An integrated approach to profiling haematological disorders
Genetic alterations in haematology

It is well known that most haematological disorders are caused by genomic changes such as point mutations, chromosomal rearrangements, copy number variations (CNVs) or a combination of these. Technological advances have enabled us to uncover disease-driving mutations and translate these findings to actionable targets. This has contributed greatly to the development of precision medicine — personalised treatment based on a patient’s genetic makeup.

An integrated approach to detection

For accurate detection of all types of genetic aberrations, various technologies are used. By combining information from multiple technologies, researchers can analyse complex samples and get the most complete overview of disease-driving mutations.

Oxford Gene Technology (OGT) offers an integrated portfolio of products providing clinical researchers with the most advanced tools available to study haematological disorders. Our products are backed by deep technical expertise and dedicated customer support. We partner with leading clinical researchers to advance discoveries by providing proven, high-quality solutions. Custom product capabilities are also available.

- Fluorescence In Situ Hybridisation (FISH) — Cytocell Probes (page 2)
- Next Generation Sequencing (NGS) — SureSeq Products (page 4)
- Microarrays — CytoSure Cancer +SNP Arrays (page 6)

**FISH probes**
- Haematology
- Haematopathology
- Translocations and inversions
- Deletions and insertions
- Gains and amplifications

**NGS products**
- Hybridisation-based targeted panels
- Library preparation kit
- Analysis software
- Single nucleotide variants (SNVs)
- Small insertions and deletions (indels)

**Array products**
- Haematological cancer +SNP array
- Labelling kit
- Analysis software
- Genome wide copy number variations (CNVs)
- Loss of heterozygosity (LOH)
Cytocell® Haematology FISH Probes

OGT’s comprehensive Cytocell range of high-quality FISH probes delivers reliable, tight, bright signals (Figure 1) which can significantly reduce scoring times, improve efficiency and reduce running costs. Cytocell offers one of the largest portfolios of FISH products with over 400 catalogue probes and provides custom FISH probes through our myProbes® service.

Specific Cytocell FISH probes are available for a number of haematological disorders. These probes are directly labelled, and are developed to allow co-denaturation of the FISH probe and target DNA. The probes are available in two formats:

**Aquarius® liquid format** — probes are ready to use in hybridisation buffer and available in economical five, and larger ten, test kits.

**Chromoprobe Multiprobe® System** — probes are reversibly bound to the surface of a glass device and dissolve back into solution once in contact with hybridisation buffer. Provided in a convenient kit format for two, five or ten tests.

The probe mixtures are designed for use on interphase nuclei and metaphase chromosomes from cultured peripheral blood cells or cultured bone marrow samples.

**Chromoprobe Multiprobe Haematology Panels**

Panels are available for the following diseases:
- ALL
- CLL
- AML/MDS

For a list of all probes within each disease panel, visit www.cytocell.com.

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Fluorescence *in situ* hybridisation (FISH), using locus-specific probes has become a routine diagnostic test in clinical genetics laboratories, providing essential information for the management of leukaemia patients. FISH probes are particularly useful when the gene of interest is strongly suspected and for the detection of balanced translocations, which cannot easily be detected using arrays or NGS.

**Figure 1.** Cytocell probes deliver strong signals for fast and accurate scoring. Shown here P53/TP53/ATM Combination probe, ATM labelled in green and TP53 in red.

Cytocell FISH probes deliver:
- Exceptional value without compromising on performance
- Bright signals with minimal background for fast and accurate scoring with low retest rates
- Cost-effective formats to suit your throughput requirements
- Excellent customer service and support in over 60 countries worldwide
- Convenient, directly labelled products premixed in hybridisation solution
Catalogue FISH probes

