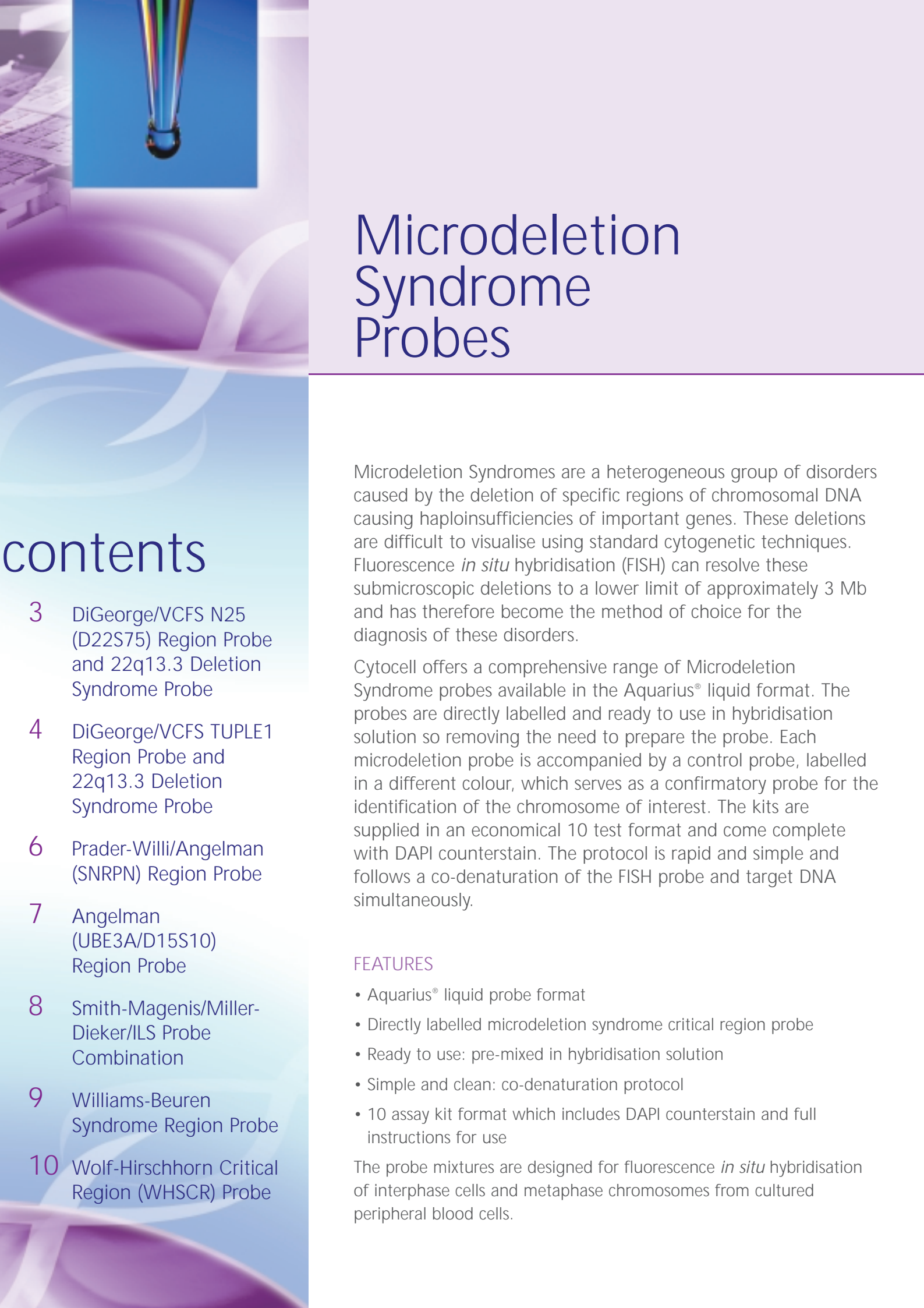


# Microdeletion Syndrome probes

The background of the advertisement features a soft, abstract design with flowing, wavy lines in shades of purple and blue. In the upper left corner, there is a small inset image showing a glass pipette with a single drop of liquid hanging from its tip, set against a solid blue background. The overall aesthetic is clean and professional, typical of a scientific or medical product brochure.

