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Protocol

Check for updates

Optimized whole-genome sequencing workflow for tumor diagnostics in routine pathology practice

In the format provided by the authors and unedited

REAGENTS

PART 9 (step 1-51)

- Qiasymphony DSP DNA Midi kit 1000ul (Qiagen GmbH, cat. no. 997002)
- Qubit™ dsDNA BR assay kit (broad range; e.g., Thermo Fisher, cat.no. Q32850)

PART 10 (step 52 – 118)

- QIAsymphony® DSP DNA Mini kit 200ul (Qiagen GmbH, cat. no. 937236)
- Dry ice
- ATL buffer (Qiagen GmbH, cat. no. 939011)
- Proteinase K (Qiagen GmbH, cat. no. 19133)
- beta-mercapto ethanol (Acros, cat. no. 125470100)
- DNAse+RNAse free water (Acros, cat. no. 327390010)
- Qubit™ dsDNA BR assay kit (broad range; e.g., Thermo Fisher, cat.no. Q32850)

EQUIPMENT

PART 9 (step 1-51)

- QIAsymphony® SP instrument (Qiagen GmbH, cat. No. 9001297) • Qiasymphony Accessory through (Qiagen GmbH, cat. no. 997012)
- Streck cell-free DNA BCT preservatives tubes (10 ml; STRECK, cat.no. 218997)
- Piercing lid, part of the DSP DNA Midi kit (Qiagen GmbH, cat. no. 997002)
- Reuse seal set, part of the DSP DNA Midi kit (Qiagen GmbH, cat. no. 997002)
- Qiasymphony pipette tips, 200 µl and 1500 µl (Qiagen GmbH, cat. no. 990332 and cat. no. 997024))
- Cartridges (8 wells) (Qiagen GmbH, cat. no. 997002)
- Covers (8 rods) (Qiagen GmbH, cat. no. 997004)
- Barcode tubes (1 ml) and rack (Sopachem, 68-1002-11N)
- Screw skirted tubes, 2 ml (Sarstedt, cat.no. 72.694.005)
- Whatman® paper (e.g., Benchkote, cat. no. 20905901)

- Plate centrifuge (Eppendorf, centrifuge 5810, cat. no. 5810000010)
- Refrigerated centrifuge (Eppendorf, centrifuge 5810R, cat. no. 5811000015)
- Vortex (e.g., Vortex Genie 2, cat. no. LLG7930060)
- Cooling adaptor, SBS Universal (Qiagen GmbH, cat. no. 9243384)

PART 10 (step 52 – 118)

- QIAsymphony® SP instrument (Qiagen GmbH, cat. no. 9001297)
- Qiasymphony Accessory through (Qiagen GmbH, cat. no. 997012)
- TissueLyser II system (Qiagen GmbH, cat.no. 85300)
- DNA LoBind® Tubes, 2 ml (Eppendorf, cat. no. 525-0131)
- Piercing lid, part of the DSP DNA Mini kit (Qiagen GmbH, cat. no. 997002)
- Reuse seal set, part of the DSP DNA Mini kit (Qiagen GmbH, cat. no. 997002)
- Qiasymphony pipette tips, 200 µl and 1500 µl (Qiagen GmbH, cat. no. 990332 and cat. no. 997024)
- Cartridges (8 wells) (Qiagen GmbH, cat. no. 997002)
- Covers (8 rods) (Qiagen GmbH, cat. no. 997004)
- Cooling adaptor, SBS Universal (Qiagen GmbH, cat. no. 9243384)
- Barcode tubes (1 ml) and rack (Sopachem, cat.no. 68-1002-11N)
- Screw skirted tubes, 2 ml (Sarstedt, cat.no. 72.694.005)
- Stainless steel beads, 5 mm (Qiagen GmbH, cat. no. 69989)
- Pipette Tips RT LTS 1000µL W 768A/8, wide tips (Mettler Toledo, cat. no. 30389217)
- Filtered pipette tips (20 ul, 200 ul and 1000 ul, Mettler Toledo, cat. no. 30389226, 30389240 and 30389213)
- Qubit Fluorometer 4.0 (Thermofisher, cat. no. Q33238)
- Qubit Assay Tubes (Invitrogen, cat. no. Q32856)
- Centrifuge (e.g., Micro star 17, cat. no. 521-1646)
- Vortex (e.g., Vortex Genie 2, cat. no. LLG7930060)
- Mini cooler (-20°C) (e.g., Brand, cat.no. 479-1242)
- Mini cooler (4°C) (e.g., Brand, cat.no. 479-1241)
- Grant – Bio ThermoShaker (PHMT, cat. no. PHMT-PSC24)

PROCEDURE

Continuing from part 8 (step 64) in the main article

PART 9: AUTOMATED BEADS-BASED DNA ISOLATION FROM BLOOD AT THE EXTERNAL WGS PROVIDER

Timing 150 minutes

Receiving blood samples for DNA isolation

Timing 5 minutes

1. Check the mail delivery every morning for new blood samples (these will usually be delivered in a biological bag). If not immediately processed for DNA isolation, store fresh blood tubes at 4°C for maximum 3 days or frozen blood tubes at -20°C (no maximum), until further processing. DNA from blood samples can be isolated per single sample or per batches of maximum 24 samples.
2. Confirm that the required information (patient hospital number or other identifying number as registered by the hospital and tube barcodes) is complete on the digital registration form (Supplementary Figure 1) and convert it to a CSV file for import into the Lab Information Management System (LIMS). Personal information and information that is not needed information must be destroyed or anonymized when applicable.

Blood sample registration

Timing 5 minutes

3. Log in to LIMS and import the digital registration form.
4. Place a Whatman paper or another filter paper (500 mm x 600 mm) on the workbench before placing the received blood tubes on it, for absorbing potential spillovers or splashes.
5. Scan the barcode on the received blood tube to retrieve the corresponding patient information from LIMS and place them in the rack that will be placed in the

QIAsymphony later on (step 21)

6. Validate that the blood tube barcode corresponds to the barcode of the respective patient in the validation field. The barcode turns green when correct and red when incorrect.
7. Once the barcodes have been validated, click ‘Received’ followed by ‘Add to LIMS’. LIMS automatically adds a date to the digital report corresponding to the date the samples were received.

Create an experiment on the QIAsymphony SP instrument

Timing 5 minutes

CRITICAL The external WGS facility we work with uses the QIAsymphony SP instrument for DNA isolation. The following steps describe how to set up the workflow.

8. Click on the ‘Blood isolation’ button to start a new experiment.
9. Click on ‘Compose new’ to find all samples waiting for isolation.
10. Click ‘Add’ to assign the new experiment number (IYY-XXXX; can be retrieved from LIMS) to the samples listed on the screen.
11. Click ‘Export’. An export file containing all the necessary information of the composed experiment will appear on your desktop. Use the information from this file to fill out the specific experiment form for blood isolation (excel file with sample names and barcodes and the measured concentration) and save the document in a folder with the experiment number.
12. Gather the blood samples stored at 4°C or -20°C and let the latter thaw at 4°C or room temperature.
13. Prepare new 1-ml barcoded tubes and register the tube codes directly on the digital registration form.

