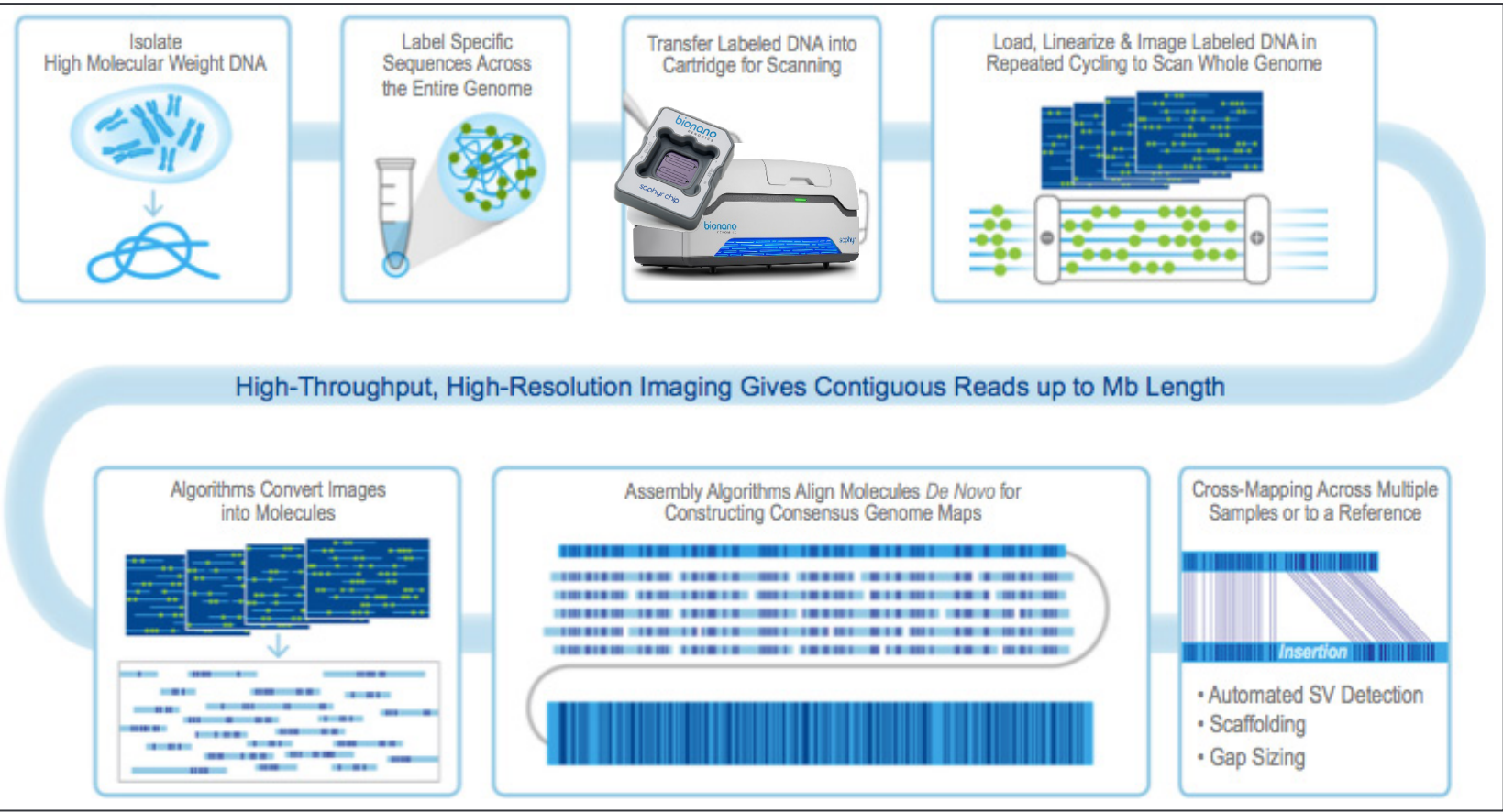




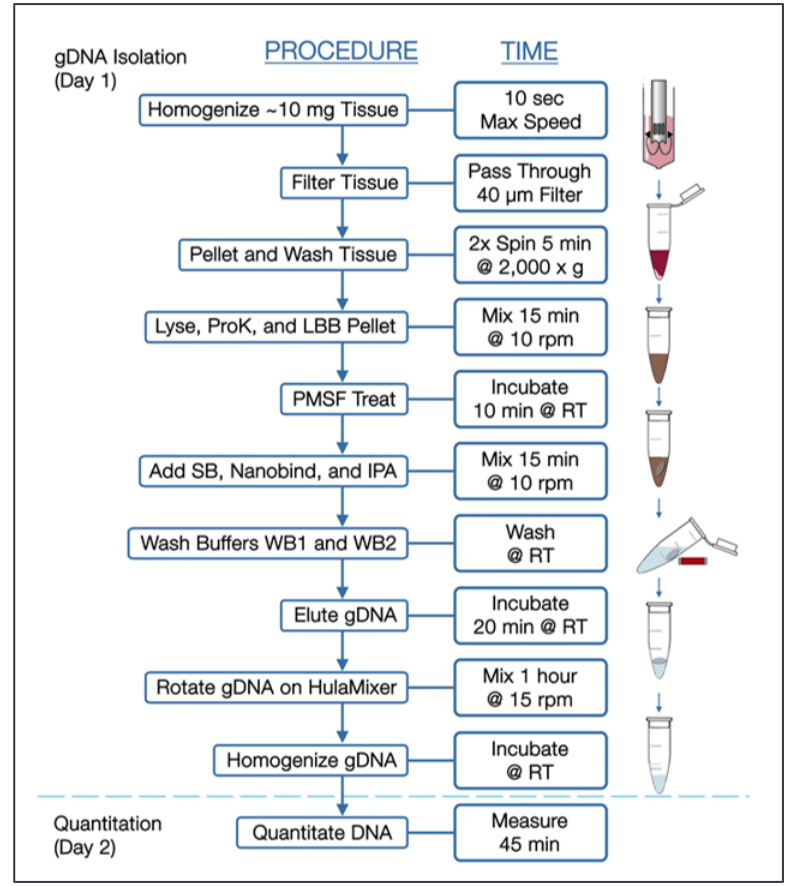
Rapid Isolation of high quality ultra-high molecular weight (UHMW) genomic DNA (gDNA) from blood, bone marrow aspirates (BMAs) and fresh frozen human tumors

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1. Bionano Sample to Answer Workflow on the Saphry System



2. Bionano Prep SP Tissue & Tumor DNA Isolation



3. Characteristics of the Freshly Frozen Human Tumors from BioIVT

| Tissue | Amount (mg) | Clinical Diagnosis | Tumor Grade | Stage | STMR | SNML | SNCR | SOTR | RIN | Date of Excision | Age at Excision | Sex |
|----------|-------------|--------------------------|---------------------|-------|------|------|------|------|------|------------------|-----------------|--------|
| Bladder | 4882 | Urothelial Carcinoma | High Grade | II | 89 | 0 | 8 | 3 | 9.27 | 2016 | 64 | Male |
