Haematology Probes for Multiple Myeloma
Multiple myeloma (MM) is a plasma cell neoplasm, characterised by the accumulation of clonal plasma cells in the bone marrow and by very complex cytogenetic and molecular genetic aberrations. The modal chromosome number in newly diagnosed symptomatic patients is usually either hyperdiploid, with multiple trisomies of chromosomes 3, 5, 7, 9, 11, 15, 19 and 21, or hypodiploid with immunoglobulin heavy chain (Ig) translocations. At disease progression, several genetic progression factors have been identified as deletions of 13q, deletions of 17p and deletion of 1p and/or amplification of 1q\(^1,2,3\).

Cytogenetic abnormalities are detected by conventional cytogenetics in about one third of the cases, but FISH increases the proportion of chromosomal abnormality detection to >90\(^%\)\(^4\).

References:
1. Fonseca et al., Leukemia 2009;23(12):2210-2221
2. Sawyer, Cancer Genetics 2011;204(1):3-12
4. Swerdlow et al., Editors, WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, Lyon, France, IARC:2008

CKS1B/CDKN2C (P18)
Amplification/Deletion

The CKS1B (CDC28 protein kinase regulatory subunit 1B) gene is located at 1q21.3 and the CDKN2C (cyclin-dependent kinase inhibitor 2C) gene is located at 1p32.3.

Gain of the 1q21 region including CKS1B is one of the most frequently occurring chromosomal aberrations seen in MM\(^1\). Over expression of the CKS1B gene up-regulates cell cycle progression, resulting in a more proliferative disease\(^2\). This is related to the advanced phenotype of MM and may therefore be associated with poor prognosis and disease progression\(^1,2,3\). Gain of 1q21 has been linked to inferior survival and further amplification is observed in disease relapse.

CDKN2C is a tumour suppressor gene that is up-regulated by cytokine IL-6 in multiple myeloma and homozygous deletion of the gene is associated with a more proliferative disease\(^4\). Cytogenetic analyses have shown that abnormalities of 1p32-36 occur in around 16% of human MM and are associated with worse overall survival\(^2,3,4,5\).

The CKS1B/CDKN2C (P18) product consists of a 180kb probe, labelled in red, covering the entire CKS1B gene and flanking regions, including the PYGO2 and ZBTB7B genes, and a green probe covering a 168kb region, including the entire CDKN2C gene, the D1S1661 marker and the centromeric end of the FAF1 gene.

References:
2. Fonseca et al., Leukemia 2009;23(12):2210-2221
3. Sawyer, Cancer Genetics 2011;204(1):3-12
5. Kulkarni et al., Leukemia 2002;16:127-34
P53 (TP53) Deletion

The TP53 (tumour protein p53) gene at 17p13.1 is a tumour suppressor gene that has been shown to be deleted in a wide range of human malignancies.

TP53 is one of most important tumour suppressor genes; it acts as a potent transcription factor with a fundamental role in the maintenance of genetic stability. TP53 loss in patients with MM is a late event, seen as a marker of disease progression and is associated with a very poor prognosis.

The P53 (TP53) Deletion Probe, labelled in red, covers the whole TP53 gene, extending 66kb telomeric to the gene and covering a region centromeric to the gene, to just beyond the marker D17S655. The probe mix also contains a control probe for the 17 centromere (D17Z1), labelled in green.

References:
1. Fonseca et al., Leukemia 2009;23(12):2210-21
13q Deletion Probes

Chromosome 13q aberrations occur in 16-40% of MM cases - most of them being complete monosomy 13 (85%), whilst the remaining 15% constitute deletion of 13q1.2,3. A case study of MM patients narrowed down the critical deleted region to 13q14.4. Historically, deletions of 13q have been associated with poor prognosis in MM, but now it is believed that its prognostic relevance may be related to its association with other concurrent genetic lesions.3

References:
1. Bullrich et al., Cancer Res 2001;61:6640-8
3. Sawyer, Cancer Genetics 2011;204:3-12
5. Fonseca et al., Leukemia 2009;23:2210-2221

13q14.3 Deletion

The 13q14.3 Deletion probe, labelled in red, covers the D13S319 and D13S25 markers. The 13qter subtelomere specific probe (clone 163C9), labelled in green, allows identification of chromosome 13 and acts as a control probe.
13q Deletion Probes

D13S319 Plus Deletion

The D13S319 probe, labelled in red, covers a 156kb region including the entire DLEU1 and most of the DLEU2 genes and the D13S319, D13S272 and RH47934 markers. The 13qter subtelomere specific probe, labelled in green, allows identification of chromosome 13 and acts as a control probe.

