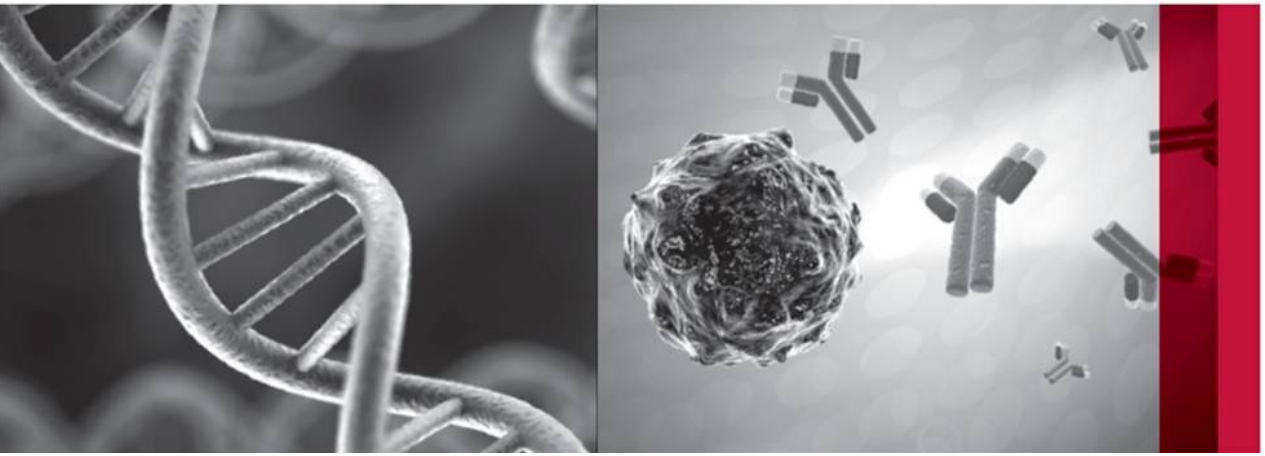
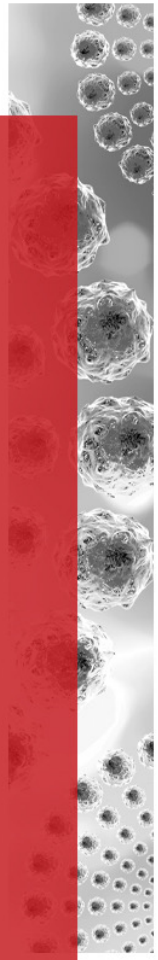


HOT Topics in OncoScan: Somatic Mutations

2014-03 Carsten Bruckner



AGENDA

- What are somatic mutations
- How to review results
- Factors affecting data quality

Basic concepts (thanks Wikipedia!)

- Somatic mutation: acquired (not inherited) mutation not usually transmitted to descendants
- Oncogene: gene that has the potential to cause cancer. In tumor cells, they are often mutated or expressed at high levels.
- Tumor suppressor gene: gene that protects a cell from one step on the path to cancer. Loss-of-function mutations can play a role in formation of tumor cells.

Somatic Mutation Example

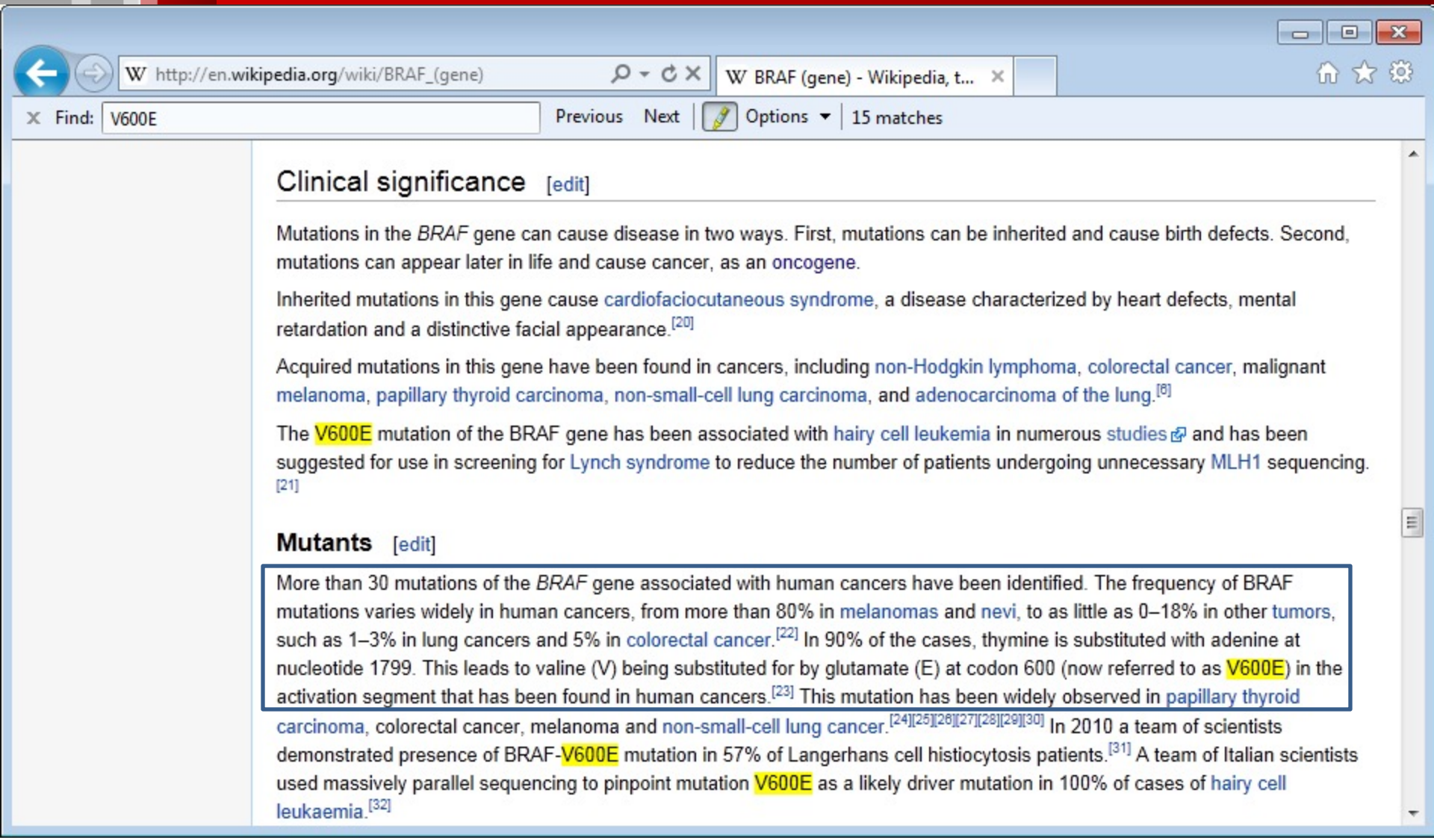
BRAF V600E **Wild** Type	catcgagatttcActgtagctagaccaa
BRAF V600E **Mutant**	catcgagatttcTctgtagctagaccaa

Single Base substitution from A to T leads to the activation of the BRAF oncogene.

~90% of BRAF reported mutation events are V600E. This makes it a juicy target for drug development: The downstream protein made by this oncogene is targeted by a drug vemurafenib.

~85% of OncoScan SM are single base changes, with the remainder being multiple base substitutions, insertions, deletions

OncoGene BRAF



Clinical significance [edit]

Mutations in the *BRAF* gene can cause disease in two ways. First, mutations can be inherited and cause birth defects. Second, mutations can appear later in life and cause cancer, as an oncogene.

Inherited mutations in this gene cause cardiofaciocutaneous syndrome, a disease characterized by heart defects, mental retardation and a distinctive facial appearance.[20]

Acquired mutations in this gene have been found in cancers, including non-Hodgkin lymphoma, colorectal cancer, malignant melanoma, papillary thyroid carcinoma, non-small-cell lung carcinoma, and adenocarcinoma of the lung.[6]

The V600E mutation of the BRAF gene has been associated with hairy cell leukemia in numerous studies and has been suggested for use in screening for Lynch syndrome to reduce the number of patients undergoing unnecessary MLH1 sequencing.[21]

Mutants [edit]

More than 30 mutations of the *BRAF* gene associated with human cancers have been identified. The frequency of BRAF mutations varies widely in human cancers, from more than 80% in melanomas and nevi, to as little as 0–18% in other tumors, such as 1–3% in lung cancers and 5% in colorectal cancer.[22] In 90% of the cases, thymine is substituted with adenine at nucleotide 1799. This leads to valine (V) being substituted for by glutamate (E) at codon 600 (now referred to as V600E) in the activation segment that has been found in human cancers.[23] This mutation has been widely observed in papillary thyroid carcinoma, colorectal cancer, melanoma and non-small-cell lung cancer.[24][25][26][27][28][29][30] In 2010 a team of scientists demonstrated presence of BRAF-V600E mutation in 57% of Langerhans cell histiocytosis patients.[31] A team of Italian scientists used massively parallel sequencing to pinpoint mutation V600E as a likely driver mutation in 100% of cases of hairy cell leukaemia.[32]

Certain somatic mutations are routinely tested (and reimbursed)

- BRAFv600E (predictive in melanoma)
  - Zelboraf® (**vemuraf**enib) is a BRAF inhibitor that is able to block the function of the **V**600**E**-**mu**tated B**RAF** protein
  - Vemurafenib received FDA approval for the treatment of late-stage melanoma on August 17, 2011,[2] Health Canada approval on February 15, 2012[3] and on February 20, 2012, the European Commission approved vemurafenib as a monotherapy for the treatment of adult patients with BRAF V600 mutation positive unresectable or metastatic melanoma, the most aggressive form of skin cancer.

