

# NGS Panels applied to Hereditary Cancer Syndromes

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**Introduction:** Cancer is among the leading causes of morbidity and mortality worldwide (Okur et al, 2017). Germline pathogenic variants for monogenic, highly penetrant cancer susceptibility genes are observed in 5%–10% of all cancers (Lu et al, 2014). Hereditary cancers due to monogenic causes are characterized by earlier age of onset, other associated cancers, and often a family history of specific cancers. From the clinical perspective, it is important to recognize the affected individuals to provide them the best clinical management (Hennessy et al, 2010; Ledermann et al, 2014; Pennington et al, 2014) and to identify at-risk family members who will benefit from predictive genetic testing and enhanced surveillance, including early detection and/or risk reduction measures (Kurian et al, 2010; Okur et al, 2017). Germline variants identified in major cancer susceptibility genes associated with hereditary breast or ovarian cancer (HBOC) or hereditary colorectal cancer (HCRC), also account for 5-10% of the patients with these cancers. In the last years, new susceptibility genes, with different penetrance degrees, have been identified. Variants in any of those genes are rare and classical methodologies (e.g. Sanger sequencing - SS) are time consuming and expensive. Next-generation sequencing (NGS) has several advantages compared to SS, including the simultaneous analysis of many samples and sequencing of a large set of genes, higher sensitivity (down to 1% vs 15-20% in SS), lower cost and faster turnaround time, reasons that make NGS the best approach for molecular diagnosis.

It is possible nowadays to choose between whole-genome sequencing (WGS), whole-exome sequencing (WES) and NGS limited to a set of genes (NGS-Panel). In cases where a suspected genetic disease or condition has been identified, targeted sequencing of specific genes or genomic regions is preferred (Grada et al, 2013). For that reason, we use NGS-Panel approach using TruSight Cancer (Illumina) to sequence DNA extracted from blood samples of patients with personal and/or familial history of cancer. This hereditary cancer gene panel sequences 94 genes associated with both common (e.g., breast, colorectal) and rare hereditary cancers and allows the creation of virtual gene panels according to each phenotype or disease under study.

NGS workflow analysis (Figure 1) includes five steps: quality assessment of raw data, read alignment to a reference genome, variant identification/calling, variant annotation and data visualization (Pabinger et al, 2013). The establishment of the most appropriate bioinformatics pipeline is crucial in order to achieve the best results. NGS data allows the identification of several types of variants like single nucleotide variants (SNVs), small insertions/deletions, inversions and also copy number variants (CNVs).

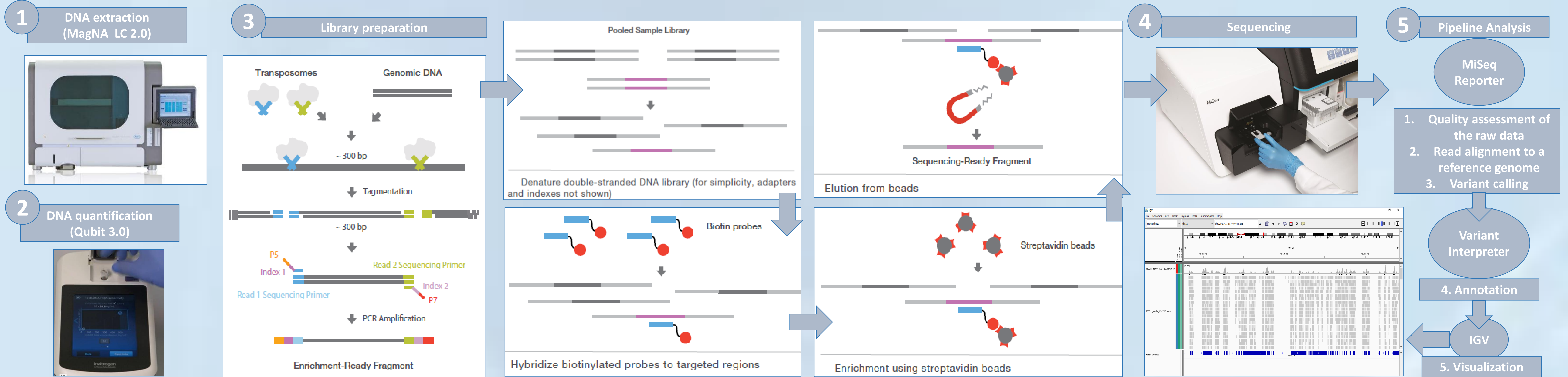


Figure 1. Main steps of NGS workflow. Courtesy of Illumina, Inc.