DNA isolation using the QIAsymphony SP

CRITICAL Standardized protocols for the QIAsymphony SP instrument are provided by QIAGEN. Modified QIAGEN protocols or custom protocols for QIAsymphony SP can be developed to meet specific requirements. The procedure provided below is the manufacturer's version.

Timing 120 minutes

14. Switch on the QIAsymphony SP and log on to the instrument if necessary.
15. Start the UV light (duration 15 minutes) and press 'OK'.
16. Press 'OK' to continue once the UV-light illumination is completed.
17. Click on 'Tools' followed by 'Sample preparation' and wait until the initialization procedure is complete.
18. Click on the fork spanner/hammer icon.
19. Click 'Done' to complete the maintenance and press 'OK'.
20. Open the 'eluate' drawer of the QIAsymphony SP and select slot 1 on the screen.
21. Place a rack with scanned barcoded tubes on the cooling adaptor and load the adaptor into the 'eluate' drawer of the QIAsymphony SP. Ensure the caps are removed from the barcoded tubes before loading the rack into the QIAsymphony SP. Verify that the cooling slot is on as follows:
    - Press 'Deep well' and select FL_TubeRack #68-1002-10*T1.0.
    - Press 'Rack ID' and create a rack ID (IYY-XXXX) and press 'OK'.
    - Close the 'eluate' drawer and press 'OK'.
22. Open the 'waste' drawer and ensure at least half of the waste drawer is empty. Replace the tip disposal bin if full, and press 'Scan'.
23. Open the 'reagent' and 'consumables' drawers.
24. Prepare one or several DSP Midi kit reagent cartridges:
    - Vigorously vortex the trough containing magnetic beads until fully resuspended.

- Check that buffers QSL1 and QSB1 from the QIAsymphony DSP DNA kit are clear and do not contain salt flakes (precipitates). If salt flakes are present, remove the buffers from the reagent cartridge, incubate at 37°C, and shake to dissolve the precipitates.
- Place the cartridge in the appropriate holder. Ensure the piercing lid is placed on the reagent cartridge and the lid of the magnetic bead trough has been removed. If using a previously used reagent cartridge, remove the reuse seal strips before placing the cartridges in the holder. Open the enzyme tubes.

25. Load the Qiasymphony SP with:
    - The prepared reagent cartridge
    - Tips (200 ul and 1500 ul)
    - 8-rod covers
    - 8-well cartridges
26. Close the 'reagent'' and 'consumable' drawers and select 'Scan'.
27. Mix the sample tubes briefly by vortexing.
28. Validate the tube barcodes on the DNA isolation registration form.
29. Carefully remove the tube caps and place the tubes containing the blood samples into the appropriate tube carrier orienting the tubes in such a way that the barcodes face the barcode reader on the left side of the QIAsymphony SP.
    A. When the blood tube contains less than 2 ml of blood transfer 1 ml blood into a 2-ml Sarstedt tube.Place a Sarstedt holder in the tube carrier. This is done to make sure the QiaSymphony will pick up the blood.
    B. Frozen blood is usually stored in different tube types as compared to the original blood tube. When frozen blood was received, transfer 1 ml of thawed blood into a 2-ml Sarstedt tube. Place a Sarstedt holder in the tube carrier. This is done to make sure the QiaSymphony will pick up the blood
30. Open the 'sample' drawer.
31. Load the tube carrier into position 1, 2, 3, or 4 of the 'sample' drawer.

CRITICAL STEP Change the positions of the tube carrier in the drawer between each use to minimize wear and tear.

32. Select ‘SP batch’ on the QIAsymphony SP.
33. Check the sample ID. If the sample ID is incorrect, select the incorrect sample ID, press ‘Sample ID’, add the correct ID, and click ‘OK’. A representation of a hand appears next to the sample ID.
34. Select ‘NEXT’.
35. Select all samples and couple them to the isolation protocol ‘Blood_1000_custom’ under ‘Custom protocols’ (when the sample and isolation protocol are coupled, a hand appears on the screen).
36. Select ‘NEXT’.
37. Select slot 1. Elution volume is by default 200 µl.
38. Click ‘QUEUED’.
39. If proceeding with more than one tube carrier (i.e., more than 24 samples), repeat the procedure from step 85 for the remaining tube carriers.
40. Press the ‘Run’ button to start the isolation procedure.
41. After isolation, remove the elution rack with purified DNA from the ‘eluate’ drawer.
42. Click ‘Remove’ followed by ‘Yes’ when the ‘Do you want to remove the rack’ pop-up appears. Close the ‘eluate’ drawer and press ‘OK’.
43. If the reagent cartridge is partially used, seal it with the provided reuse seal strips and close the enzyme tubes with screw caps immediately after the end of the protocol run to avoid evaporation.
44. Remove full waste containers and the tube carrier.

Quality control of DNA isolation

Timing 10 minutes

45. Measure the DNA concentration of the samples from step 105 using the Qubit dsDNA BR assay kit (broad range).
46. Fill in the DNA concentration (ng/µl) in the corresponding digital registration form. After isolation, the final DNA concentration should be more than 2 ng/µl. Repeat the isolation

step (starting at step 104) once if the quality values are out of the range of the kit or DNA concentration is less than 2 ng/µl. Contact the hospital and request new blood if isolation fails twice.

Finishing the DNA isolation experiment from blood using the QIAsymphony SP instrument

Timing 5 minutes

47. When isolation is successful, and blood should be stored for biobank purposes transfer the remaining blood from the blood tubes into appropriate plastic tubes and store. Note the tube position, corresponding blood barcode, and sample name. If the storage box is full, perform a rack scan to confirm the sample position. Store the barcoded blood tubes at -20°C. When DNA isolation was not successful, cap the blood tube and store at 4°C for up to three days for a second attempt.
48. To finish an experiment, log in to LIMS.
49. Click 'Blood isolation'.
50. Select the corresponding isolation number in the column to the left of the screen (it turns black when selected).
51. Click on 'Import file' and upload sample barcodes from the blood registration form and DNA concentrations. Confirm that all barcodes and DNA concentrations are assigned to the respective sample IDs. LIMS completes the experiment upon confirmation.

PART 10: AUTOMATED BEADS-BASED DNA ISOLATION FROM TUMOR MATERIAL AT AN EXTERNAL WGS PROVIDER

Timing 295 minutes

Receiving tumor samples for DNA isolation

Timing 5 minutes

CRITICAL Upon receiving or when handling tumor material, always keep the samples on dry ice or at -80°C to prevent samples from thawing and degrading.

52. Upon arrival, confirm that the box containing the samples is intact and the corresponding information from the lab that sent them (e.g., shipment addressed and if the sender is known) is correct.

53. Confirm that the digital registration form includes all the required information (such as the patient hospital number or other identifying number as registered by the hospital and tube barcodes) and convert it into a CSV file for import. Personal information and information that is not necessary must be destroyed or anonymized when applicable.

Tissue sample registration

Timing 5 minutes

54. Log in to LIMS and import the digital registration form.

55. Scan the barcode of the received tissue-containing tube. LIMS highlights the corresponding patient ID.

56. Validate the tissue tube in the validation field. The barcode turns green when correct and red when incorrect.

57. Click 'Received' followed by 'Add to LIMS'. LIMS automatically adds a date to the digital report corresponding to the date the samples were received.