OncoScan™ FFPE Assay Kit
Somatic mutations content

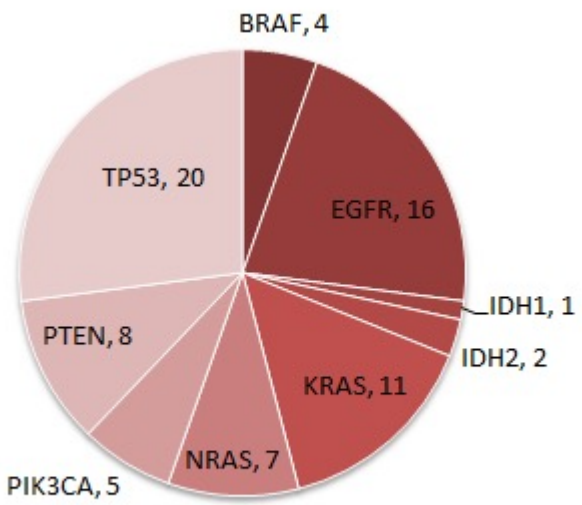
Somatic mutation panel

- 64 probesets respond to 74 mutations
- 9 genes - BRAF, KRAS, EGFR, IDH1, IDH2, PTEN, PIK3CA, NRAS, TP53
- Concordance with orthogonal methods observed for multiple somatic mutations
- Tool provided for visualization of mutant call versus reference (wild type)

Sensitivity

- Sensitivity claim validated by spike-in studies (oligos with mutant spiked in at various mutant % levels in normal FFPE DNA)
- Majority of mutations were detected at 20% sensitivity
- In real world samples, there were examples of detection down to ~10%

Distribution of 74 mutations by gene



Somatic probe design with mutation in gap fill position

Description	type or probeset	Sequence	Hom strand	Coding strand
KRASp.G12	wild	tgaatataaacttgtggtagttggagctgGtggcgtaggcaagagtgccttgacgatac	R	-
KRASp.G12Vc.35G>T	mutant	tgaatataaacttgtggtagttggagctgTtggcgtaggcaagagtgccttgacgatac	R	-
KRASp.G12Dc.35G>A	mutant	tgaatataaacttgtggtagttggagctgAtggcgtaggcaagagtgccttgacgatac	R	-
KRAS:p.G12D/V:c.35G>A/T	93107489A	TGTGGTAGTTGGAGCTG TGGCGTAGGCAAGAGTG	R	-

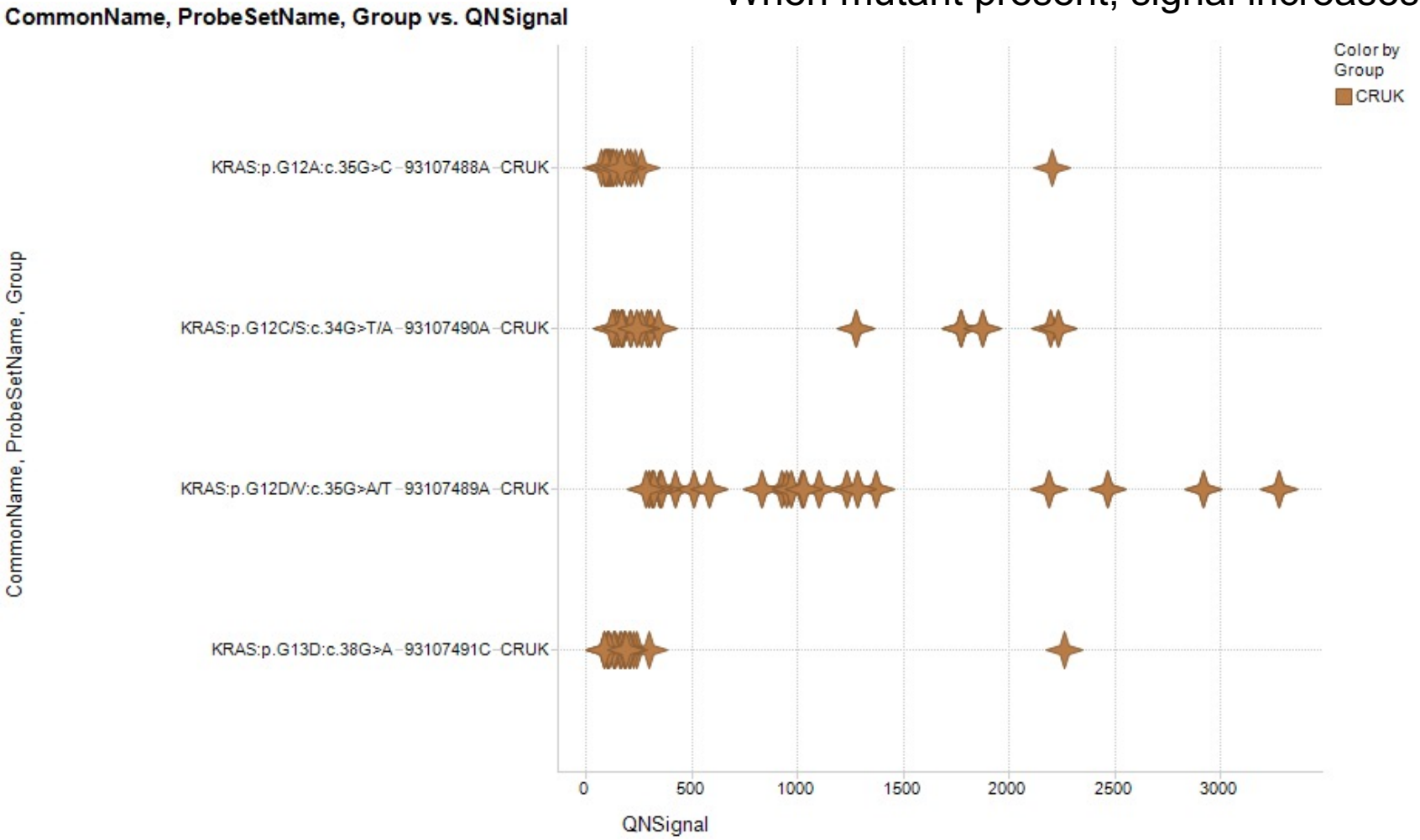
Molecular Inversion Probe

- The dNTP fills in the gap fill position
- When the G>A or G>T mutation is present, the signal in the AT channel signal increases

- In general, the MIP design and readout channel are chosen so that MIP shouldn't amplify if mutant not present

