Hereditary Cancer Panel (HCP)
# Customer FAQs

Oxford Nanopore Technologies

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## 1. Sample

**Q: Have you tested sample types other than blood and cell lines?**

**A:** No. However, we know that the PGx Adaptive Sampling panel uses saliva as starting input so this would likely also work for HCP.

## 2. DNA

**Q: I use a different DNA extraction kit and don't want to use Puregene. How should I proceed?**

**A:** Other DNA extraction kits may work – we have tested the Qiagen QIAamp DSP Blood Mini Kit and know it works. In addition, some of our beta testers successfully used DNA previously extracted with these kits: NEB Monarch HMW DNA kit, QIAsymphony DNA midi kit and Revvity Chemagic DNA Blood.

Please note that since different kits use different reagents, have different contaminants, and create DNA fragments of different sizes, we cannot guarantee that other kits will work.

**Q: Do we know what other DNA extraction kits might work?**

**A:** We have tested the Qiagen DSP Blood Mini kit.

**Q: I have <1 µg of DNA – can I proceed with less?**

**A:** We have shown that you can successfully generate libraries with down to 600ng of DNA input but in some cases, lower starting input libraries do not achieve the recommended mean 30x target coverage.

## 3. Panel Design

**Q: How was the panel designed?**

**A:** The panel covers 258 genes associated with increased cancer risk. The panel design uses gene annotation boundaries plus 1kb upstream depending upon direction to try and capture promotor, then 10 kb padding either side.

**Q: Can I add or subtract genes from the Hereditary Cancer Panel target list?**

**A:** You can use the gene list as a starting point and create a custom panel, then use the Bed Bugs Tool to create your own bed file and run wf-human-variation for their analysis.

Custom panels will need to establish performance metrics independently.

## 4. Kits

**Q: What are the SKUs & kit sizes?**

**A:** SKU: HCP18 (18 sample kit) and SKU: HCP72 (72 sample kit). The bundles include the library prep reagents and FCs sufficient for the number of samples in the kit.

**Q: Does I need to purchase the bed file for HCP?**

**A:** No, this is included with the purchase of the HCP bundle and will be distributed via email after purchase. If you have purchased the HCP bundle and not received the bed file, please contact your local Clinical Specialist, or email Support@nanoporetech.com to request it.

## 5. Batching

**Q: How many samples per Prom FC should my customer run?**

**A:** We recommend 3 samples per FC to achieve the recommended 30x mean target coverage.

**Q: Can I multiplex more than 3 samples per FC?**

**A:** We do not expect >3 sample per FC pooling to achieve 30X mean target coverage so this would be an unsupported workflow.

**Q: Can I run a single sample on a FC?**

**A:** Yes. The sample will need ~15Gb to provide mean 30x coverage of targets. We estimate that this will be achieved in ~7-8 hrs.

**Q: Can I run this assay on a MinION FC?**

**A:** No, you will not achieve sufficient output to get the coverage required.

## 6. Sequencing

**Q: I have a P2Solo – can I run HCP and use the Grid for compute?**

**A:** We have not validated this. Internal experiments show that basecalling will not keep up, but the system did not crash.

**Q: Can I run HCP on a GridION?**

**A:** We have not tested this. You would need to run a single sample per FC and would be unlikely to reach 30x mean target coverage.

**Q: Can I stagger FC runs?**

**A:** Yes. P2i and P24 FCs function independently. You could load FCs one day, then process and load additional FCs the next. The only caveat is that you cannot kick off EPI2ME analysis until all sequencing has finished.

**Q: Can I wash and reload the FC to get higher coverage?**

**A:** This protocol is written to generate libraries from 1µg of DNA input and load the whole library onto the FC (as a 3plex). If you follow the protocol, you will not have any additional library to enable wash and reload.

**Q: Do I need to run for 72 hrs?**

**A:** We recommend running for 72 hrs to ensure you achieve 30x mean target coverage, however most of the data is produced within the first 48 hrs. If you decide to run for 48 hrs instead of 72, you will likely get good data but may not reproducibly hit 30x mean target coverage.

**Q: I am not going to look at CNVs – can I run with Basecall on-target reads only: ON?**

**A:** Yes. If you will not assess Copy Number Variants, you do not need to basecall off-target reads.

## 7. Performance

**Q: What does barcode balance look like?**

**A:** We typically see a 10 – 20% variance in reads across barcodes, depending on shearing and quantity.

**Q: Where can I find HCP data to review?**

**A:** Data is available for download here.

## 8. MinKNOW

**Q: Do I have to run MinKNOW 25.03.07?**

**A:** No. MinKNOW 25.03.07 represents the fully validated software for HCP, we expect newer versions of MinKNOW to work as well or better but have not tested this. If you wish to run a newer version, you should test to confirm performance themselves.

**Q: How do I access the MinKNOW bed file and reference?**

**A:** Upon purchase, you should receive an email containing download instructions. If you have not received this please contact your local Clinical Specialist or email Support@nanoporetech.com to request it.

## 9. EPI2ME

**Q: How do I access the EPI2ME bed file?**

**A:** This is not needed – the analysis bed file is already hard-coded into wf-hereditary-cancer for ease of use.

**Q: Can I run EPI2ME wf-hereditary-cancer via command line?**

**A:** We are working to enable a Command Line option. Please email Support@nanoporetech.com and your email will be forwarded to the internal team to provide assistance.

**Q: How should I use the EPI2ME wf-hereditary-cancer Alignment Report?**

**A:** The Alignment Report provides high level performance metrics such as N50 and Sample Mean Coverage. In addition, the "Coverage Summary" (Exon and Gene) report allows users to review coverage performance of genes of interest, while the "Regions below target coverage" (Exon and Gene) report shows all the low coverage regions from the sample.

## 10. Variant Calling

**Q: What variant types do we call?**

**A:** We call single nucleotide variants (SNVs), insertions & deletions (indels), structural variants (SVs), phasing and methylation.

## 11. Interpretation

**Q: What 3rd Party Interpretation partners do we recommend?**

**A:** At launch, Geneyx are our compatible product partner for HCP, but we expect to add more partners quickly.