D13S25 Deletion

The D13S25 probe, labelled in red, covers a 306kb region including most of the DLEU7 gene and the D13S25 marker. The 13qter subtelomere specific probe (clone 163C9), labelled in green, allows identification of chromosome 13 and acts as a control probe.
IGH Breakapart and IGH \textit{Plus} Breakapart

Rearrangements of the IGH (immunoglobulin heavy locus) gene at 14q32.33 with a number of different gene partners are a frequent finding in patients with MM. These include: t(4;14)(p16;q32) translocations involving IGH with FGFR3 and NSD2 (WHSC1); t(6;14)(p21;q32) translocations involving IGH and CCND3; t(11;14) (q13;q32) translocations involving IGH and CCND1; t(14;16)(q32;q23) translocations involving IGH and MAF, and t(14;20)(q32;q12) translocations involving IGH and MAFB.\textsuperscript{1-2} 

OGT provides two different IGH Breakapart probe designs to suit your needs.

References:

\textbf{IGH Breakapart} 

The IGH probe consists of a 124kb probe, labelled in red, covering part of the IGH Constant region and a green probe, covering a 617kb region within the Variable segment of the gene.

\textbf{IGH \textit{Plus} Breakapart} 

The IGH \textit{Plus} probe consists of a 359kb probe, labelled in red, proximal to the IGH Constant region and a green probe, covering a 617kb region within the Variable segment of the gene.
Approximately 50-60% of MM cases are associated with translocations involving IGH and one of several partners including CCND1, NSD2 (WHSC1) and FGFR3, CCND3, MAF or MAFB. Our IGH Translocation, Dual Fusion probes all consist of two green probes that cover the IGH region, positioned proximal to the constant region and within the Variable segment. The second part of the probe, labelled in red, covers the gene associated with the respective translocation.

References:
1. Fonseca et al., Cancer Res 2004;64:1546-58

IGH/CCND3 Plus Translocation, Dual Fusion

The CCND3 (cyclin D3) gene is located at 6p21.1 and IGH (immunoglobulin heavy locus) at 14q32.33.

The t(6;14)(p21;q32) translocation is a recurrent translocation seen in approximately 4% of cases of MM. CCND3 has been identified as a putative oncogene that is dysregulated as a consequence of the t(6;14)(p21;q32) translocation.

References:
IGH Translocation, Dual Fusion Probes

IGH/FGFR3 Plus Translocation, Dual Fusion

The FGFR3 (fibroblast growth factor receptor 3) gene is located at 4p16.3 and IGH (immunoglobulin heavy locus) at 14q32.33.

The t(4;14) (p16;q32) translocation is a recurrent translocation seen in 15% of MM cases. This translocation results in the dysregulation of two genes at 4p16; NSD2 (Nuclear receptor binding SET domain protein 2) and FGFR3. The consequence of the translocation is increased expression of FGFR3 and NSD2. The translocation can be unbalanced, with 25% of cases losing the derivative chromosome 14, associated with the loss of FGFR3 expression1,2.

This t(4;14) translocation is often cytogenetically cryptic and was poorly described before the advent of FISH techniques. The translocation has been associated with poorer survival in MM patients1,2.

References:
1. Fonseca et al., Leukemia 2009;23(12):2210-2221
2. Sawyer, Cancer Genetics 2011;204(1):3-12
IGH/MAF Plus Translocation, Dual Fusion

The MAF (MAF bZIP transcription factor) gene is located at 16q23.2 and IGH (immunoglobulin heavy locus) at 14q32.33.

The t(14;16)(q32;q23) translocation is a recurrent translocation seen in approximately 5% of MMs. MM patients harbouring the t(14;16) appear to have a more aggressive clinical outcome.

The majority of the breakpoints occur within the last intron of WWOX (WW domain containing oxidoreductase), centromeric to MAF. These breakpoints have a dual impact of positioning the IGH enhancer near MAF and disrupting the WWOX gene. Gene expression profiling of myeloma cell lines revealed that MAF caused transactivation of cyclin D2 (a promoter of cell cycle progression), thus enhancing proliferation of myeloma cells.

References:
1. Fonseca et al., Cancer Research 2004;64:1546-1558
2. Fonseca et al., Leukemia 2009;23(12):2210-2221
3. Sawyer, Cancer Genetics 2011;204(1):3-12
4. Walker et al., Blood 2013;121 (17);3413-3419
5. Chang et al., Leukemia 2007;1572-1574
IGH/MAFB Plus Translocation, Dual Fusion

The MAFB (MAF bZIP transcription factor B) gene is located at 20q12 and IGH (immunoglobulin heavy locus) at 14q32.33.

The t(14;20)(14q32;20q12) translocation is a recurrent translocation seen in around 2% of MMs. The reciprocal rearrangement brings a truncated form of the IGH μ-enhancer (Eμ, located between the joining (J) segments and the constant region of the IGH gene) in close contact with the MAFB gene. The resultant fusion and the up-regulated transcription product has been shown to cause dysregulation of cyclin D2.