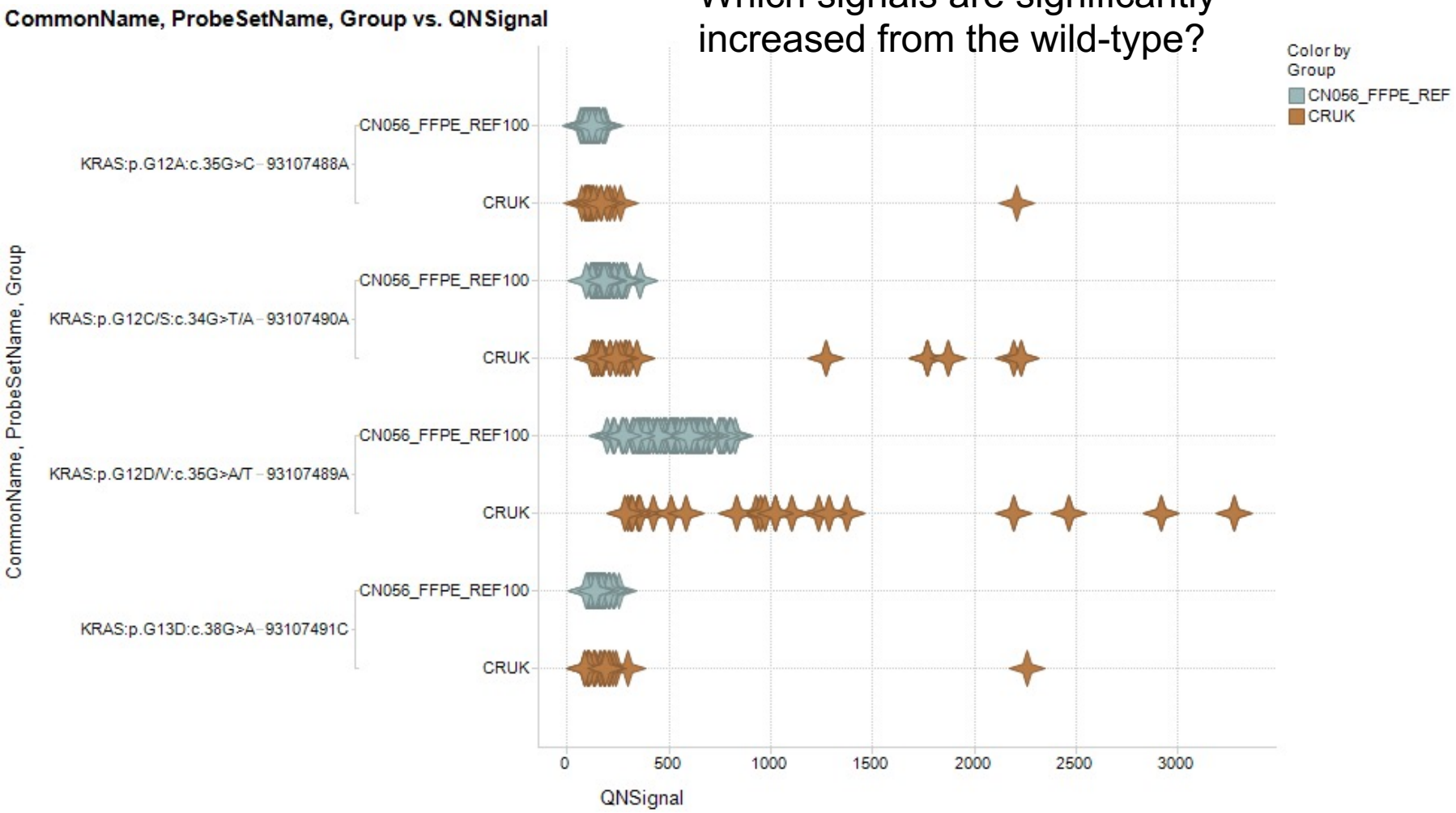
Some data for 4 somatic mutation probesets 24 samples/probeset: Which have mutation?

When mutant present, signal increases



Add wild-type signal distribution from .SOM_REF_MODEL

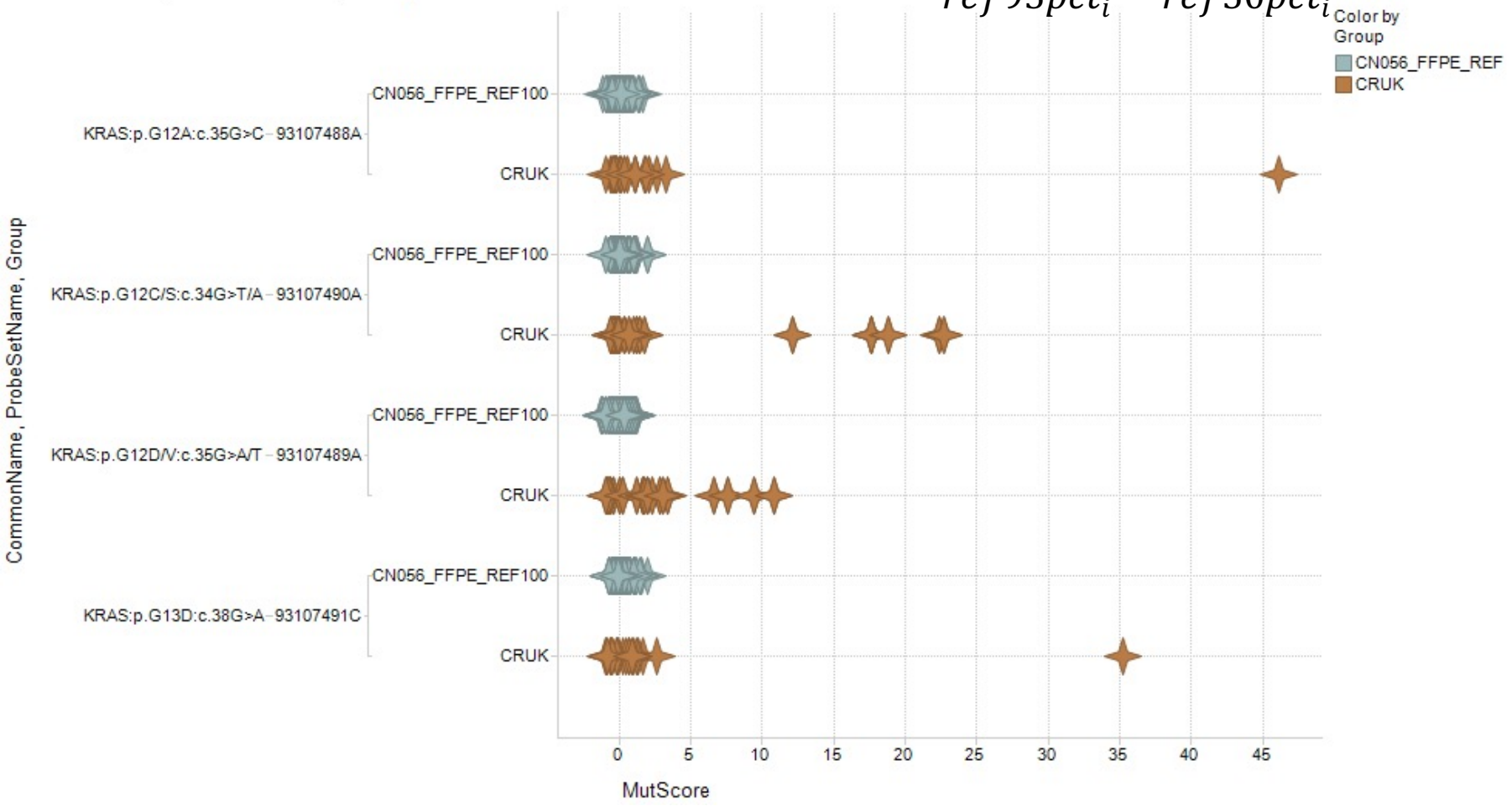
Which signals are significantly increased from the wild-type?



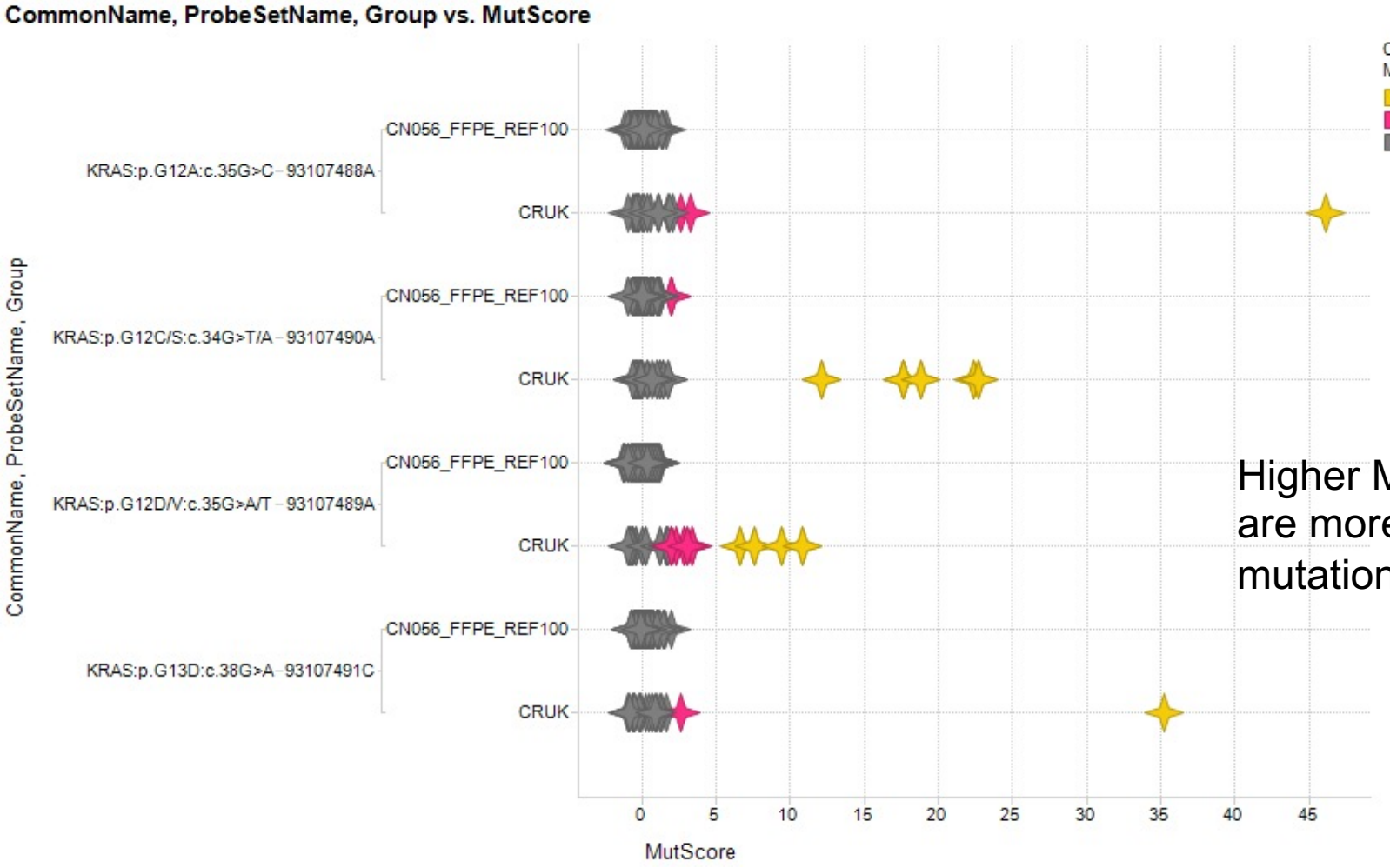
Mutation Score normalizes signal across markers

