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Integrated Genomic Scar Analysis for Homologous Recombination Deficiency (HRD): A Solution for Clinical Research Labs

Homologous recombination deficiency (HRD) represents a major area of research that is focused on demonstrating the utility and characterization of genomic scar analysis for implementation into routine care settings.

Bionano's VIA™ Analysis software provides labs an automated means to analyze genomic scars associated with HRD. Through participation in the Friends of Cancer Research (FOCR) **HRD Harmonization Project**, Bionano is contributing to community efforts to standardize the analytical approaches to HR status assessment to support its use as an effective biomarker for certain cancer types and treatment options.

Here, we briefly explore the background and current understandings of HRD, its potential clinical research applications, **VIA**, provides powerful new capabilities for research labs looking to calculate HRD scores.

What is HRD?

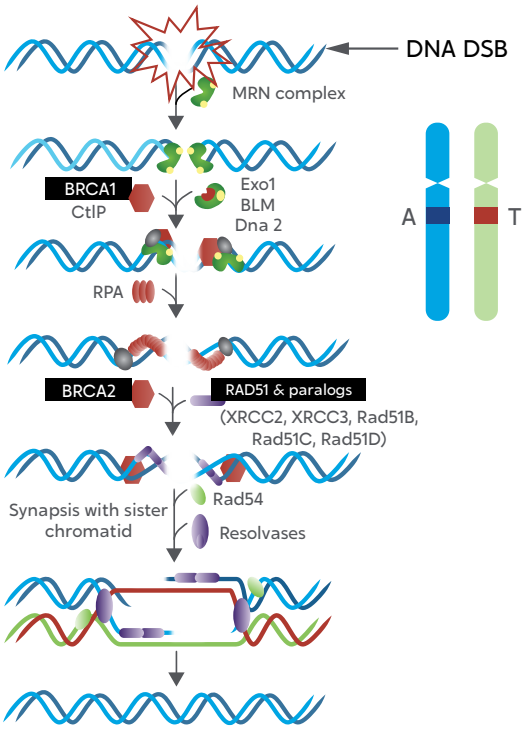
HRD is the inability to repair DNA double-strand breaks using the Homologous Recombination Repair (HRR) pathway.

This repair deficiency results in increased accumulation of chromosomal structural variants in the form of deletions, duplications, loss of heterozygosity (LOH), translocations, insertions, and inversions.

HRD is a common hallmark of cancer, and is of high interest for clinical research due to the sensitivity of homologous recombination-deficient cells to poly (ADP-ribose) polymerase (PARP) inhibitors and platinum-based therapies.[1]

Stover, et al offers a more detailed definition of HRD:

"Homologous recombination DNA repair deficiency (HRD) is a functional defect in homologous recombination DNA repair, arising from germline or somatic mutations in BRCA1/2 or other mechanisms. Cells with HRD are more sensitive to platinum and poly (ADP-ribose) polymerase inhibitors (PARPi). HRD generates permanent changes in the genome with specific, quantifiable patterns ('genomic scars')."



Overview of HR Repair
(Graphic adapted from Iliakis et al[2])

[1] Stover EH, Fuh K, Konstantinopoulos PA, Matulonis UA, Liu JF. Clinical assays for assessment of homologous recombination DNA repair deficiency. Gynecol Oncol. 2020;159(3): 887-898. doi:10.1016/j.ygyno.2020.09.029 [2] Iliakis G, Murmann T, Soni A. Alternative end-joining repair pathways are the ultimate backup for abrogated classical non-homologous end- joining and homologous recombination repair: Implications for the formation of chromosome translocations. Mutat Res Genet Toxicol Environ Mutagen. 2015 Nov;793:166-75. doi: 10.1016/j.mrgentox.2015.07.001. Epub 2015 Jul 4. PMID: 26520387.

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Harmonizing HR Status and Its Use as a Biomarker in Clinical Research Settings

Homologous recombination repair is one of the major mechanisms of defective DNA repair and frequently occurs in cancer. It's emerging as a promising biomarker with treatment implications for some disease types. The genomic scarring often left by homologous recombination is now the subject of intense research but there are diverse approaches to define, measure, and report HR status.

