*Supplementary information*

# Comprehensive Custom NGS Panel Validation for the Improvement of the Stratification of B-Acute Lymphoblastic Leukemia Patients

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**Table S1. Genetic characteristics of the patients.** This table shows the genetic characteristics of the B-ALL patients included in the study. In the first column the genetic subtype, followed by the patient identifier, the karyotype, the FISH, the findings observed by MLPA and changes greater than 5Mb detected by aCGH. Not done or Not data (ND), Male (M), Female (F).

| Genetic subtype | ID | Sex/ Age (years) | Karyotype | FISH | MLPA | aCGH >5Mb |
|---|---|---|---|---|---|---|
| ***BCR/ABL1*** | ID1 | M/70 | Karyotype failure | LSI BCR/ABL1 fusion (54%) | *IKZF1* | +19 |
| | ID2 | M/76 | 55-60,XY,+Y,+4,+5,+7,+8,t(9;22)(q34;q11),+9,+11,add(12)(p12)[15] | LSI BCR/ABL1 Clonal - 9q34/ABL1 gain (89%), BCR/ABL1 fusion (89%), LSI MLL-r clonal - 11q23/MLL gain (28%) | ND | +4, +18, +X, -19 |
| | ID3 | M/34 | 46,XY,t(9;22)(q34;q11)[7]/46,XY[5] | ND | *IKZF1* | +17, +X |
| | ID4 | M/50 | 46,XY,t(9;22)(q14;q11)[10]/46,XY[5] | LSI BCR/ABL1 fusion (97%) | ND | ND |
| | ID5 | M/47 | 46,XY,t(9;22)(q34;q11)[7]/47,XY,+8,t(9;22)(q34;q11)[2]/46,XY[5] | LSI BCR/ABL1 fusion (65%) | Normal | +8 |
| | ID6 | M/ND | 46,XY[6] | LSI BCR/ABL1 fusion (82.5%) | *IKZF1, CDKN2A, PAX5* | -9 |
| | ID7 | M/70 | 46,XY,t(9;22)(q34;q11)[8]/46,XY[2] | LSI BCR/ABL1 fusion (87%) | *CDKN2A* | Normal |
| | ID8 | F/72 | 46,XX,t(9;22)(q34;q11)[2]/46,XX[10] | LSI BCR/ABL1 fusion (26%) | ND | ND |
| | ID9 | M/70 | 46,XY,t(9;22)(q34;q11)[13]/46,XY[8] | LSI BCR/ABL1 fusion (93%) | *IKZF1* | Normal |
| | ID10 | F/45 | Karyotype failure | LSI BCR/ABL1 fusion (98%) | *BTG1* | +2, +4, +6, +10, +14, +21, -12p |
| ***ETV6/RUNX1*** | ID11 | M/14 | 46,XY[15] | ETV6/RUNX1 fusion (80%), LSI 12p13/ETV6 gain (33%), 21q22/RUNX1 gain (76%), LSI 11q23/MLL gain (26%), LSI 9q34/ABL1 gain (48%), 22q11.2/BCR gain (60%) | *CDKN2A, PARP1* | +21 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | ID12 | F/3 | 46,XX[12] | ETV6/RUNX1 fusion (12%), LSI - 21q22/RUNX1 gain (63%) | *PAX5, ETV6* | +21 |
| | ID13 | F/5 | Karyotype failure | LSI ETV6/RUNX1 fusion (81%) | *PARP1* | -X |
| | ID14 | M/3 | 46,XY[10] | LSI ETV6/RUNX1 fusion (95,5%) | Normal | Normal |
| | ID15 | M/2 | 48,XY,+11,+17[3]/44,XY,-13,-15[2]/46,XY[14] | Normal. ETV6/RUNX1 fusion positive by RT-PCR | *ETV6* | +X, -12p |
| | ID16 | M/2 | 46,XY[10] | LSI ETV6/RUNX1 fusion (94%) | Normal | Normal |
| | ID17 | F/5 | 46,XX[10] | LSI ETV6/RUNX1 fusion (96%) | *BTG1, ETV6* | +19 |
| | ID18 | M/2 | Karyotype failure | LSI ETV6/RUNX1 fusion (90%) | ND | ND |
| | ID19 | F/15 | 47,XX,+21c[15]/46,XX[3] | LSI ETV6/RUNX1 fusion (88,5%), LSI 21q22/RUNX1 gain (100%) | Normal | Normal |
| | ID20 | F/14 | Karyotype failure | LSI ETV6/RUNX1 fusion (96%) | *PAX5, ETV6* | +21, -6q |
| ***MLLr*** | ID21 | F/47 | 46,XX,t(4;11)(q21;q23)[14] | Normal | Normal | Normal |
| | ID22 | M/46 | Karyotype failure | LSI MLL-r (95,5%) | Normal | Normal |
| | ID23 | F/0 | 46,XX,inv(11)(q12q23),del(12)(p13)[7]/46,XX[13] | LSI MLL-r (93%) | Normal | Normal |
| | ID24 | M/60 | Karyotype failure | LSI MLL-r (73%) | *PARP1* | +6 |
| | ID25 | F/31 | 46,XX,t(4;11)(q21;q23)[10] | Normal | Normal | Normal |
| | ID26 | M/16 | 46,XY,t(4;11)(q21;q23)[7]/46,XY[3] | LSI MLL-r (83%) | *IKZF1, PAX5* | -17, -19, -22 |
| | ID27 | M/55 | 46,XY,t(4;11)(q21;q23)[3]/46,XY[10] | LSI MLL-r (75%) | *CDKN2A* | Normal |
| | ID28 | F/0 | 47,XX,t(4;11)(q21;q23),+mar[23] | LSI MLL-r (83%) | Normal | Normal |
| | ID29 | F/0 | 40-42,XX[6]/46,XX[6] | LSI MLL-r (18%) | Normal | Normal |
| | ID30 | M/0 | ND | LSI MLL-r (65%) | ND | ND |
| | ID31 | F/5 | 51-58,XX,+5,+6,+9,+10,+21[14]/46,XX[6] | LSI 21q22/RUNX1 gain (89%) | Normal | +4, +6, +14, +17q, +18, +21, +X, -17p |

| | | | | | | |
|---|---|---|---|---|---|---|
| **High hyperdiploid** | ID32 | F/4 | 48-52,XX,+2,+6,+8,+10,+12,+21 [12]/46,XX[3] | ND | ND | ND |
| | ID33 | F/3 | 51,XX,+21,4mar[4]/46,XX[17] | LSI 21q22/RUNX1 gain (94%), LSI 9q34/ABL1 gain (51%), LSI 11q23/MLL gain (25,5%) | Normal | +10, +18, +21 |
| | ID34 | F/4 | 50-52,XX,+10,+17,+18,+21,+mar[3]/46,XX[8] | LSI 21q22/RUNX1 gain (48.5%) | *IKZF1* | +10, +14, +21 |
| | ID35 | F/2 | Karyotype failure | LSI 12p13.2/ETV6 gain (16%), LSI 21q22.12/RUNX1 gain (55%) | Normal | +4, +5, +6, +8, +10, +14, +21, +X, -19, -20, -22 |
| | ID36 | F/47 | 46,XX[10] | LSI 2p13.2/ETV6 gain (90%), LSI 21q22.12/RUNX1 gain (90%), LSI MYC gain (62%), LSI IGH gain (60%) | Normal | +6, +8, +13, +14, +19, +21, -17q, -X |
| | ID37 | F/4 | 46,XX[10] | Normal | *IKZF1* | +1q, +4, +6, +7q, +9, +10, +14, +17, +21, -7p |
| | ID38 | F/6 | 46,XX [6]/46,XX,add(3)(q)(21)[2] | ND | *IKZF1, ETV6* | +10, +14, +17, +21 |
| | ID39 | M/16 | 46,XY [10] | Normal | Normal | +10q, +14, +17, +18, +21, +22, +X |
| | ID40 | F/4 | 60,XY,+X,+4,+5,+6,+8,+10,+10,+16,+17,+18,+21,.MOB+22,+mar[14] | ND | ND | ND |
| **Hypodiploid** | ID41 | M/34 | 39-42,XY[cp12] | Normal | Normal | Normal |
| | ID42 | ND | 39,XX,-3,-7,-13,-15,-16,-17,-20[18]/46,XX[2] | Normal | ND | ND |
| | ID43 | F/71 | 33-40,XX,add(4)(q33)[9]/46,XX[1] | ND | *CDKN2A* | Normal |
| **iAMP21** | ID44 | M/13 | Karyotype failure | LSI 21q22/RUNX1 gain (98%) | ND | ND |
| | ID45 | F/8 | Karyotype failure | LSI 21q22/RUNX1 gain (90%) | ND | ND |
| | ID46 | F/11 | Karyotype failure | LSI 21q22/RUNX1 gain (55%) | ND | ND |
| **B-Other** | ID47 | ND | 47,XX,t(X;14)(p22;q32),+der(X)t(X;14),inc[6]/47,idem,i(17)(q10)[1] | LSI CRFL2/IGH fusion (90%) | ND | ND |
| | ID48 | F/58 | 46,XX[20] | LSI CRFL2/IGH fusion (87%) | ND | ND |
| | ID49 | M/3 | Karyotype failure | LSI 21q22/RUNX1 gain (82%) | Normal | X+ |

