRAS	0(0.0%)	0(0.0%)	0(0.0%)
PDGFRA	1(0.8%)	1(1.3%)	0(0.0%)
PIK3CA	0(0.0%)	0(0.0%)	0(0.0%)
PTEN	1(0.8%)	0(0.0%)	1(2.2%)
PTPN11	0(0.0%)	0(0.0%)	0(0.0%)
RB1	0(0.0%)	0(0.0%)	0(0.0%)
RET	0(0.0%)	0(0.0%)	0(0.0%)
SMAD4	0(0.0%)	0(0.0%)	0(0.0%)
SMARCB1	0(0.0%)	0(0.0%)	0(0.0%)
SMO	0(0.0%)	0(0.0%)	0(0.0%)
SRC	0(0.0%)	0(0.0%)	0(0.0%)
STK11	0(0.0%)	0(0.0%)	0(0.0%)
TP53	1(0.8%)	1(1.3%)	0(0.0%)
VHL	1(0.8%)	0(0.0%)	1(2.2%)

Supplementary Table 2. Frequencies of point mutations, insertion and delection mutations in 737 loci of 45 genes in 121 GISTs.

Gene Mutations	Number of samples with this mutation site	Number of samples with this mutation gene	Mutation Frequency	Gene mutation frequency in publications	Site mutation frequency in gene in publications	If reported in GIST in COSMIC database	If reported in GIST in MyCancerGenom
BRAFc.1799T>A	1	1	0.8%	<1% (mycancergenome)	-	YES	YES
KITc.1667_1672del6;c.1669_1674del6	7	33	27.3%	~85%（mycancergenome）	~85%*~70% (11 exon)（mycancergenome）	YES	NO
KITc.1679T>A	4					YES	NO
KITc.1675_1677delGTT;c.1678_1680delGTT;c.1679_1681delTTG	3					YES	NO
KITc.1669T>C	5					YES	NO
KITc.1674_1676AGGT>G	1					NO	NO
KITc.1672_1680del9	1					YES	NO
KITc.1675_1683del9;c.1676_1684del9;c.1673_1681delAGGTTGTTG	2					YES	NO
KITc.2466T>A	2					YES	NO
KITc.1694_1717GAAACAATTATGTTTACATAGACC>CCT	1					NO	NO
KITc.1676T>A	4					YES	NO
KITc.1676T>G	2					YES	NO
KITc.1660_1674del15;c.1661_1675del15	1					YES	NO
KITc.1670_1674TGGAAG>ACT	1					NO	NO

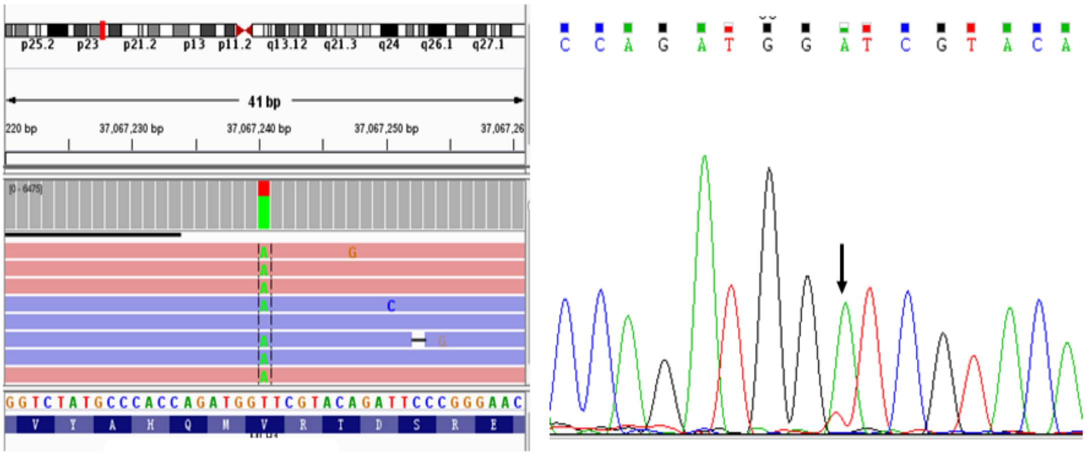
KITc.1735_1737delGAT;c.1733_1735delATG	5					NO	NO
KITc.1679T>G	2					YES	NO
KITc.1727T>C	2					YES	NO
KITc.2464A>T	1					YES	NO
KITc.1728_1729insCCTTATGATCACAAA	1					NO	NO
KITc.1668_1721del54	1					YES	NO
KITc.1667_1673CAGTGGAA>CCCAC	1					YES	NO
KITc.1676T>C	1					YES	NO
KITc.1669T>A	2					YES	NO
KITc.1679_1680TT>AG	1					YES	NO
KITc.1669T>G	1					YES	NO
KITc.1669_1710del42	1					YES	NO
KITc.1653_1676del24	1					YES	NO
KITc.1670_1678del9	1					YES	NO
KITc.1689_1733del45	1					NO	NO
KITc.1670_1675del6	1					YES	NO
KITc.2153C>G	1					NO	NO
KITc.1673_1693del21	1					YES	NO
KITc.1671_1672GA>TG	1					NO	NO
KITc.1667_1673>CC	1					YES	NO
KRASc.34G>C	1	1	0.8%	~5%[4]	-	NO	NO
PDGFRAc.2525A>T	1	1	0.8%	~5%(mycancergenome)	~5%*~5%(18 exon)（mycancergenome）	YES	NO