Create an experiment on the QIAsymphony SP instrument

Time 5 minutes

58. Log in to LIMS and click on 'Tissue isolation'.

59. Click 'Compose new' to find all samples waiting for isolation.

60. Click 'Add' to assign the new experiment number (IYY-XXXX; can be retrieved from LIMS) to the samples listed on the screen.

61. Click ‘Export’. An export CSV file containing all the necessary information of the composed experiment will appear on your desktop. Use the information in the file to fill out the specific experiment form for tissue isolation (excel file with sample names and barcodes and in the end the DNA barcodes and measured concentrations) and save the document in a folder with the experiment number.
62. Assign biopsy numbers (internal number for every biopsy that is isolated) to the respective samples and register the numbers in the experiment form.
63. Assign DNA tubes with unique barcodes to the respective samples by registering them in the experiment form.

Preparation of DNA isolation buffer

Timing 10 minutes

64. Print stickers with the corresponding biopsy numbers and barcodes and attach them to 2-ml Sarstedt tubes and set them aside for step 87.
65. Print -80°C stickers with the corresponding BI numbers and barcodes and attach them to separate 2-ml Sarstedt tubes. Add a 5-mm Stainless steel bead in each tube.
66. Keep the prepared -80°C Sarstedt tubes in a -20°C cooler or on dry ice.
67. Check the ATL buffer for precipitates. If necessary, warm the buffer while swirling under a lukewarm tap to dissolve precipitates.
68. Prepare the DNA-isolation buffer in the fume hood following the table below:

number of samples	1	8
Buffer ATL (µl)	154.5	1390.5
Proteinase K (µl)	15.5	139.5

Total (µl)	170	1530

The volume of buffer for 8 samples is calculated with an excess of 1.

69. Invert the buffers several times to ensure they are thoroughly mixed. CRITICAL STEP Do not vortex the tubes.
70. Add 170 µl of the prepared DNA isolation buffer from step 68 into each of the barcoded 2-ml Sarstedt tubes.

Sample preparation for DNA isolation

Timing 180 minutes

CRITICAL If there are multiple tumor samples per patient, only process one at a time. Store the second sample at -80°C as a back-up if the first sample fails.

CRITICAL: It is essential to work quickly to keep the samples as cool as possible.

71. Collect the samples from the shipment box or -80°C freezer (see step 52).
72. Prepare the samples in batches (a maximum of 12 samples per batch) to prevent them from becoming too warm.

CRITICAL STEP Keep the samples in a -20°C cooler or on dry ice before starting the purification process.

Processing whole biopsies, sections, or cell pellets

73. For whole biopsies, follow Option A, for sections, follow Option B, and for cell pellets, follow Option C.

A. Whole biopsies (5 min per biopsy)

i. Verify the barcodes of the tissue tubes on the experiment form (see step 63).

ii. Transfer the complete biopsy into the barcoded 2-ml Sarstedt tube with a stainless steel bead (Step 65). iii. Add 210 µl DNAse/RNAse-free water (4°C) to each Sarstedt tube.

iv. Place the samples in a 4°C cooler.

CRITICAL STEP If biopsies are tiny, use a broad tip for pipetting them up into the FluidX tissue tubes with 210 µl DNAse/RNAse-free water.

Transfer the water with the respective biopsy into the barcoded 2-ml Sarstedt tube. If biopsies are too large for pipetting with broad tips, use a scalpel, tweezer, and petri dish on dry ice to cut out a piece (2-3 mm^2) of the most likely tumor part (assessed macroscopically). When in doubt, isolate 2 parts from a single biopsy.

B. <u>Sections (1 min per tube)</u>

i. Sections are delivered in barcoded 2-ml Sarstedt tubes containing a stainless steel bead. Verify that the 2-ml Sarstedt tube barcodes correspond to the patient ID on the experiment form.

ii. Add 110 µl DNAse/RNAse-free water (4°C) to the coupes or sections.

iii. Place the samples in a 4°C cooler.

C. <u>Cell pellets (2 min per tube)</u>

i. Cell pellets are delivered in Eppendorf tubes. Verify that the tube barcodes correspond to patient IDs on the experiment form.

ii. Add 110 µl DNAse/RNAse-free water (4°C) to the cell pellets, mix thoroughly by pipetting, and, using a pipette, transfer the total volume to a barcoded Sarstedt tube containing a stainless steel bead. Keep the samples in a 4°C cooler until further processing.

74. Load the samples into the tube adaptors of the TissueLyser. Ensure the TissueLyser's adaptor sets are balanced by evenly distributing the reaction tubes across the tube adaptors. Place the tube adaptors in the TissueLyser and start Program 1 (2 min at 25Hz).
75. Quick spin the tubes immediately after the TissueLyser has completed the program and place the tubes in a 0°C cooler.
76. Transfer the 0°C cooler containing the tubes with lysed products to the fume hood.
77. Transfer about 50 µl of the lysed product to the DNA isolation buffer tubes from step 70 and mix by pipetting up and down. Store the remaining TissueLysed material (+/- 100 µl from the barcoded Sarstedt tubes) at -80°C in an appropriate storage box.
78. Vortex the samples for DNA isolation and incubate them at 56°C for 2 hours to lyse the cells completely.
79. If further processing is not desired, store lysates in ATL buffer for 2 hours at 4°C.
80. After completing the lysis step, spin down the samples by a short spin to make sure there is nothing left in the lid. Keep the samples at 4°C until loaded in the QIAsymphony SP (step 95).

DNA isolation using the QIAsymphony SP

Timing 75 minutes

81. Switch on the QIAsymphony SP and log on to the instrument if necessary.
82. Start the UV light (duration 15 minutes) and press 'OK'.
83. Press 'OK' to continue once the UV-light procedure is completed.
84. Click on 'Tools' followed by 'Sample preparation' and wait until the initialization procedure is complete.
85. Click on the fork spanner/hammer icon.
86. Click 'Done' to complete the maintenance and press 'OK'.
87. Open the 'eluate' drawer of the QIAsymphony SP and select slot 1. Load the rack with barcoded tubes from step 64, scan the rack and place it on the cooling adaptor. Load the adaptor into the 'eluate' drawer of the QIAsymphony SP. Ensure the caps from the barcoded tubes are removed before loading the rack into the QIAsymphony SP. Verify that

the cooling slot is on by pressing ‘Deep well’ and selecting FL_TubeRack #68-1002-10*T1.0.

88. Press ‘Rack ID’, create a rack ID (IYY-XXXX), and press ‘OK’.

89. Close the ‘eluate’ drawer and press ‘OK’.

90. Open the ‘waste’ drawer and ensure at least half of the waste drawer is empty. Replace the tip disposal bin if full and press ‘Scan’.

91. Open the ‘reagent’ and ‘consumables’ drawers.

92. Prepare one or several DSP Mini kit reagent cartridges:

    i. Vigorously vortex the trough containing magnetic beads until they are fully resuspended.

    ii. Place the cartridge in the appropriate holder. Ensure the piercing lid is placed on the reagent cartridge and the lid of the magnetic beads trough has been removed.

    iii. If using a previously used reagent cartridge, remove the reuse seal strips before placing the cartridges in the holder. Open the enzyme tubes.