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|---|---|---|---|---|---|---|
| | ID50 | F/6 | Karyotype failure | Normal | Normal | Normal |
| | ID51 | F/39 | 46,XX[22] | Normal | *CDKN2A* | +1q, +10p, +19p, -9p, -17p |
| | ID52 | F/7 | 46,XX[4]/46,XX,del(6)(q15q23) [6] | ND | *CDKN2A, ETV6* | -9p |
| | ID53 | M/16 | 46,XX[12] | Normal | *IKZF1* | +14 |
| | ID54 | F/10 | 46,XX[8] | Normal | *BTG1* | Normal |
| | ID55 | F/6 | 46,XX[22] | Normal | *ETV6* | -13 |
| | ID56 | M/4 | 46,XY[20] | Normal | *RB1* | Normal |
| | ID57 | M/7 | 46,XY[22] | Normal | *CDKN2A, PAX5* | -9p |
| | ID58 | F/1 | 46,XX[14] | Normal | *PAX5* | +1q |
| | ID59 | M/4 | Karyotype failure | Normal | *IKZF1, CDKN2A, PAX5* | -9p, -20q |
| | ID60 | M/61 | 46,XY[13] | Normal | *IKZF1* | ND |
| | ID61 | M/5 | 46,XY,add(12)(q22)[16]/46,XY[4] | ND | *BTG1* | Normal |
| | ID62 | F/1 | 46,XX[5]/46,XX[15] | Normal | *PAX5* | -7p |
| | ID63 | M/8 | 46,XY[11] | ND | ND | ND |
| | ID64 | F/2 | 46,XX[10] | ND | Normal | -X |
| | ID65 | F/15 | 46,XX[2]/46,XX,t(3;17)(q13;p13)[10] | ND | Normal | -X |
| | ID66 | F/6 | 46,XX[10] | Normal | Normal | Normal |
| | ID67 | M/1 | 47,XY,+21[3]/46,XY[18] | Normal | Normal | Normal |
| | ID68 | M/25 | 46,XY [17]/46,XY,del(14)(q12)[8] | Normal | *IKZF1, BTG1* | -8p |
| | ID69 | F/5 | 46,XY[8] | ND | Normal | Normal |
| | ID70 | M/5 | Karyotype failure | Normal | ND | ND |
| | ID71 | F/ND | Karyotype failure | Normal | *CDKN2A* | ND |
| | ID72 | M/1 | 46,XY[12] | ND | *IKZF1, JAK2, CDKN2A, PAX5* | -9p, -X |

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|---|---|---|---|---|---|---|
| | ID73 | M/20 | Karyotype failure | Normal | *IKZF1, CDKN2A, BTG1, ETV6* | +X |
| | ID74 | M/3 | 47,XY,+21c | ND | ND | ND |
| | ID75 | F/4 | 49,XX,+10,+19,+20[7]/46,XX[3] | Normal | Normal | Normal |

**Table S2. Clinical and demographic characteristics of the patient cohort.**

| **Parameter** | **N** | **%** | **Median** (range) |
|---|---|---|---|
| **Gender** (n=73) | | | |
| Male | 35 | 47.9 | |
| Female | 38 | 52.1 | |
| **Age (years)** | | | 7 (0 - 76) |
| Age <18 | 50 | 66.7 | |
| Age >18 | 25 | 33.3 | |
| **Biochemical data** | | | |
| Hb (g/L) | | | 84 (26 - 144) |
| Platelets (x $10^9$/L) | | | 55 (7 - 580) |
| WBC >30 (x$10^9$/L) (n=55) | 28 | 50.1 | |
| Blast in BM | | | 85 (10 - 98.3) |
| MRD >0.01% (n=59) | 27 | 45.8 | |

**Table S3. List of genes included in panel design for mutation analysis.**

| ID gene | Exon | Transcript |
|---|---|---|
| *ABL1* | 4 - 10 | NM_005157.5 |
| *ABL2* | Full CDS | NM_001136000 |
| *ADARB2* | 5 - 7 | NM_018702.3 |
| *AFF3* | 14, 17 | NM_002285.2 |
| *ASMTL* | 4, 7, 10 - 12 | NM_004192.3 |
| *ASXL1* | 13 | NM_015338.5 |
| *ATM* | 3 | NM_000051.3 |
| *ATP10A* | 10 | NM_024490.3 |
| *ATRX* | 8, 17, 22, 30 | NM_138270.3 |
| *BCOR* | 4, 13 | NM_017745.5 |
| *BIRC3* | 4 | NM_001165.4 |
| *BLNK* | 17 | NM_013314.3 |
| *BRAF* | 1, 3, 10, 11, 15, 17, 18 | NM_004333.5 |
| *CBL* | 8, 9 | NM_005188.3 |
| *CCT6B* | 4, 8 | NM_006584.3 |
| *CDCP1* | 3 | NM_022842.4 |
| *CDH17* | 16 | NM_004063.3 |
| *CDKN2A* | 1, 2 | NM_000077.4 |
| *CDX2* | 3 | NM_001265.5 |
| *CENPE* | 24 | NM_001813.2 |
| *CLCA4* | 6 | NM_012128.3 |
| *CREBBP* | 1, 4, 6, 10, 14, 16 - 19, 21, 24 - 28, 30, 31 | NM_004380.2 |
| *CRLF2* | Full CDS | NM_022148 |
| *CSF3R* | 14 | NM_000760.3 |
| *CTCF* | 6 | NM_006565.3 |
| *DCK* | 2, 3 | NM_000788.2 |
| *DIS3* | Full CDS | NM_014953 |
| *DNAH2* | 71 | NM_020877.3 |
| *DNM2* | Full CDS | NM_004945 |

| | | |
|---|---|---|
| *DNMT3A* | 13 - 23 | NM_022552.4 |
| *DOT1L* | 5, 15 | NM_032482.2 |
| *DRD3* | 2, 3 | NM_000796.5 |
| *DTX1* | 6 | NM_004416.2 |
| *EBF1* | 3, 13 | NM_024007.4 |
| *ECT2* | 20 | NM_018098.5 |
| *ECT2L* | Full CDS | NM_001077706 |
| *EED* | 8, 12 | NM_003797.4 |
| *EP300* | 2, 11, 12, 14, 30 | NM_001429.3 |
| *EPOR* | 2, 3 | NM_000121.3 |
| *ERG* | 11 | NM_004449.4 |
| *ETV6* | Full CDS | NM_001987 |
| *EZH2* | 1, 3, 5, 7, 13 - 19 | NM_152998.2 |
| *FANCD2* | 6 | NM_033084.4 |
| *FAT1* | 2, 8 | NM_005245.3 |
| *FAT3* | 6, 23 | NM_001008781.2 |
| *FBL* | 2 | NM_001436.3 |
| *FBXW7* | Full CDS | NM_033632 |
| *FLT3* | 5, 8, 9, 12 - 16, 19 - 21 | NM_004119.2 |
| *FOXO4* | 1 | NM_005938.3 |
| *GATA2* | 3 | NM_032638.4 |
| *GATA3* | 5, 6 | NM_002051.2 |
| *GATA4* | 6 | NM_002052.4 |
| *GSTM1* | Full CDS | NM_000561 |
| *GSTP1* | 5 | NM_000852.3 |
| *HDAC2* | 6 | NM_001527.3 |
| *HES1* | 3 | NM_005524.3 |
| *HPRT1* | 7 | NM_000194.2 |
| *HRAS* | 2 | NM_005343.2 |
| *IDH1* | 4, 7 | NM_005896.3 |
| *IDH2* | 4 | NM_002168.3 |
| *IKZF3* | Full CDS | NM_012481 |

| | | |
|---|---|---|
| *IL7R* | 3, 5, 6 | NM_002185.4 |
| *IRF8* | 7, 8 | NM_002163.2 |
| *JAK1* | 9, 10, 12 - 23 | NM_002227.3 |
| *JAK2* | 14, 16, 20, 21, 24 | NM_004972.3 |
| *JAK3* | 2, 4, 5, 10 - 13, 16, 18, 19 | NM_00215.3 |
| *KLHL6* | 6 | NM_130446.3 |
| *KMD6A* | 15, 16, 24, 26, 28 | NM_021140.3 |
| *KMT2C* | 7, 14, 18, 53 | NM_170606.2 |
| *KMT2D* | 11, 28, 38, 43, 53 | NM_003482.3 |
| *KRAS* | 1 - 4 | NM_004985.4 |
| *LEF1* | 3, 4 | NM_016269.4 |
| *LLGL1* | 14 | NM_004140.3 |
| *MAPK1* | 4, 9 | NM_002745.4 |
| *MDM4* | 3 | NM_002393.4 |
| *MOV10L1* | 18 | NM_018995.2 |
| *MPL* | 10, 12 | NM_005373.2 |
| *MST1* | 14 | NM_020998.3 |
| *MYBL2* | 6, 7 | NM_002466.3 |
| *MYC* | 2 | NM_002467.5 |
| *NANOG* | 3 | NM_024865.3 |
| *NBN* | 6 | NM_002485.4 |
| *NCOR1* | 5, 15 | NM_006311.3 |
| *NF1* | 9, 10, 12, 18, 19, 21, 23, 25 - 29, 31, 33, 34, 36 - 38, 41, 42, 44, 49, 52 | NM_00267.3 |
| *NOTCH1* | 7, 11, 17, 25 - 28, 33, 34 | NM_017617.4 |
| *NOTCH2* | 11, 34 | NM_024408.3 |
| *NOTCH3* | 33 | NM_00435.2 |
| *NR3C1* | 2, 6 - 8 | NM_000176.2 |
| *NR3C2* | 2 | NM_000901.4 |
| *NRAS* | 1- 3 | NM_002524.4 |
| *NSD2* | 4 - 20 | NM_133335.3 |
| *NT5C2* | 4, 11, 15 | NM_012229.4 |

