



HAL
open science

ShallowHRD: detection of homologous recombination deficiency from shallow whole genome sequencing

Alexandre Eeckhoutte, Alexandre Houy, Elodie Manié, Manon Reverdy, Ivan Bièche, Elisabetta Marangoni, Oumou Goundiam, Anne Vincent-Salomon, Dominique Stoppa-Lyonnet, François-Clément Bidard, et al.

► To cite this version:

Alexandre Eeckhoutte, Alexandre Houy, Elodie Manié, Manon Reverdy, Ivan Bièche, et al.. ShallowHRD: detection of homologous recombination deficiency from shallow whole genome sequencing. *Bioinformatics*, 2020, 36 (12), pp.3888-3889. 10.1093/bioinformatics/btaa261 . inserm-03034570

HAL Id: inserm-03034570

<https://inserm.hal.science/inserm-03034570>

Submitted on 1 Dec 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Genome analysis

ShallowHRD: detection of homologous recombination deficiency from shallow whole genome sequencing

Alexandre Eeckhoutte ^{1,2,*}, Alexandre Houy^{1,2}, Elodie Manié^{1,2}, Manon Reverdy^{1,2}, Ivan Bièche³, Elisabetta Marangoni^{2,4}, Oumou Goundiam^{2,4}, Anne Vincent-Salomon⁵, Dominique Stoppa-Lyonnet^{1,6}, François-Clément Bidard^{7,8}, Marc-Henri Stern^{1,2,3} and Tatiana Popova^{1,2}

¹DNA Repair and Uveal Melanoma (D.R.U.M.), Inserm U830, Institut Curie, Paris 75248, France, ²Institut Curie, PSL Research University, Paris 75005, France, ³Department of Genetics, Institut Curie, Paris 75248, France, ⁴Department of Translational Research, Institut Curie PSL Research University, Paris 75248, France, ⁵Department of Biopathology, Institut Curie PSL Research University, Paris 75005, France, ⁶Faculty of Medicine, University of Paris, Paris, France, ⁷Department of Medical Oncology, Institut Curie PSL Research University, Paris 75248, France and ⁸Versailles Saint Quentin en Yvelines University, Paris Saclay University, Versailles 78035, France

*To whom correspondence should be addressed.

Associate Editor: Inanc Birol

Received on January 3, 2020; revised on March 25, 2020; editorial decision on April 13, 2020; accepted on April 14, 2020

Abstract

Summary: We introduce *shallowHRD*, a software tool to evaluate tumor homologous recombination deficiency (HRD) based on whole genome sequencing (WGS) at low coverage (shallow WGS or sWGS; ~1X coverage). The tool, based on mining copy number alterations profile, implements a fast and straightforward procedure that shows 87.5% sensitivity and 90.5% specificity for HRD detection. *shallowHRD* could be instrumental in predicting response to poly(ADP-ribose) polymerase inhibitors, to which HRD tumors are selectively sensitive. *shallowHRD* displays efficiency comparable to most state-of-art approaches, is cost-effective, generates low-storable outputs and is also suitable for fixed-formalin paraffin embedded tissues.

Availability and implementation: *shallowHRD* R script and documentation are available at <https://github.com/aeckhou/hou/shallowHRD>.

Contact: alexandre.eeckhoutte@curie.fr

Supplementary information: [Supplementary data](#) are available at *Bioinformatics* online.

1 Introduction

Aggressive subtypes of breast and ovarian cancers are frequently associated with homologous recombination deficiency (HRD) making these tumors sensitive to poly(ADP-ribose) polymerase inhibitors (Coleman *et al.*, 2019). HRD arises upon inactivation of *BRCA1/2*, *RAD51C* or *PALB2* and is characterized by specific tumor genome instability (Nik-Zainal *et al.*, 2016; Staaf *et al.*, 2019). Even though HRD genes are mostly known, exhaustive testing of their inactivation is difficult. This motivates developing surrogate genomic markers of HRD. Recent developments based on high throughput sequencing, HRDetect, Signature 3, SigMA, scarHRD, achieved excellent capacity to evaluate HRD (Davies *et al.*, 2017; Gulhan *et al.*, 2019; Polak *et al.*, 2017; Sztupinski *et al.*, 2018). However, these methods are technically complex, time- and data-storage consuming, often need a matched normal sample and can be costly.

