Introduction of an NGS gene panel into the Haemato-Oncology MPN service

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INTRODUCTION

- The *JAK2* V617F mutation is very common in the three classic *BCR-ABL1*-negative chronic myeloproliferative neoplasms (MPN), occurring in 90–95% of cases of polycythemia vera (PV), and in 40–60% of cases of essential thrombocythaemia (ET) and primary myelofibrosis (PMF).

- The British Committee for Standards in Haematology guidelines for PMF, ET and PV advise that all suspected cases of MPN should have their clinical diagnosis confirmed by molecular analysis of the *JAK2*, *CALR* and *MPL* genes.

CURRENT SERVICE

- Quantitative analysis of *JAK2* V617F is provided as a core service and *CALR* exon 9 mutation analysis as a fee-per-test service.

- Additional sequencing analysis is performed in approximately 8% of *JAK2* V617F negative suspected MPN cases (12 cases per month).

- Testing is performed in a sequential manner with each analysis taking an average of 21 days (*CALR* then *MPL* and/or *JAK2* exon 12).

- These tests are not available in-house and are sent away to another laboratory.

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Why move to include an NGS panel test?

- Reduced turnaround time (single test rather than sequential testing)
- Improved sensitivity for detection of *CALR*, *MPL*, and *JAK2* exon 12 mutations compared to high resolution melt curve analysis (5-10%)
- Comprehensive molecular mutation profiling has greater value than the current service for triple-negative MPN cases (e.g. *CALR*-, *ASXL1*+, associated with poor prognosis).
- *JAK2* V617F sub-clinical (<1%) and low level positive (1-15%) cases should be clinically interpreted with caution. The patient's haematological symptoms may not be due to a minor *JAK2* V617F positive sub-clone and further investigation may identify the major clone present.
- A commercial hybridisation-based enrichment NGS panel of 25 genes (OGT SureSeq™ Myeloid Panel) is currently being validated with a range of known positives and negative controls, using the Illumina MiSeq sequencing platform.

NGS MYELOID PANEL GENE CONTENT

ASXL1	*EGLN1*	*IDH2*	*NRAS*	*SRSF2*
CBL	*EPAS1*	*JAK2*	*RUNX1*	*TET2*
CALR	*EPOR*	*KIT*	*SETBP1*	*TP53*
CSF3R	*EZH2*	*KRAS*	*SF3B1*	*U2AF1*
DNMT3A	*IDH1*	*MPL*	*SH2B3*	*VHL*

- *JAK2, CALR, MPL and KIT* mutations are relevant for diagnosis, prognosis, targeted therapy choices of MPN [1, 2]

- *EPOR, EPAS1, EGLN1, VHL* mutations are seen in families with hereditary MPN-like conditions[3]

- *ASXL1, CSF3R, DNMT3A, EZH2, IDH1, IDH2, SF3B1, SRSF2, TET2, TP53, U2AF1* - genes mutated in MPN with possible prognostic value [4,5]

- *ASXL1, CSF3R, DNMT3, EZH2, IDH1, IDH2, SF3B1, SRSF2, TET2, TP53, U2AF1, JAK2, CBL, KRAS, NRAS, SH2B3,RUNX1* – genes mutated in Myelodysplastic Syndrome (MDS) [6]

1.Arber et al. Blood. 2016 May 19;127(20):2391-405,
2. Tefferi et al., Am J Hematol. 2016 Jan;91(1):50-8)
3. Hussein et al., Eur J Hum Genet. 2012 May;20(5)
4. Tefferi A et al., Leukemia. 2014 Jul 1;28(7):1494-500
5. Tefferi et al., Leukemia. 2014 Jul;28(7):1407-13
6. Papaemmanuil et al., Blood. 2013 Nov 21;122(22):3616-27