| | | |
|---|---|---|
| *NTRK3* | 3, 13 | NM_002530.3 |
| *NUDT15* | 1, 3 | NM_018283.3 |
| *OBSCN* | 37 | NM_052843.3 |
| *PAG1* | 7 | NM_018440.3 |
| *PAX5* | 2 - 5, 7 - 9 | NM_016734.2 |
| *PDGFRA* | 10 | NM_006206.5 |
| *PDGFRB* | 3, 18 | NM_002609.3 |
| *PHF6* | Full CDS | NM_032458 |
| *PLEKHG1* | 2 | NM_001329801.1 |
| *PMS2* | 2, 9, 14 | NM_000535.6 |
| *PRF1* | 3 | NM_005041.5 |
| *PRKD1* | 6 | NM_002742.2 |
| *PRKN* | 9 | NM_004562.2 |
| *PRPS1* | 2 - 5, 7 | NM_002764.3 |
| *PTEN* | 2, 5, 7 | NM_000314.6 |
| *PTPN11* | 3, 4, 7, 8, 11, 13 | NM_080601.2 |
| *PTPN14* | 10 | NM_05401.4 |
| *RAG1* | Full CDS | NM_000448 |
| *RAG2* | Full CDS | NM_001243786 |
| *RANBP2* | 27 | NM_006267.4 |
| *RB1* | 4, 8, 9, 13, 16, 19, 20, 23 | NM_00321.2 |
| *RELN* | 29, 33, 40, 55, 59 | NM_005045.3 |
| *RFPL4B* | 3 | NM_001013734.2 |
| *RHOBTB2* | 3 | NM_015178.2 |
| *RHOH* | 3 | NM_004310.4 |
| *RIT1* | 5 | NM_006912.5 |
| *RUNX1* | 3 - 9 | NM_001754.4 |
| *SAE1* | 3 | NM_005500.2 |
| *SBNO2* | 17, 20 | NM_014963.2 |
| *SETD2* | 1 - 17, 19, 20 | NM_014159.6 |
| *SF1* | Full CDS | NM_201995 |
| *SF3A1* | 13 | NM_005877.5 |

| | | |
|---|---|---|
| *SH2B3* | 2 - 8 | NM_005475.2 |
| *SLC25A6* | 4 | NM_001636.3 |
| *SMAD5* | 8 | NM_005903.6 |
| *SMARCA1* | 22 | NM_003069.4 |
| *SOS1* | 6 | NM_005633.3 |
| *SOX3* | 1 | NM_005634.2 |
| *SP140* | 16 | NM_007237.4 |
| *SPI1* | 3 | NM_003120.2 |
| *SPRED1* | 3 | NM_152594.2 |
| *STAG2* | Full CDS | NM_001042749 |
| *STIM2* | 7 | NM_020860.3 |
| *SUZ12* | 14, 15 | NM_015355.3 |
| *SYNE1* | 14, 16, 35, 41, 62 | NM_033071.3 |
| *TBL1XR1* | 5 - 9 | NM_024665.5 |
| *TCF12* | 8 | NM_207040.1 |
| *TCF3* | Full CDS | NM_003200 |
| *TET2* | Full CDS | NM_001127208 |
| *TP53* | 2 - 11 | NM_000546.5 |
| *TRAPP* | 61 | NM_003496.3 |
| *TRIM13* | 3 | NM_052811.3 |
| *TYK2* | 8, 20, 22 | NM_003331.4 |
| *WDR72* | 14 | NM_182758.3 |
| *WEE1* | 5 | NM_003390.3 |
| *WNK3* | 2 | NM_020922.4 |
| *WT1* | 5 - 9 | NM_00378.5 |
| *XPO1* | 15 | NM_003400.3 |

**Table S4. Chromosomic regions for fusion genes detection.**

| Fusion gene | Exon/Intron | Genomic position GRCh38/hg38 |
|---|---|---|
| *ETV6*/*RUNX1* t(12;21)(p13;q22) | Intron 5 of *ETV6* | chr12: 11869929 - 11884409 |
| *BCR*/*ABL1* t(9;22) (q34;q11) | Intron 1 of *BCR* | chr22: 23182170 - 23253663 |
| BCR/ABL1 t(9;22)(p24;q11.2) | Exon 12-16 of *BCR* | chr22: 23288058 - 23295133 |
| *MLL*r (*KMT2A*r) | Intron 9 to exon 12 of *MLL* | chr11: 118484173 - 118489862 |
| *TCF3*/*PBX1* t(1;19)(q23;p13)and *TCF3*/*HLF* t(17;19)(q22;p13) | Intron 16 of *TCF3* | chr19: 1619457 - 1620958 |
| *CRLF2*/*IGH* t(X;14)(p22; q32) | Upstream region of *CRLF2* exon 1 | chrX: 1228511 - 1228820 |

**Table S5. Chromosomes mapped to aneuploidy detection.**

| Aneuploidy | Chromosome |
|---|---|
| High hyperdiploidy | 4 |
| High hyperdiploidy | 8 |
| High hyperdiploidy | 10 |
| High hyperdiploidy | 21 |
| Low hypodiploidy | 7 |
| Low hypodiploidy | 17 |

**Table S6. Genes for CNVs detection.**

| ID gene | Exon | Transcript |
|---|---|---|
| *IKZF1* | Full CDS | NM_006060.6 |
| *CDKN2A* | Full CDS | NM_000077.4 |
| *PAX5* | 2 - 9 | NM_016734.2 |
| *ETV6* | Full CDS | NM_001987 |
| *RB1* | *4, 8, 9, 13, 16, 19, 20, 23* | NM_00321.2 |
| *BTG1* | Full CDS | NM_001731.3 |
| *ERG* | Full CDS | NM_004449.4 |

**Table S7. Pharmacogenomic SNPs included.**

| ID gene | rs number or AA change |
|---|---|
| *A2BP1* | rs9924075 |
| *ABCB1* | rs3770102, rs4728709, rs1128503, rs10264856 |
| *ABCC1* | rs246240 |
| *ABCC2* | rs3740065, rs3740066, rs717620 |
| *ABCC3* | rs9895420 |
| *ABCC4* | rs9516519, rs17268122, rs9556455 |
| *ACTG1* | rs1135989 |
| *ADORA2A* | rs2236624 |
| *APEX1* | rs2307486 |
| *ARID5B* | rs4948502, rs4948496, rs4948487, rs6479778, rs2893881, rs4948488, rs2393782, rs10821938, rs7923074, rs6479779, rs17215180 |
| *ATP6AP2* | rs5917990 |
| *BCL2L11 (BIM)* | rs724710 |
| *C3orf6* | rs13358399 |
| *C5orf3, MFAP3* | rs707184, rs1438588 |
| *CCDC24* | rs368182 |
| *CDH12* | rs10473594 |
| *CTLA-4* | rs3087243, rs231775 |
| *CYP2C19* | rs4244285, rs1057910 |
| *CYP3A5* | rs776746 |
| *CYP4F2* | rs2108622 |
| *DPYD* | rs3918290 |
| *DROSHA* | rs639174 |
| *FAM8A6P* | rs1040637 |
| *FCHSD1* | rs251177, rs6773449, rs6007758, rs41488548 |
| *FRMD4B* | rs11707515, rs6549198 |
| *G6PD* | rs5030868 |

| | |
|---|---|
| *GALNT10* | rs11167667, rs12523441, rs7737215, rs10875583, rs6890748, rs6863455 |
| *GART* | rs2070388 |
| *GATA3* | rs3824662 |
| *GIT1* | rs17808412 |
| *GNG2* | rs12886319 |
| *GRIA1* | rs2055083 |
| | rs707176, rs17356099, rs13354399, rs10072570, rs11167640, rs6889909, rs4958676, rs6890057, rs10070447, rs4958351, rs4424038, rs7711124, rs7708391 |
| *HIVEP2, AIG1* | rs200148 |
| *IMPDH1* | rs4731448 |
| *ITPA* | 94C >A, rs41320251, rs1127354, rs7270101 |
| *KCNMA1* | rs11001976, rs17480656, rs12765834, rs17389791, rs11001997 |
| *KIF13A* | rs73726531 |
| *LOC642340* | rs10170236 |
| *MAPK4* | rs11662176, rs9953685 |
| *MCL1* | rs3738485, 256G>C , 194T>G |
| *MOCOS* | rs3744900 |
| *MTHFR* | rs786204016, rs1801131, rs1801133 |
| *MYRIP* | rs17079534 |
| *NBN* | 1197A>G |
| *NFATC2* | rs6021191 |
| *NME1* | rs2215290 |
| *NME1, NME2* | rs3760467, rs1558254 |
| *NUDT15* | rs746071566, rs766023281, 103A > G, rs116855232, rs186364861, rs147390019, rs554405994 |
| *P2RX1* | rs17795186 |
| *PACSIN2* | rs2413739 |
| *PAG1* | rs877419 |
| *PDE4B* | rs6683977, rs1402612, rs7578361, rs524770, rs641262, rs4265132, rs16965335, rs7141601, rs2613079,rs12751530 |
| *PTPRS* | rs17763463, rs7600852 |