| Brain | 960 | Astrocytoma Anaplastic | III | III | 100 | 0 | 0 | 0 | 9.52 | 2013 | 39 | Female |
| Breast | 2886 | Ductal Carcinoma In Situ | High Grade | IIB | 97 | 0 | 1 | 2 | 10.0 | 2013 | 49 | Female |
| Colon | 2620 | Adenoma Invasive | Well Differentiated | UNK | 97 | 3 | 0 | 0 | 9.71 | 2015 | 78 | Male |
| Kidney | 3285 | Renal Cell Carcinoma | II | II | 100 | 0 | 0 | 0 | 9.71 | 2012 | 61 | Female |
| Liver | 2806 | Hepatocellular Carcinoma | Mod Differentiated | I | 88 | 0 | 0 | 12 | 9.73 | 2016 | 71 | Male |
| Lung | 4440 | Adenosquamous Carcinoma | Moderate to Poor | IIA | 100 | 0 | 0 | 0 | 9.72 | 2012 | 36 | Male |
| Ovary | 3587 | SerousCarcinoma | NR | IA | 100 | 0 | 0 | 0 | 9.54 | 2013 | 39 | Female |
| Prostate | 372 | Adenocarcinoma Invasive | 4+4=8 | IV | 92 | 0 | 0 | 8 | 9.07 | 2016 | 61 | Male |
| Thyroid | 2860 | Papillary Carcinoma | Well Differentiated | I | 100 | 0 | 0 | 0 | 9.57 | 2018 | 36 | Female |