93. Load the QIAsymphony SP with:

    i. The prepared reagent cartridge

    ii. Tips (200 ul and 1500 ul)

    iii. 8-rod covers iv.

    iv. 8-well cartridges

94. Close the ‘reagent’ and ‘consumable’ drawers and select scan.

95. Open the sample drawer and load the sample tubes from step 80.

96. Validate the barcodes on the DNA isolation registration form.

97. Load the tube carrier into position 1, 2, 3, or 4 of the ‘sample’ drawer.

    CRITICAL STEP Change the positions of the tube carrier in the drawer between each use to minimize wear and tear.

98. Select ‘SP batch’ on the QIAsymphony SP.

99. Check the box ‘SAR-FIX_#72.694 T2.0 ScrewSkirt’.

100. Check the sample ID. If it is incorrect, select the incorrect sample ID, press ‘Sample ID’, add the correct ID, and press ‘OK’. A representation of a hand appears next to the sample ID.

101. Select ‘NEXT’.

102. Select all samples and couple them onto the isolation protocol 'Tissue_LC_200' under 'DNA tissue' (when the sample and isolation protocol are coupled, a hand appears on the screen).

103. Select 'NEXT'.

104. Select slot 1. Elution volume is by default 50 µl.

105. Press 'QUEUED'.

106. If processing more than one tube carrier (i.e., more than 24 samples), repeat the procedure from step 151 for the remaining tube carrier(s).

107. Click 'Run' to start the purification procedure. A pop-up will appear before starting the procedure asking you to confirm that you are using FluidX tubes. Select 'Yes' when the correct tube type has been specified.

108. After isolation, remove the elution rack containing the purified DNA from the 'eluate' drawer.

109. Click 'Remove' followed by 'Yes' when the 'Do you want to remove the rack' pop-up appears. Close the 'eluate' drawer and press 'OK'. If a reagent cartridge is only partially used, seal it with the provided reuse seal strips and close the enzyme tubes with screw caps immediately after the end of the protocol run to avoid evaporation.

110. Discard the used sample tubes in the disposal tip bin.

Quality control of DNA isolation

Timing 10 minutes

111. Measure the DNA concentration of the samples from step 172 using the Qubit dsDNA BR assay kit (broad range).

112. Fill in the DNA concentration (ng/µl) in the experiment form. After isolation, the final DNA concentration should be more than 1 ng/µl. Repeat the isolation step (see step 166) once if the DNA concentration is less than 1 ng/µl. An isolation failed report will be made if the concentration remains less than 1 ng/µl after repeating the isolation step and a spare biopsy is unavailable.

113. Discard the DNA if the final concentration is less than 1 ng/µl.

Finishing the DNA isolation experiment on the QIAsymphony SP instrument

Timing 5 minutes

114. Place the FluidX tubes with isolated DNA in a storage box. Note the position, corresponding tissue barcode, and sample name. If the storage box is full, perform a rack scan to confirm the sample positions.
115. To finish the experiment, log in to LIMS.
116. Click 'Tissue isolation'.
117. Select the corresponding isolation number in the column to the left of the screen (it turns black when selected).
118. Click 'Import DNA + finish' in LIMS and upload the experiment form with sample names, barcodes and concentrations. Confirm that all the DNA barcodes and concentrations are assigned to sample IDs. LIMS completes the experiment upon confirmation.

TIMING

PART 9: AUTOMATED BEADS-BASED DNA ISOLATION FROM BLOOD AT THE EXTERNAL WGS PROVIDER

Steps 1-3, receiving blood samples for DNA isolation: 5 minutes

Steps 4-7, blood sample registration: 5 minutes

Steps 8-13, QIAsymphony SP: Create an experiment: 5 minutes

Steps 14-44: QIAsymphony SP: DNA isolation: 120 minutes

Steps 45-46, quality control of DNA isolation: 10 minutes

Steps 47-51, QIAsymphony SP: Finishing the DNA isolation experiment: 5 minutes

PART 10: AUTOMATED BEADS-BASED DNA ISOLATION FROM TUMOR MATERIAL AT AN EXTERNAL WGS PROVIDER

Steps 52-53, receiving tumor samples for DNA isolation: 5 minutes

Steps 54-57, tissue sample registration: 5 minutes

Steps 58-63, QIAsymphony SP: Create an experiment: 5 minutes

Steps 64-70, preparation of DNA isolation buffer: 10 minutes

Steps 71-72, sample preparation for DNA isolation: 180 minutes

Steps 73Ai-73Aiv, processing whole biopsies: 5 minutes per biopsy

Steps 73Bi-73Biii, processing sections: 1 minutes per tube

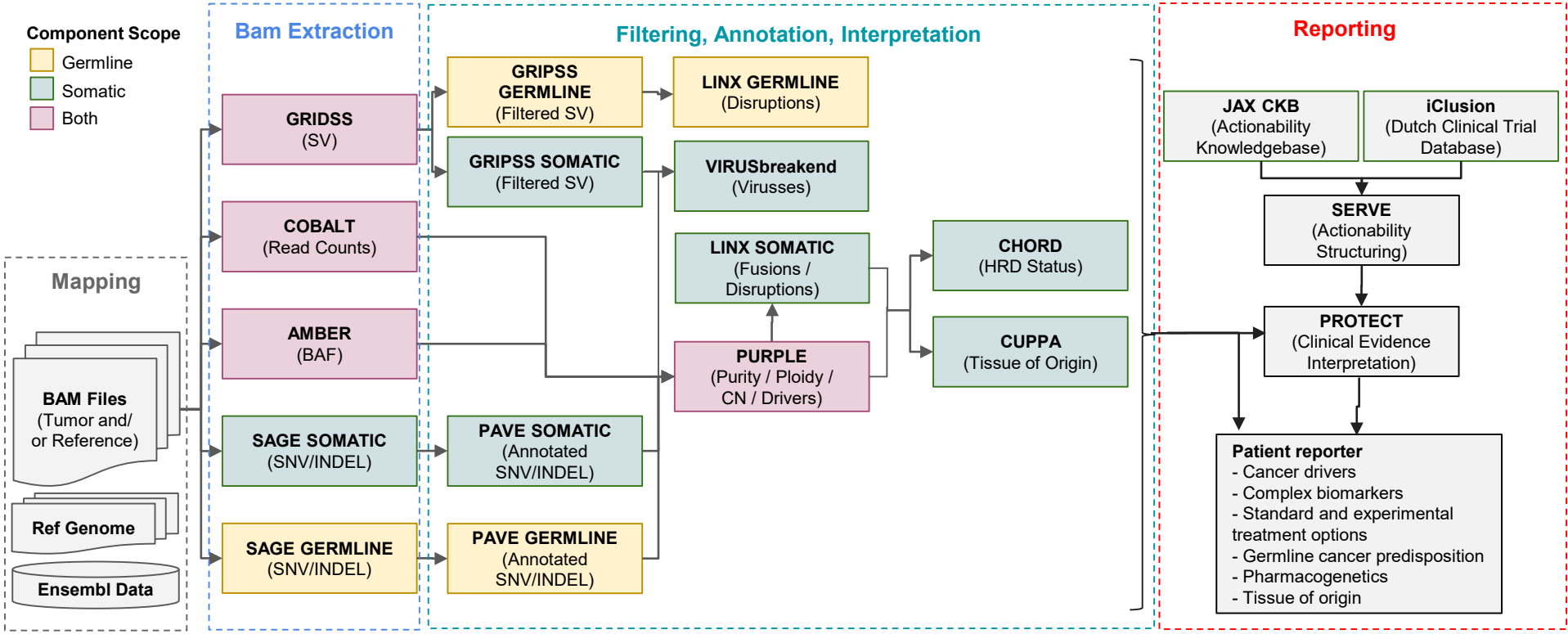
Steps 73Ci-73Cii, processing cell pellets: 2 minutes per tube