Supplementary Table 3. Sanger results

#	Sample ID	Cosmic ID	Gene	CDS change	Amino acid change	Variant frequency (%)	Sanger result Consistent
1	13	26085	MLH1	c.1151T>A	p.V384D	66.81	YES
2	95	26085	MLH1	c.1151T>A	p.V384D	46.96	YES
3	25	28026	KIT	c.1621A>C	p.M541L	50.38	YES
4	31	--------	KIT	c.1653_1676del24	p.551del_frameshift	50.89	YES
5	112	1210; 1217	KIT	c.1667_1672del6;c.1669_1674del6	p.W557_K558del	45.39	YES
6	95	1210;1217	KIT	c.1667_1672del6;c.1669_1674del6	p.W557_K558del	35.82	YES
7	76	---------	KIT	c.1670_1674TGGAAG>ACT	p.W557_K558>T	39.18	YES
8	109	---------	KIT	c.1667_1673CAGTGGAA>CCCAC	p.Q556_K558>PT	33.01	YES
9	76	---------	KIT	c.1670_1674TGGAAG>ACT	p.W557_K558>T	39.18	YES
10	25	1221	KIT	c.1669T>G	p.W557G	36.7	YES
11	27	---------	KIT	c.1674_1676AGGT>G	p.K558_V559>S	86.18	YES
12	101	1240	KIT	c.1673_1693del21	p.558del_frameshift	75.45	YES
13	107	1250;1254	KIT	c.1675_1683del9;c.1676_1684del9;c.1673_1681delAGGTTGTTG	p.V559_E561del	48.06	YES
14	106	1252	KIT	c.1676T>A	p.V559D	42.72	YES
15	91	1257	KIT	c.1679T>A	p.V560D	37.31	YES
16	90	1290	KIT	c.1727T>C	p.L576P	51.25	YES
17	107	1290	KIT	c.1727T>C	p.L576P	42.21	YES
18	111	1294;255168	KIT	c.1735_1737delGAT;c.1733_1735delATG	p.D579del	59.83	YES
19	92	19109	KIT	c.2464A>T	p.N822Y	27.88	YES
20	49	1321	KIT	c.2466T>A	p.N822K	27.55	YES
21	106	710	MET	c.1124A>G	p.N375S	50.73	YES