4. Average Tumor Single Molecule Quality Report (MQR) Metrics

| Tumor (#Samples-#Operators) | Input (mg) | DNA [] (ng/μl) | DNA Yield (μg/mg) | N50 Kbp (>20Kbp) | N50 Kbp (>150Kbp) | Labels/100 Kbp | Map Rate (%) | Gbp/Scan |
|-----------------------------|------------|-----------------|-------------------|------------------|-------------------|----------------|--------------|----------|
| Bladder (3-3) | 9.7 | 245 | 1.64 | 313 | 357 | 15.2 | 91.8 | 66 |
| Brain (2-2) | 10.5 | 168 | 1.06 | 228 | 292 | 14.6 | 90.2 | 42 |
| Breast (3-2) | 13.3 | 183 | 1.04 | 317 | 395 | 14.2 | 84.1 | 77 |
| Colon (3-2) | 11.3 | 231 | 1.33 | 263 | 330 | 14.9 | 88.6 | 42 |
| Kidney (3-2) | 10 | 96 | 0.63 | 201 | 269 | 14.6 | 83.5 | 41 |
| Liver (2-2) | 9.0 | 196 | 1.41 | 265 | 306 | 14.9 | 89.3 | 84 |
| Lung (5-3) | 9.6 | 128 | 0.86 | 248 | 304 | 15.0 | 90.4 | 51 |
| Ovary (2-2) | 10.5 | 168 | 1.05 | 228 | 292 | 14.6 | 90.2 | 42 |
| Prostate (4-3) | 10.3 | 113 | 0.72 | 273 | 361 | 14.8 | 85.1 | 62 |
| Thyroid (4-3) | 10 | 126 | 0.77 | 213 | 294 | 14.5 | 87.6 | 64 |

5. Total Structural Variation Detected (RVP)

| Sample | Deletion | Insertion | Duplication | Inversion | Interchr. translocation | Intrachr. translocation |
|------------|----------|-----------|-------------|-----------|-------------------------|-------------------------|
| bladder_1 | 317 | 471 | 57 | 52 | 1 | 0 |
| bladder_2 | 332 | 527 | 62 | 63 | 1 | 1 |
| bladder_3 | 334 | 503 | 62 | 52 | 0 | 0 |
| brain_1 | 354 | 483 | 64 | 83 | 74 | 45 |
| brain_2 | 363 | 469 | 75 | 102 | 105 | 69 |
| breast_1 | 314 | 462 | 57 | 90 | 58 | 65 |
| breast_2 | 335 | 491 | 80 | 95 | 61 | 87 |
| breast_3 | 326 | 492 | 73 | 97 | 61 | 81 |
| colon1 | 366 | 467 | 62 | 81 | 0 | 1 |
| colon3 | 370 | 481 | 65 | 83 | 0 | 1 |
| kidney_1 | 281 | 432 | 51 | 45 | 0 | 0 |
| kidney_2 | 434 | 653 | 68 | 77 | 16 | 18 |
| kidney_3 | 290 | 443 | 47 | 43 | 0 | 0 |
| liver_1 | 317 | 486 | 75 | 71 | 1 | 4 |
| liver_3 | 390 | 580 | 74 | 69 | 2 | 5 |
| lung_1 | 363 | 492 | 93 | 96 | 30 | 49 |
| lung_2 | 392 | 514 | 101 | 95 | 51 | 55 |
| lung_3 | 413 | 556 | 91 | 101 | 27 | 35 |
| ovary_1 | 364 | 593 | 103 | 68 | 76 | 28 |
| ovary_2 | 375 | 590 | 90 | 66 | 70 | 25 |
| prostate_1 | 412 | 584 | 65 | 86 | 40 | 28 |
| prostate_2 | 363 | 506 | 60 | 72 | 25 | 14 |
| prostate_3 | 327 | 464 | 53 | 71 | 24 | 14 |
| thyroid_1 | 315 | 452 | 27 | 49 | 1 | 0 |
| thyroid_2 | 350 | 490 | 55 | 62 | 1 | 0 |
| thyroid_3 | 338 | 478 | 56 | 64 | 0 | 1 |