<table>
<thead>
<tr>
<th>Haematology Probes</th>
<th>D13S319/13qter/12cen Deletion/Enumeration</th>
<th>IGH/cMYC (MYC) Translocation, DF</th>
<th>P53 (TP53) Deletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML1 (RUNX1) Breakapart</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AML1/ETO (RUNX1/RUNX1T1) Translocation, DF</td>
<td>Del(5q) Deletion</td>
<td>IGH/FGFR3 Translocation, DF</td>
<td>P53(TP53)/ATM Probe Combination</td>
</tr>
<tr>
<td>ATM Deletion</td>
<td>Del(7q) Deletion</td>
<td>IGH/MAF Translocation, DF</td>
<td>PDGFRB Breakapart</td>
</tr>
<tr>
<td>BCR/ABL (ABL1) Translocation, DF</td>
<td>Del(20q) Deletion</td>
<td>IGH/MAFB Translocation, DF</td>
<td>Fast RARα (RARA) Translocation, DF</td>
</tr>
<tr>
<td>BCR/ABL (ABL1) Plus Translocation, DF</td>
<td>E2A (TCF3) Breakapart</td>
<td>IGH/MYE0V Translocation, DF</td>
<td>PML/RARα (RARA) Translocation, DF</td>
</tr>
<tr>
<td>BCL6 Breakapart</td>
<td>E2A/PBX1 Translocation, DF</td>
<td>IGK Breakapart</td>
<td>RARα (RARA) Breakapart</td>
</tr>
<tr>
<td>CBF3/MYH11 Translocation, DF</td>
<td>E2A/PBX1/HLF Translocation, DF</td>
<td>IGL Breakapart</td>
<td>TCL1 Breakapart</td>
</tr>
<tr>
<td>CKS1B/CDKN2C (P18) Amplification/Deletion</td>
<td>EV1 (MECOM) Breakapart</td>
<td>MLL (KMT2A) Breakapart</td>
<td>TCRAD Breakapart</td>
</tr>
<tr>
<td>CML PROFILER Kit</td>
<td>RIP1L1/CHIC2/PDGFRA Deletion/Fusion</td>
<td>MLL/AFF1 Translocation, DF</td>
<td>TCRB Breakapart</td>
</tr>
<tr>
<td>CML Plus Screening Panel</td>
<td>IGH Breakapart</td>
<td>MLL/MLLT1, MLL/MLLT3, MLL/MLLT4</td>
<td>TEL/AML1 (ETV6/RUNX1) Translocation, DF</td>
</tr>
<tr>
<td>cMYC (MYC) Breakapart</td>
<td>IGH/BCL2 Translocation, DF</td>
<td>MYB Deletion</td>
<td>TLX1 Breakapart</td>
</tr>
<tr>
<td>CRLF2 Breakapart and P2RY8 Deletion</td>
<td>IGH/CCND1 Translocation, DF</td>
<td>P16 (CDKN2A) Deletion</td>
<td>TLX3 Breakapart</td>
</tr>
<tr>
<td>Deletion 13q14.3, D13S319 Plus and D13S25</td>
<td>IGH/CCND3 Translocation, DF</td>
<td></td>
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</tr>
</tbody>
</table>

Table 1: A selection of high-quality Cytocell FISH probes. For the latest products and information, visit www.cytocell.com.

myProbes custom FISH probes

Designed to your specifications, myProbes® custom probes utilise Cytocell’s proprietary BAC clone collection to produce fully quality-assured custom FISH probes. Options include a simple modification, such as a change in fluorophore label, through to the custom production of a truly unique product.

Optimised buffers and reagents for excellent results

In addition to FISH probes, the Cytocell range includes the reagents and materials required to ensure that your FISH experiment is hassle free. From microscope filters, DAPI, hybridisation solutions and rubber cement, through to hybridisation chambers and surface thermometers, we offer a comprehensive choice of products to meet your requirements.

For more information, visit www.cytocell.com

“We have successfully used Cytocell haematology probes over the last five years and were looking for the same quality and consistency for our FISH pathology screening. OGT worked closely with us to help our lab evaluate — and later validate — Cytocell pathology probes. Cytocell is now our primary FISH probe supplier. Results have been excellent and we were able to consolidate our workflow to follow a single, streamlined protocol.”

Dr. Mary Nordberg, Director of Molecular Pathology, Delta Pathology Group LLC, Louisiana, USA
SureSeq™ NGS Products

OGT offers an expanding portfolio of high-quality NGS products, including targeted sequencing panels, library preparation reagents and user-friendly analysis software.

SureSeq NGS panels deliver:

- The latest evidence-based content
- Highly accurate determination of allele frequency — Hybridisation enrichment allows the removal of PCR-based bias and duplications
- Excellent coverage uniformity — Sensitive and reproducible variant detection even in heterogeneous samples
- Fast and easy workflow — Streamlined library preparation
- Powerful variant analysis software included (Figure 2)

The application of next generation sequencing (NGS) technologies to haematological disorders over the past several years has provided novel insights into disease initiation, progression and response to therapy. This powerful technique allows for accurate analysis of nucleotide-level aberrations such as single nucleotide variants (SNVs) and small insertions and deletions (indels). The advent of targeted sequencing approaches, with associated cost, time and analysis advantages is further increasing the adoption of NGS in the clinical research lab.

Figure 2: SureSeq Interpret Software is provided with all SureSeq NGS Panel purchases and enables simple and rapid identification of meaningful results, without the requirement for additional bioinformatic support.
**SureSeq Myeloid Panel**

Designed in collaboration with recognised cancer experts, the 25-gene myeloid disorders hybridisation-based NGS enrichment panel allows for mutation analysis in key genes implicated in MPNs and MDS such as JAK2, CALR, MPL and KIT.