# Microdeletion Syndrome Probes

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Microdeletion Syndromes are a heterogeneous group of disorders caused by the deletion of specific regions of chromosomal DNA causing haploinsufficiencies of important genes. These deletions are difficult to visualise using standard cytogenetic techniques. Fluorescence *in situ* hybridisation (FISH) can resolve these submicroscopic deletions to a lower limit of approximately 3 Mb and has therefore become the method of choice for the diagnosis of these disorders.

Cytocell offers a comprehensive range of Microdeletion Syndrome probes available in the Aquarius® liquid format. The probes are directly labelled and ready to use in hybridisation solution so removing the need to prepare the probe. Each microdeletion probe is accompanied by a control probe, labelled in a different colour, which serves as a confirmatory probe for the identification of the chromosome of interest. The kits are supplied in an economical 10 test format and come complete with DAPI counterstain. The protocol is rapid and simple and follows a co-denaturation of the FISH probe and target DNA simultaneously.

### FEATURES

- Aquarius® liquid probe format
- Directly labelled microdeletion syndrome critical region probe
- Ready to use: pre-mixed in hybridisation solution
- Simple and clean: co-denaturation protocol
- 10 assay kit format which includes DAPI counterstain and full instructions for use

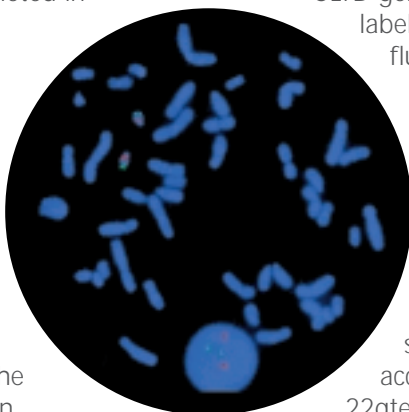
The probe mixtures are designed for fluorescence *in situ* hybridisation of interphase cells and metaphase chromosomes from cultured peripheral blood cells.

# DiGeorge/VCFS N25 (D22S75) Region Probe and 22q13.3 Deletion Syndrome Probe

Cat. No. LPU 010

**N25 (D22S75): Red Fluorophore**  
**22q Subtelomere Specific Probe (clone N85A3): Green Fluorophore**

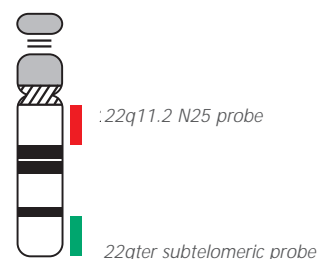
A region of 2 Mb referred to as the DiGeorge Critical Region (DGCR) is most commonly deleted in up to 90% of patients.<sup>1,2,3</sup> Within the DGCR a minimal critical region of 480–575 Kb has been described<sup>4,5</sup> containing several genes including the Citrate Transport Protein (CTP) and the Clathrin heavy chain genes (CLTD)<sup>5</sup> which may be implicated in the aetiology of VCFS.



approximately 90 Kb and is sited in the CTP gene<sup>6</sup> and possibly also the CLTD gene.<sup>7</sup> The probe is labelled with a red fluorophore (Texas Red spectrum). This type of probe may be used to identify deletions of band 22q11.2 found in DiGeorge and associated syndromes. It is accompanied by the 22qter subtelomere specific probe (clone N85A3) labelled with a green fluorophore (FITC spectrum), which allows identification of chromosomes 22.

