NxClinical™ 6.2
Release Notes

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The N_xClinical™ Release Notes document was written at BioDiscovery, LLC., 715 North Douglas Street, El Segundo, CA 90245.

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PATENTS

Several patents are pending.

CONTENTS

NEW FEATURES IN VERSION 6.2

Note: Some features are license dependent so may not apply to your specific installation of N_xClinical. E.g. CNV from NGS and Seq Var analysis capabilities are only available to those with a license that includes NGS (Array + NGS license).

GENOMIC INSTABILITY SCORING FOR HRD

Homologous recombination deficiency (HRD) is the inability to repair double-stranded DNA breaks using the HRR cellular pathway, which consequentially results in an acquired chromosomal breakage. Clinical research has shown cells with HRD are more sensitive to certain therapies and a measurement of HRD can be an effective pharmacogenetic biomarker across various tumor types. As a means to provide a functional evaluation of HR status, HRD scarring is an analysis approach to assess three specific quantifiable signatures of HRD genomic instability: HRD-LOH, TAI, and LST. N_xClinical includes a measurement of these three "genomic scars" to aid with HRD status assessment in cancer samples across technology types (where Test Type parameter in Sample Types = Oncology).

1. **Loss of heterozygosity (LOH)** - number of regions representing one parental allele via LOH (copy number neutral, or loss) longer than a specified min LOH event size, but shorter than the whole chromosome.
2. **Telomeric Allelic Imbalance (TAI)** - number of regions with CNV or allelic imbalance longer than the specified min TAI event that extend to one of the telomeres, but do not cross the centromere.
3. **Large-Scale State Transitions (LST)** - number of chromosomal break points between adjacent regions of change in copy number or allelic content longer than a specified minimum LST segment size. Adjacent events with a gap less than the maximum LST gap size are merged. State changes at centromeres and telomeres are excluded.

The calculations for these scars involve three processing steps:

1. **Merging** the CN Event and Zygosity tracks (used for all three scars)
2. **Smoothing** the resulting merged track to combine similar event types and smooth across small gaps as well as the centromere (performed independently for each scar)
3. **Selecting** the resulting calls or breakpoints that comply with each scar's specifications (performed independently for each scar)

A config file specifies parameters used in calculation of the genomic scars:

- Minimum LST segment size and maximum LST gap size
- Minimum LOH event size and Maximum LOH gap size

- Minimum TAI event size and Maximum TAI gap size

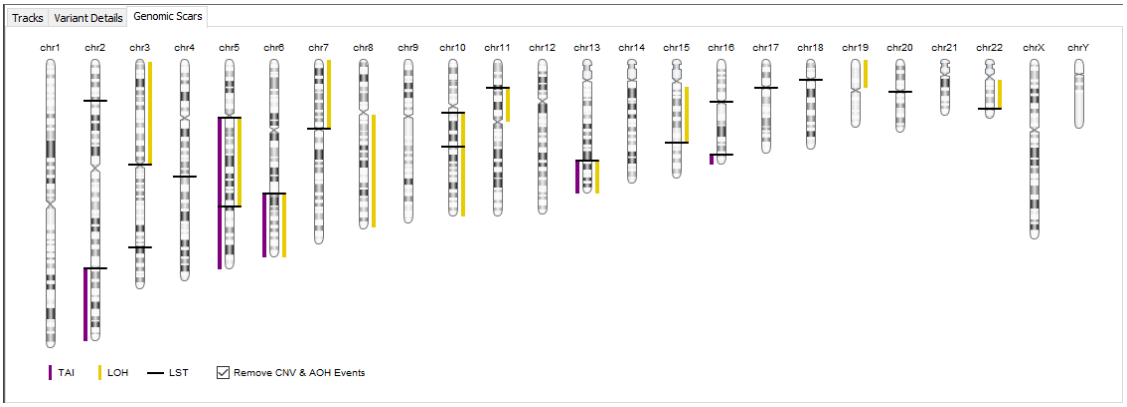
New features in N_xClinical associated with genomic scar scoring include

1. Calculation of genomic scar values during sample processing
2. Display of genomic scar values on the home page
3. Display of genomic scar values in the sample info window and listing in the table export with breakpoints/genomic regions listed for each measure.
   Info. window displaying genomic scar scores:

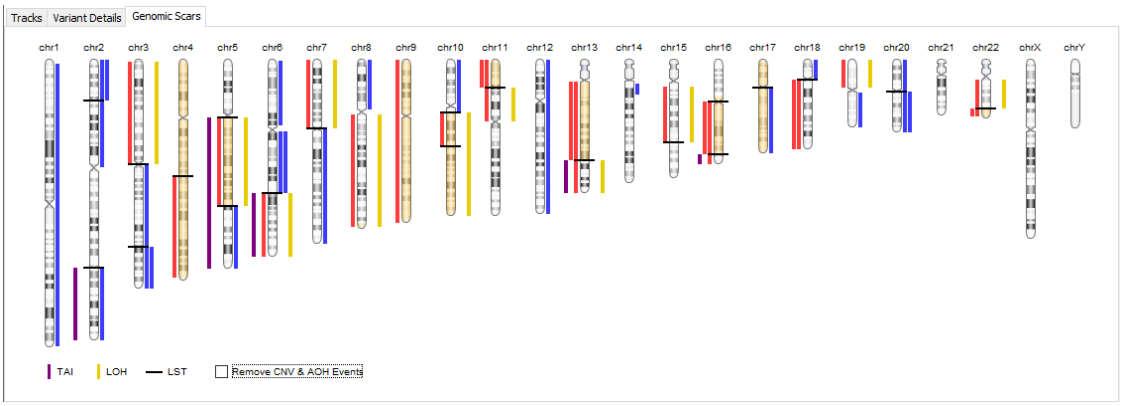
Genomic Scars

Minimum LST segment size:	10.0 Mb
Maximum LST gap size:	3.0 Mb
Minimum LOH event size:	15.0 Mb
Maximum LOH gap size:	3.0 Mb
Minimum TAI event size:	3.0 Mb
Maximum TAI gap size:	3.0 Mb
Large Scale Transition (LST) breakpoints:	12
	chr3:49,328,275 chr4:97,657,933 chr5:14,687,246
Loss of Heterozygosity (HRD-LOH) regions:	8
	chr5:14,687,246-163,147,253 chr6:64,965,826-170,982,559 chr8:31,279-40,333,918
Total Allelic Imbalance (TAI) regions:	6
	chr6:149,686-45,010,222 chr8:95,558,140-146,293,414 chr9:37,747-21,910,836

4. A visual representation of the genomic scar measures plotted on chromosome ideograms in new tab called “Genomic Scars” with the ability to view the scars alone or along with CNV and AOH.
   Genomic scars only:



Genomic scars along with CNV and AOH:



5. HRD calculation is enabled by the Admin based on the Sample Type level by selecting the new parameter “Perform Genomic Scar Calculation” in the Sample Type tab.

Other New Features

1. [Array+NGS license] There is a slight change to the transcript picking algorithm for selecting the default transcript in the table for seqvar events. For a seqvar event with multiple transcripts annotated by Nirvana, if one of these transcripts matches the canonical transcript in the NxClinical server, then this is the default selected transcript in the events table. If there is no match, then the existing transcript picking algorithm is used to make the selection.
2. A new batch upload utility that runs from the command line takes as input a descriptor file to load samples or both load and process samples without manual intervention.
3. The parameter “M vs. F, X Loss (0:2)” has been added to Analysis settings for setting thresholds for male ChrX loss. Previously, this threshold was set internally using the “Big Loss (0:2)” parameter for chrX loss (as the reference for SNP arrays is two copies). Now the male ChrX loss will be based on this new setting and this also allows setting a mosaic threshold (different threshold from big loss threshold) for chrX loss.
4. The Gene Panel filter in the pipeline can now be applied independently to each modality; e.g. for a sample with CNV and seq var. variants, the selected panel can be applied to CNVs but not the other modalities. Note, that different panels cannot be applied to different modalities. A selected panel can be turned on or off individually for each modality.