| | |
|---|---|
| *PYGL* | rs7142143 |
| *RAD51AP2* | rs424827, rs665312, rs7017705, rs13273490 |
| *SLC24A3* | rs3748483 |
| *SLC25A37* | rs2775139, rs2775134, rs1332944, rs2585498, rs2585499 |
| *SLC28A3* | rs17428030, rs4588940, rs4305983, rs7043257, rs7035753, rs17087144 |
| *SLC36A3* | rs7717132 |
| *SLCO1B1* | rs11045879, rs4149081, rs4149056, rs2306283 |
| *SMARCB1* | 228G>T |
| *SOD2* | rs4880 |
| *TPMT* | rs1800462, rs1800460, rs1142345, rs1800584, rs115106679, rs144041067, rs12201199, rs1142345, rs75543815, rs144041067, rs115106679 |
| *VKORC1* | rs9923231 |
| *VSNL1* | rs2710688 |
| *XDH* | rs494852 |
| *XRCC1* | 26304C>T |
| *XRCC3* | rs1799794 |
| *ZP4* | rs1565430 |

**Table S8. Genes include in targeted TruSeqCustom Amplicon (TSCA) panel for ALL (pre-beta Test Plan for Illumina)**

| Genes | | | |
|---|---|---|---|
| *ABL1* | *EZH2* | *KRAS* | *RELN* |
| *ADARB2* | *FBXW7* | *LEF1* | *RUNX1* |
| *ASMTL* | *FLT3* | *MAPK1* | *SAE1* |
| *BRAF* | *GATA3* | *MYBL2* | *SETD2* |
| *CDKN2A* | *IDH1* | *NF1* | *SH2B3* |
| *CREBBP* | *IDH2* | *NOTCH1* | *SLC25A6* |
| *CRLF2* | *IKZF3* | *NR3C1* | *STAG2* |
| *DNM2* | *IL7R* | *NRAS* | *SUZ12* |
| *DNMT3A* | *JAK1* | *PAX5* | *TBL1XR1* |
| *ECT2L* | *JAK2* | *PHF6* | *TCF3* |
| *EED* | *JAK3* | *PTEN* | *TP53* |
| *EP300* | *KDM6A* | *PTPN11* | *WHSC1* |
| *ETV6* | *KMT2D* | *RB1* | *WT1* |

**Table S9. Oligonucleotide pair for fusion gene detection.** Oligonucleotide pairs used in the PCR to confirm the presence of the fusion gene in the different samples. ID52, forward: intron 5 of *ETV6* (NM_001987.5) and reverse: intron 3 *RUNX1* (NM_001754.4). ID61, forward: intron 5 of *ETV6* (NM_001987.5) and reverse: intron 2 *RUNX1* (NM_001754.4). ID63, forward: intron 1 *BCR* (NM_004327.4) and reverse: intron 1 *ABL1* (NM_007313.2).

| | **Fusion gene** | **Forward 5′-3′** | **Reverse 5′-3′** |
|---|---|---|---|
| **ID52** | *ETV6*/*RUNX1* | TGTGTGCAGCAGTACTTGACA (I.5 *ETV6*) | AAACGTTCTGGTTCTGCGGAT (I.3 *RUNX1*) |
| **ID61** | *ETV6*/*RUNX1* | TTCATGTAAAATAACCCTGGGG (I.5 *ETV6*) | GGCTCATATTTCAGCTCTAGAT (I.2 *RUNX1*) |
| **ID63** | *BCR*/*ABL1* | ATGTTGGTTCCACGTCCAAAC (I.1 *BCR*) | TGTTGCAGCATCCAGTTCATC (I.1 *ABL1*) |

**Table S10. List of variants detected in duplicate sequenced samples.** VAF obtained in the sequencing of each of the samples (VAF1) and the one obtained the second time they were sequenced (VAF2).

| *Sample* | *Gene* | **Exon, AA change & Transcript** | **VAF 1 (%)** | **VAF 2 (%)** |
|---|---|---|---|---|
| S1 | *PAX5* | exon5:c.589_590insACTACCC:p.R197fs | 37.66 | 37.55 |
| S2 | *PAX5* | exon6:c.748_749insCT:p.F250fs | 39.89 | 35.69 |
| | *PAX5* | exon3:c.T395C:p.V132A | 10.99 | 6.86 |
| | *KCNE2* | exon2:c.T170C:p.I57T | 47.22 | 38.46 |
| S3 | *TCF3* | exon11:c.C923T:p.T308M | 52.57 | 46.99 |
| | *NOTCH1* | exon34:c.T7328G:p.V2443G | 20.35 | 20.99 |

**Table S11. Sensitivity and specificity of detection of different types of alterations.** Sensitivity = true positives / (true positives + false negatives) x100 (%), specificity = true negatives / (true negatives + false positives) x100 (%).

| **Type of alteration** | **Sensitivity** (%) | **Specificity** (%) |
|---|---|---|
| SNV/INDELS | 96.3 | 90 |
| CNVs | 95.5 | 100 |
| Aneuploidies | 93.3 | 100 |
| Fusion genes | 89.7 | 100 |

**Table S12. List of detected mutations.** In the first column the sample identifier (ID), followed by the gene, VAF, function, exonic consequence, exon and AA change, cosmic82 identifier and avsnp144 identifier.