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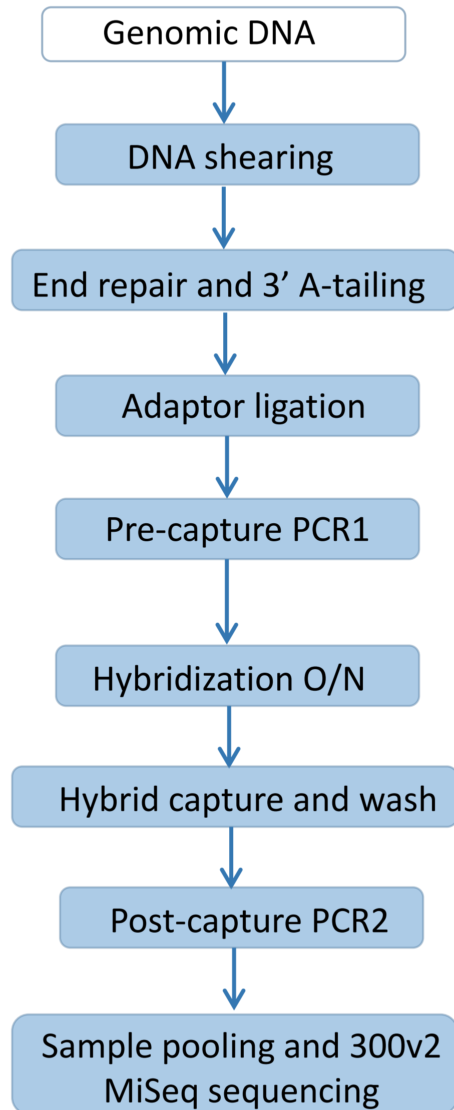
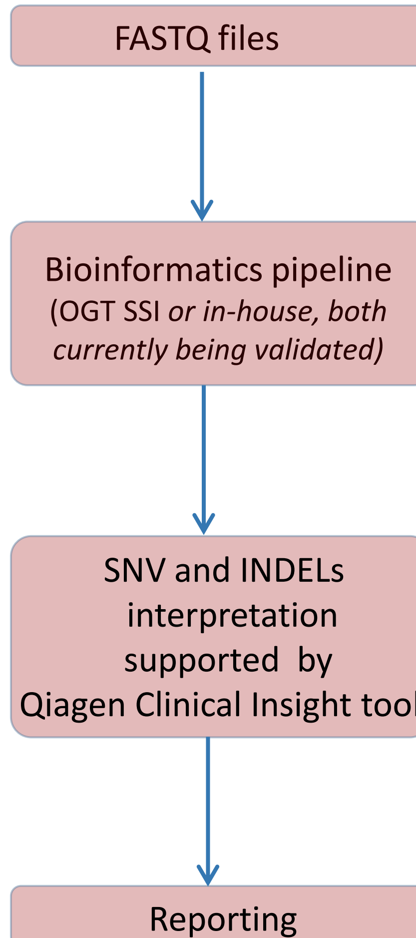
ANALYSIS WORKFLOW and RUN SUMMARY

Wet lab

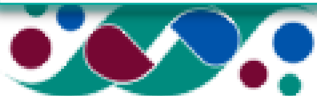


Dry lab



Metrics

- 16 samples per MiSeq run
- 4 runs so far
- 1824x –mean coverage
- Uniformed coverage of ROI
- Sensitivity down to 1% allele frequency (AF)
- But presence of chemistry specific/false positives/ artefacts at AF <5%
- 100 % intra-run concordance (at AF>5%)



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VARIANT DETECTION OF OGT SURESEQ™ MYELOID PANEL

29 samples with 30 mutations were analysed during the validation of the OGT SureSeq™ Myeloid Panel.
All 30 mutations were previously detected by other methods (droplet digital PCR, Sanger sequencing). The allele frequency (where applicable) was concordant with the NGS data.

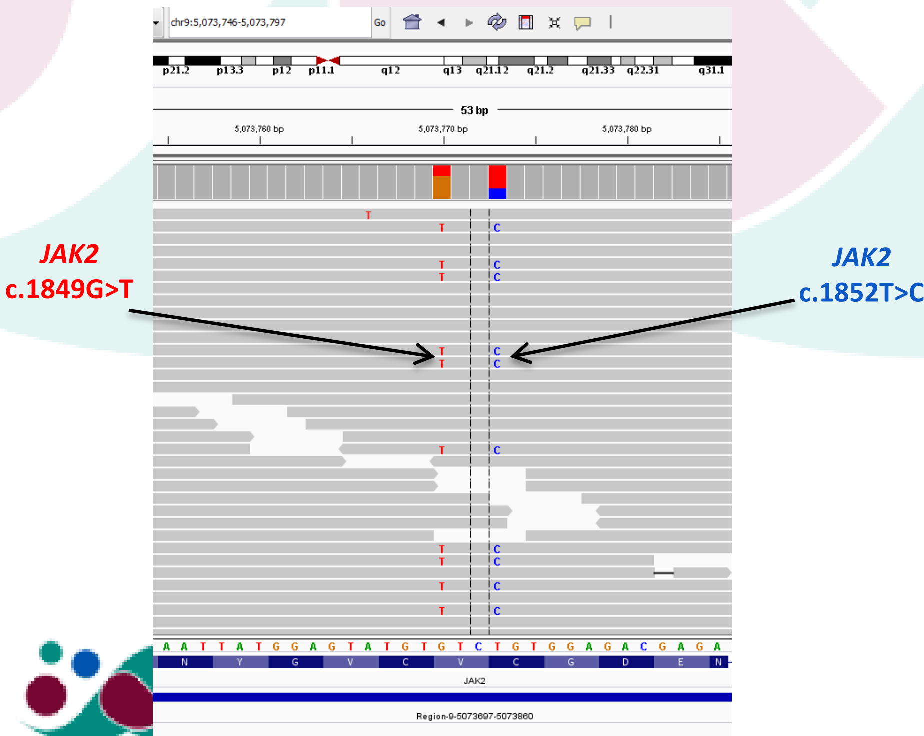
- **10 *JAK2 V617F* mutations** (AF 1.2%-91%) c.1849C>T p.(Val617Phe)
- **2 mutations in *JAK2* exon12**, both in-frame deletions: c.1625_1630delATGAAG p.(Glu543_Asp544del), c.1611_1616delTCACAA p.(Phe537_Lys539del)
- **12 mutations in *CALR* exon9** including Type I and Type II and the detection of both an in-frame deletion and a frame-shift deletion in one individual.
- **4 missense mutations in *KIT*** (AF 2.3%-50%): c.2447A>T p.(Asp816Val), c.1526A>T p.(Lys509Ile)
- **2 mutations in *MPL*:** c.1543_1544TG>AA p.(Trp515Arg), c.1544G>T p.(Trp515Leu)