**Material and Methods:** NGS-Panels were created based on literature, disease-specific and general databases like, LOVD, ClinVar, HGMD Professional, HPO, Genecards, GARD, etc. Biomart - Ensembl was also used to extract data from several databases (OMIM, ORPHANET, e.g). DNA extracted automatically (MagNA LC 2.0, Roche) from peripheral blood samples was sequenced in MiSeq platform (Illumina) using the Trusight Cancer Sequencing Panel (Illumina), according to the manufacturer’s protocol. Bioinformatics analysis of NGS data (Figure 1) is performed with MiSeq Reporter and Variant Interpreter, from Illumina, and Integrative Genomics Viewer (IGV) from Broad Institute. Variant Annotation and Filter Tool (VarAFT, Desvignes JP, 2018) can also be used for annotation and is also useful for coverage analysis. Analysis of CNVs is performed with Panelcn.MOPS (Povysil et al, 2017). Assessment of variant pathogenicity involves query of disease-specific and population variation databases (e.g. HGMD Professional, LOVD, ClinVar) and *in silico* prediction of the biological impact of variants with several prediction tools (e.g. Alamut Visual, Human Splicing Finder, UMD-Predictor, VarSome, Mutation Taster). All pathogenic, likely pathogenic and variants of uncertain significance (VUS) must be confirmed by SS (in case of SNVs or small variants) or by MLPA/microarray/CGH (in case of CNVs).

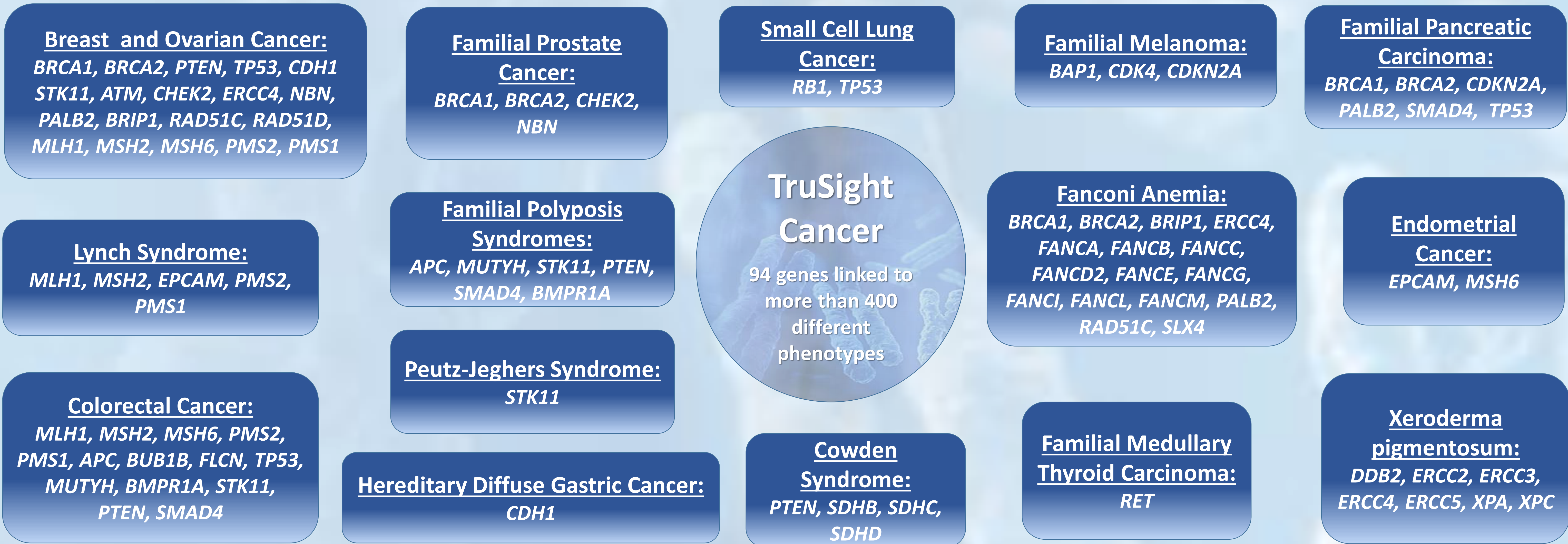


Figure 2. Examples of virtual NGS Panels based on TruSight Cancer panel from Illumina.