6. Shared Among Duo/Trio Samples (%)

| Sample | Deletion | Insertion | Duplication | Inversion | Interchr. translocation | Intrachr. translocation |
|------------|------------|------------|-------------|-----------|-------------------------|-------------------------|
| bladder_1 | 304 (95.9) | 452 (96.0) | 47 (82.5) | 47 (90.4) | 0 (0.0) | 0 (0.0) |
| brain_1 | 324 (91.5) | 428 (88.6) | 54 (84.4) | 73 (88.0) | 53 (71.6) | 27 (60.0) |
| breast_1 | 292 (93.0) | 440 (95.2) | 51 (89.5) | 83 (92.2) | 54 (62.8) | 61 (93.8) |
| colon1 | 357 (97.5) | 445 (95.3) | 54 (87.1) | 79 (97.5) | 0 (0.0) | 1 (100) |
| kidney_1 | 255 (90.7) | 401 (92.8) | 39 (76.5) | 35 (77.8) | 0 (0.0) | 0 (0.0) |
| liver_1 | 308 (97.2) | 471 (96.9) | 61 (81.3) | 62 (87.3) | 1 (100) | 3 (75.0) |
| lung_1 | 330 (90.9) | 465 (94.5) | 64 (68.8) | 76 (79.2) | 21 (70.0) | 29 (59.2) |
| ovary_1 | 346 (95.1) | 533 (89.9) | 84 (81.6) | 63 (92.6) | 69 (90.8) | 25 (89.3) |
| prostate_1 | 310 (75.2) | 437 (74.8) | 40 (61.5) | 64 (74.4) | 19 (47.5) | 13 (46.4) |
| thyroid_1 | 302 (95.9) | 425 (94.0) | 22 (81.5) | 43 (87.8) | 0 (0.0) | 0 (0.0) |

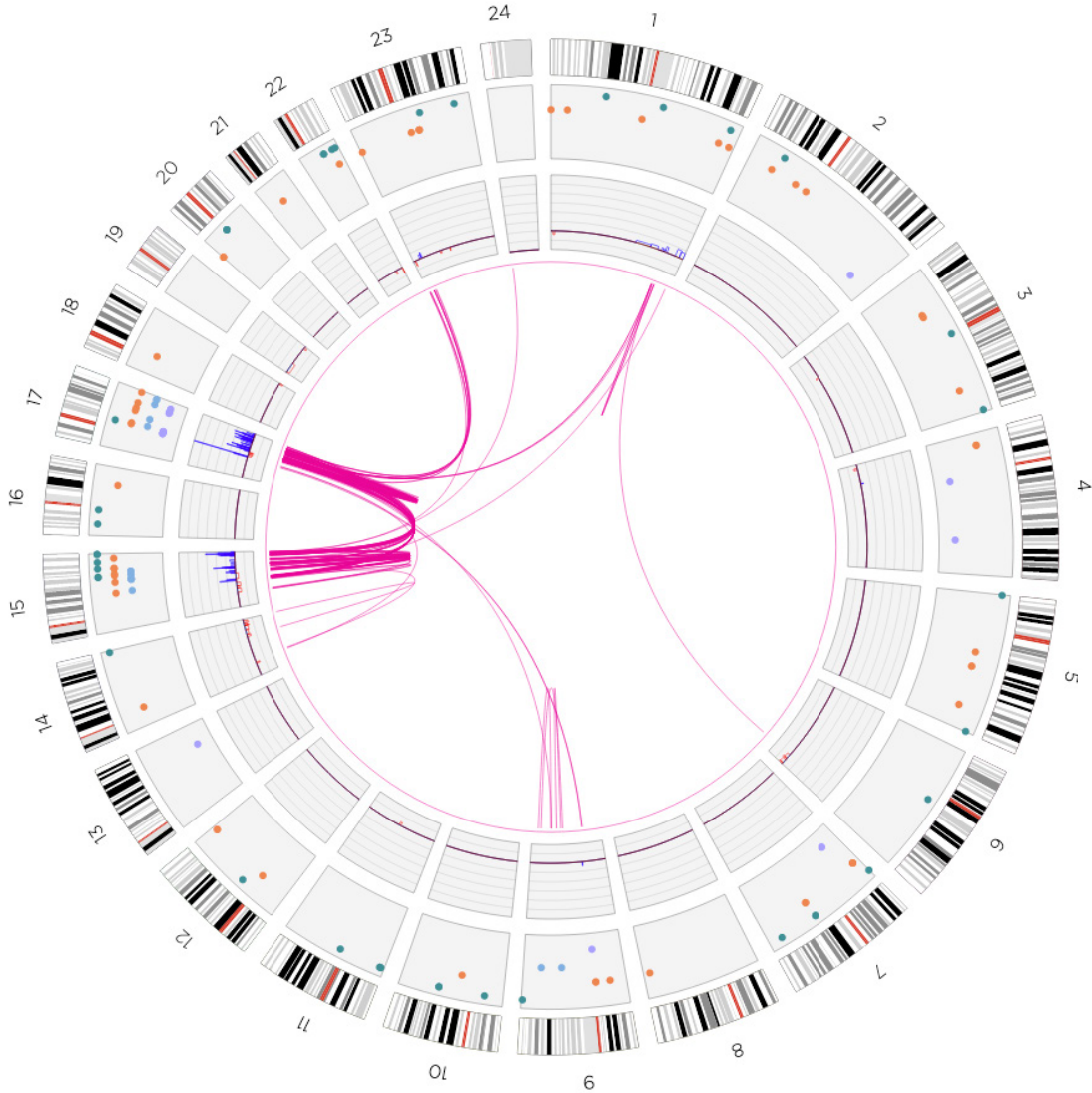
*All calls are clustered. Removal of redundancy.