The DiGeorge/VCFS N25 probe is

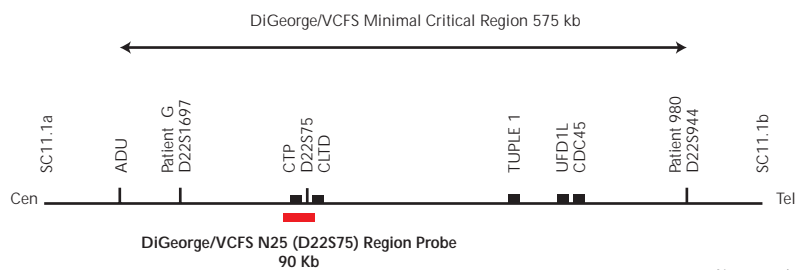
22



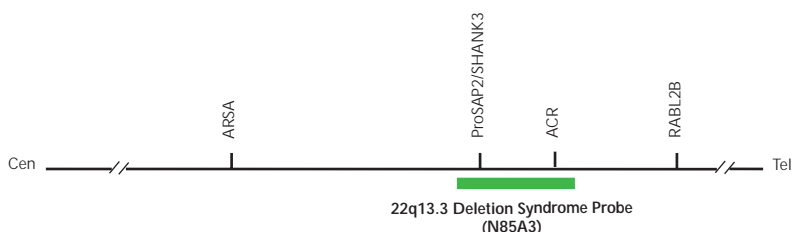
**Note: 22q13.3 deletion syndrome.** See "DiGeorge/VCFS TUPLE1 Region Probe" paragraph for more details.

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1. Driscoll, D.A. *et al* (1992) *J Med Genet* **50**: 924-933
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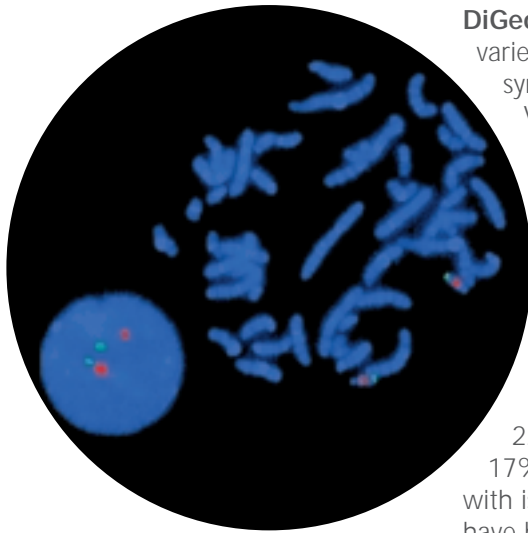
Not to scale



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# DiGeorge/VCFS TUPLE1 Region Probe and 22q13.3 Deletion Syndrome Probe

**TUPLE1: Red Fluorophore**  
**22q Subtelomere Specific Probe, (clone N85A3): Green Fluorophore**



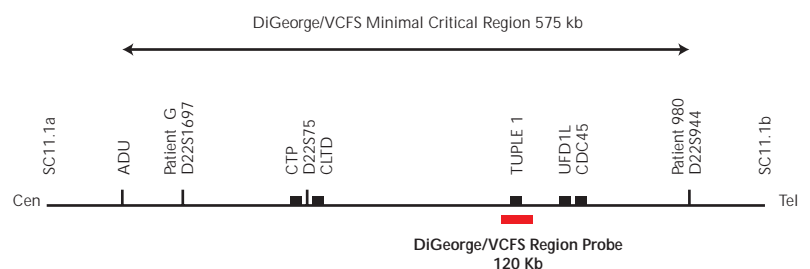
**DiGeorge syndrome**<sup>1</sup> and a variety of congenital malformation syndromes, including Velocardiofacial (VCFS)<sup>2</sup> and Conotruncal Anomaly Face syndromes<sup>3</sup> share the phenotypic features covered by the acronym CATCH22 (cardiac defects; abnormal facies; thymic hypoplasia; cleft palate; hypocalcaemia) and deletion of chromosome 22 at 22q11.2.<sup>4,5,6</sup> In addition around 17% of nonsyndromic patients with isolated conotruncal defects have been shown to have a 22q11.2 microdeletion.<sup>7</sup> The incidence of these anomalies is estimated to be 1:4000 live births<sup>8</sup> and therefore the deletion 22q11.2 is now thought to be the most common microdeletion syndrome.