The panel is optimised to deliver sensitive and reproducible detection of variants, down to 1% MAF in DNA derived from whole blood (Table 2). See a complete list of genes at www.ogt.com.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Known Mutation</th>
<th>Mean target coverage</th>
<th>% MAF detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1a</td>
<td>52bp deletion in CALR exon 9</td>
<td>1315</td>
<td>8.3%</td>
</tr>
<tr>
<td>Sample 2a</td>
<td>5bp insertion in CALR exon 9</td>
<td>1079</td>
<td>38%</td>
</tr>
<tr>
<td>Sample 3a</td>
<td>JAK2 V617F</td>
<td>1450</td>
<td>3%</td>
</tr>
<tr>
<td>Sample 4a</td>
<td>JAK2 V617F</td>
<td>1232</td>
<td>2%</td>
</tr>
<tr>
<td>Sample 5a</td>
<td>MPL W515R</td>
<td>1621</td>
<td>21%</td>
</tr>
<tr>
<td>Sample 6a</td>
<td>MPL W515R</td>
<td>1719</td>
<td>22%</td>
</tr>
<tr>
<td>Sample 7a</td>
<td>KIT D816V</td>
<td>1772</td>
<td>2.3%</td>
</tr>
<tr>
<td>Sample 8a</td>
<td>KIT D816V</td>
<td>1894</td>
<td>19.8%</td>
</tr>
</tbody>
</table>

Table 2: Data obtained using the SureSeq Myeloid Panel on clinical research samples containing known CALR, JAK2, MPL and KIT mutations. Samples marked a: 24 samples per MiSeq lane; 500 ng input DNA (samples provided by the Wessex National Genetics Reference Laboratory, UK). Samples marked b: 16 samples per MiSeq lane, 500 ng input DNA (samples processed by the West Midlands Regional Genetics Laboratory, UK). Samples marked c: 16 samples per MiSeq lane, 3000 ng input DNA (samples processed by the Bristol Genetics Laboratory, UK).

**SureSeq Library Preparation Kit**

With fewer hands-on steps, the SureSeq Library Preparation Kit saves an entire day from the typical library preparation workflow and reduces the potential for handling errors. Extensive optimisation of enzymes and buffers has enabled the number of steps to be reduced over standard methods, while still delivering libraries of the highest quality.

For the latest information on the expanding SureSeq NGS product range, visit www.ogt.com/sureseq

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“The currently the genetic characterisation of MPNs and related hereditary conditions can be time consuming and complex. The SureSeq panel should allow research into these disorders to become a much more standard procedure.”

Professor Nick Cross, Director of the Wessex National Genetics Reference Laboratory, UK
CytoSure™ Cancer +SNP Arrays

OGT’s CytoSure Cancer +SNP arrays combine long oligo aCGH probes for superior copy number detection with fully research-validated single nucleotide polymorphism (SNP) content for accurate identification of LOH without concurrent changes in gene copy number.

Three array designs are available, each targeting specific regions known to be important for researching disease progression (Table 3).

<table>
<thead>
<tr>
<th>Array</th>
<th>Cancer type</th>
<th>Copy number resolution</th>
<th>LOH Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Backbone</td>
<td>Average gene resolution (Hg19)</td>
</tr>
<tr>
<td>CytoSure Haematological</td>
<td>Haematological</td>
<td>1 probe every 117kb</td>
<td>1 probe every 68kb</td>
</tr>
<tr>
<td>Cancer +SNP (8x60k)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CytoSure Cancer +SNP (4x180k)</td>
<td>Haematological and solid tumour</td>
<td>1 probe every 44kb</td>
<td>1 probe every 25kb</td>
</tr>
<tr>
<td>CytoSure Consortium Cancer +SNP (4x180k)</td>
<td>Haematological and solid tumour</td>
<td>1 probe every 36kb</td>
<td>1 probe every 23kb</td>
</tr>
</tbody>
</table>

Table 3: CytoSure Cancer +SNP arrays selection guide. For a complete list of genes covered by each array, email products@ogt.com.

OGT’s CytoSure Cancer +SNP arrays deliver:

- Confident detection of CNV and LOH on a single array
- High signal to noise ratios
- Increased specificity and sensitivity compared to BAC arrays
- Flexible choice of reference sample allowing comparison of matched cancer and “normal” samples
- Exclusive CytoSure Interpret Software to translate data into meaningful results

For more information on CytoSure arrays, visit www.ogt.com/cytosure

Copy number variation (CNV) and copy neutral loss of heterozygosity (LOH) have been shown to be associated with a variety of haematological malignancies. As the gold-standard technique for CNV analysis, array comparative genomic hybridisation (aCGH) is an indispensable tool for research into disease predisposition and progression, while further enabling the identification of new therapeutic targets.

“The major advantage of the OGT array…is that it enables one to use a matched reference. This enables the detection of the acquired abnormalities, copy number aberrations as well as copy neutral loss of heterozygosity, and allows [one] to distinguish it from benign germline aberrations. This is very important information when interpreting the results. With all other platforms, two hybridisations would be needed to get this result, which doubles the costs and the workload.”

Professor Jacqueline Schoumans — Head of the Cancer Cytogenetic Unit in Lausanne University Hospital, Switzerland
About Oxford Gene Technology

Founded by Professor Sir Edwin Southern, Oxford Gene Technology (OGT) provides world-class genetics research solutions to leading clinical and academic research institutions. Cytocell FISH probes, CytoSure array products, and SureSeq next generation sequencing (NGS) products deliver high-quality genetic analysis to enable accurate identification and confirmation of the causative variation underlying genetic disease.

For more information on OGT products and services, please visit www.ogt.com.


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