Sanger figures

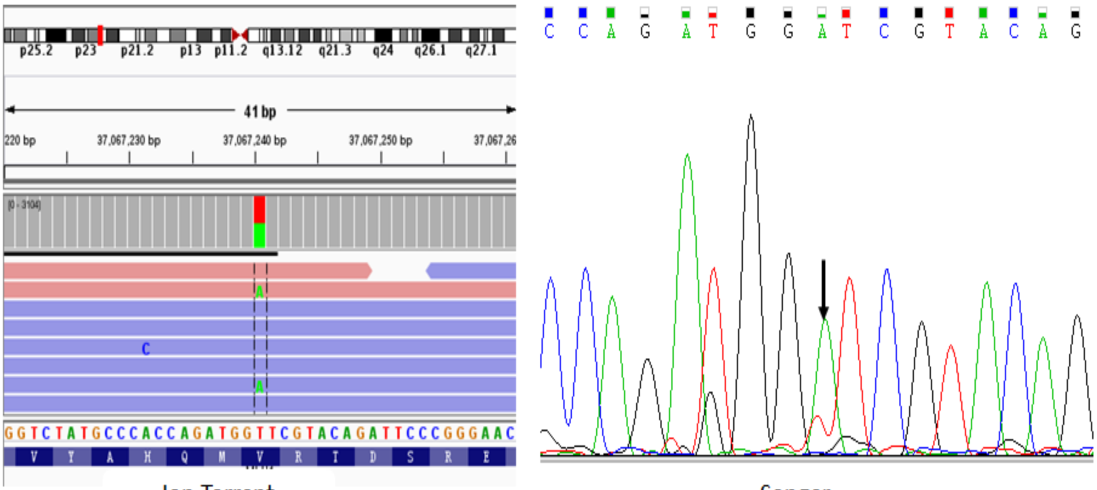
#1



Ion Torrent

Sanger

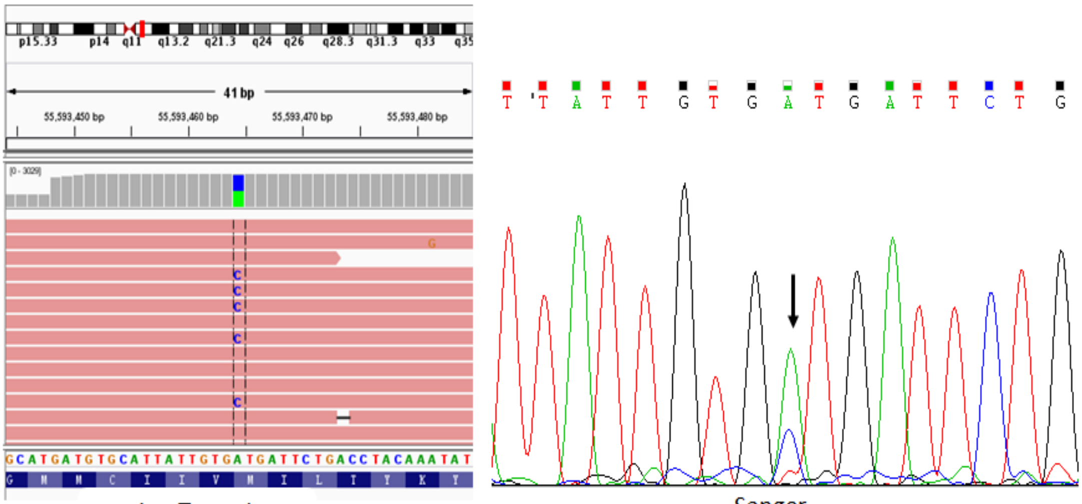
#2



Ion Torrent

Sanger

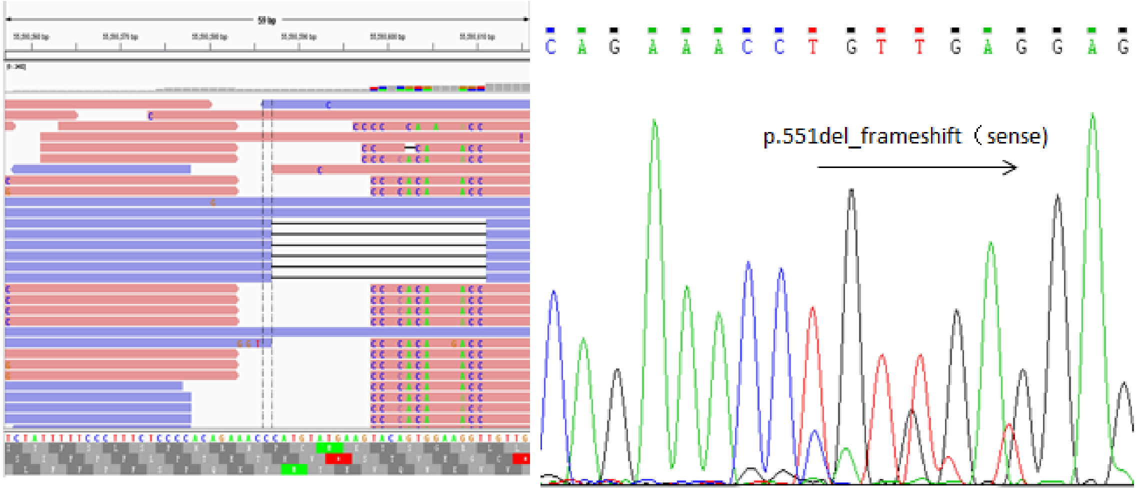
#3



Ion Torrent

Sanger

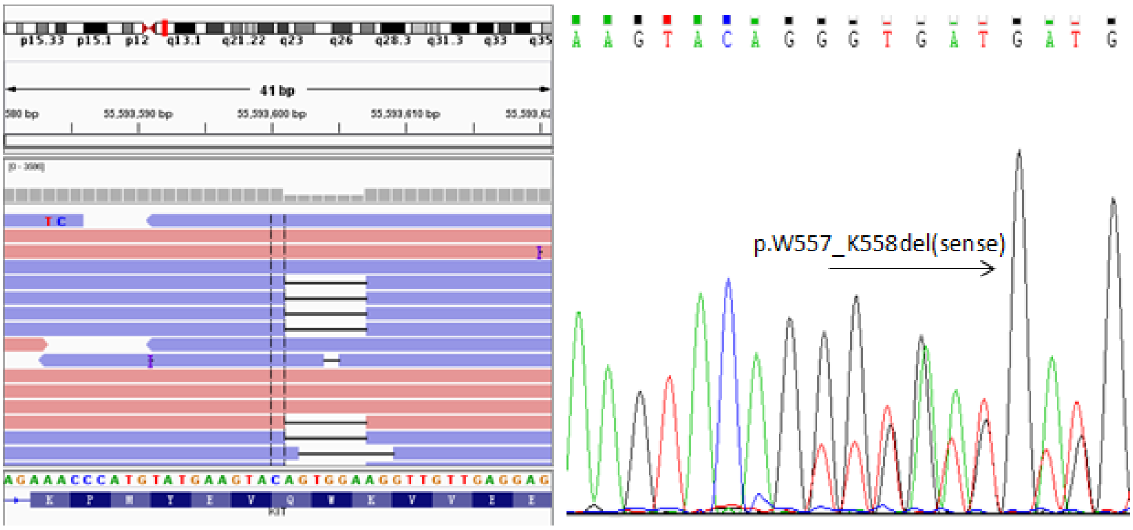
#4



Ion Torrent

Sanger

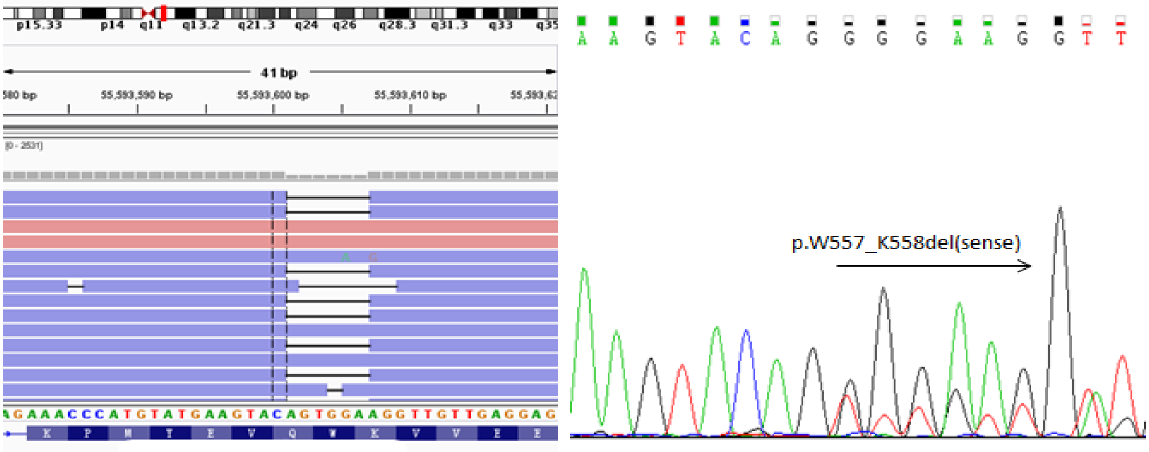
#5