| ID | Gene | VAF (%) | Function | Exonic consequence | Exon and AA change | cosmic82 | avsnp144 |
|---|---|---|---|---|---|---|---|
| ID1 | *EP300* | 49.25 | exonic | nonsynonymous SNV | exon14:c.C2773A:p.P925T | COSM88779 | rs148884710 |
| | *IKZF1* | 74.63 | exonic | nonsynonymous SNV | exon5:c.G468C:p.Q156H | NA | NA |
| | *PHF6* | 21.43 | exonic | frameshift deletion | exon7:c.586_587del:p.R196fs | NA | NA |
| | *PHF6* | 51.52 | exonic | nonframeshift insertion | exon9:c.901_902insCGGGGG:p.Y301delinsSGD | NA | NA |
| ID2 | *CBL* | 69.58 | exonic | nonsynonymous SNV | exon9:c.C1298T:p.P433L | COSM4669519 | rs140627020 |
| | *RFPL4B* | 65.1 | exonic | nonsynonymous SNV | exon3:c.G679A:p.V227I | NA | NA |
| | *TCF12* | 49.6 | exonic | nonsynonymous SNV | exon8:c.T860C:p.V287A,TCF12 | NA | NA |
| ID3 | *IKZF3* | 31.11 | exonic | nonsynonymous SNV | exon8:c.G1516A:p.A506T | COSM5972169 | rs749495184 |
| | *SF1* | 45.17 | exonic | nonsynonymous SNV | exon6:c.G582C:p.Q194H | NA | NA |
| | *TCF3* | 42.73 | exonic | nonsynonymous SNV | exon7:c.C472T:p.R158W | NA | rs749247091 |
| ID5 | *RUNX1* | 40.76 | exonic | nonsynonymous SNV | exon4:c.G320A:p.R107H | COSM4385193 | NA |
| ID8 | *IKZF1* | 17.18 | exonic | frameshift insertion | exon7:c.732dupT:p.T244fs | NA | NA |
| ID9 | *TET2* | 50 | exonic | nonsynonymous SNV | exon7:c.C3813G:p.C1271W | COSM120176 | NA |
| ID10 | *TET2* | 35.6 | exonic | nonsynonymous SNV | exon3:c.G1909A:p.E637K | NA | NA |
| ID11 | *KMT2C* | 41.61 | exonic | nonsynonymous SNV | exon14:c.A2233G:p.I745V | NA | rs769543160 |
| | *NF1* | 19.67 | exonic | frameshift insertion | exon18:c.2027dupC:p.T676fs | COSM1235317 | rs587781807 |
| | *NRAS* | 2.02 | exonic | nonsynonymous SNV | exon2:c.G35T:p.G12V | COSM566 | rs121913237 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | *NRAS* | 6.14 | exonic | nonsynonymous SNV | exon2:c.G38A:p.G13D | COSM573 | rs121434596 |
| ID13 | *RB1* | 36.51 | exonic | nonsynonymous SNV | exon9:c.C920T:p.T307I | NA | rs183898408 |
| ID14 | *CREBBP* | 19.54 | exonic | nonsynonymous SNV | exon30:c.T5050C:p.S1684P | NA | rs587783503 |
| | *NRAS* | 21.58 | exonic | nonsynonymous SNV | exon2:c.G35A:p.G12D | COSM564 | rs121913237 |
| ID15 | *NR3C2* | 28.14 | exonic | stopgain | exon2:c.C785G:p.S262X | NA | NA |
| ID16 | *PAX5* | 37.66 | exonic | frameshift insertion | exon5:c.589_590insACTACCC:p.R197fs | NA | NA |
| ID18 | *BRAF* | 29.35 | exonic | nonsynonymous SNV | exon15:c.G1780A:p.D594N | COSM27639 | rs397516896 |
| ID20 | *NRAS* | 4.49 | exonic | nonsynonymous SNV | exon2:c.G35A:p.G12D | COSM564 | rs121913237 |
| ID21 | *CDKN2A* | 3.63 | exonic | stopgain | exon2:c.C216A:p.C72X | COSM13567 | NA |
| | *KRAS* | 1.97 | exonic | nonsynonymous SNV | exon4:c.A351T:p.K117N | COSM1562192 | NA |
| | *KRAS* | 16.87 | exonic | nonsynonymous SNV | exon2:c.G35T:p.G12V | COSM1140133 | rs121913529 |
| | *SLC25A6* | 41.01 | exonic | nonsynonymous SNV | exon4:c.T856A:p.F286I | NA | NA |
| ID22 | *CRLF2* | 62.96 | exonic | nonsynonymous SNV | exon5:c.C485A:p.S162Y | NA | NA |
| | *NR3C1* | 48.75 | exonic | nonsynonymous SNV | exon2:c.A695G:p.D232G | NA | NA |
| | *NRAS* | 5.11 | exonic | nonsynonymous SNV | exon2:c.G38A:p.G13D | COSM573 | rs121434596 |
| | *TET2* | 44.56 | exonic | nonsynonymous SNV | exon3:c.A2580T:p.Q860H | NA | NA |
| ID23 | *FLT3* | 12.86 | exonic | nonsynonymous SNV | exon20:c.G2503T:p.D835Y | COSM783 | rs121913488 |
| | *FLT3* | 41.24 | exonic | nonsynonymous SNV | exon19:c.G2329A:p.E777K | NA | NA |
| | *JAK3* | 41.18 | exonic | nonsynonymous SNV | exon2:c.C23T:p.T8M | NA | rs145500023 |
| | *KRAS* | 14.68 | exonic | nonsynonymous SNV | exon2:c.G35T:p.G12V | COSM1140133 | rs121913529 |
| ID25 | *PAX5* | 23.08 | exonic | nonsynonymous SNV | exon3:c.T399A:p.S133R | NA | NA |
| ID26 | *SH2B3* | 51.58 | exonic | nonsynonymous SNV | exon2:c.C464T:p.P155L | COSM1685385 | rs531156627 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ID27 | *BCOR* | 99.46 | exonic | nonsynonymous SNV | exon4:c.G1780A:p.V594I | COSM5030822 | rs764515953 |
| | *CDKN2A* | 56.94 | exonic | frameshift insertion | exon2:c.225dupC:p.A76fs | NA | NA |
| | *NRAS* | 6.79 | exonic | nonsynonymous SNV | exon2:c.G35A:p.G12D | COSM564 | rs121913237 |
| | *RANBP2* | 52.4 | exonic | nonsynonymous SNV | exon27:c.T8800C:p.F2934L | NA | NA |
| ID28 | *CDKN2A* | 51.67 | exonic | nonsynonymous SNV | exon2:c.G397A:p.A133T | NA | NA |
| | *RUNX1* | 49.09 | exonic | nonsynonymous SNV | exon8:c.G849C:p.Q283H | NA | NA |
| ID29 | *NOTCH2* | 50 | exonic | nonsynonymous SNV | exon34:c.C6094A:p.H2032N | COSM1581340 | rs143236410 |
| | *NRAS* | 5.13 | exonic | nonsynonymous SNV | exon2:c.G35A:p.G12D | COSM564 | rs121913237 |
| ID30 | *FLT3* | 10.25 | exonic | nonsynonymous SNV | exon20:c.A2504T:p.D835V | COSM784 | rs121909646 |
| ID31 | *ASXL1* | 45.98 | exonic | nonsynonymous SNV | exon12:c.A2957G:p.N986S | COSM96383 | rs145132837 |
| | *KRAS* | 39.7 | exonic | nonsynonymous SNV | exon4:c.A351C:p.K117N | COSM1256061 | rs770248150 |
| | *TET2* | 61.75 | exonic | nonsynonymous SNV | exon3:c.G1285A:p.G429R | COSM5095018 | rs201642693 |
| ID32 | *NRAS* | 1.9 | exonic | nonsynonymous SNV | exon2:c.G35C:p.G12A | COSM565 | rs121913237 |
| | *NRAS* | 5.95 | exonic | nonsynonymous SNV | exon2:c.G38A:p.G13D | COSM573 | rs121434596 |
| | *PTPN11* | 33.76 | exonic | nonsynonymous SNV | exon3:c.G226A:p.E76K | COSM13000 | rs121918464 |
| ID33 | *KRAS* | 5.85 | exonic | nonsynonymous SNV | exon2:c.G38A:p.G13D | COSM1140132 | rs112445441 |
| ID34 | *KRAS* | 13.39 | exonic | nonsynonymous SNV | exon2:c.G35T:p.G12V | COSM1140133 | rs121913529 |
| | *PAX5* | 48.12 | exonic | nonsynonymous SNV | exon9:c.T1032A:p.S344R | NA | NA |
| ID35 | *DIS3* | 20.49 | splicing | NA | NA | NA | rs750580353 |
| | *NRAS* | 3.62 | exonic | nonsynonymous SNV | exon3:c.C181A:p.Q61K | COSM580 | rs121913254 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ID36 | *CREBBP* | 68.71 | exonic | nonsynonymous SNV | exon26:c.C4336T:p.R1446C | COSM88749 | rs398124146 |
| | *IKZF1* | 7.77 | exonic | nonsynonymous SNV | exon8:c.G1381C:p.V461L | NA | NA |
| | *NRAS* | 10.2 | exonic | nonsynonymous SNV | exon3:c.A183C:p.Q61H | COSM586 | NA |
| | *SETD2* | 74.13 | exonic | stopgain | exon3:c.A1126T:p.K376X | NA | NA |
| ID37 | *FLT3* | 30.59 | exonic | nonframeshift deletion | exon20:c.2508_2510del:p.836_837del | COSM19836 | rs121913490 |
| | *PTPN11* | 2.96 | exonic | nonsynonymous SNV | exon3:c.G226C:p.E76Q | COSM13016 | rs121918464 |
| ID38 | *SH2B3* | 48.11 | exonic | nonsynonymous SNV | exon2:c.C639A:p.S213R | COSM1685386 | rs111360561 |
| ID39 | *ETV6* | 19.07 | exonic | nonsynonymous SNV | exon6:c.G1080C:p.W360C | NA | NA |
| | *IRF8* | 48.95 | exonic | nonsynonymous SNV | exon7:c.T895G:p.C299G | NA | NA |
| | *KRAS* | 16.67 | exonic | nonsynonymous SNV | exon4:c.G436A:p.A146T | COSM1165198 | rs121913527 |
| | *KRAS* | 21.86 | exonic | nonsynonymous SNV | exon2:c.G35T:p.G12V | COSM1140133 | rs121913529 |
| ID40 | *ETV6* | 35.19 | exonic | frameshift deletion | exon7:c.1165_1166del:p.M389fs | NA | NA |
| | *FLT3* | 36.51 | exonic | nonsynonymous SNV | exon14:c.T1733C:p.M578T | COSM5945297 | NA |
| ID41 | *PAX5* | 44.13 | exonic | nonsynonymous SNV | exon3:c.C239G:p.P80R | COSM85953 | NA |
| | *PAX5* | 50.66 | exonic | nonsynonymous SNV | exon3:c.A215G:p.Y72C | COSM455949 | NA |
| | *PTPN11* | 4.86 | exonic | nonsynonymous SNV | NM_080601:exon3:c.G181T:p.D61Y | COSM13011 | NA |
| | *PTPN11* | 30.87 | exonic | nonsynonymous SNV | NM_080601:exon3:c.A227G:p.E76G | COSM13017 | rs121918465 |