5. Default max memory allocation for the processing server has been increased to 4096M for new installs; when updating via processing server update installer, memory allocation is not changed.
6. Processing and Server installers have been updated to Java version 1.8.0_202
7. The Client installer for Win 32 has the default max memory usage set to less than 2G; if set to 2G or greater, the Client may not run.
8. Checkboxes in the “Select” column of the Table Details are grayed out in the table unless Edit mode is on.
9. [Array+NGS] There was a small change to the criteria for excluding alignment records from the BAF calculations. Specifically, secondary alignments (read mapping to more than one location) are still excluded but supplementary alignments (part of read aligned in one location and part of read aligned somewhere else) are now included; this aligns with the criteria for CNV detection which includes supplementary alignments but excludes secondary alignments.
10. Addition of a “StopProcessing” executable to the Win64 and Linux processing server installs that allows an Admin to stop the Processing Server by running the executable.
11. Improved the displayed metrics for the parent of origin calculation likelihood ratio by including the count of the fully-informative maternal and paternal probes identified within a region. These counts have been added to the Table and the Variant details.

BUG FIXES IN VERSION 6.2

Note: Lists bugs that were present in previous version(s) and older builds of version 6.2 and are fixed in the current release of version 6.2.

1. During sample upload via Sample Descriptor, the “Upload Sample Data” window did not have the relevant variant modality checkboxes marked off even though the sample would load and process correctly. Now the appropriate checkboxes (CNV, SeqVar, BAM) are marked off based on the samples and modalities being loaded.
2. Exporting all events from a Home page query now allows events from all samples in the query result to be exported; previously this was being limited to only the first 100 samples.
3. Fixed issue where some gnomAD frequencies in VCF files annotated with VEP 86/87/90 were not mapped correctly with their respective populations.
4. Fixed issue where SNP Rank Segmentation algorithm was overlapping adjacent events by one bp.
5. Fixed issue where a significantly larger max contiguous probe spacing parameter was causing segments to overlap by one base at the centromere.
6. Addressed a thread safety issue with parent of origin calculations originating from a change in the visibility of the parent of origin column vs. that initiated when manually editing an event or manually running the UPD calculation.
7. [Array+NGS license] B-allele frequency calculation from BAM files now uses SNP probe frequencies obtained from dbSNP for A and B alleles whereas previously it was counting the SNPs from BAM files and taking the most common as B-allele.
8. Fixed the issue where sometimes %AOH was reporting a negative value and missing from the Home page results.
9. Fixed a race condition issue where Sample Types were not being fully loaded when sample import via the Sample Importer utility was initiated causing errors stating that the Sample Type didn't exist.
10. Fixed a vulnerability to prevent the page's content from being rendered by another site when using the frame or iframe HTML tags that could potentially expose the site to a clickjacking or UI redress attack.
11. Fixed a potential denial of service vulnerability where the license reset feature on the server did not require authentication.
12. Fixed the issue where events near the centromere were overlapping by one bp if max consecutive probe spacing was set to a sufficiently high value when using SNP-FASST2 Segmentation and “inner probes” as the “Segment Boundaries” Settings parameter; segments are no longer overlapping with these settings.
13. Fixed the issue where re-ordering of attributes via drag and drop in the Sample Types - Sample Attributes tab was not working.
14. Fixed an issue with the ACMG Calculator where a score of -0.9 was incorrectly displaying VUS; now it correctly displays “Likely Benign”.

15. For genomic scarring, addressed bug where two events >10 mb separated by a region less than 10mb had two LST events called. Now no LST events are called here as there are no breakpoints between regions of at least 10Mb between the two events.
16. For genomic scarring, previously, an event considered to be touching the centromere or telomere needed to contain the last probe in the arm; if the last probe were not within the event, a user had to manually adjust the event to contain the probe. Now the user does not need to do any adjusting; if an event is within the boundaries of the centromere or telomere regions, even without touching the last probe, it will be considered to be touching the centromere or telomere.
17. If a BAM file has moved from its original location, the prompt to select a new path displayed the Java object for the file path rather than the actual file path on the storage device; now the path is displayed correctly.
18. Resolved an issue with the Probe coloring on BAF track for CN loss so that now the probe coloring scheme on the BAF track follows the inheritance logic of the allelic event
19. Resolved an issue that caused an exception and hindered other actions requiring users to re-log in whenever a CN event that overlapped an AOH event was deleted

KNOWN ISSUES IN VERSION 6.2

Known issues in the current release of version 6.2 with information where available on whether it's addressed in a future version.

1. When attempting to open a sample after creating a Sample Type in a build not previously used, an exception is thrown upon clicking on an event. To be fixed in a future version. Current workaround: The Admin should log out and log in again after creating a sample type in a build not used previously by another sample type.