ADVANTAGES OF NGS MYELOID PANEL

Case study 1 (2 mutations in *JAK2* exon14)

- *JAK2* V617F (c.1849G>T) was not detected by ddPCR in one patient but NGS identified this mutation at AF 32% and also another mutation in the same exon - c.1852T>C p.(C618R) at AF 31.5%. Both mutations are located on the same allele (figure below).
- The close proximity of the c.1852T>C mutation appears to have prevented ddPCR probe binding (designed to detect c.1849C>T) resulting in failure to detect the c.1849G>T mutation by ddPCR.

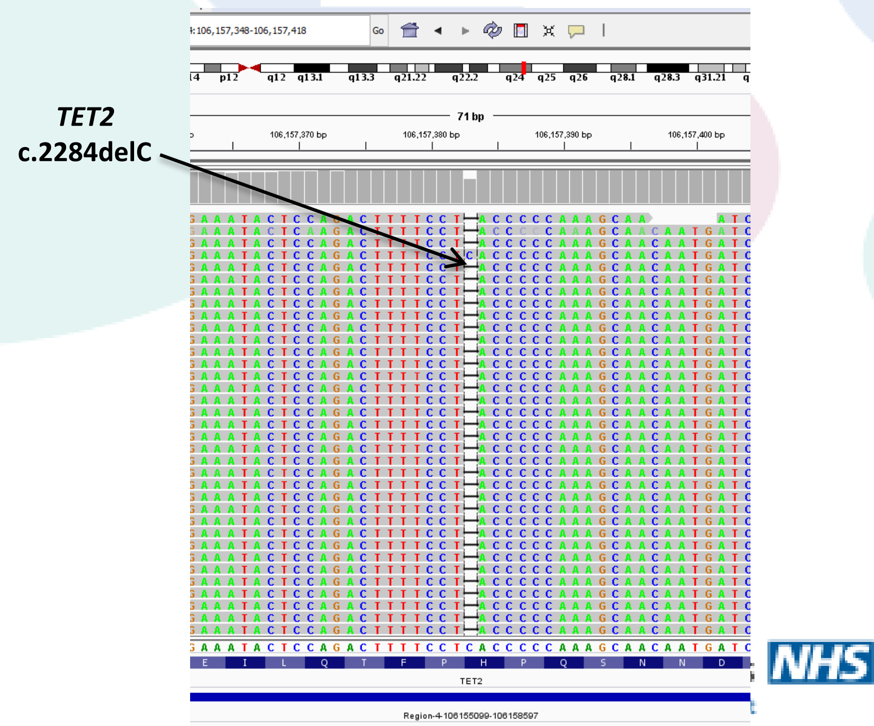
NGS results in IGV genome viewer



Case study 2 (low level *JAK2* mutation)

- Low level *JAK2* V617F was detected by ddPCR (AF 3%) and also by NGS (AF 3%)
- In addition, NGS identified a potential driver mutation in *TET2* gene which was present at AF26% - c.2284delC p.(His762ThrfsTer51).
- *TET2* mutations have been reported to occur in a range of myeloproliferative disorders suggesting that disruption of this gene can be an early event in a disease development (Delhommeau et al N Engl J Med 2009;360:2289-2301).

NGS results in IGV genome viewer



SEQUENCE VARIANTS INTERPRETATION

- Any novel variants found require a literature search to determine their pathogenicity.

- To aid the interpretation of variants found in triple-negative MPN cases, which do not possess a common mutation in *JAK2*, *CALR* or *MPL*, the utility of the QIAGEN Clinical Insight (QCI) software has been evaluated.
  https://www.qiagenbioinformatics.com/products/qiagen-clinical-insight/

SUMMARY and INTRODUCTION INTO CLINICAL SERVICE

- The NGS panel performs technically very well and has detected all known positives with excellent sensitivity (approximately 1%).
- Whether a variant detected at less than 5-10% would be reported is dependent on the level of confidence of the pathogenicity of the variant.
- The NGS panel will enter clinical service during Q2 of 2016.
- Please contact Jane Bryon for further details (jane.bryon@bwnft.nhs.uk).

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