A region of 2 Mb referred to as the DiGeorge Critical Region (DGCR) is most commonly deleted in up to 90% of patients.<sup>5,9,10</sup> Within the

DGCR a minimal critical region of 480–575 Kb has been described<sup>11,12</sup> containing several genes including the TUPLE1 (HIRA) gene, a proposed candidate partly responsible for the observed phenotype.<sup>11</sup>

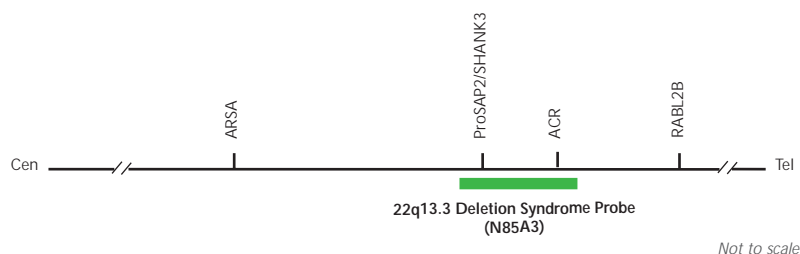
The DiGeorge/VCFS TUPLE1 Region probe is approximately 120 Kb encompassing the entire *TUPLE1* gene and flanking DNA and may be used to identify deletions of band 22q11.2 found in DiGeorge and associated syndromes. It is directly labelled with a red fluorophore (Texas Red spectrum). The accompanying subtelomere specific probe (clone N85A3) is labelled with a green fluorophore (FITC spectrum) and allows identification of chromosomes 22.

The **22q13.3 deletion syndrome** presents a recognisable phenotype characterised by hypotonia, delay or absence of expressive speech, moderate to profound mental retardation, normal to accelerated



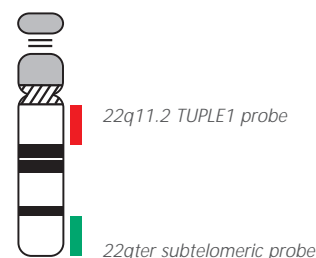
*Not to scale*

Cat. No. LPU 004



Not to scale

22



growth and mild dysmorphic features.<sup>13</sup> Some deletions of the terminal region of chromosome 22q are cytogenetically visible. However, a few cases of cryptic deletions have been reported,<sup>13,14</sup> suggesting that the actual incidence of 22q telomere deletion may be higher than previously thought.

Several observations of patients with 22q13.3 deletion showed that the ProSAP2/SHANK3 gene, coding for a structural protein of the postsynaptic density of excitation synapses and expressed in the cortex and cerebellum of the brain<sup>15</sup> was disrupted<sup>15,16,17</sup> or

deleted<sup>18</sup>, making it a good candidate gene for this syndrome. The deletion varies widely in size, from 130kb to 9Mb.<sup>18,19,20</sup> Recent findings recommend the use of 22q subtelomeric probes distal to the ARSA gene for examining all 22q13.3 deletions.<sup>20,21</sup>

The ProSAP2/SHANK3 gene maps within the 22qter subtelomere specific probe (clone n85a3).<sup>20</sup> It allows identification of the most distal 22q13.3 deletions. The DiGeorge/VCFS TUPLE1 probe allows identification of chromosome 22 and acts as a control probe.