| | *IKZF1* | 41.93 | exonic | frameshift insertion | exon3:c.97_98insTCGC:p.I33fs | NA | NA |
| ID42 | *NF1* | 69.35 | exonic | stopgain | exon34:c.C4537T:p.R1513X | COSM24466 | rs760703505 |
| | *SH2B3* | 49.28 | exonic | nonsynonymous SNV | exon2:c.G622C:p.E208Q | COSM1235390 | rs202080221 |
| | *TP53* | 88.82 | exonic | nonsynonymous SNV | exon6:c.G638A:p.R213Q | COSM131469 | rs587778720 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ID43 | *ANKRD11* | 26.24 | exonic | nonsynonymous SNV | exon9:c.G6076A:p.A2026T | NA | rs752781169 |
| | *SETD2* | 27.67 | exonic | nonsynonymous SNV | exon15:c.A6472G:p.N2158D | NA | NA |
| | *TP53* | 42.27 | splicing | NA | NA | COSM18655 | NA |
| ID44 | *EP300* | 53.17 | exonic | nonsynonymous SNV | exon14:c.C2773A:p.P925T | ICOSM88779 | rs148884710 |
| | *IKZF1* | 6.42 | exonic | nonsynonymous SNV | exon5:c.G472A:p.G158S | COSM303844 | NA |
| | *KMT2C* | 9.54 | exonic | nonsynonymous SNV | exon18:c.G2926A:p.A976T | COSM3304323 | rs779599464 |
| | *NRAS* | 35.68 | exonic | nonsynonymous SNV | exon2:c.G35A:p.G12D | ICOSM564 | rs121913237 |
| | *PAX5* | 35.29 | exonic | nonsynonymous SNV | exon3:c.C224T:p.T75I | COSM4993960 | NA |
| | *SH2B3* | 38.41 | exonic | stopgain | exon6:c.1200_1201insTAGGGGT:p.E400_Y401delinsEX | NA | NA |
| ID45 | *ABL2* | 46.67 | exonic | nonsynonymous SNV | exon12:c.A1945T:p.T649S | NA | rs141450341 |
| | *NRAS* | 39.82 | exonic | nonsynonymous SNV | exon2:c.G38A:p.G13D | COSM573 | rs121434596 |
| ID46 | *EP300* | 51.46 | exonic | nonsynonymous SNV | exon14:c.C2773A:p.P925T | COSM88779 | rs148884710 |
| | *IKZF1* | 10 | exonic | nonsynonymous SNV | exon5:c.G472A:p.G158S | COSM303844 | NA |
| ID47 | *ETV6* | 41.37 | exonic | frameshift insertion | exon5:c.479_480insGA:p.R160fs | NA | NA |
| | *JAK2* | 6.74 | exonic | nonsynonymous SNV | exon16:c.A2044T:p.I682F | COSM303887 | NA |
| | *JAK2* | 10.06 | exonic | nonsynonymous SNV | exon20:c.G2617A:p.D873N | COSM303882 | NA |
| | *NRAS* | 12.27 | exonic | nonsynonymous SNV | exon3:c.C181A:p.Q61K | COSM580 | rs121913254 |
| | *NRAS* | 16.22 | exonic | nonsynonymous SNV | exon3:c.A182T:p.Q61L | COSM583 | rs11554290 |
| ID48 | *JAK2* | 3.04 | exonic | nonsynonymous SNV | exon16:c.A2049T:p.R683S | COSM29302 | NA |
| | *JAK2* | 12.13 | exonic | nonsynonymous SNV | exon16:c.T2081C:p.F694S | NA | NA |
| | *JAK2* | 29.87 | exonic | nonsynonymous SNV | exon16:c.A2047G:p.R683G | COSM29300 | NA |
| | *NRAS* | 4.22 | exonic | nonsynonymous SNV | exon2:c.G35A:p.G12D | COSM564 | rs121913237 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ID49 | *JAK2* | 25.97 | exonic | nonsynonymous SNV | exon20:c.C2624A:p.T875N | COSM23940 | NA |
| | *MPL* | 42.68 | exonic | nonsynonymous SNV | exon9:c.T1432A:p.S478T | NA | rs781129632 |
| ID51 | *NRAS* | 7.58 | exonic | nonsynonymous SNV | exon2:c.G35A:p.G12D | COSM564 | rs121913237 |
| ID52 | *KRAS* | 9.02 | exonic | nonsynonymous SNV | exon2:c.G34C:p.G12R | COSM518 | rs121913530 |
| ID53 | *PEAK1* | 53.49 | exonic | nonsynonymous SNV | exon5:c.A2689G:p.T897A | NA | NA |
| ID54 | *FAT1* | 49.12 | exonic | nonsynonymous SNV | exon2:c.G1555A:p.V519M | NA | NA |
| ID55 | *ASXL1* | 48.55 | exonic | nonsynonymous SNV | exon12:c.G1831A:p.A611T | COSM2889263 | rs372418554 |
| | *KMT2C* | 10.71 | exonic | nonsynonymous SNV | exon18:c.G2914A:p.G972R | NA | rs746739227 |
| | *NRAS* | 21.83 | exonic | nonsynonymous SNV | exon2:c.G38A:p.G13D | COSM573 | rs121434596 |
| ID57 | *NRAS* | 45.12 | exonic | nonsynonymous SNV | exon2:c.G35A:p.G12D | COSM564 | rs121913237 |
| | *XPO1* | 51.52 | exonic | nonsynonymous SNV | exon15:c.G1711A:p.E571K | COSM96797 | NA |
| ID58 | *SPI1* | 31.92 | exonic | frameshift insertion | exon3:c.186_187insTCCCTCC:p.E63fs | NA | NA |
| ID59 | *KRAS* | 23.36 | exonic | nonsynonymous SNV | exon2:c.G34A:p.G12S | COSM1152506 | rs121913530 |
| | *NRAS* | 8.03 | exonic | nonsynonymous SNV | exon2:c.G35A:p.G12D | COSM564 | rs121913237 |
| | *PTPN11* | 4.93 | exonic | nonsynonymous SNV | NM_080601:exon3:c.C215A:p.A72D | COSM13035 | NA |
| ID60 | *CRLF2* | 2.84 | exonic | nonsynonymous SNV | exon6:c.T695G:p.F232C | COSM41268 | NA |
| | *DNMT3A* | 2.91 | exonic | nonsynonymous SNV | exon23:c.C2711T:p.P904L | COSM87007 | rs149095705 |
| | *JAK2* | 6.67 | exonic | nonsynonymous SNV | exon16:c.A2047G:p.R683G | COSM29300 | NA |
| | *JAK2* | 16.85 | exonic | nonsynonymous SNV | exon20:c.C2624A:p.T875N | COSM23940 | NA |
| ID61 | *EZH2* | 3.2 | exonic | nonsynonymous SNV | exon17:c.C1948T:p.H650Y | COSM53040 | rs193921147 |
| | *SETD2* | 5.11 | exonic | nonsynonymous SNV | exon3:c.G1369A:p.E457K | NA | NA |
| | *SETD2* | 5.17 | exonic | frameshift insertion | exon3:c.1367dupG:p.R456fs | NA | NA |
| | *SETD2* | 18.45 | exonic | stopgain | exon3:c.C995G:p.S332X | NA | NA |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | *SETD2* | 19.49 | exonic | nonsynonymous SNV | exon3:c.C805G:p.Q269E | COSM5986197 | NA |
| ID63 | *ZNF384* | 44.84 | exonic | nonsynonymous SNV | exon8:c.T1007A:p.L336H | NA | NA |
| ID64 | *FLT3* | 43.79 | exonic | nonsynonymous SNV | exon5:c.G580A:p.V194M | COSM28039 | rs146030737 |
| ID65 | *CSF2RA* | 25 | splicing | NA | NA | NA | NA |
| | *IL7R* | 25.71 | exonic | nonsynonymous SNV | exon3:c.G291T:p.K97N | COSM1067537 | NA |
| | *NRAS* | 3.64 | exonic | nonsynonymous SNV | exon2:c.G35A:p.G12D | COSM564 | rs121913237 |
| ID66 | *COG1* | 37.93 | exonic | nonsynonymous SNV | exon7:c.A1741G:p.I581V | NA | rs757072904 |
| ID67 | *NRAS* | 26.62 | exonic | nonsynonymous SNV | exon2:c.G38A:p.G13D | COSM573 | rs121434596 |
| | *PTPN11* | 2.24 | exonic | nonsynonymous SNV | exon3:c.T211C:p.F71L | COSM13039 | rs397507512 |
| ID68 | *CREBBP* | 42.81 | exonic | nonsynonymous SNV | exon31:c.A5617G:p.N1873D | NA | NA |
| | *ETV6* | 35.39 | exonic | nonsynonymous SNV | exon7:c.G1223C:p.R408T | NA | NA |
| | *NRAS* | 31.07 | exonic | nonsynonymous SNV | exon2:c.G38A:p.G13D | COSM573 | rs121434596 |
| | *RAG1* | 31.27 | exonic | nonframeshift insertion | exon2:c.775_776insGCT:p.S259delinsSC | NA | NA |
| ID69 | *KMT2D* | 50 | exonic | nonsynonymous SNV | exon11:c.C3793T:p.P1265S | NA | NA |
| | *NOTCH1* | 48.37 | exonic | nonsynonymous SNV | exon34:c.G6392A:p.G2131D | NA | NA |
| | *PAX5* | 33.51 | exonic | stopgain | exon5:c.601_602insCTT:p.E201delinsAX | NA | NA |
| | *PAX5* | 36.04 | exonic | nonsynonymous SNV | exon5:c.G601C:p.E201Q | NA | NA |
| ID70 | *JAK2* | 15.84 | exonic | nonsynonymous SNV | exon16:c.A2049T:p.R683S | COSM29302 | NA |
| ID71 | *GRM1* | 42 | exonic | nonsynonymous SNV | exon8:c.C2756T:p.T919I | NA | rs536808733 |
| | *PHF6* | 18.42 | exonic | stopgain | exon5:c.C385T:p.R129X | COSM4775093 | NA |
| | *RUNX1* | 48.68 | exonic | nonsynonymous SNV | exon4:c.G136A:p.A46T | NA | NA |
| ID72 | *EP300* | 42.98 | exonic | nonsynonymous SNV | exon14:c.C2773A:p.P925T | COSM88779 | rs148884710 |
| | *NRAS* | 3.97 | exonic | nonsynonymous SNV | exon2:c.G35A:p.G12D | COSM564 | rs121913237 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | *CRLF2* | 25.69 | exonic | nonsynonymous SNV | exon6:c.T695G:p.F232C | COSM41268 | NA |
| ID73 | *JAK2* | 14.96 | exonic | nonsynonymous SNV | exon16:c.A2049T:p.R683S | COSM29302 | NA |
| | *PAX5* | 17.27 | exonic | frameshift insertion | exon6:c.621_622insCTGGCGCCTTGGCCTG:p.V208fs | NA | NA |
| ID74 | *JAK2* | 41.1 | exonic | nonsynonymous SNV | exon16:c.A2047G:p.R683G | COSM29300 | NA |
| ID75 | *NRAS* | 34 | exonic | nonsynonymous SNV | exon2:c.G34A:p.G12S | COSM563 | rs121913250 |