Ion Torrent

Sanger

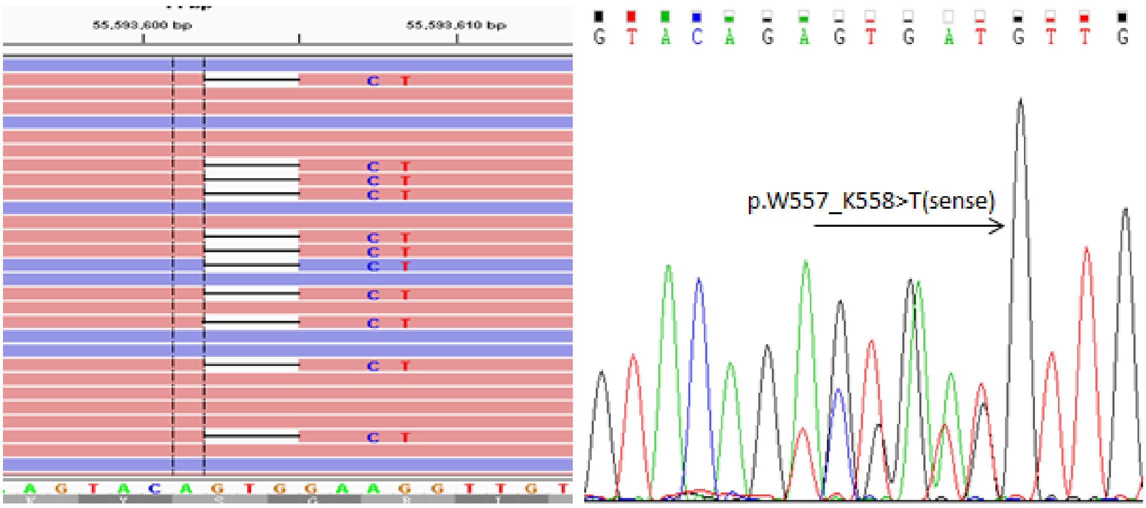
#6



Ion Torrent

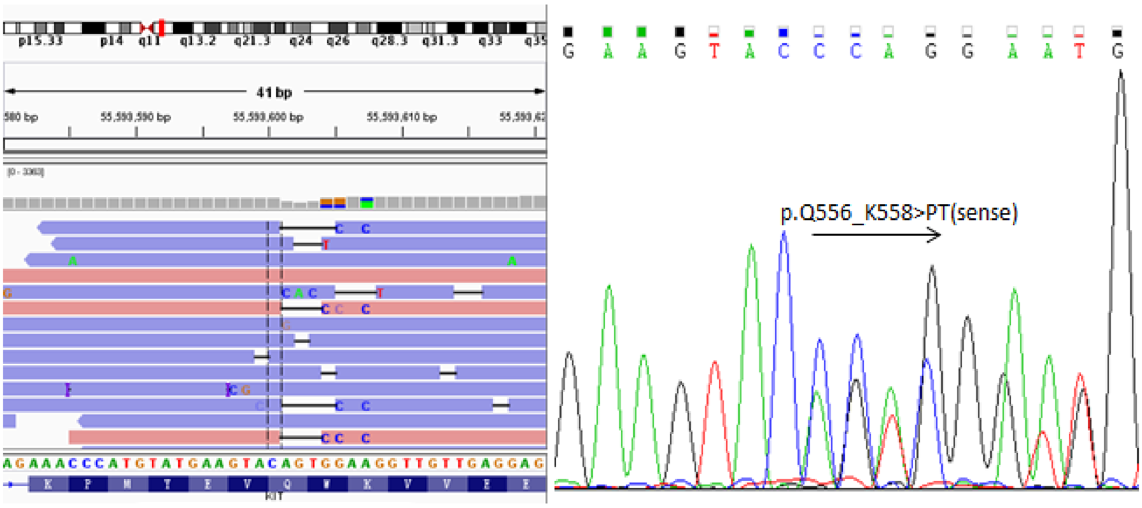
Sanger

#7



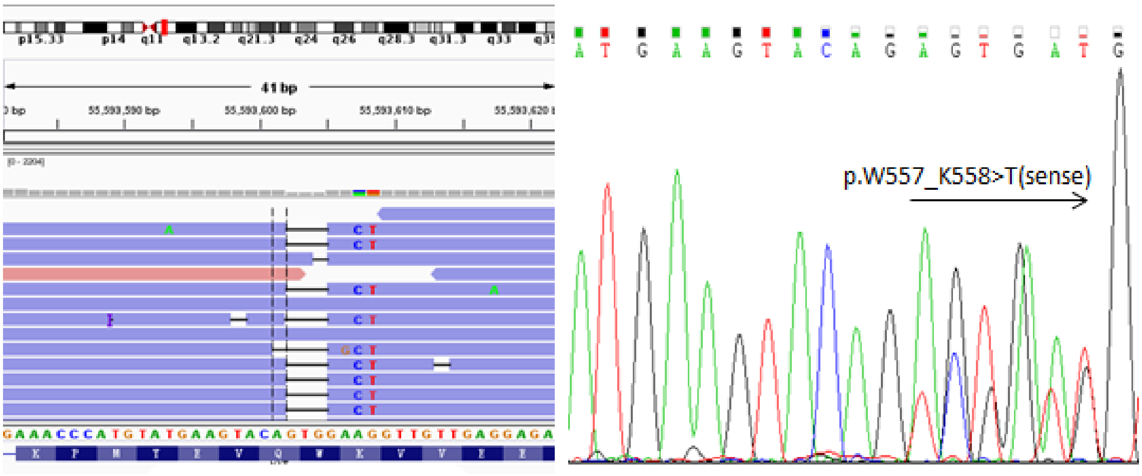
Ion Torrent Sanger

#8



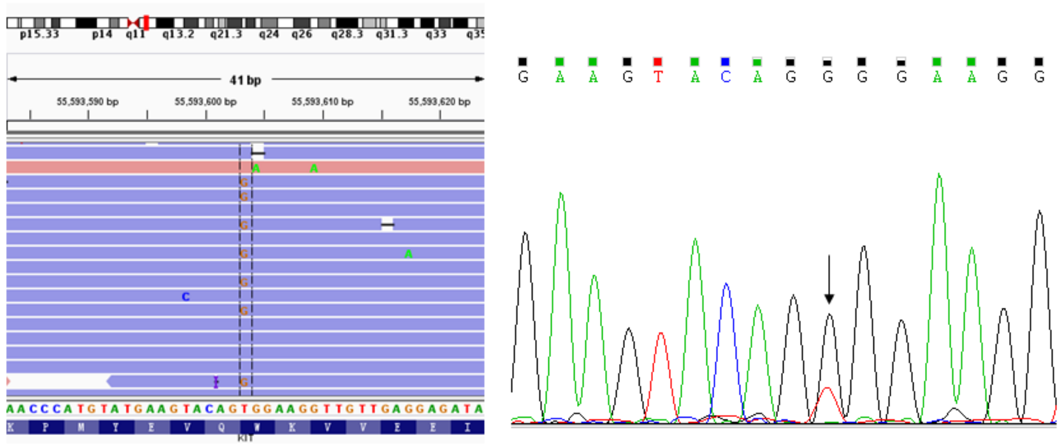
Ion Torrent Sanger

#9



Ion Torrent Sanger

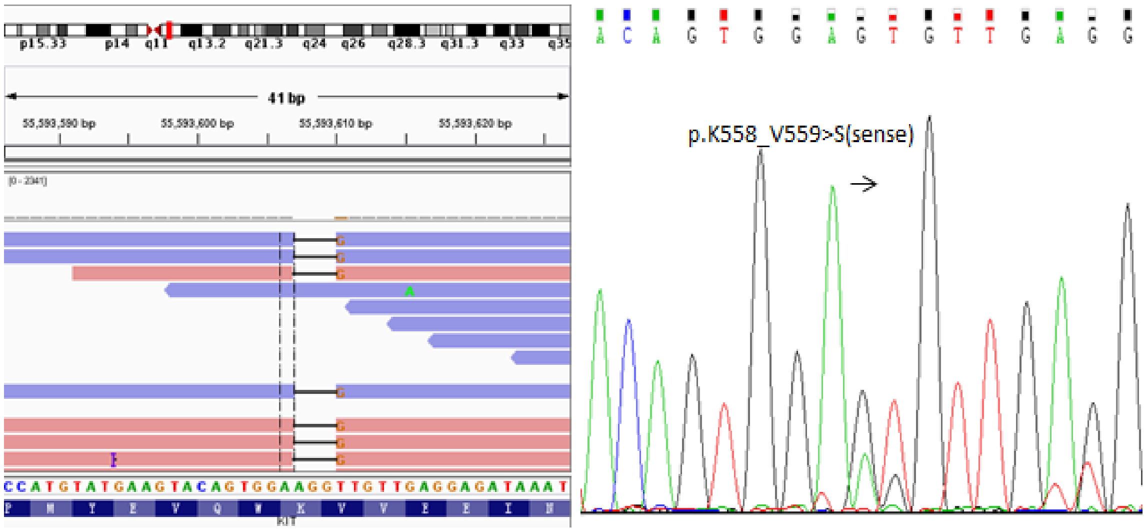
#10