$$MutScore_{i,j} = \frac{QNsignal_{i,j} - ref50pct_i}{ref95pct_i - ref50pct_i}$$

i^{th} marker
j^{th} sample

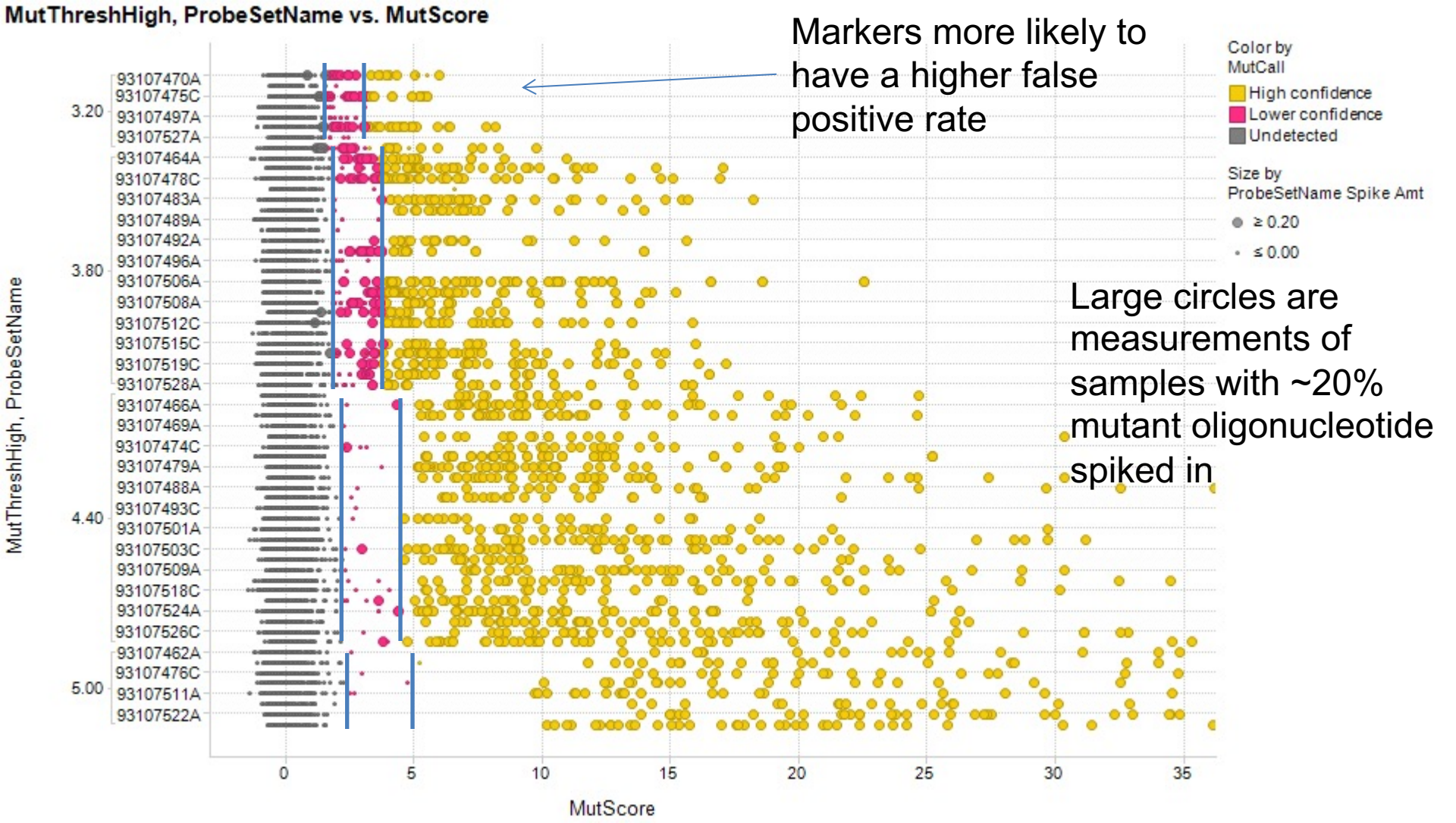


Apply MutScore thresholds to assign calls

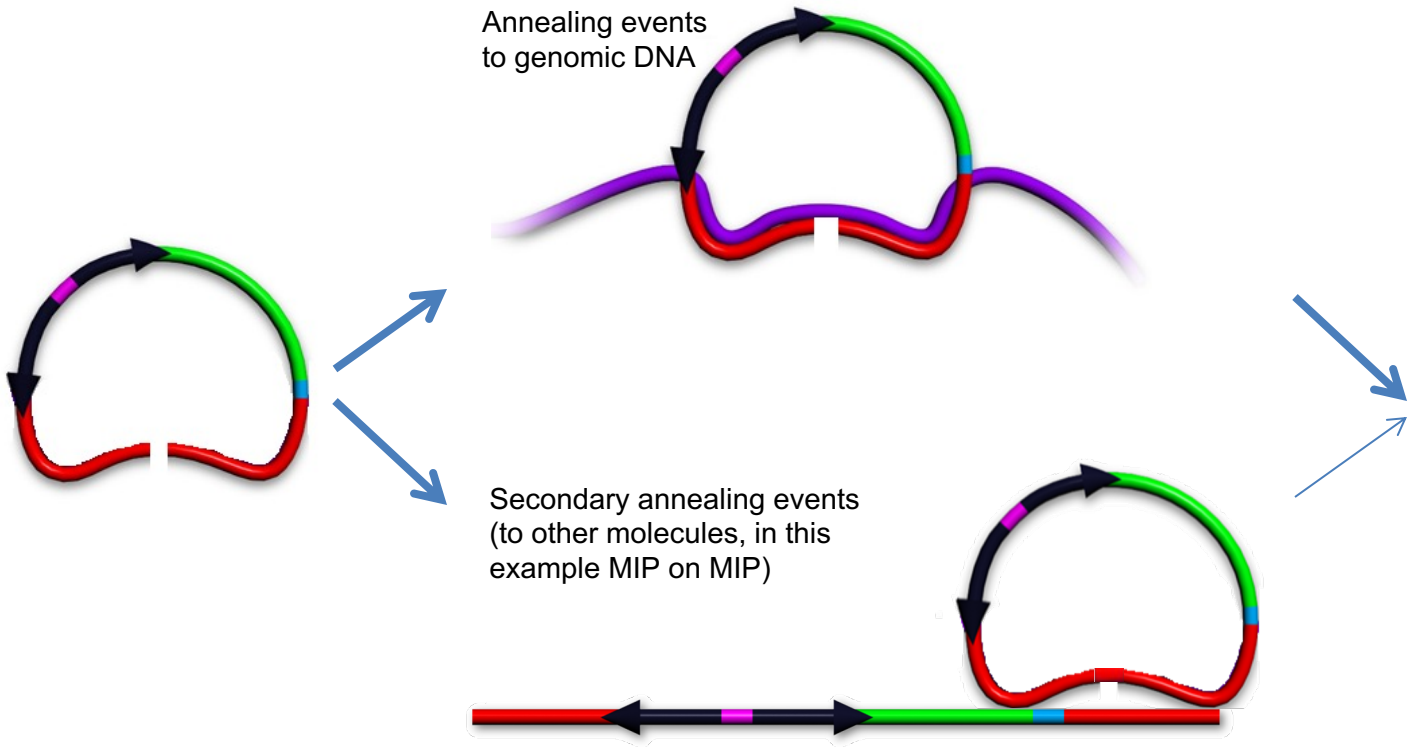


Higher MutScores are more significant mutation calls

4 MutScore calling threshold bins, probesets assigned to bin based on apparent sensitivity to mutation



Competitive annealing model

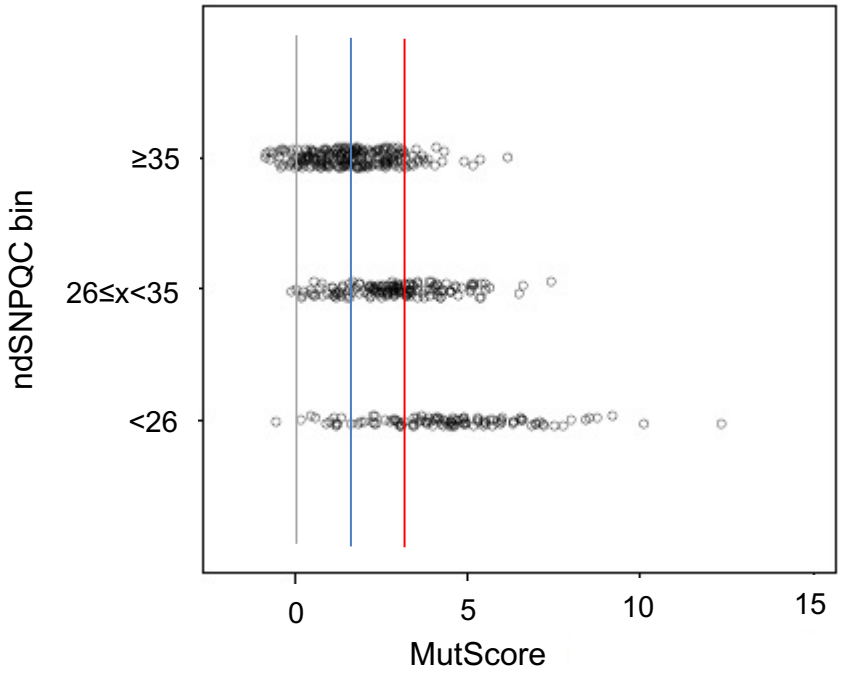


Something WILL be amplified during PCR. As accessible genomic DNA decreases, circularized MIPs from secondary annealing events will be an increasing proportion of input to PCR.

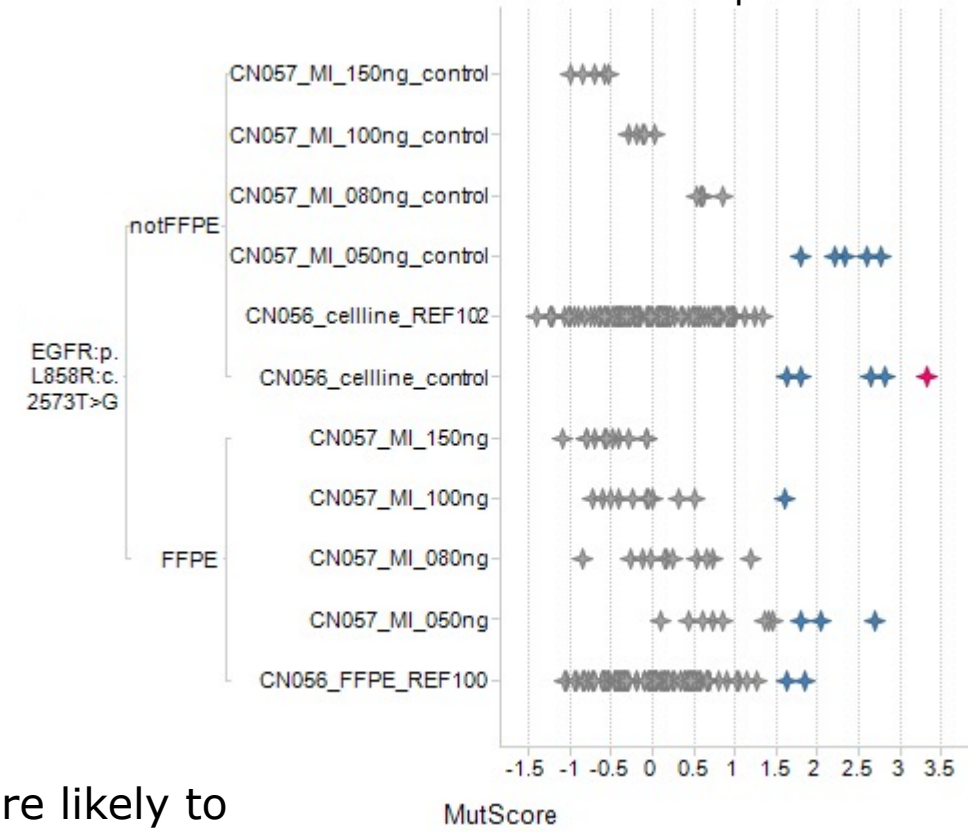
Some markers show MutScore trend vs ndSNPQC, and vs input mass

- EGFR:p.L858R:c.2573T>G

Example customer study (each data point a sample)



DNA mass titration experiment



- Perhaps MIPs for some markers more likely to have secondary annealing events, leading to increased background signal

You have a bunch of "High confidence" calls. Now what?

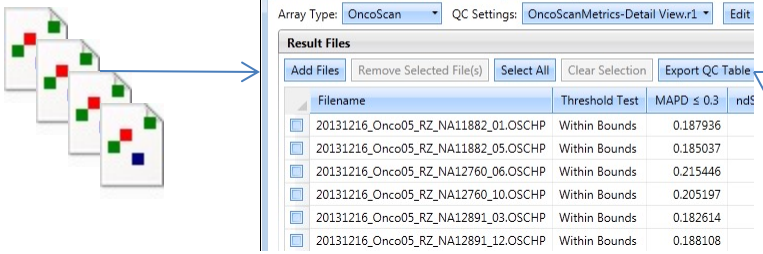
- For "in bounds" samples,
  - IF there are 0-2 real mutations per tumor sample out of these 64 measurements,
  - product specification targets a specificity of 99% (1 of 100 measurements is allowed to be false positive call),
- ...perhaps half of "High confidence" somatic calls can be false positives.

- Somatic calling algorithm is "single sample": it doesn't leverage information from other samples to set calling thresholds
  - By viewing somatic mutation data across samples, can sometimes make better calls to correct for any batch effects

- So....you should use Somatic Mutation Viewer to get a better feel for the data, and possibly re-call some measurements