We introduce *shallowHRD*, a software for HRD testing based on the number of large-scale genomic alterations (LGA) obtained from

whole genome sequencing (WGS) at low coverage (shallow WGS or sWGS; ~1X). sWGS robustly detect copy number alterations (CNAs), even in fixed-formalin paraffin embedded (FFPE) samples and liquid biopsies (Van Roy *et al.*, 2017) at low cost and with easy-storable outputs. The concept of LGAs follows single-nucleotide polymorphism (SNP) array approaches, exploiting an increased number of large-scale intra-chromosomal CNAs characteristic of HRD (Abkevich *et al.*, 2012; Birkbak *et al.*, 2012; Popova *et al.*, 2012).

2 Materials and methods

2.1 Data

In-house sWGS of breast and ovarian cancers (26 primary tumors, 39 patient-derived xenografts from frozen blocks and 4 primary tumors FFPE) and down-sampled to ~1X WGS (108 normal tissues, 79 primary tumors from the TCGA breast cancer) were processed

by Control-FREEC (v11.5) (Boeva *et al.*, 2012) (Supplementary Material).

2.2 shallowHRD

The tool takes as input ‘sample_name.bam_ratio.txt’, which includes CNA profile $\{x, g\}_{1, N}$ where x is normalized read counts in a sliding window, g is genomic coordinate and the profile segmentation with S_i, Z_i segment median and size (in megabases, Mb).

2.2.1 Workflow

- CNA cut-off is detected and the profile segmentation is optimized as follows: Segments are defined as ‘large’ if $Z_i \geq (Q1 + Q3)/2$, where $Q1, Q3$ are quartiles of Z_i ($Z_i > 3$ Mb) distribution. M is detected as the first local minimum of $(S_i - S_j)$ density, where i, j are large segments (Supplementary Fig. S1). CNA cut-off = $\min(\max(0.025, M), 0.45)$. Adjacent segments are merged if $(S_i - S_{i+1}) < \text{CNA cut-off}$; starting from the largest segment.
- LGAs, defined as intra-chromosome arm CNA breaks with adjacent segments $Z_i, Z_{i+1} \geq 10$ Mb, are counted after removing segments < 3 Mb.
- The sample is annotated as ‘non-HRD’ (LGA < 15), ‘borderline’ ($15 \leq \text{LGA} \leq 19$) or ‘HRD’ (LGA > 19).
- Sample quality is defined by M and $cMAD$, $cMAD = \text{median}((x - S_x))$, where S_x corresponds to the segment enclosing x , before optimization: ‘bad’ ($cMAD > 0.5$ | $cMAD > 0.14$ and $M > 0.45$), ‘average’ ($cMAD > 0.14$ and $M < 0.45$ | $cMAD < 0.14$ and $M > 0.45$) or ‘normal or highly contaminated’ ($M < 0.025$) (Supplementary Material and Fig. S2).
- CCNE1 amplification is called if $S_c \geq 4 \cdot \text{CNA cut-off}$, where c is the segment enclosing the gene (4 was set arbitrarily).

shallowHRD output contains: (A) Tumor genome profile. (B) Density plot for CNA cut-off. (C) CNA segmentation summary. (D) Sample quality and HRD diagnostics (Supplementary Fig. S3).

3 Results

In-house sWGS and down-sampled WGS of normal samples (TCGA) were employed to develop the sWGS methodology similar to the large-scale state transitions (LST) in SNP-arrays (Popova *et al.*, 2012) (Section 2). LGAs inferred from sWGS corresponded well to the LSTs with identical HRD calls for 8 primary tumors tested (76–97% match in segments ≥ 10 Mb) (Supplementary Fig. S4). sWGS coverage $> 0.3X$ provide adequate quality, also for FFPE (Supplementary Figs. S2 and 5).

Validation by down-sampled WGS (TCGA) showed LGA to be coherent to SNP-arrays LST ($r = 0.92$; slope = 0.88; $P < 2.2e-16$, Pearson) with increased discrepancy in average quality samples

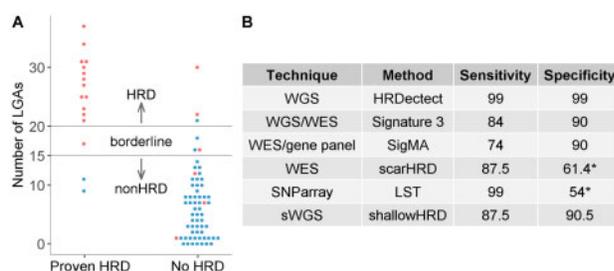


Fig. 1. shallowHRD validation in down-sampled WGS of the TCGA (A) and performance (B). Proven/No HRD: cases with/without inactivation of BRCA1/2, RAD51C or PALB2 (Supplementary Material); HRD (red) and non-HRD (blue) cases in SNP-arrays; LGAs: large-scale genomic alterations; WES: whole exome sequencing. *Low specificity could be due to non-complete annotation of HRD