The prognostic outcome of t(14;20)(14q32;20q12) is assumed to be the same as the t(14;16) (q32;q23).

References:
1. Fonseca et al., Leukemia 2009;23(12):2210-2221
2. Sawyer, Cancer Genetics 2011;204(1):3-12
4. Fonseca et al., Cancer Research 2004;64:1546-1558
IGH/MYEOV Plus Translocation, Dual Fusion

The MYEOV (myeloma overexpressed) gene is located at 11q13.3 and IGH (immunoglobulin heavy locus) at 14q32.33.

The t(11;14)(q13;q32) translocation is the most common translocation in MM, where it is seen in approximately 15% of cases\(^1,2\).

The breakpoints in MM cases are dispersed within a 360kb region between CCND1 (cyclin D1) and MYEOV at 11q13. MYEOV is a putative oncogene, located 360kb centromeric to CCND1\(^3\). Overexpression of MYEOV is a possible prognostic factor in MM\(^4\). The t(11;14)(q13;q32) is associated with a favourable outcome in most series and therefore is regarded as neutral with regard to prognosis\(^2\).

References:
1. Fonseca et al., Leukemia 2009;23(12):2210-2221
2. Sawyer, Cancer Genetics 2011;204(1):3-12
The Cytocell® Advantage

- Excellent detection and accurate scoring: robust, easy to analyse probes
- Confidence in your results: excellent contrast with minimal background and specific high-intensity signals
- Easy to use: probes are pre-mixed, minimising errors and saving time
- Ready-to-use in five and ten test pack sizes: ensure optimal stock levels are kept with minimal wastage and re-validation

Multiple Myeloma Probe Range

<table>
<thead>
<tr>
<th>Probe Name</th>
<th>Chromosome Region</th>
<th>Probe Type</th>
<th>Control Probe</th>
<th>No. Tests</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>13q14.3</td>
<td>13q14.2-q14.3</td>
<td>Deletion</td>
<td>D13S1825</td>
<td>5 or 10</td>
<td>LPH 006</td>
</tr>
<tr>
<td>CKS1B/CDKN2C (P18)</td>
<td>1q21.3/1p32.3</td>
<td>Amplification/Deletion</td>
<td>-</td>
<td>5 or 10</td>
<td>LPH 039</td>
</tr>
<tr>
<td>D13S319 Plus</td>
<td>13q14.2-14.3</td>
<td>Deletion</td>
<td>LAMP1</td>
<td>5 or 10</td>
<td>LPH 068</td>
</tr>
<tr>
<td>D13S26</td>
<td>13q14.3</td>
<td>Deletion</td>
<td>D13S1825</td>
<td>5 or 10</td>
<td>LPH 043</td>
</tr>
<tr>
<td>IGH</td>
<td>14q32.33</td>
<td>Breakapart</td>
<td>-</td>
<td>5 or 10</td>
<td>LPH 014</td>
</tr>
<tr>
<td>IGH Plus</td>
<td>14q32.33</td>
<td>Breakapart</td>
<td>-</td>
<td>5 or 10</td>
<td>LPH 070</td>
</tr>
<tr>
<td>IGH/CCND3 Plus Dual Fusion</td>
<td>14q32.33/6p21</td>
<td>Translocation</td>
<td>-</td>
<td>5 or 10</td>
<td>LPH 075</td>
</tr>
<tr>
<td>IGH/FGFR3 Plus, Dual Fusion</td>
<td>14q32.33/4p16.3</td>
<td>Translocation</td>
<td>-</td>
<td>5 or 10</td>
<td>LPH 074</td>
</tr>
<tr>
<td>IGH/MAF Plus, Dual Fusion</td>
<td>14q32.33/16q23.1-q23.2</td>
<td>Translocation</td>
<td>-</td>
<td>5 or 10</td>
<td>LPH 073</td>
</tr>
<tr>
<td>IGH/MAFB Plus, Dual Fusion</td>
<td>14q32.33/20q12.2</td>
<td>Translocation</td>
<td>-</td>
<td>5 or 10</td>
<td>LPH 077</td>
</tr>
<tr>
<td>IGH/MYEOV Plus, Dual Fusion</td>
<td>14q32.33/11q13.3</td>
<td>Translocation</td>
<td>-</td>
<td>5 or 10</td>
<td>LPH 078</td>
</tr>
<tr>
<td>P53 (TP53)</td>
<td>17P13.1</td>
<td>Deletion</td>
<td>D17Z1</td>
<td>5 or 10</td>
<td>LPH 017</td>
</tr>
</tbody>
</table>

*For 5 test kit add -S to catalogue number, e.g: LPH ###-S

View our complete haematology FISH range at www.cytocell.com