Steps 74-80, preparing for automated DNA isolation: 254 minutes

Steps 81-110, QIAsymphony SP: DNA isolation: 75 minutes

Steps 111-113, quality control of DNA isolation: 10 minutes

Steps 114-118 QIAsymphony SP: Finishing the DNA isolation experiment: 5 minutes



Supplementary Figure 1. Schematic overview of the bioinformatics pipeline.
The pipeline analyses tumor (somatic) and blood (germline) sequencing data and reports single variants (SNV), insertions and deletions (INDEL) copy number (CN) variants, structural variant (SV), gene disruption, gene fusions, integrated viruses, pharmacogenomics, tumor ploidy, but also more complex signatures like tissue of origin prediction, homologous recombination deficiency (HRD) signature, microsatellite instability and tumor mutational burden.

Hartwig MEDICAL FOUNDATION

Sample Registration Form
HMF-FOR-097

Please fill in blue cells correct and complete

HMF patient information

Workflow type:	
Source:	
Hospital:	
4 digits patient study number:	
HMF patient ID:	
HMF sample ID:	
Matching other HMF patient ID:	If applicable
Cohort:	If applicable

Hospital patient information

Hospital patient ID:	
Date of birth:	

Contact information

Requester name:	
Requester email:	

Tissue information

Hospital PA sample ID:	If applicable
Primary tumor type:	*When CUP (PTO) choose "Carcinoma of Unknown Primary" from dropdown
Tumor subtype:	If applicable
Biopsy sample type:	
Biopsy site:	
Other biopsy site:	If applicable

Reporting information

Option reporting germline findings:	
Option data storage in database:	
Pharmaco genetics:	

General information

Sampling date:	
Remarks:	

Barcode tube:	
Concentration ng/uL:	
Barcode tube 2:	

Save | E-mail | Save,Print & E-mail | Save (HMF) | Export form

Supplementary Figure 2: Sample registration form. Blue fields are filled by the user. Drop-down menus help users to accurately complete the form.

Hartwig MEDICAL FOUNDATION

Hartwig Medical OncoAct

DNA Analysis Report

Summary (1/2)

HMF SAMPLE ID
PNT00012345T

REPORT DATE
18-May-2022

HOSPITAL
HMF Testing Center

PRIMARY TUMOR LOCATION
Skin

BIOPSY LOCATION
Skin

PRIMARY TUMOR TYPE
Melanoma

The information regarding 'primary tumor location', 'primary tumor type' and 'biopsy location' is based on information received from the originating hospital.

Clinical Conclusion

Melanoma sample showing:
- activating BRAF mutation that is associated with response to BRAF-inhibitors (in combination with a MEK-inhibitor)
- complete inactivation of CDKN2A, indicating potential benefit of CDK4/6 inhibitors
- complete inactivation/loss of PTEN likely resulting in an activation of the PI3K-AKT-mTOR pathway and indicating potential benefit of mTOR/PI3K inhibitors
- high mutational burden (mutational load (ML) of 180, tumor mutation burden (TMB) of 13.6) that is potentially associated with an increased response rate to checkpoint inhibitor immunotherapy

An overview of all detected oncogenic DNA aberrations can be found in the report

Special Remark

This is a special remark

Treatment options (tumor-type specific)

Number of alterations with therapy indication	**2 | 7 (A, B) treatment(s)**
Number of alterations with clinical trial eligibility	**3 | 6 trial(s)**

Tumor characteristics

Tumor purity	**100%**
Molecular tissue of origin prediction	**Melanoma (likelihood=99.6%)**
Tumor mutational load	**High (186 mut/genome)**
Microsatellite (in)stability	**Stable (0.12)**
HR Status	**Proficient (0)**
Integrated Virus	**NONE**

Hartwig MEDICAL FOUNDATION

Hartwig Medical OncoAct

DNA Analysis Report

Summary (2/2)

HMF SAMPLE ID
PNT00012345T

REPORT DATE
18-May-2022

HOSPITAL
HMF Testing Center

Genomic alterations in cancer genes

Genes with driver mutation	CDKN2A (p16), TERT, BRAF
Number of reported variants	5
Amplified gene(s)	NONE
Deleted gene(s)	PTEN
Homozygously disrupted genes	NONE
Gene fusions	NONE

Pharmacogenetics

Genes with haplotypes	DPYD
Number of reported haplotypes	1
Functions of the haplotypes	Normal Function

Therapy details (Tumor type specific)

Tumor type specific evidence

TREATMENT	MATCH	LEVEL	RESPONSE	GENOMIC EVENT	EVIDENCE LINKS
Cobimetinib + Vemurafenib	Hotspot	A	▲	BRAF p.V600E	
Dabrafenib	Hotspot	A	▲	BRAF p.V600E	
Dabrafenib + Trametinib	Hotspot	A	▲	BRAF p.V600E	1
Trametinib	Hotspot	A	▲	BRAF p.V600E	
Vemurafenib	Hotspot	A	▲	BRAF p.V600E	1
Buparlisib + Carboplatin + Paclitaxel	Deletion	B	▲	PTEN partial loss	1
RO4987655	Codon 600	B	▲	BRAF p.V600E	1

Tumor type specific clinical trials (NL)

TRIAL	MATCH	GENOMIC EVENT
Array 818-103	Codon 600	BRAF p.V600E
BASKET OF BASKETS (VHIO17002)	Signature	High tumor mutation load
DRUP	Signature Activation, Codon 600 Deletion, Inactivation	High tumor mutation load BRAF p.V600E PTEN partial loss
EBIN (EORTC-1612-MG)	Codon 600	BRAF p.V600E
KEYNOTE-158	Signature	High tumor mutation load
NASAM	Hotspot	BRAF p.V600E

Potential eligibility for DRUP is dependent on tumor type details therefore certain tumor types may not be eligible for the DRUP.

The iClusion knowledgebase is used to annotate DNA aberrations for potential clinical study eligibility. Please note clinical study eligibility depends on multiple patient and tumor characteristics of which only the DNA aberrations are considered in this report.

The Clinical Knowledgebase (CKB) is used to annotate variants of all types with clinical evidence. Only treatment associated evidence with evidence levels ((A FDA approved therapy and/or guidelines; B late clinical trials; C early clinical trials) can be reported. Potential evidence items with evidence level (D case reports and preclinical evidence) are not reported.