Ion Torrent

Sanger

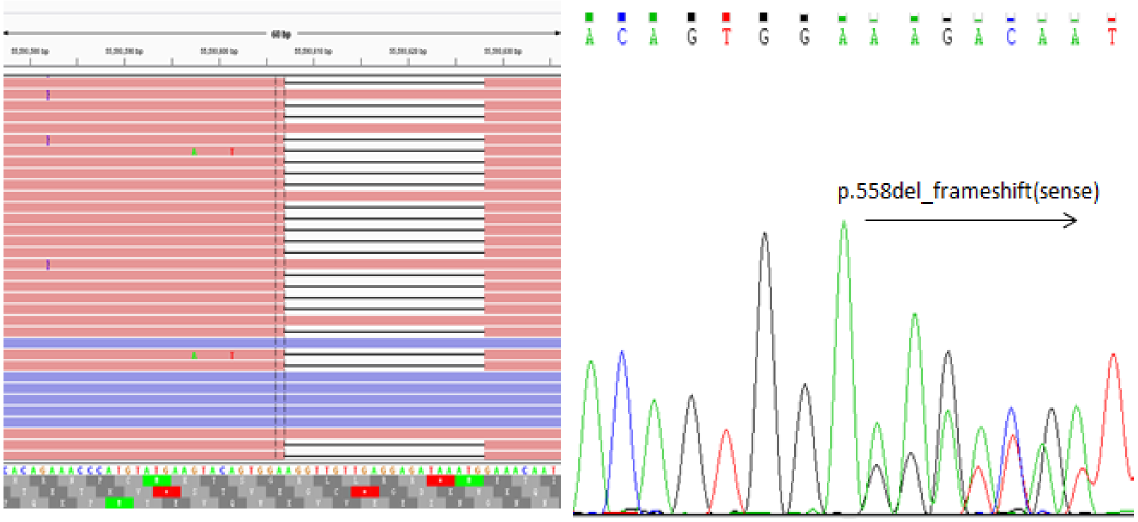
#11



Ion Torrent

Sanger

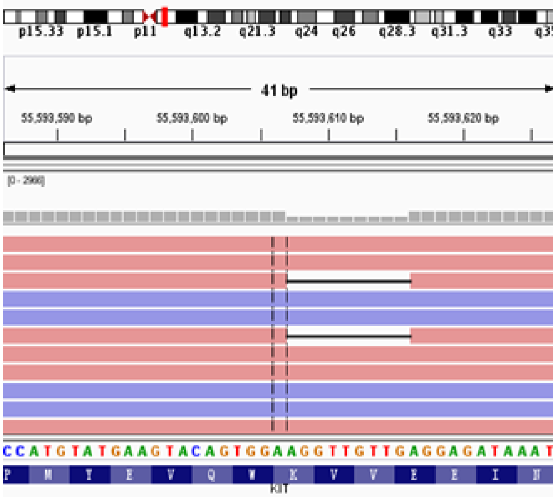
#12



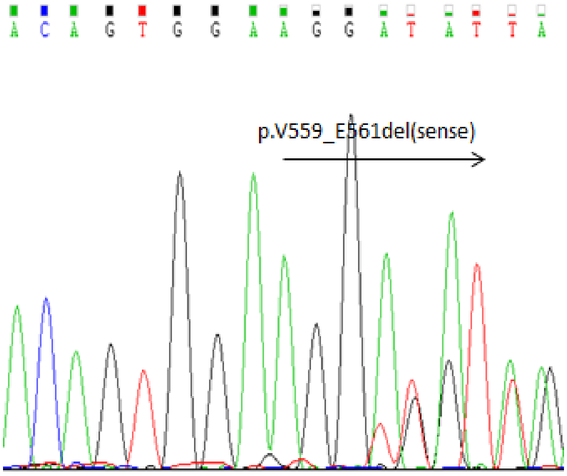
Ion Torrent

Sanger

#13

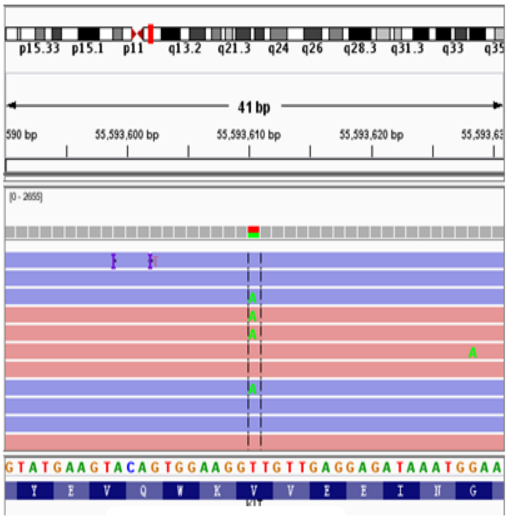


Ion Torrent

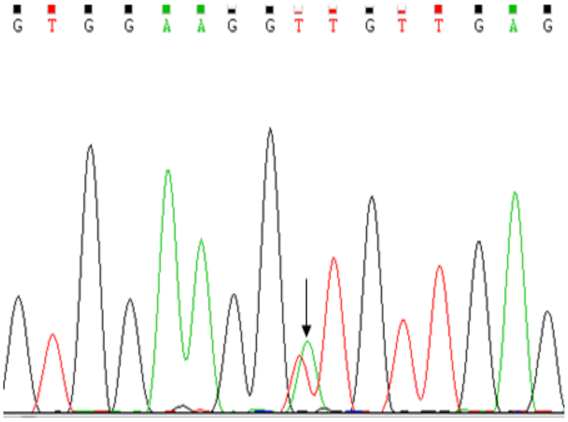


Sanger

#14

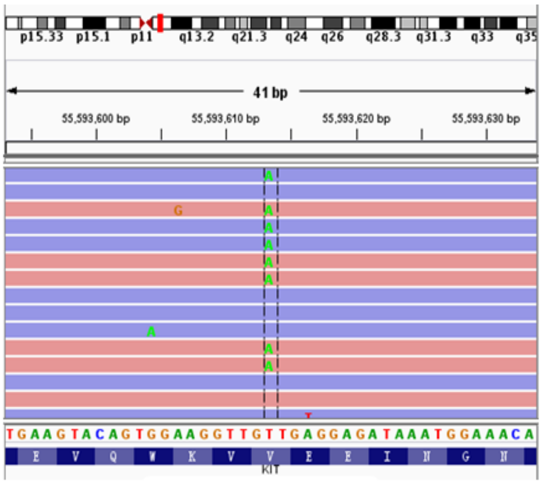