Review-only analysis pipeline

.CEL

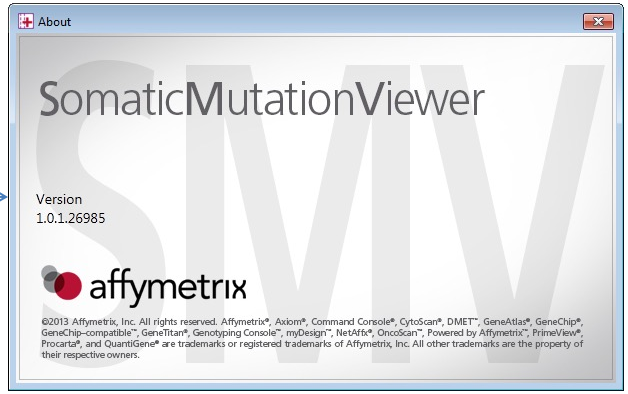


OncoScan Console

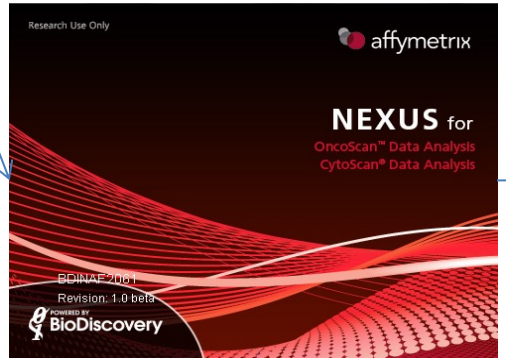
.OSCHP

.txt

for somatic mutation review



for CN aberration review

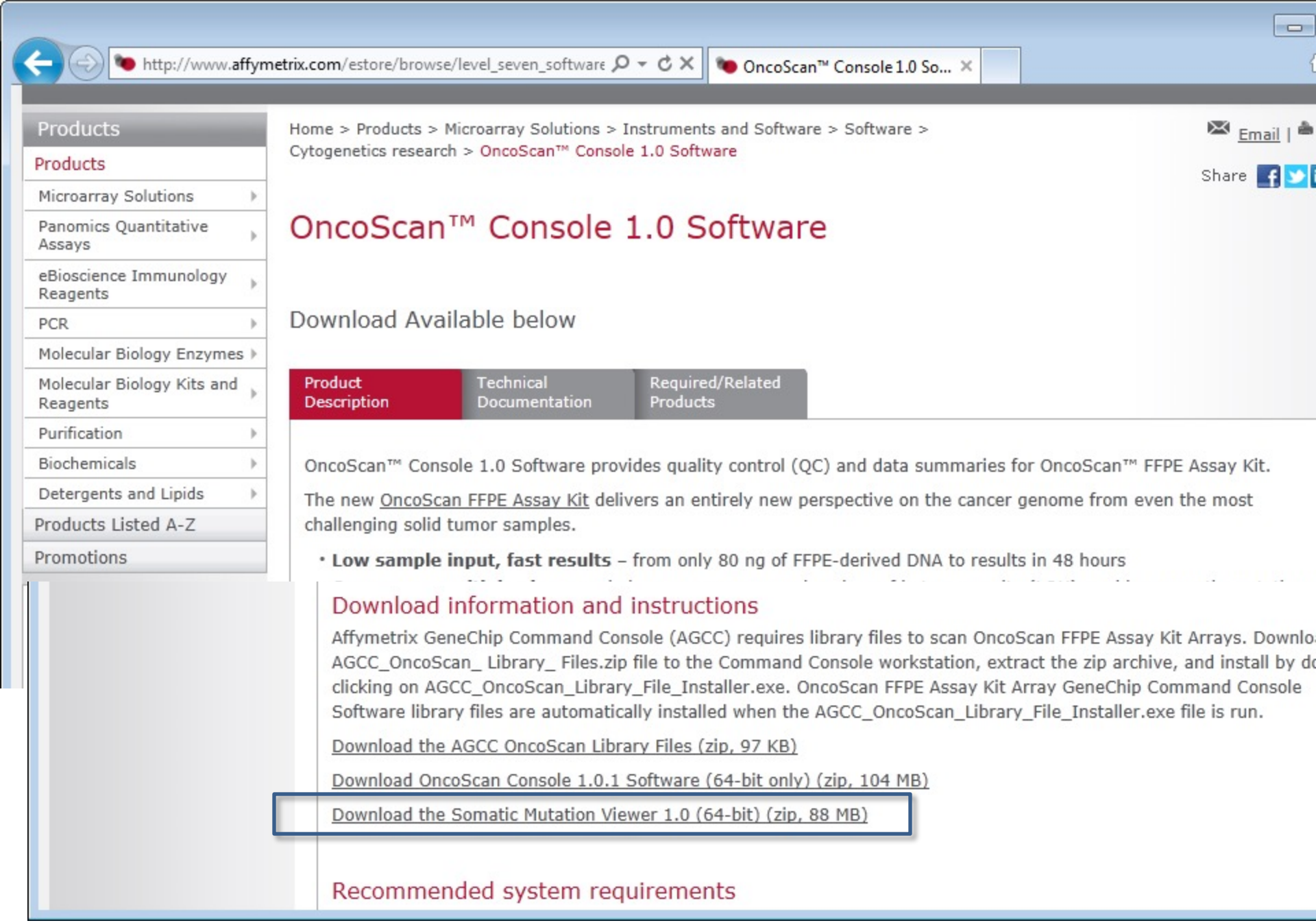


.txt

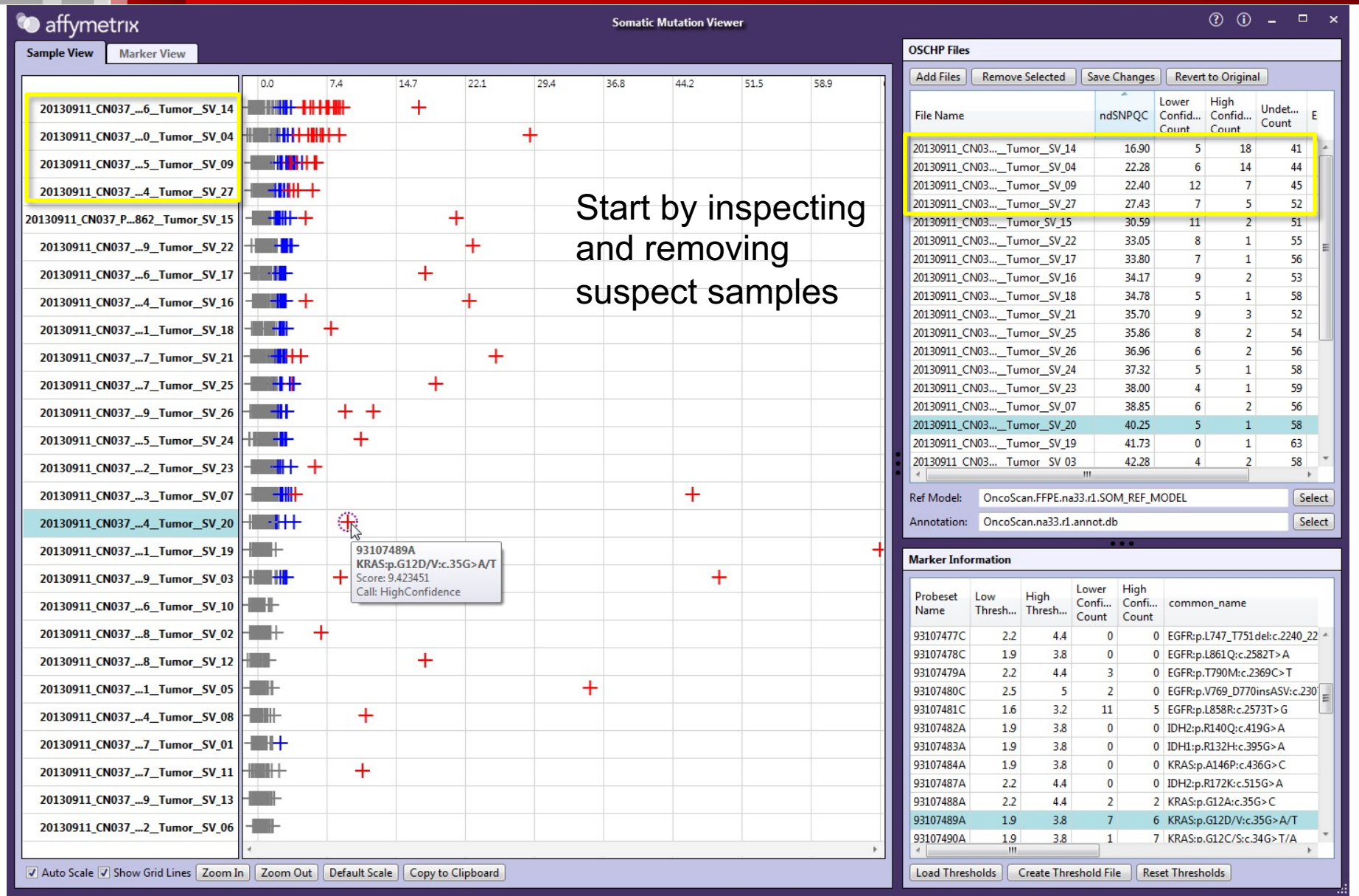
Nexus Express for OncoScan
(partnership with BioDiscovery)

<u>Steps</u>

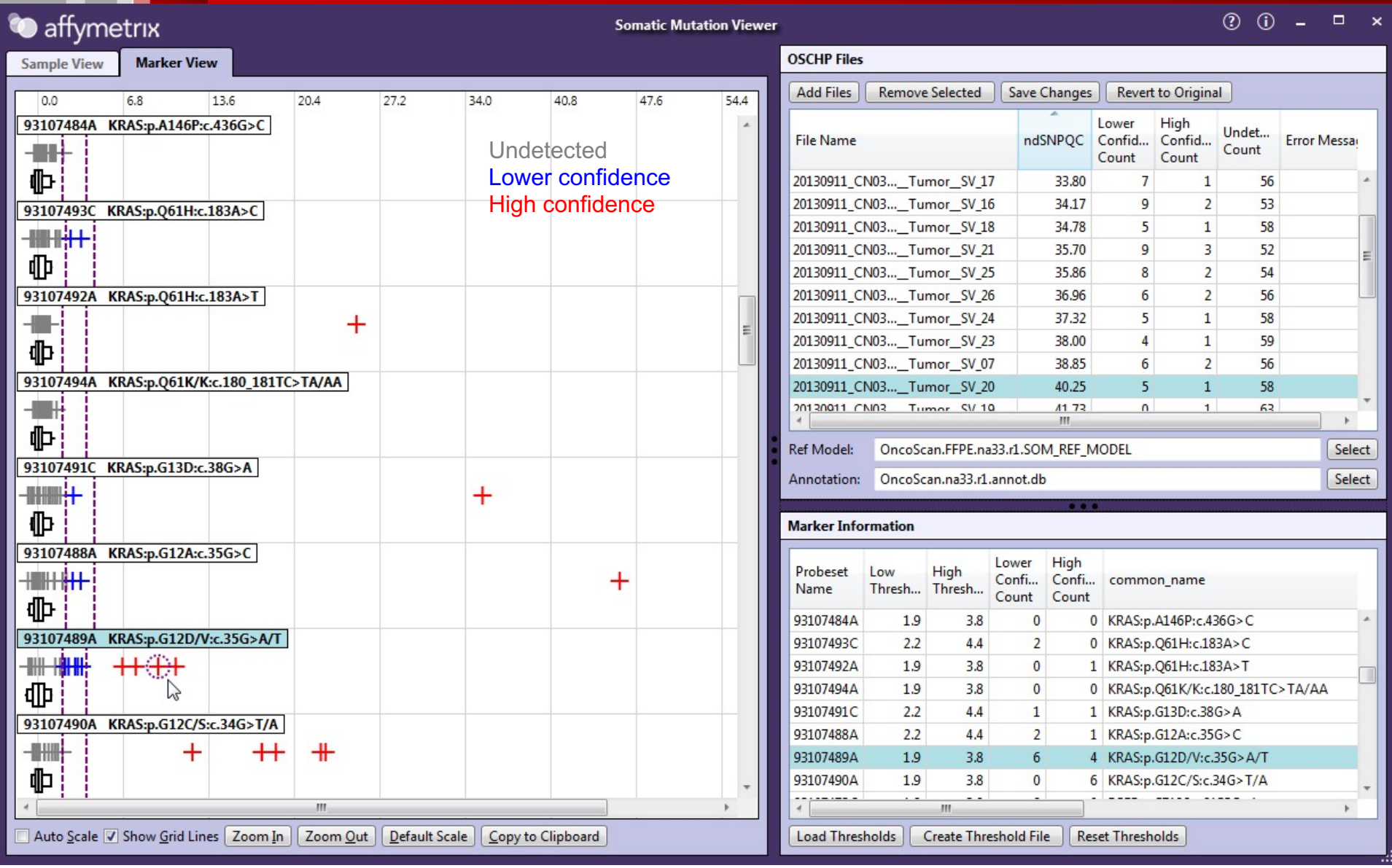
OSC_1: CELs -> OSCHPs
OSC_2: export SOM to txt
SMV_1: review SOM results
NEX_1: load OSCHPs, review CN results
NEX_2: export CN results to txt