The symbol (▲) means that the evidence is responsive. The symbol (▼) means that the evidence is resistant. The abbreviation (P mentioned after the level of evidence) indicates the evidence is predicted responsive/resistent. More details about CKB can be found in their Glossary Of Terms

If evidence matching based on a mutation is not in a hotspot, evidence should be interpreted with extra caution.

Therapy details (Other tumor types)

Evidence on other tumor types

TREATMENT	MATCH	LEVEL	RESPONSE	GENOMIC EVENT	EVIDENCE LINKS
Anti-EGFR monoclonal antibody	Deletion, Inactivation	B	▼	PTEN partial loss	1, 2
Bevacizumab	Hotspot	B	▼	BRAF p.V600E	1, 2
CI-1040	Hotspot	B	▲	BRAF p.V600E	1, 2
Cetuximab	Hotspot	B	▼	BRAF p.V600E	1, 2, 3, 4, 5, 6
	Deletion	B	▼	PTEN partial loss	1
Cetuximab + Irinotecan + Vemurafenib	Hotspot	B	▲	BRAF p.V600E	1
Everolimus	Deletion	B	▼	PTEN partial loss	1
Fluorouracil	Hotspot	B	▼	BRAF p.V600E	1
Irinotecan	Hotspot	B	▼	BRAF p.V600E	1
Lapatinib + Trastuzumab	Deletion	B	▼	PTEN partial loss	1
Oxaliplatin	Hotspot	B	▼	BRAF p.V600E	1
Panitumumab	Hotspot, Codon 600	B	▼	BRAF p.V600E	1, 2, 3, 4
Selumetinib	Hotspot	B	▲	BRAF p.V600E	1
Sorafenib	Hotspot	B	▲	BRAF p.V600E	1, 2
Trastuzumab	Deletion	B	▼	PTEN partial loss	1, 2

The Clinical Knowledgebase (CKB) is used to annotate variants of all types with clinical evidence. Only treatment associated evidence with evidence levels ((A) FDA approved therapy and/or guidelines; (B) late clinical trials; (C) early clinical trials) can be reported. Potential evidence items with evidence level ((D) case reports and preclinical evidence) are not reported.

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If evidence matching based on a mutation is not in a hotspot, evidence should be interpreted with extra caution.

Genomic alteration details (1/2)

Tumor purity & ploidy

Tumor purity	100%
Average tumor ploidy	3.1

Tumor specific variants

GENE	POSITION	VARIANT	PROTEIN	READ DEPTH	COPIES	TVAF	BIALLELIC	HOTSPOT	DRIVER
BRAF	7:140453136	c.1799T>A	p.Val600Glu	**150** / 221	6	68%	No	Yes	High
CDKN2A (p16)	9:21971153	c.203_204delCG	p.Ala68fs	**99** / 99	2	100%	Yes	Near	High
TERT	5:1295228	upstream		**56** / 65	2	87%	Yes	Yes	High
SF3B1	2:198266779	c.2153C>T	p.Pro718Leu	**74** / 111	3	67%	No		Low
TP63	3:189604330	c.1497G>T	p.Met499Ile	**47** / 112	4	42%	No		Low

Tumor specific gains & losses

CHROMOSOME	REGION	GENE	TYPE	MIN COPIES	MAX COPIES	CHROMOSOME ARM COPIES
10	q23.31	PTEN	partial loss	0	2	2

Tumor specific gene fusions

NONE

Tumor specific homozygous disruptions

Complete loss of wild type allele

NONE

Tumor specific gene disruptions

LOCATION	GENE	DISRUPTED RANGE	TYPE	CLUSTER ID	DISRUPTED COPIES	UNDISRUPTED COPIES
10q23.31	PTEN	Intron 5 -> Intron 6	DEL	67	2	0

Tumor specific viral insertions

NONE

Genomic alteration details (2/2)

Pharmacogenetics

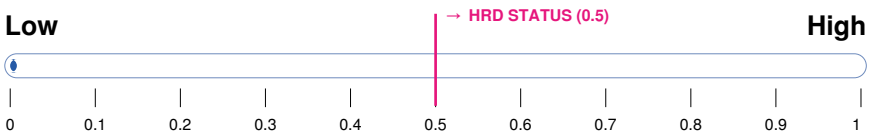
GENE	GENOTYPE	FUNCTION	LINKED DRUGS	SOURCE
DPYD	*1_HOM	Normal Function	5-Fluorouracil;Capecitabine;Tegafur	PHARMGKB

Tumor characteristics (1/2)

HR-Deficiency score

Proficient 0

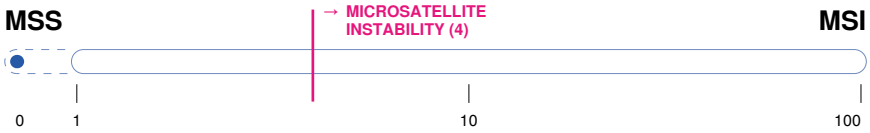
The HR-deficiency score is determined by CHORD, a WGS signature-based classifier comparing the signature of this sample with signatures found across samples with known BRCA1/BRCA2 inactivation.
Tumors with a score greater or equal than 0.5 are considered HR deficient by complete BRCA inactivation.



Microsatellite status

Stable 0.12

The microsatellite stability score represents the number of somatic inserts and deletes in (short) repeat sections across the whole genome of the tumor per Mb. This metric can be considered as a good marker for instability in microsatellite repeat regions. Tumors with a score greater than 4.0 are considered microsatellite unstable (MSI).



Tumor mutational load

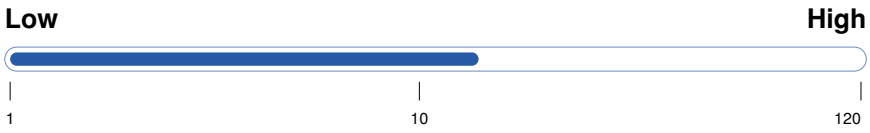
High 186

The tumor mutational load represents the total number of somatic missense variants across the whole genome of the tumor. Patients with a mutational load over 140 could be eligible for immunotherapy within the DRUP study.