7. Shared Among Duo/Trio Samples (%), Novel to Bionano control database.

| Sample | Deletion | Insertion | Duplication | Inversion | Interchr. translocation | Intrachr. translocation |
|------------|-----------|------------|-------------|-----------|-------------------------|-------------------------|
| bladder_1 | 31 (96.9) | 27 (87.1) | 3 (60.0) | 4 (100) | 0 (0.0) | 0 (0.0) |
| brain_1 | 39 (86.7) | 18 (72.0) | 14 (73.7) | 8 (33.3) | 53 (71.6) | 27 (60.0) |
| breast_1 | 39 (84.8) | 31 (93.9) | 16 (88.9) | 42 (91.3) | 54 (93.1) | 61 (93.8) |
| colon1 | 56 (96.6) | 37 (82.2) | 10 (90.9) | 3 (50.0) | 0 (0.0) | 1 (100) |
| kidney_1 | 20 (74.1) | 23 (82.1) | 4 (40.0) | 2 (50.0) | 0 (0.0) | 0 (0.0) |
| liver_1 | 40 (87.0) | 42 (89.4) | 9 (52.9) | 4 (28.6) | 1 (100) | 3 (75) |
| lung_1 | 50 (69.4) | 33 (78.6) | 26 (53.1) | 54 (62.8) | 21 (70) | 29 (59.2) |
| ovary_1 | 82 (88.2) | 140 (83.8) | 40 (78.4) | 4 (40.0) | 69 (90.8) | 25 (89.3) |
| prostate_1 | 27 (51.9) | 21 (42.9) | 2 (22.2) | 8 (44.4) | 19 (47.5) | 13 (46.4) |
| thyroid_1 | 21 (84.0) | 22 (73.3) | 2 (100) | 2 (100) | 0 (0.0) | 0 (0.0) |