Ion Torrent

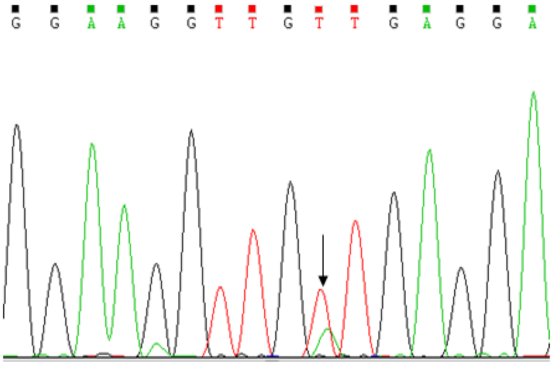


Sanger

#15

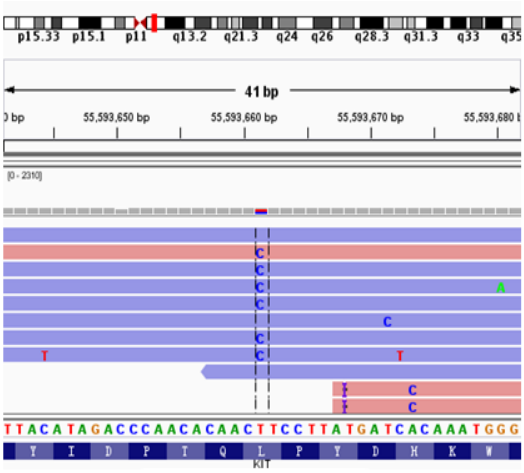


Ion Torrent

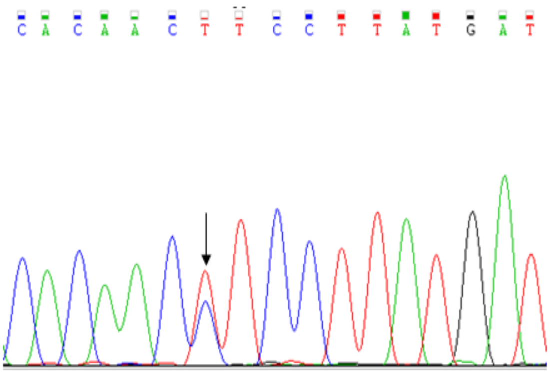


Sanger

#16



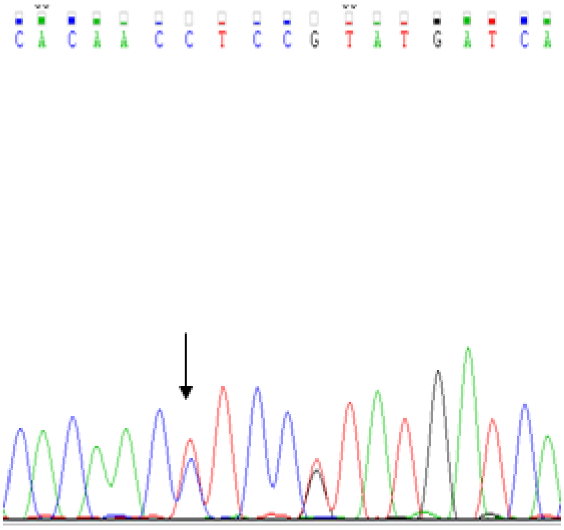
Ion Torrent



Sanger

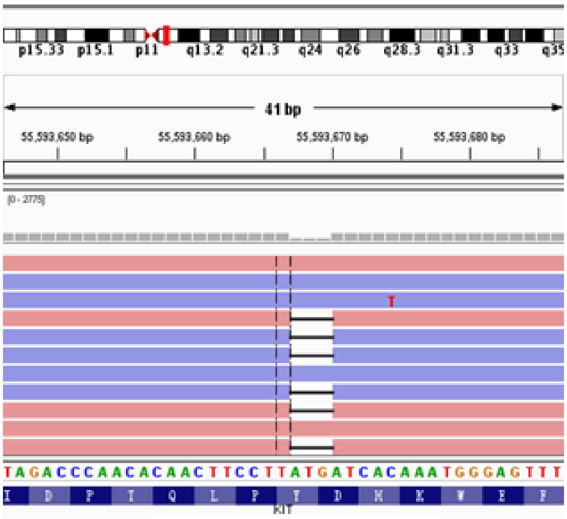
#17

Ion Torrent