http://www.affymetrix.com/estore/browse/level_seven_software
OncoScan™ Console 1.0 So...
Products
Products
Microarray Solutions
Panomics Quantitative Assays
eBioscience Immunology Reagents
PCR
Molecular Biology Enzymes
Molecular Biology Kits and Reagents
Purification
Biochemicals
Detergents and Lipids
Products Listed A-Z
Promotions
Home > Products > Microarray Solutions > Instruments and Software > Software > Cytogenetics research > OncoScan™ Console 1.0 Software
Email
Share
OncoScan™ Console 1.0 Software
Download Available below
Product Description
Technical Documentation
Required/Related Products
OncoScan™ Console 1.0 Software provides quality control (QC) and data summaries for OncoScan™ FFPE Assay Kit.
The new OncoScan FFPE Assay Kit delivers an entirely new perspective on the cancer genome from even the most challenging solid tumor samples.
• Low sample input, fast results – from only 80 ng of FFPE-derived DNA to results in 48 hours
Download information and instructions
Affymetrix GeneChip Command Console (AGCC) requires library files to scan OncoScan FFPE Assay Kit Arrays. Downlo
AGCC_OncoScan_ Library_ Files.zip file to the Command Console workstation, extract the zip archive, and install by do
clicking on AGCC_OncoScan_Library_File_Installer.exe. OncoScan FFPE Assay Kit Array GeneChip Command Console
Software library files are automatically installed when the AGCC_OncoScan_Library_File_Installer.exe file is run.
Download the AGCC OncoScan Library Files (zip, 97 KB)
Download OncoScan Console 1.0.1 Software (64-bit only) (zip, 104 MB)
Download the Somatic Mutation Viewer 1.0 (64-bit) (zip, 88 MB)
Recommended system requirements