**Table S13. Mutations described in REH cell line.** The following table shows a number of mutations detected in the REH cell line and two clones established from it. In the first column the gene, altered nucleotide (CDS mutation), altered amino acid (AA mutation) and variant allele frequency (VAF) detected in REH cells, clon 1 and clon 2.

| Gene | CDS mutation | AA mutation | VAF (%) | | |
|---|---|---|---|---|---|
| | | | REH | Clon 1 | Clon 2 |
| ***TBLX1R1*** | c.G497A | p.R166Q | 53.3 | 62.36 | 48.94 |
| ***IKZF1*** | c.G1079A | p.R360H | 44.14 | 46.96 | 49.76 |
| ***NOTCH1*** | c.C7568T | p.S2523L | 26 | 0 | 0 |
| ***IL27*** | c.G100A | p.G34R | 32.32 | 29.9 | 34.43 |
| ***TP53*** | c.C541T | p.R181C | 29.3 | 0 | 0 |
| **GATA3** | c.C953T | p.A318V | 41.85 | 47.19 | 50.78 |
| ***BCL11B*** | c.G1251A | p.T417T | 40.71 | 44.01 | 42.41 |
| ***BCL11B*** | c.C915T | p.F305F | 52.26 | 46.63 | 45.93 |

**Table S14. Frequency of pharmacogenetic SNPs detected in the patient cohort.**

| rs number | Gene | Heterozygosis frequency (%) | Homozygosis frequency (%) | Total frequency (%) |
|---|---|---|---|---|
| **rs1128503** | *ABCB1* | 18.7 | 8.0 | 29.3 |
| **rs4728709** | *ABCB1* | 1.3 | 0.0 | 1.3 |
| **rs3740066** | *ABCC2* | 17.3 | 2.7 | 20.0 |
| **rs3740065** | *ABCC2* | 13.3 | 0.0 | 13.3 |
| **rs717620** | *ABCC2* | 12.0 | 0.0 | 12.0 |
| **rs17216310** | *ABCC2* | 1.3 | 0.0 | 1.3 |
| **rs4793665** | *ABCC3* | 21.3 | 10.7 | 32.0 |
| **rs9895420** | *ABCC3* | 4.0 | 1.3 | 5.3 |
| **rs9516519** | *ABCC4* | 6.7 | 0.0 | 6.7 |
| **rs9556455** | *ABCC4* | 4.0 | 1.3 | 5.3 |
| **rs1139405** | *ACTG1* | 13.3 | 20.0 | 33.3 |
| **rs1135989** | *ACTG1* | 16.0 | 5.3 | 21.3 |
| **rs1048945** | *APEX1* | 6.7 | 0.0 | 6.7 |
| **rs2307486** | *APEX1* | 1.3 | 0.0 | 1.3 |
| **rs10821936** | *ARID5B* | 40.0 | 18.7 | 58.7 |
| **rs7073837** | *ARID5B* | 36.0 | 18.7 | 54.7 |
| **rs10994982** | *ARID5B* | 38.7 | 10.7 | 49.3 |
| **rs10740055** | *ARID5B* | 37.3 | 10.7 | 48.0 |
| **rs10821935** | *ARID5B* | 36.0 | 10.7 | 46.7 |
| **rs7089424** | *ARID5B* | 38.7 | 5.3 | 44.0 |
| **rs6479778** | *ARID5B* | 12.0 | 22.7 | 34.7 |
| **rs4614389** | *ARID5B* | 12.0 | 22.7 | 34.7 |
| **rs2893881** | *ARID5B* | 12.0 | 22.7 | 34.7 |
| **rs2393782** | *ARID5B* | 13.3 | 18.7 | 32.0 |
| **rs4948496** | *ARID5B* | 22.7 | 8.0 | 30.7 |
| **rs7923074** | *ARID5B* | 14.7 | 14.7 | 29.3 |
| **rs10821938** | *ARID5B* | 14.7 | 14.7 | 29.3 |
| **rs4948488** | *ARID5B* | 12.0 | 16.0 | 28.0 |
| **rs17215180** | *ARID5B* | 21.3 | 5.3 | 26.7 |
| **rs6479779** | *ARID5B* | 16.0 | 9.3 | 25.3 |
| **rs4948502** | *ARID5B* | 16.0 | 4.0 | 20.0 |
| **rs7075591** | *ARID5B* | 14.7 | 4.0 | 18.7 |
| **rs4948487** | *ARID5B* | 12.0 | 4.0 | 16.0 |

| | | | | |
|---|---|---|---|---|
| **rs2393783** | *ARID5B* | 8.0 | 6.7 | 14.7 |
| **rs77918077** | *ARID5B* | 5.3 | 0.0 | 5.3 |
| **rs10994973** | *ARID5B* | 2.7 | 0.0 | 2.7 |
| **rs77708105** | *ARID5B* | 1.3 | 0.0 | 1.3 |
| **rs188676594** | *ARID5B* | 1.3 | 0.0 | 1.3 |
| **rs149113357** | *ARID5B* | 1.3 | 0.0 | 1.3 |
| **rs776746** | *CYP3A5* | 4.0 | 26.7 | 30.7 |
| **rs2108622** | *CYP4F2* | 13.3 | 4.0 | 17.3 |
| **rs17376848** | *DPYD* | 2.7 | 0.0 | 2.7 |
| **rs3918290** | *DPYD* | 1.3 | 0.0 | 1.3 |
| **rs3918289** | *DPYD* | 1.3 | 0.0 | 1.3 |
| **rs639174** | *DROSHA* | 1.3 | 0.0 | 1.3 |
| **rs251177** | *FCHSD1* | 18.7 | 0.0 | 18.7 |
| **rs11707515** | *FRMD4B* | 9.3 | 1.3 | 10.7 |
| **rs7737215** | *GALNT10* | 16.0 | 12.0 | 28.0 |
| **rs6890748** | *GALNT10* | 17.3 | 9.3 | 26.7 |
| **rs12523441** | *GALNT10* | 13.3 | 9.3 | 22.7 |
| **rs11167667** | *GALNT10* | 13.3 | 8.0 | 21.3 |
| **rs10875583** | *GALNT10* | 13.3 | 8.0 | 21.3 |
| **rs153440** | *GALNT10* | 8.0 | 0.0 | 8.0 |
| **rs139021674** | *GALNT10* | 1.3 | 0.0 | 1.3 |
| **rs422628** | *GATA3* | 34.7 | 61.3 | 96.0 |
| **rs3839918** | *GATA3* | 20.0 | 40.0 | 60.0 |
| **rs1058240** | *GATA3* | 20.0 | 40.0 | 60.0 |
| **rs9746** | *GATA3* | 22.7 | 4.0 | 26.7 |
| **rs3824662** | *GATA3* | 17.3 | 5.3 | 22.7 |
| **rs11567941** | *GATA3* | 9.3 | 1.3 | 10.7 |
| **rs552157419** | *GATA3* | 1.3 | 0.0 | 1.3 |
| **rs12886319** | *GNG2* | 9.3 | 24.0 | 33.3 |
| **rs707176** | *GRIA1* | 16.0 | 2.7 | 18.7 |
| **rs6889794** | *GRIA1* | 12.0 | 5.3 | 17.3 |
| **rs4958351** | *GRIA1* | 13.3 | 0.0 | 13.3 |
| **rs10070447** | *GRIA1* | 13.3 | 0.0 | 13.3 |
| **rs6890057** | *GRIA1* | 10.7 | 0.0 | 10.7 |
| **rs6889909** | *GRIA1* | 10.7 | 0.0 | 10.7 |
| **rs4958676** | *GRIA1* | 10.7 | 0.0 | 10.7 |
| **rs13354399** | *GRIA1* | 10.7 | 0.0 | 10.7 |