The Friends of Cancer Research, in partnership with a large working group of academic sites and industry partners, which Bionano is a member, is currently working on the **HRD Harmonization Project** to solve that problem. This project aims to understand the differences in the current assays available today for HRD and ultimately aligning methods for measuring HR status as a clinical biomarker.[2]

Bionano is contributing to the analysis of HRD genomic scars through the deployment of the genomic data analysis solution, **VIA™**. FOCR released results from the in silico data analysis phase of the HRD Harmonization project that highlights the variance of approaches and positivity rate of particpants emphsaizing the need for developing best practices.[3] The project will continue with an analysis of freshly extracted formalin-fixed paraffin-embedded human archival ovarian tumor samples to further understand similarities and differences among HRD assays.

Approaches to HRD Measurement and Scoring

Measurement of HR status employs two key approaches: a causative assessment of sequence variants within genes associated with the homologous recombination repair cellular pathway, and an assessment of the functional loss of HRR mechanism. A functional assessment of the tumor's genomic instability has previously been challenging due to the limitation of technologies to confidently assess structural changes of the tumor. Comprehensive assessment of HR status should include:

- The assessment of **sequence variants,** particularly germline or somatic pathogenic variants (PVs) in HR pathway genes (primarily BRCA1/BRCA2) that have been well documented in clinical research studies.
- Measurement of the **genomic instability** that results from HRD by looking at three specific genomic measurements of DNA repair competency: loss of heterozygosity (LOH), telomere allelic imbalance (TAI), and large-scale state transitions (LST).

NGS and SNP arrays are two commonly employed methodologies to assess HRD. Optical genome mapping (OGM) is capable of detecting classes of structural variation that are missed by these technologies and therefore has potential to provide additional insight to the chromosomal aberrations resulting from the HR pathway disruption.

There are differing approaches to evaluate the functional impact of HRD, such as a measurement for the total percentage of genomic loss of heterozygosity. It has been reported that a combination of LOH, TAI, and LST, which has been demonstrated to have a higher value than the LOH score alone.[4]

[2] The Oncologist, Volume 27, Issue 3, March 2022, Pages 167–174

[3] Stires H. Assessing Variability Across HRD Assays: Findings from the Friends HRD Harmonization Project. Poster presented at: The Association of Molecular Pathology Annual Meeting; Nov, 2022; Phoenix, AZ.

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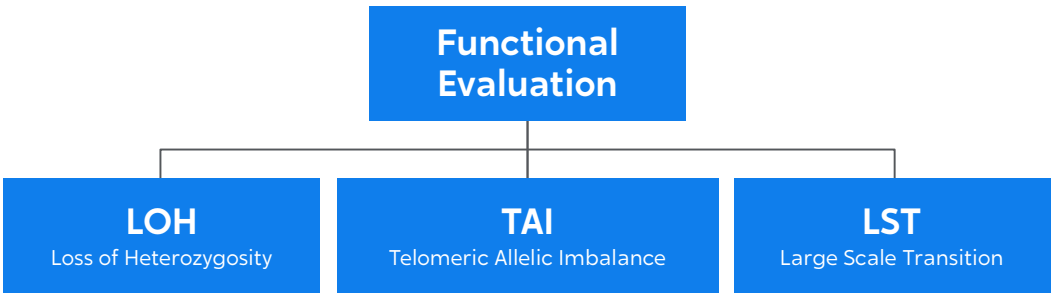
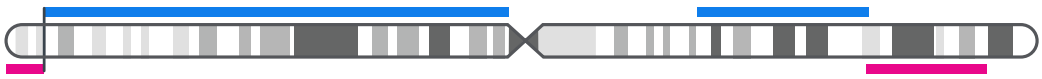


Solid tumor tissue storage layers on additional technical challenges that can further complicate the prospect of HRD analysis. This limitation needs to be considered when including genomic scar analysis into a workflow. However, **VIA™** is capable of conducting a genomic scar analysis from methods adapted for FFPE tissues.