**Results:** Data extracted from Biomart allowed the identification of more than 400 phenotypes associated with the 94 genes included in the TruSight Cancer panel. The creation of virtual panels (Figure 2) gives the opportunity to study several genes associated with specific phenotypes. We are currently using panels for HBOC and HCRC,(Lynch Syndrome and Familial Polyposis Syndromes) hat were successfully validated (412 exonic and intronic variants, previously detected by SS, in 64 samples, were identified by NGS with high analytical sensitivity (100%) and specificity (99.3%)). These NGS-Panels detected several variants with clinical significance in other genes not commonly analysed in a first diagnostic approach (eg. *ATM* in HBOC). We identified a double heterozygous patient for two pathogenic variants in *ATM* and *BRCA1* genes (*BRCA1*: c.2037delinsCC, p.(Lys679Asn\*4) and *ATM*: c.3802delG, p.(Val1268\*)), confirmed by SS. We also identified two patients with possible mosaicism in *TP53* gene (c:724T>G; p.(Arg175His) and c:764T>G; p.(Ile255Ser)), confirmed by ARMS-PCR and SS. With the classical approach these cases could have been misdiagnosed. The use of NGS-Panel approach allowed us faster results and lower costs compared with classical methodology). The inclusion of software Panelcn.MOPS in our pipeline allows the identification of CNVs (that must be confirmed by other techniques as MLPA) and contributes to reduce the costs. Pseudogenes (e.g. *PMS2*) can be problematic in this type of analysis. The creation of new virtual gene panels, with appropriate validation, will enable us to study other hereditary cancer syndromes (Figure 2).

**Discussion:** Our experience confirms the clinical usefulness of using single or multiple cancer gene panels. The identification of a double heterozygous HBOC patient for two pathogenic variants in the *BRCA1* and *ATM* genes would have been missed if a standard methodology limited to the *BRCA1* and *BRCA2* genes was used. The two cases of possible *TP53* mosaicism are also elucidative of the usefulness of NGS. This approach can contribute to discover new variants which pathogenicity must be characterised by additional research. NGS provides a shorter turnaround time and a more comprehensive and cost-effective method for the molecular diagnosis of hereditary cancers. With genetic counselling and specialized medical management/surveillance (including predictive genetic testing) families will benefit from this new technology with high impact in public health. The creation of virtual panels based on TruSight Cancer will allow us to study more patients with a diverse spectrum of tumors associated with hereditary conditions that are mainly linked to tumor suppressor genes.

References:  
1. Desvignes JP, Bartoli M, Delague, Krahn M, Miltgen M, Bérout C, Salgado D. 2018. VarAFT: a variant annotation and filtration system for human next generation sequencing data. NARS Volume 46, Issue W1, W545–W553.  
2. Grada A and Weibrecht K. 2013. Next-Generation Sequencing: Methodology and Application. Journal of Investigative Dermatology 133, e11.  
3. Hennessy BT, Timms KM, Carey MS, Gutin A, Meyer LA, Flake DD II, Abkevich V, Potter J, Pruss D, Glenn P, et al. 2010. Somatic mutations in BRCA1 and BRCA2 could expand the number of patients that benefit from poly (ADP ribose) polymerase inhibitors in ovarian cancer. J Clin Oncol 28: 3570–3576.  
4. Kurian AW, Sigal BM, Plevritis SK. 2010. Survival analysis of cancer risk reduction strategies for BRCA1/2 mutation carriers. J Clin Oncol 28: 222–231.  
5. Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, Scott CL, Meier W, Shapira-Frommer R, Safra T, et al. 2014. Olaparib maintenance therapy in patients with platinum-sensitive relapse serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2trial. Lancet Oncol 15: 852–861.  
6. Lu KH, Wood ME, Daniels M, Burke C, Ford J, Kauff ND, Kohlmann W, Lindor NM, Mulvey TM, Robinson L, et al. 2014. American Society of Clinical Oncology Expert Statement: collection and use of a cancer family history for oncology providers. J Clin Oncol 32: 833–840.  
7. Okur V and Chung. 2017. The impact of hereditary cancer gene panels on clinical care and lessons learned. Cold Spring Harb Mol Case Stud 3: a002154.  
8. Pabinger S, Dander A, Fischer M, Snajder R, Sperk M, Erremova M, Krabichler B, Speicher MR, Zschocke J and Zlatko Trajanoski. 2013. A survey of tools for variant analysisof next-generation genome sequencing data. Briefings in Bioinformatics Vol 15, No 2, 256-278.  
9. Povysil G, Tziika A, Vogt J, Haunschmid W, Messiaen L, Zschocke J, Klambauer G, Hochreiter S and Wimmer K. Panelcn.MOPS: Copy-number detection in targeted NGS panel data for clinical diagnostics. Hum Mutat . 2017 Jul; 38(7): 889–897.