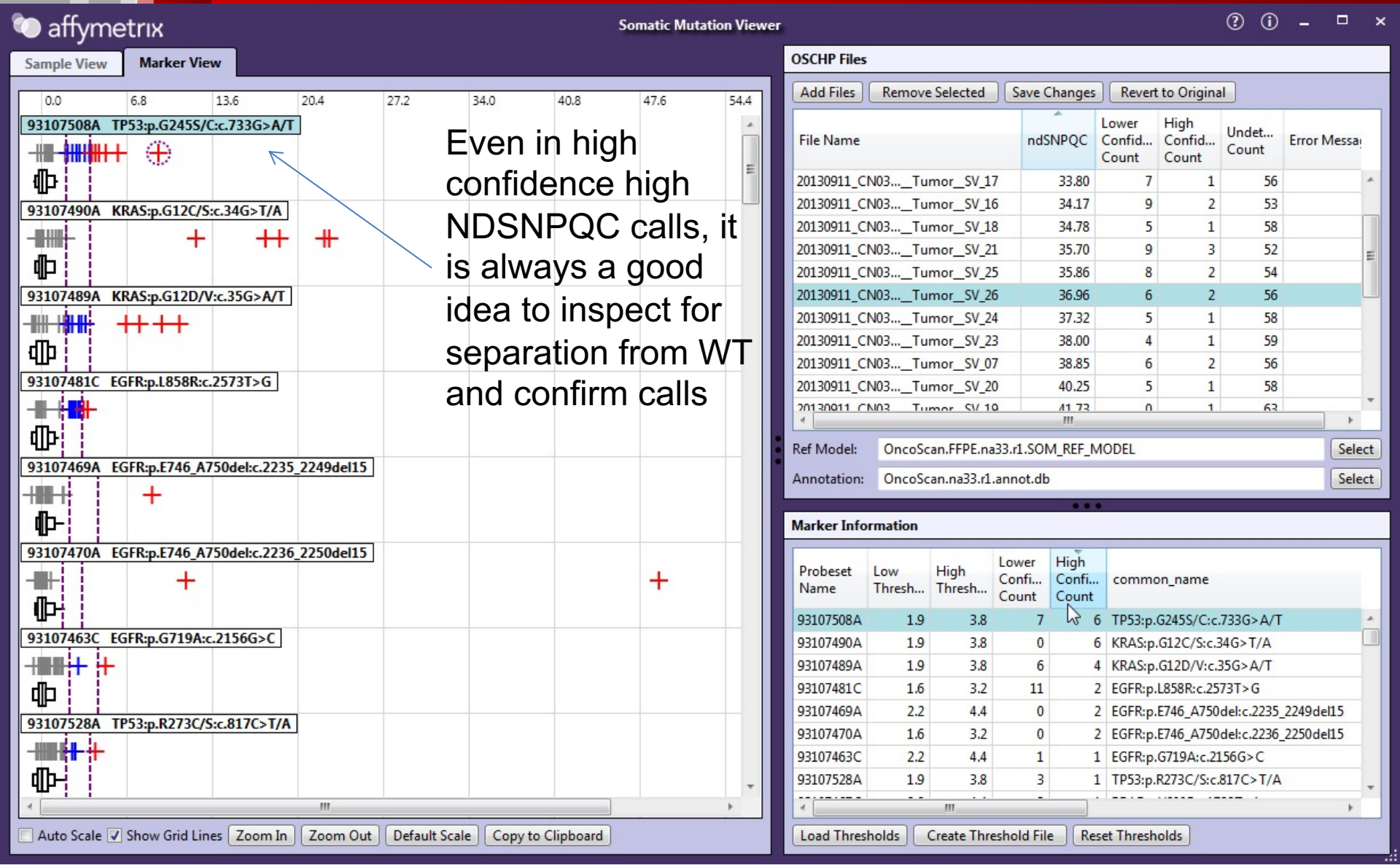
Somatic Mutation Viewer



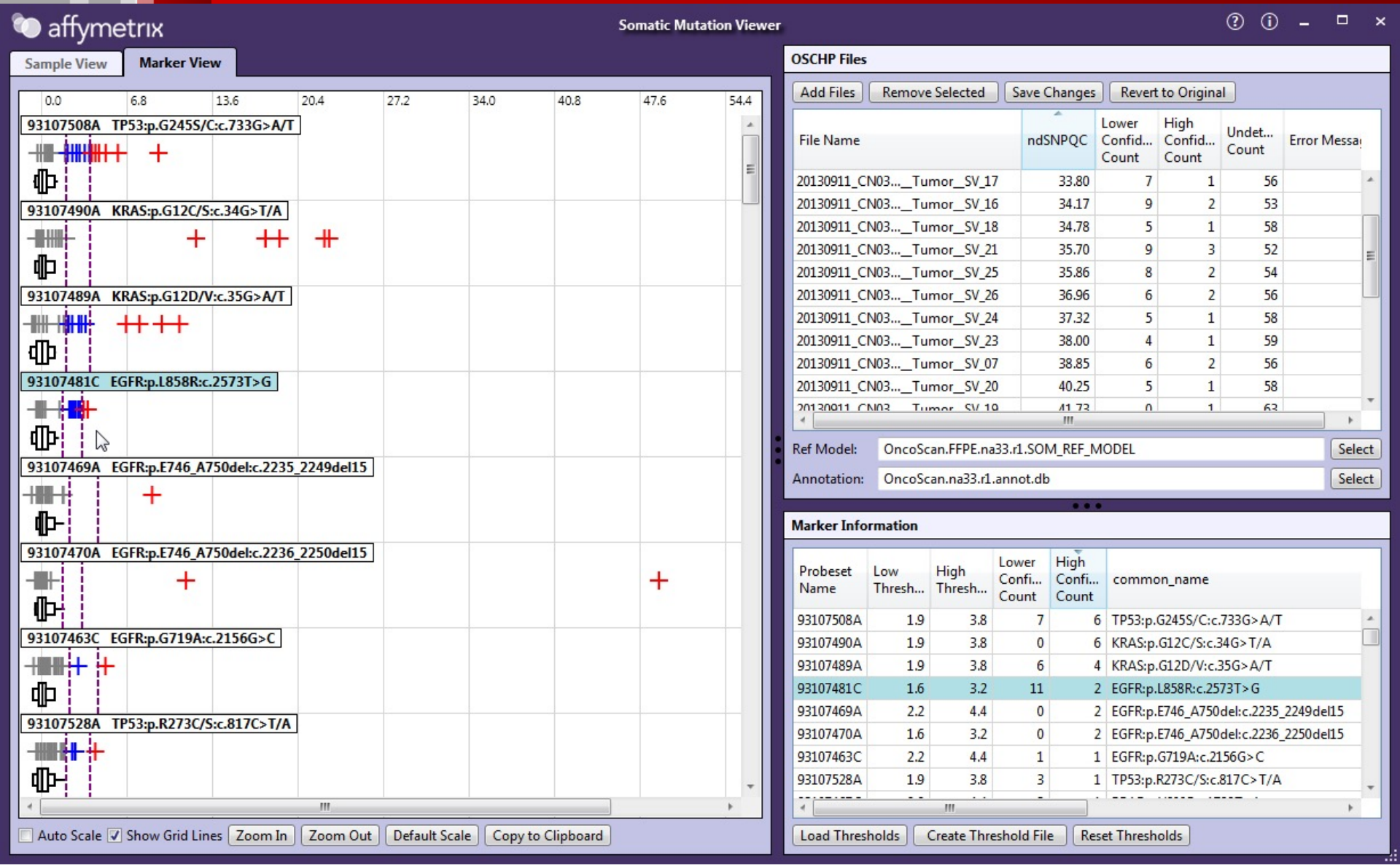
Switch to Marker View



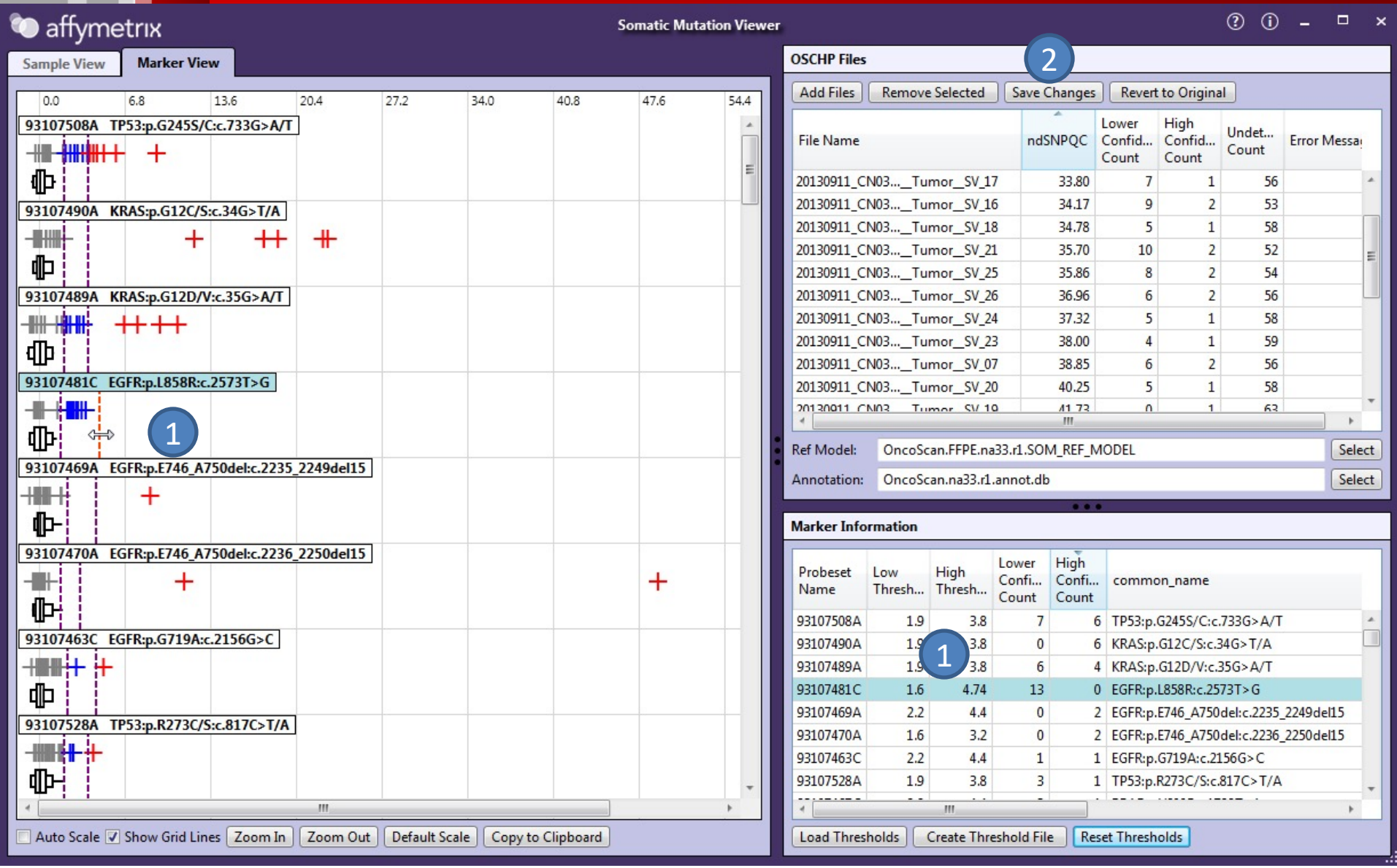
Sort Marker Information table by High Confidence Count



Could edit and save changes to OSCHP file

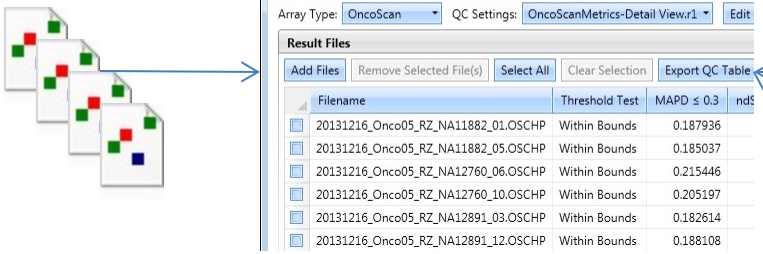


Could edit and save changes to OSCHP file



Edit-SOM-results analysis pipeline

.CEL

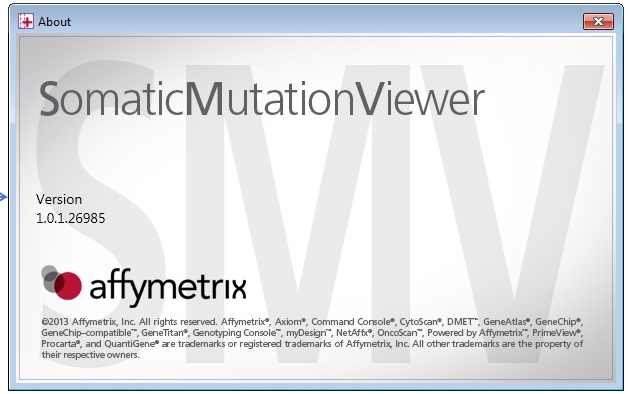


OncoScan Console

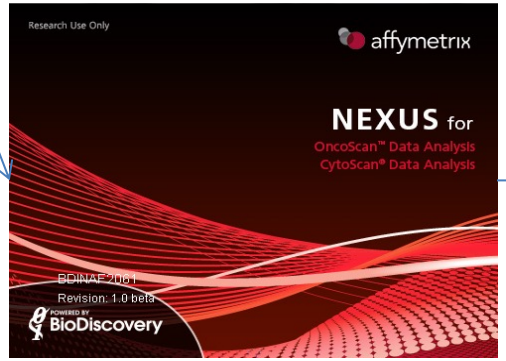
.OSCHP

.txt

for somatic mutation review



for CN aberration review



.txt

Nexus Express for OncoScan
(partnership with BioDiscovery)

<u>Steps</u>

OSC_1: CELs -> OSCHPs
SMV_1: review SOM results
SMV_2: optionally save modified calls to OSCHPs
OSC_2: load OSCHPs, export SOM to txt
NEX_1: load OSCHPs, review CN results
NEX_2: export CN results to txt

Factors affecting the false positive rate
(or: Why so many "High confidence" calls?)

- Some markers with calling thresholds closer to MutScore=0 will have higher false positive rate

- Sample QC metrics like MAPD and ndSNPQC are "out of bounds" (ndSNPQC<26 or MAPD>0.3)
  - Chip signal too weak
  - Insufficient DNA mass or poor quality DNA
  - Reagent Lot issues

- Sample QC metrics are "in bounds"
  - Some markers have more MutScore variability across data sets and/or reagent lots
  - Recommend using Somatic Mutation Viewer to review and adjust calling thresholds as needed, and confirm calls

Summary

- ndSNPQC more important for SM than CN
- when ndSNPQC is out of bounds SM calls are not reliable
- Good practice to examine calls in the viewer
  - You will observe that data quality improves with higher ndSNPQC eg., the number of lower confidence calls disappear