($n = 13$), and HRD diagnostics discordant in three and borderline in four cases (Fig. 1A; Supplementary Material, Supplementary Figs. S6 and 7, Supplementary Table S1). CCNE1 amplification was found in four non-HRD cases, in-line with previous observations of almost mutual exclusivity with HRD (Goundiam *et al.*, 2015). Thus, sWGS LGAs is suitable to take over the SNP-array LSTs, which is a clinically validated method for HRD detection.

Tumor content for sWGS limits to > 0.3 as estimated from the TCGA and *in silico* dilution series (Supplementary Material, Supplementary Figs. S8 and 9).

Fifteen and 20 LGAs represent soft and stringent cut-offs with sensitivity of 87.5% and 81.25% (16 cases HRD) and specificity of 90.5% and 95.2% (63 non-HRD cases), respectively, which is compatible with other state-of-the-art approaches (Fig. 1B).

To conclude, shallowHRD implements a fast and straightforward evaluation of tumor HRD in breast, ovarian and other cancers such as pancreatic or prostatic, performing similar to most state-of-the-art approaches, the technique is cheap and suitable for all type of samples.

Acknowledgements

The results here are in part-based upon data generated by the TCGA Research Network: <http://cancergenome.nih.gov/>. The authors thank The Seven Bridges Cancer Genomics Cloud for computational facilities.

Funding

This work was supported by the Ligue Nationale Contre le Cancer (to A.E.).

Conflict of Interest:

E. Manić, T. Popova and M.-H. Stern are co-inventors of the LST method (US20170260588, US20150140122 and exclusive Licence to Myriad Genetics).

References

- Abkevich, V. *et al.* (2012) Patterns of genomic loss of heterozygosity predict homologous recombination repair defects in epithelial ovarian cancer. *Br. J. Cancer*, 107, 1776–1782.
- Birkbak, N.J. *et al.* (2012) Telomeric allelic imbalance indicates defective DNA repair and sensitivity to DNA-damaging agents. *Cancer Discov.*, 2, 366–375.
- Boeva, V. *et al.* (2012) Control-FREEC: a tool for assessing copy number and allelic content using next-generation sequencing data. *Bioinformatics*, 28, 423–425.
- Coleman, R.L. *et al.* (2019) Veliparib with first-line chemotherapy and maintenance therapy in ovarian cancer. *N. Engl. J. Med.*, 381, 2403–2415.
- Davies, H. *et al.* (2017) HRDetect is a predictor of BRCA1 and BRCA2 deficiency based on mutational signatures. *Nat. Med.*, 23, 517–525.
- Goundiam, O. *et al.* (2015) Histo-genomic stratification reveals the frequent amplification/overexpression of CCNE1 and BRD4 genes in non-BRCAness high grade ovarian carcinoma. *Int. J. Cancer*, 137, 1890–1900.
- Gulhan, D.C. *et al.* (2019) Detecting the mutational signature of homologous recombination deficiency in clinical samples. *Nat. Genet.*, 51, 912–919.
- Nik-Zainal, S. *et al.* (2016) Landscape of somatic mutations in 560 breast cancer whole-genome sequences. *Nature*, 534, 47–54.
- Polak, P. *et al.* (2017) A mutational signature reveals alterations underlying deficient homologous recombination repair in breast cancer. *Nat. Genet.*, 49, 1476–1486.
- Popova, T. *et al.* (2012) Ploidy and large-scale genomic instability consistently identify basal-like breast carcinomas with BRCA1/2 inactivation. *Cancer Res.*, 72, 5454–5462.
- Stauf, J. *et al.* (2019) Whole-genome sequencing of triple-negative breast cancers in a population-based clinical study. *Nat. Med.*, 25, 1526–1533.
- Sztupinski, Z. *et al.* (2018) Migrating the SNP array-based homologous recombination deficiency measures to next generation sequencing data of breast cancer. *NPJ Breast Cancer*, 4, 16.
- Van Roy, N. *et al.* (2017) Shallow whole genome sequencing on circulating cell-free DNA allows reliable noninvasive copy-number profiling in neuroblastoma patients. *Clin. Cancer Res.*, 23, 6305–6314.