*All calls are clustered. Removal of redundancy. Control filtered 3

8. Circos Plot of Shared SVs in the Breast Tumor



*Redundancy Removed, Shared, Control Filtered

9. Shared Gene Amplifications in the Breast Tumor

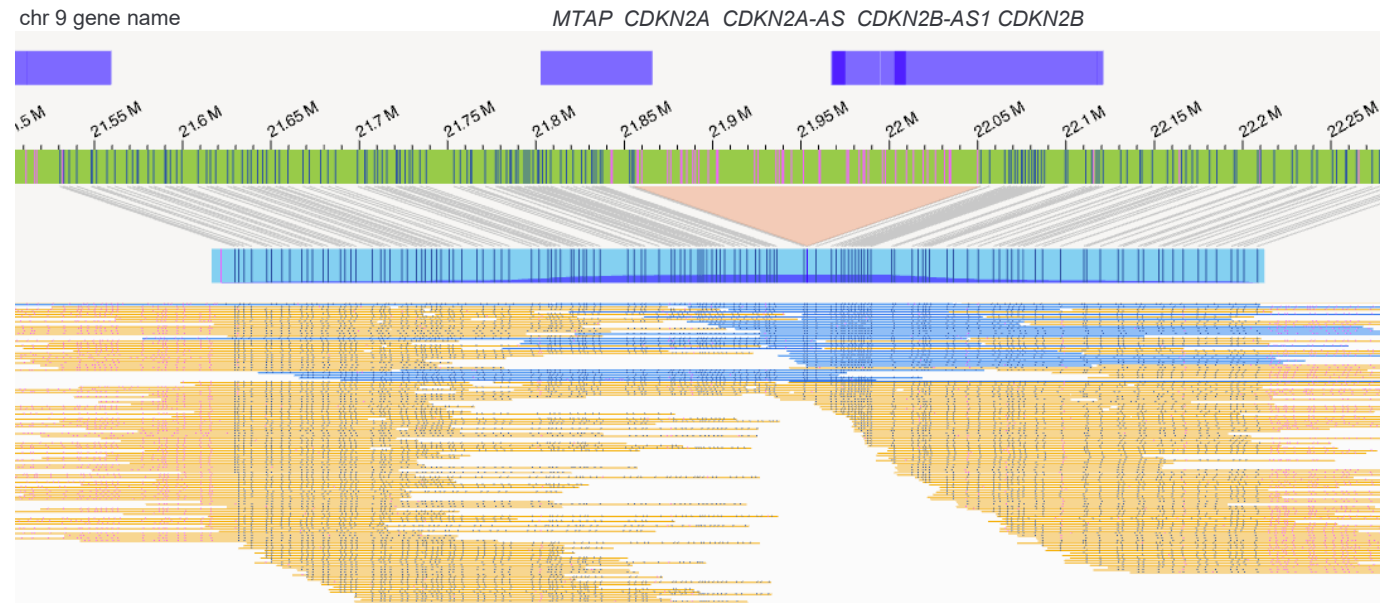
| Chromosome Region | Example Genes | Copy Number |
|-------------------|--|-------------|
| 15q21.1-15q21.2 | <i>COPS2, FGF7, MIR4712</i> | 4 |
| 15q21.2 | <i>USP8, TRMP7, SPPL2A</i> | 5 |
| 15q24.3 | <i>PEAK1, HMG20A</i> | 4 |
| 17q12-17q21.1 | <i>FBXL20, CDK12, NeuroD, ERBB2, Grb7, LRR3C</i> | 10 |
| 17q21.33 | <i>ITGA3, COL1A, ABCC3</i> | 5 |
| 17q24.2 | <i>PRKCA, CACN5, PSMD12, MIR548D2</i> | 4 |

Filter Criteria

| | |
|------------------------------|--------|
| BED Overlap Precision (Kbp): | 12 |
| SV Masking Filter: | all |
| Copy Number Type: | all |
| Copy Number Confidence: | 0.99 |
| Copy Number Min Size (bp): | 500000 |
| Self Molecule Count: | 5 |
| % in Control: | 5 |
| % in Control for Enzyme: | 5 |
| SV Chimeric Score: | all |
| Found in Parents Assembly: | both |
| Found in Parents Molecules: | both |
| Found in Self Molecules: | yes |
| Overlap Genes: | all |

| SV Filter | Confidence | Min Size (bp) |
|-----------------|------------|---------------|
| ● Insertion | 0 | |
| ● Deletion | 0 | |
| ● Inversion | 0.01 | |
| ● Duplication | -1 | |
| ● Translocation | 0 | |

10. A 19.6 Kbp Deletion of CDKN2A/2B Locus (p16^{INK4A}, p14^{ARF}, & p15^{INK4B} Tumor Suppressors)



11. A 65 Kbp Amplification of the CDK12 and ErBB2 Locus (Tumor Drivers)