| | | | | |
|---|---|---|---|---|
| **rs10072570** | *GRIA1* | 10.7 | 0.0 | 10.7 |
| **rs7711124** | *GRIA1* | 9.3 | 0.0 | 9.3 |
| **rs4424038** | *GRIA1* | 8.0 | 1.3 | 9.3 |
| **rs7708391** | *GRIA1* | 8.0 | 0.0 | 8.0 |
| **rs67708322** | *GRIA1* | 6.7 | 0.0 | 6.7 |
| **rs2055083** | *GRIA1* | 5.3 | 1.3 | 6.7 |
| **rs17356099** | *GRIA1* | 5.3 | 0.0 | 5.3 |
| **rs11167640** | *GRIA1* | 4.0 | 0.0 | 4.0 |
| **rs11743325** | *GRIA1* | 2.7 | 0.0 | 2.7 |
| **rs72804610** | *GRIA1* | 1.3 | 0.0 | 1.3 |
| **rs10954184** | *IMPDH1* | 40.0 | 17.3 | 57.3 |
| **rs10954183** | *IMPDH1* | 37.3 | 17.3 | 54.7 |
| **rs4731448** | *IMPDH1* | 9.3 | 21.3 | 30.7 |
| **rs7270101** | *ITPA* | 10.7 | 1.3 | 12.0 |
| **rs1127354** | *ITPA* | 5.3 | 0.0 | 5.3 |
| **rs10762752** | *KCNMA1* | 44.0 | 4.0 | 48.0 |
| **rs17480264** | *KCNMA1* | 9.3 | 0.0 | 9.3 |
| **rs17480656** | *KCNMA1* | 6.7 | 0.0 | 6.7 |
| **rs17389791** | *KCNMA1* | 6.7 | 0.0 | 6.7 |
| **rs12765834** | *KCNMA1* | 6.7 | 0.0 | 6.7 |
| **rs11001997** | *KCNMA1* | 6.7 | 0.0 | 6.7 |
| **rs11001976** | *KCNMA1* | 6.7 | 0.0 | 6.7 |
| **rs11662176** | *MAPK4* | 13.3 | 2.7 | 16.0 |
| **rs9953685** | *MAPK4* | 13.3 | 1.3 | 14.7 |
| **rs3738485** | *MCL1* | 14.7 | 10.7 | 25.3 |
| **rs34645101** | *MCL1* | 1.3 | 0.0 | 1.3 |
| **rs376254012** | *MOCOS* | 1.3 | 0.0 | 1.3 |
| **rs3744900** | *MOCOS* | 1.3 | 0.0 | 1.3 |
| **rs4846051** | *MTHFR* | 1.3 | 34.7 | 36.0 |
| **rs1801133** | *MTHFR* | 20.0 | 2.7 | 22.7 |
| **rs1801131** | *MTHFR* | 14.7 | 2.7 | 17.3 |
| **rs709816** | *NBN* | 20.0 | 0.0 | 20.0 |
| **rs34767364** | *NBN* | 4.0 | 0.0 | 4.0 |
| **rs61973267** | *NUDT15* | 9.3 | 0.0 | 9.3 |
| **rs138959770** | *NUDT15* | 1.3 | 0.0 | 1.3 |
| **rs2413739** | *PACSIN2* | 18.7 | 5.3 | 24.0 |
| **rs117008677** | *PACSIN2* | 1.3 | 1.3 | 1.3 |

| | | | | |
|---|---|---|---|---|
| **rs7006101** | *PAG1* | 13.3 | 1.3 | 14.7 |
| **rs1866275** | *PAG1* | 1.3 | 0.0 | 1.3 |
| **rs140643833** | *PAG1* | 1.3 | 0.0 | 1.3 |
| **rs641262** | *PDE4B* | 12.0 | 13.3 | 25.3 |
| **rs524770** | *PDE4B* | 9.3 | 16.0 | 25.3 |
| **rs12137115** | *PDE4B* | 10.7 | 13.3 | 24.0 |
| **rs12137080** | *PDE4B* | 10.7 | 2.7 | 13.3 |
| **rs71121613** | *PYGL* | 10.7 | 5.3 | 16.0 |
| **rs882860** | *PYGL* | 12.0 | 1.3 | 13.3 |
| **rs665312** | *RAD51AP2* | 9.3 | 1.3 | 10.7 |
| **rs7043257** | *SLC28A3* | 12.0 | 21.3 | 33.3 |
| **rs7035753** | *SLC28A3* | 13.3 | 18.7 | 32.0 |
| **rs4588940** | *SLC28A3* | 18.7 | 12.0 | 30.7 |
| **rs17087144** | *SLC28A3* | 14.7 | 5.3 | 20.0 |
| **rs17428030** | *SLC28A3* | 4.0 | 1.3 | 5.3 |
| **rs75316220** | *SLC28A3* | 2.7 | 0.0 | 2.7 |
| **rs4149057** | *SLCO1B1* | 17.3 | 9.3 | 25.3 |
| **rs4149056** | *SLCO1B1* | 13.3 | 1.3 | 14.7 |
| **rs11045879** | *SLCO1B1* | 12.0 | 1.3 | 13.3 |
| **rs4149081** | *SLCO1B1* | 4.0 | 0.0 | 4.0 |
| **rs4880** | *SOD2* | 21.3 | 6.7 | 28.0 |
| **rs2842949** | *TPMT* | 8.0 | 8.0 | 16.0 |
| **rs2842934** | *TPMT* | 2.7 | 1.3 | 4.0 |
| **rs12201199** | *TPMT* | 1.3 | 0.0 | 1.3 |
| **rs494852** | *XDH* | 4.0 | 4.0 | 8.0 |
| **rs1799782** | *XRCC1* | 5.3 | 0.0 | 5.3 |
| **rs2307170** | *XRCC1* | 1.3 | 0.0 | 1.3 |
| **rs1799779** | *XRCC1* | 1.3 | 0.0 | 1.3 |
| **rs861529** | *XRCC3* | 2.7 | 33.3 | 36.0 |
| **rs1799794** | *XRCC3* | 14.7 | 2.7 | 17.3 |

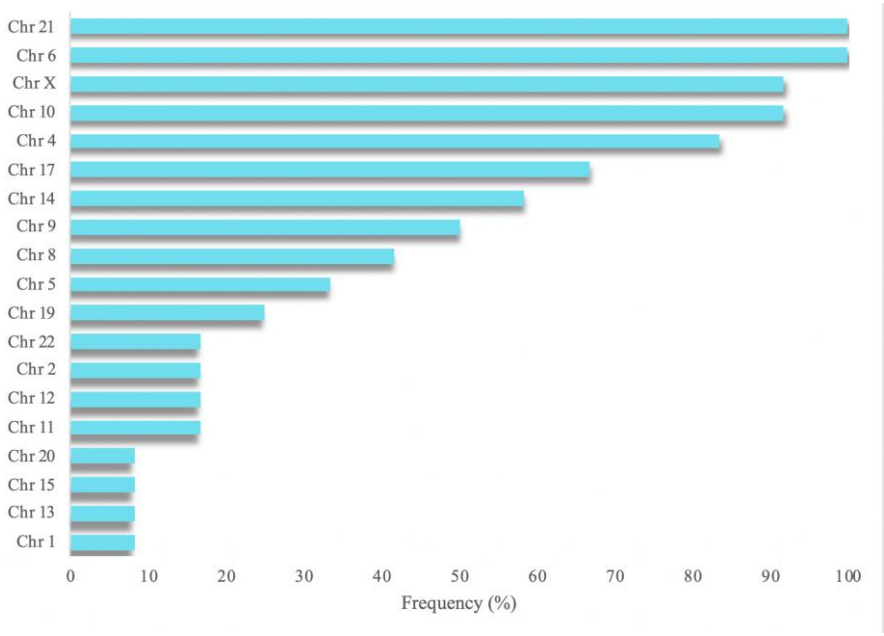


**Figure S1. Frequency of trisomies in high hyperdiploidy cases.** The following figure shows the frequency of trisomies observed in high hyperdiploidy patients included in this study in decreasing order.

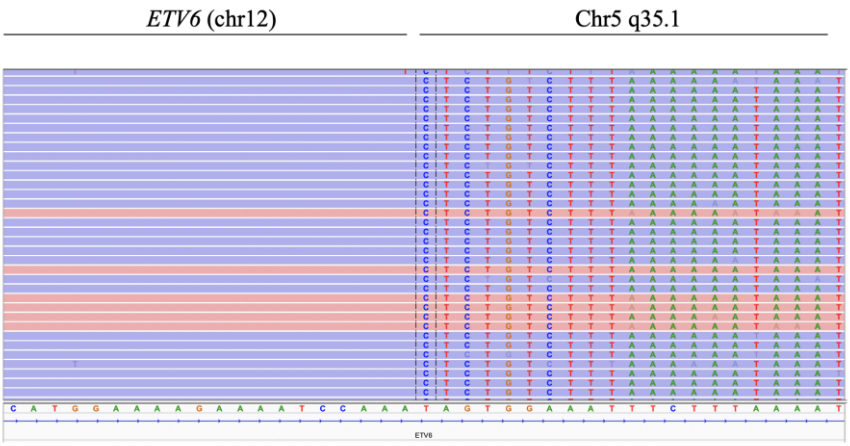


**Figure S2. t(5;12)(q35;p13) visualized with IGV.** t(5;12)(q35;p13) observed in (sample ID13). In the left region the reads aligned with intron 5 of *ETV6* and to the right the reads aligned with chromosome 5 q35.1.