Tumor mutational burden

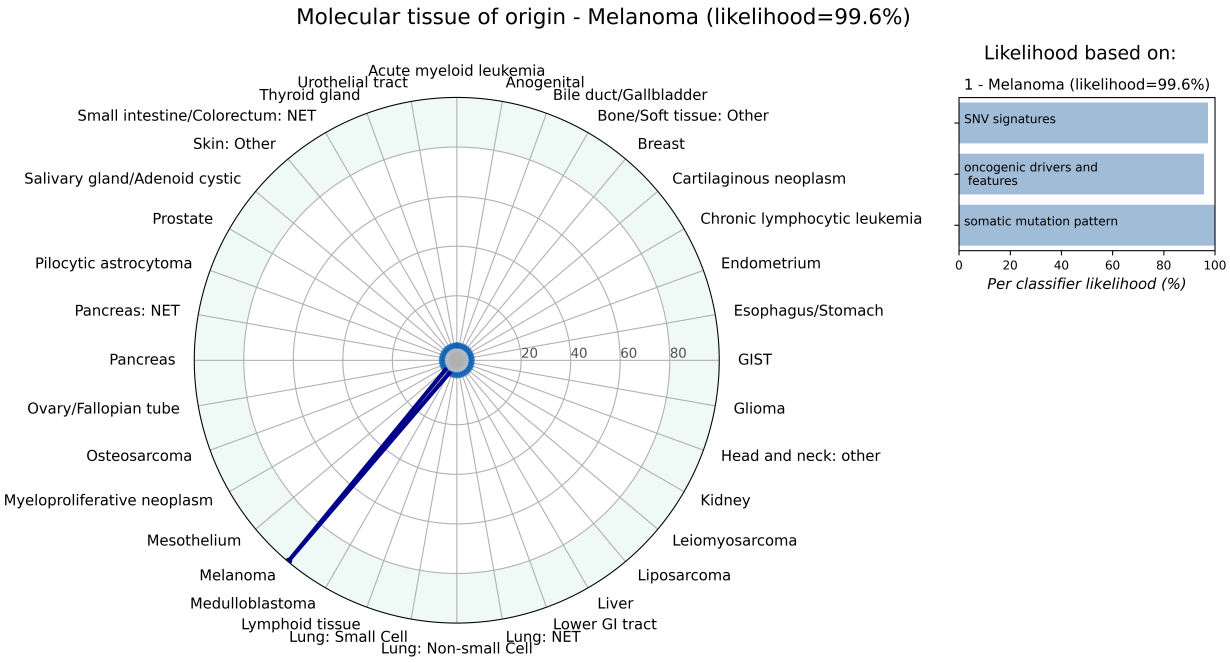
13.7 variants per Mb

The tumor mutational burden score represents the number of all somatic variants across the whole genome of the tumor per Mb.



Tumor characteristics (2/2)

Molecular tissue of origin prediction

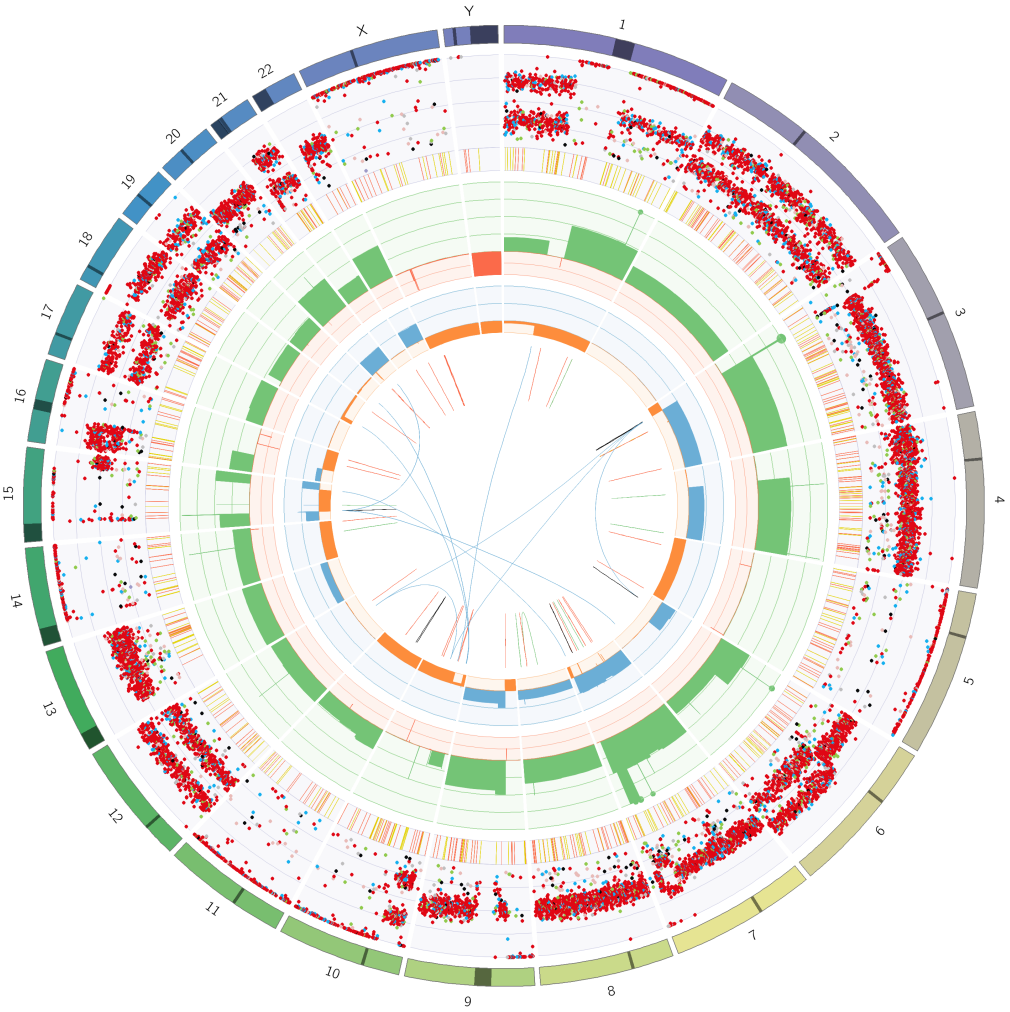


The title shows the conclusion of the prediction of the molecular tissue of origin. If none of the similarity predictions has a likelihood ≥80%, no reliable conclusion can be drawn ('results inconclusive').

The left plot shows the likelihoods (similarity) for all the origin types analyzed by the molecular tissue of origin prediction tool. Only when the likelihood is ≥80% (a peak in the green outer band of the plot), a reliable prediction (with >95% accuracy) can be drawn. Lower likelihoods (<80%) suggest there is similarity with that tissue of origin, but this is less strong and there is lower confidence.

The right plot(s) shows the breakdown of the strongest predicted likelihood(s) into the contribution of the 1) SNV types (related to those used in Cosmic signatures), 2) driver landscape and passenger characteristics (e.g. tumor-type specific drivers), and 3) somatic mutation pattern (mutation distribution across the genome).

CIRCOS plot



The outer first circle shows the chromosomes. The darker shaded areas represent large gaps in the human reference genome: i.e. regions of centromeres, heterochromatin & missing short arms.

The second circle shows all tumor specific variants (incl. exon, intron and intergenic regions) and are divided into an outer ring of single nucleotide polymorphism (SNP) allele frequencies and an inner ring of short insertion/deletion (INDEL) locations. Variant allele frequencies have been corrected for tumor purity and scale from 0 to 100%. Each dot represents a single variant and are colored according to the type of base change (e.g. C>T/G>A in red) and are in concordance with the coloring used in Alexandrov et al. 2013 Nature paper that describes the use of mutational signatures. INDELs are colored yellow and red for insertions and deletions respectively.

The third circle shows all observed tumor purity adjusted copy number changes, including both focal and chromosomal events. Copy number losses are indicated in red, green shows regions of copy number gain. The scale ranges from 0 (complete loss) to 6 (high level gains). If the absolute copy number is > 6 it is shown as 6 with a green dot on the diagram.

The fourth circle represents the observed 'minor allele copy numbers' across the chromosome. The range of the chart is from 0 to 3. The expected normal minor allele copy number is 1, and anything below 1 is shown as a loss and represents a LOH event (orange). Minor allele copy numbers above 1 indicate amplification events of both A and B alleles at the indicated locations (blue).