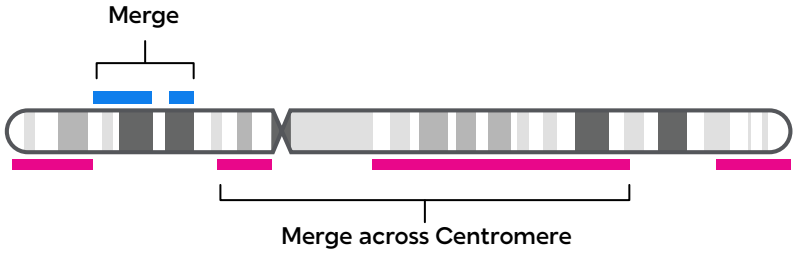
VIA's Genomic Scar Analysis and Scoring Workflow

VIA's genomic scar analysis involves the following stages. (Blue bars represent Gains, Red bars represent Losses, Vertical Lines represent LST breaks, Purple bars represent TAI, and Yellow bars represent LOH)

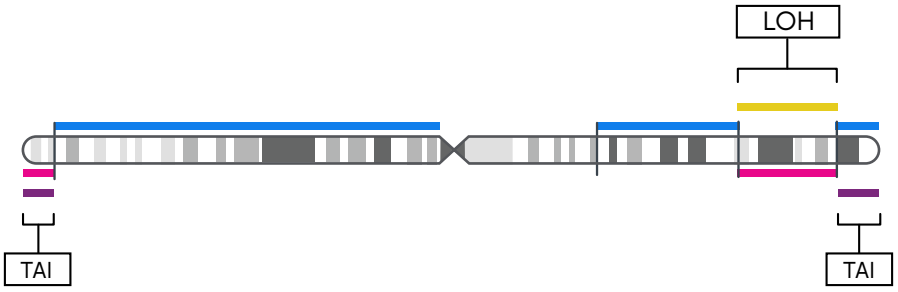
1. **Detect copy number (CN) and LOH events from arrays and NGS.** This involves merging CN and b-allele frequency (BAF) event tracks (performed once and used for all three scores: LOH, TAI, LST).



2. **Merge and smooth similar event types for scarring.** Upon creation of a unified representation, VIA smooths the resulting merged track to combine similar event types, omit small gaps, and join events spanning the centromere.



3. **Select the resulting events that meet each scar's criteria (LOH, TAI, and LST).**



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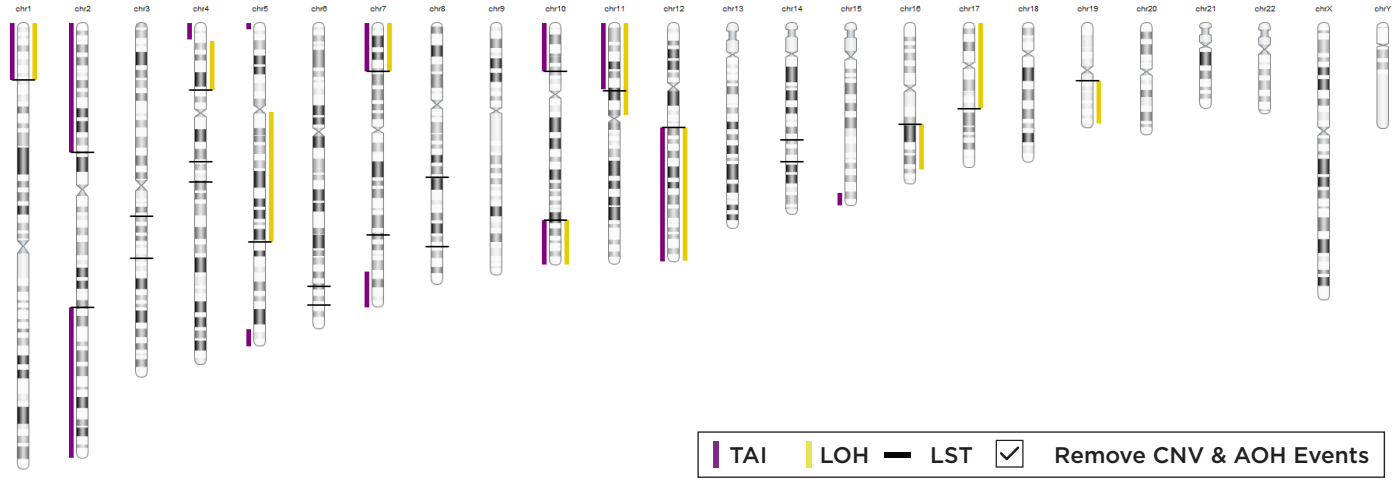
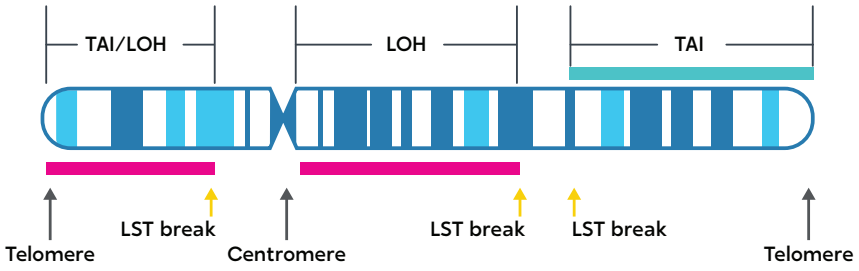
4. Visualize the genomic scars genome-wide.