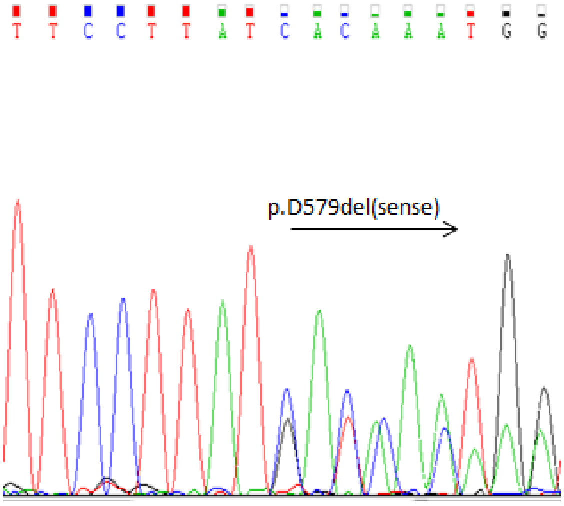


Sanger

#18

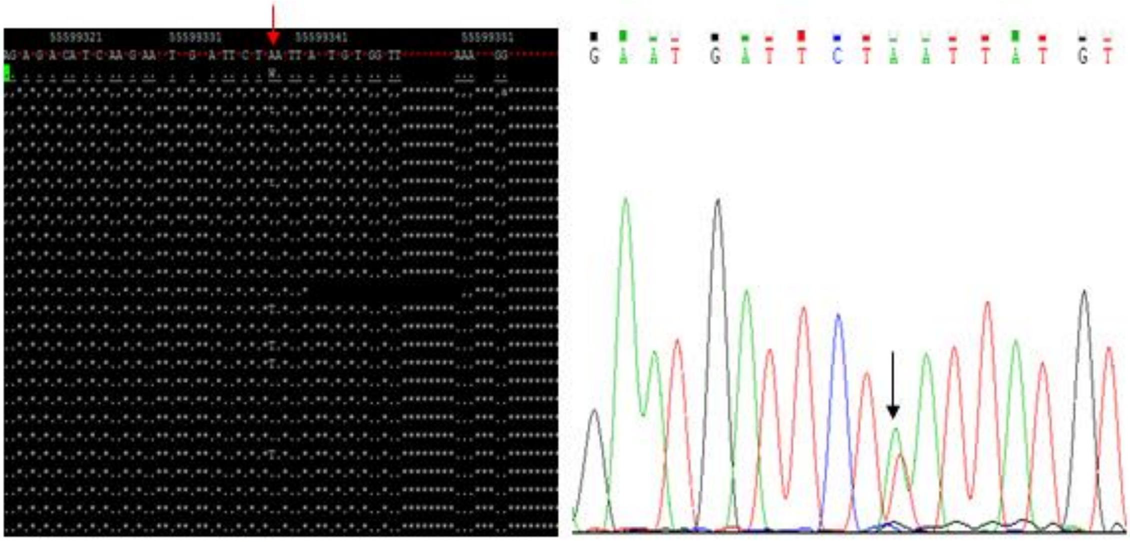


Ion Torrent



Sanger

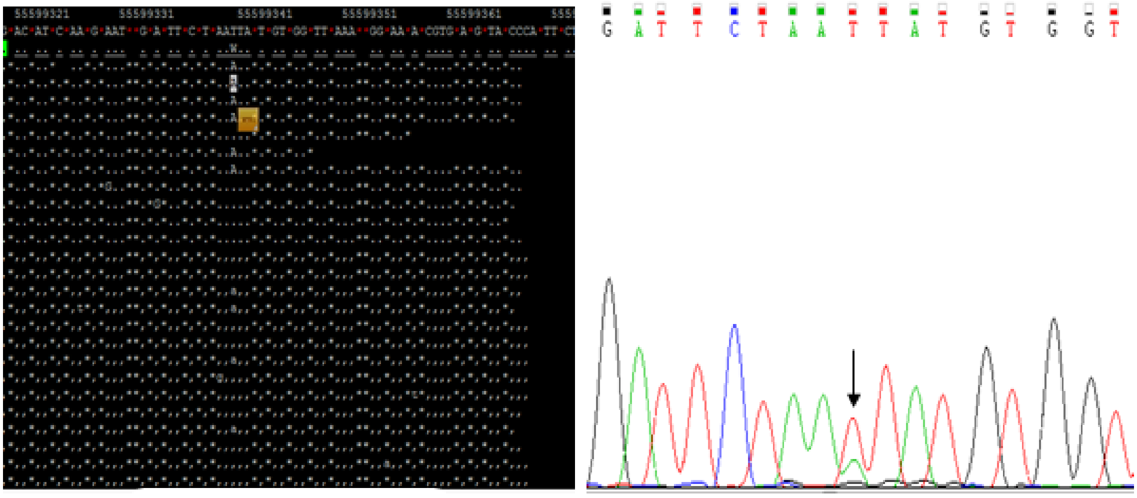
#19



Ion Torrent

Sanger

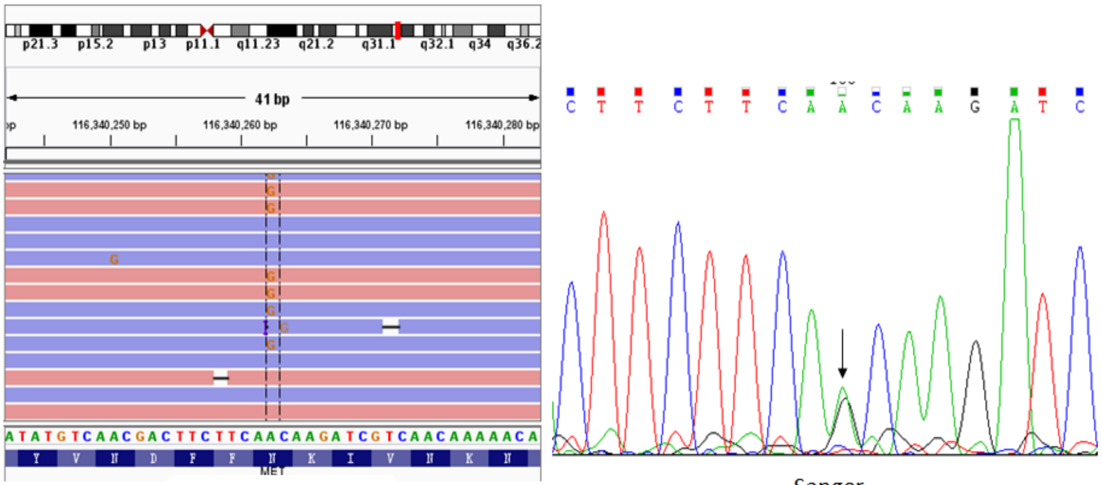
#20



Ion Torrent

Sanger

#21



Ion Torrent

Sanger

Supplementary Table 5. Clinical features of 121 GIST patients.

Characteristic		#
Gender	female	59(48.8%)
	male	62(51.2%)
Age	Average 59±11	Median:59, range:24-82
Histology	gastric	76(62.8%)
	intestinal	45(37.2%)
CD117	CD117-	3(2.5%)
	CD117+	17(14.0%)
	CD117++	23(19.0%)
	CD117+++	21(17.4%)
	unknown	57(47.1%)
CD34	CD34-	12(9.9%)
	CD34+	20(16.5%)
	CD34++	16(13.2%)
	CD34+++	17(14.0%)
	unknown	56(46.3%)