The innermost circle displays the observed structural variants within or between the chromosomes. Translocations are indicated in blue, deletions in red, insertions in yellow, tandem duplications in green and inversions in black.

Report explanation (1/2)

Details on the report in general

The analysis is based on reference genome version GRCh37.

Transcripts used for reporting can be found on https://resources.hartwigmedicalfoundation.nl in directory 'Patient-Reporting' and are generally the canonical transcripts as defined by Ensembl.

Variant detection in samples with lower tumor content is less sensitive. In case of a low tumor purity (below 20%) likelihood of failing to detect potential variants increases.

The (implied) tumor purity is the percentage of tumor cells in the tumor material based on analysis of whole genome data.

Details on the reported clinical evidence

The Clinical Knowledgebase (CKB) (https://ckbhome.jax.org/) is used to annotate variants of all types with clinical evidence, with a hyperlink to the specific evidence items when available. The evidence is gathered from CKB without further checks or interpretation. This also means that if a certain evidence item or drug-biomarker is missing from the knowledgebase it will also not be included in this report.
More details about CKB can be found in their Glossary Of Terms. (https://ckbhome.jax.org/about/glossaryOfTerms)

Clinical trials are matched against the iClusion database https://iclusion.org including a link to the specific trial.

Hartwig Medical Foundation is not responsible for the content of the knowledgebases used to generate this report. Furthermore, Hartwig Medical Foundation is not liable and cannot be held accountable for any incorrectness, incompleteness or error of any other kind in the knowledgebases, or the external software used to harmonize and curate the knowledgebases.

Details on reported somatic variants

The 'Read Depth' displays the raw number of reads supporting the variant versus the total number of reads on the mutated position.

The 'Copies' field indicates the number of alleles present in the tumor on this particular mutated position.

The 'tVAF' field displays the variant allele frequency corrected for tumor purity.

The 'Biallelic' field indicates whether the variant is present across all alleles in the tumor (and is including variants with loss-of-heterozygosity).

The 'Driver' field represents the driver probability on gene level and is calculated based on the HMF database. A variant in a gene with High driver likelihood is likely to be positively selected during the oncogenic process.

Details on reported gene copy numbers

The lowest copy number value along the exonic regions of the canonical transcript is determined as a measure for the gene's copy number.

Copy numbers are corrected for the implied tumor purity and represent the number of copies in the tumor DNA.

Any gene with less than 0.5 copies along the entire canonical transcript is reported as a full loss.

Any gene where only a part along the canonical transcript has less than 0.5 copies is reported as a partial loss.

Any gene with more copies than 3 times the average tumor ploidy along the entire canonical transcript is reporte as a full gain.

Any gene where only a part of the canonical transcript has more copies than 3 times the average tumor ploidy is reported as a partial gain.

Details on reported gene fusions

The canonical, or otherwise longest transcript validly fused is reported.

Fusions are restricted to those in the HMF known fusion list and can be found on https://resources.hartwigmedicalfoundation.nl in directory 'Patient-Reporting'.

We additionally select fusions where one partner is promiscuous in either 5' or 3' position.

The 'Driver' field is set to HIGH in case the fusion is a known pathogenic fusion, or otherwise a fusion where the promiscuous partner is fused in an exon range that is typically observed in literature.
All other fusions get assigned a LOW driver likelihood.

Details on reported gene disruptions

Genes are reported as being disrupted if their canonical transcript has been disrupted.

The range of the disruption is indicated by the intron/exon/promoter region of the break point and the direction the disruption faces.

The type of disruption can be INV (inversion), DEL (deletion), DUP (duplication), INS (insertion), SGL (single) or BND (translocation).

A gene for which no wild type exists anymore in the tumor DNA due to disruption(s) is reported in a separate section called 'homozygous disruptions'.

Report explanation (2/2)

Details on reported viral insertions

Virusses will be reported if they are present in our reporting database as clinically relevant (HPV, MCV, HBV, EBV and HHV-8) and DNA integration for the virus can be detected. If the virus is clinically relevant and no DNA integration is found, the following conditions must be met:
- Percentage covered of the viral genome is >90%
- Coverage of the virus DNA is higher than expected tumor mean coverage

Reporting of EBV is independent of tumor integration. This means that to be reportable, the viral EBV genome must be covered >90% and the coverage of the virus must be higher than the expected clonal mean coverage.

Details on reported pharmacogenetics

See the directory 'Patient Reporting' in https://resources.hartwigmedicalfoundation.nl for details on the panel and for more links to advice on treatment adjustments.

The called haplotypes for a gene are the simplest combination of haplotypes that perfectly explains all of the observed variants for that gene. If no combination of haplotypes in the panel can perfectly explain the observed variants, then 'Unresolved Haplotype' is called.

Wild type is assumed when no variants are observed.

HMF SAMPLE ID
PNT00012345T

REPORT DATE
18-May-2022

Sample details & disclaimers (1/2)

Sample details

The samples have been sequenced at **Hartwig Medical Foundation, Science Park 408, 1098XH Amsterdam**

The samples have been analyzed by Next Generation Sequencing using Whole Genome Sequencing

The HMF sample ID is: **PNT00012345T**

The results in this report have been obtained between **01-Oct-2020** and **18-May-2022**

This experiment is performed on the tumor sample which arrived on **05-Oct-2020** with internal tumor barcode **FR12345678**

This experiment is performed on the blood sample which arrived on **01-Oct-2020** with internal blood barcode **FR12123488**

The results stated in this report are based on the tested tumor and blood sample.

This experiment is performed according to lab procedures: **PREP013V23-QC037V20-SEQ008V25**

This report was generated by Lieke Schoenmaker (trained IT employee) and checked by a trained Clinical Molecular Biologist in Pathology (KMBP)

This report is addressed to: **PI, HMF Testing Center, 1000 AB AMSTERDAM**

Comments: This is a test report and is based on COLO829. Where is referred to CKB, VICC evidence is listed due to licensing restrictions.

Disclaimer

The data on which this report is based is generated from tests that are performed under ISO/ICE-17025:2017 TESTING L633 accreditation and have passed all internal quality controls.

This report is generated by patient reporter **version 7.25** based on **HMF-FOR-080.**

(basic) UDI-DI: **(01) 8720299486010(8012)v5.25.**

The OncoAct user manual can be found at https://www.oncoact.nl/manual.

This report is based on pipeline version **5.28.**

The 'primary tumor location' and 'primary tumor type' have influence on the clinical evidence/study matching. No check is performed to verify the received information.

The conclusion of this report is based solely on the results of the DNA sequencing of the tumor and the received tumor type. Final interpretation of the clinical consequence of this report should therefore always be performed by the treating physician.

Based on a tumor purity of at least 20%, the test has a sensitivity of >95% for detection of somatic variants and >95% for detection of translocations and gene copy number changes.

For feedback or complaints please contact **qualitysystem@hartwigmedicalfoundation.nl.**

For questions about the contents of this report, please contact **diagnosticssupport@hartwigmedicalfoundation.nl.**

RVA

Edwin Cuppen,
Director Hartwig Medical Foundation

Sample details & disclaimers (2/2)

— End of report —