Karyogram visualization of the copy number regions impacted by genomic scaring instability.

HRD Genomic Scar Definitions in VIA

- **Loss of Heterozygosity (LOH)**
  The number regions of representing one parental allele via LOH longer than 15 Mb but shorter than the whole chromosome[4]
- **Telomeric Allelic Imbalance (TAI)**
  The number of regions of contiguous allelic imbalance that extend to one of the subtelomeres but do not cross the centromere[5]
- **Large-Scale State Transition (LST)**
  The number of chromosomal arm breakpoints between adjacent regions greater than 10MB but not separate by less than 3MB[6]



Schematic demonstrating example measurement of a chromosome leveraging a comprehensive genomic scarring approach. It is possible for events to meet the criteria of multiple scars while other events will not count towards the scores.

[4] Abkevich, et al., Patterns of genomic loss of heterozygosity predict homologous recombination repair defects in epithelial ovarian cancer, Br. J. Cancer 107 (10) (2012)1776–1782

[5] Birkbak, et al. Telomeric allelic imbalance indicates defective DNA repair and sensitivity to DNA-damaging agents. Cancer discovery 2, no. 4 (2012): 366-375.

[6] Popova T, Manie E, Rieunier G, et al. Ploidy and large-scale genomic instability consistently identify basal-like breast carcinomas with BRCA1/2 inactivation. Cancer Res 2012;72:5454–62

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See More of The Tumor Profile

Perhaps the strongest benefit to VIA™ software for HRD analysis and scoring is the enhanced transparency and clarity of chromosomal abnormalities, which enables teams to analyze HRD more accurately.

With **VIA**, labs can look at a sample in its entirety, better understand the impact allelic copy number has on chromosomes and genomic scarring, and better visualize the impact of HRD, such as seeing the deletion of a critical oncogene. Perhaps it's not just the gene that's deleted, but rather the entire chromosome arm enabling more insights for clinicians and supporting further understanding. Labs are able to see more completely the underlying aberrations and abnormalities that are impacting the tumor genome with VIA.

Genomic Scars	
Large Scale Transition (LST)breakpoints:	20
Loss of Heterozygosity (HRD-LOH) regions:	11
Total Allelic Imbalance (TAI) regions:	5

An organized listing of the HRD events is displayed within VIA software in a sample-info pop-up window.

Extract HRD Scores From Your Existing Data and Workflow

VIA provides labs a more scalable means to include an analysis of genomic scars, reducing steps and time, from the workflow. Another massive benefit of VIA is it may be possible to extract these scores from the data and workflow you have right now.

Many sites currently running a capture-based NGS library prep today can leverage **VIA** to calculate HRD scores from genomic scars without disrupting their existing data pipeline. VIA is also compatible with major array platforms enabling the ability to implement an integrated genomic scar analysis from existing array service. This helps teams overcome a major hurdle that has limited HRD analysis for many labs.

Ready to Bring Powerful HRD Analysis and Scoring Capabilities to Your Lab?

Request a free personalized demo of VIA and see its HRD analysis workflow in action.

*This software is for research use only. It is designed to assist clinicians and it is not intended as a primary diagnostic tool. It is each lab's responsibility to use the software in accordance with internal policies as well as in compliance with applicable regulations.

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