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21. Chen C.P. *et al* (2003) *Prenat Diagn* **23**(6): 504-508

# Prader-Willi/Angelman (SNRPN) Region Probe

Cat. No. LPU 005

## SNRPN/Imprinting Centre: Red Fluorophore 15q Subtelomere Specific Control Probe (clone 154P1): Green Fluorophore

Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are distinct neurogenetic disorders, caused by the loss of function of genes on chromosome 15, bands 15q11–13 on either the paternally or maternally inherited chromosome respectively.<sup>1</sup>

In 70% of patients a large interstitial deletion of 3-4 Mb is observed<sup>1,2</sup> and in 2-4% of patients an imprinting defect is observed of which 25% involve an approximately 200 Kb deletion of the Imprinting Centre (IC).<sup>3</sup> Uniparental disomy, in which the chromosomes 15 are inherited from the same parent, accounts for most of the remaining patients with PWS and 80% of AS patients.

The SNRPN gene is one of four imprinted loci that is expressed from the paternal chromosome 15 region 15q11–13 and maps to the smallest deletion region involved in

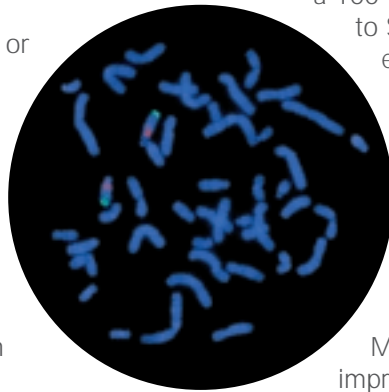
PWS. Its chromosomal location and imprinting status suggest a possible role in the aetiology of PWS.<sup>4</sup>

The imprinting centre (IC) maps to a 100 Kb region proximal to SNRPN and includes exon 1 of SNRPN.

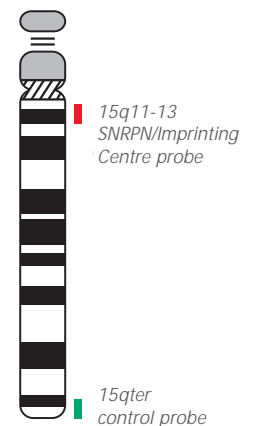
Parental deletions or mutations in the IC impair the imprinting process in 15q11–13 and cause the two distinct diseases in their offspring.<sup>5,6</sup>

Most of the PWS imprinting deletions involve SNRPN and are approximately 200 Kb. The AS imprinting deletions are small (approx. 40 Kb), involve the BD3 region and do not include SNRPN.

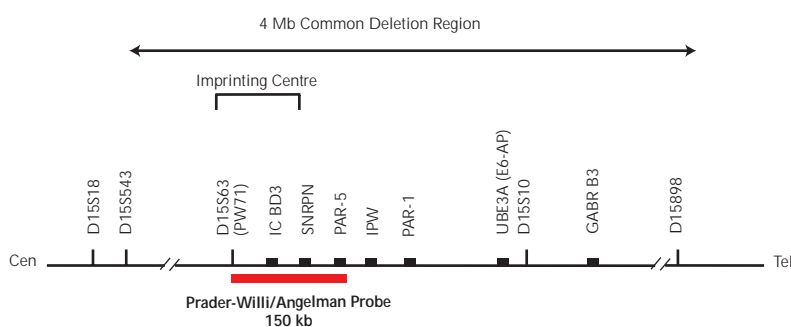
The Prader-Willi/Angelman Region probe is approximately 150 Kb of genomic DNA and targets all the SNRPN gene and all of the imprinting centre. This probe may be used to identify the 3–4 Mb deletions incorporating SNRPN common to 70% of PWS/AS and



15



the smaller deletions of approximately 200 Kb incorporating SNRPN and the Imprinting Centre found in PWS. The probe cannot be used to detect smaller deletions or mutations of the Imprinting Centre found in AS or used to detect uniparental disomy. It is directly labelled with a red fluorophore (Texas Red spectrum) whilst the accompanying subtelomere specific probe at 15qter (clone 154P1) is labelled with a green fluorophore (FITC spectrum) and allows identification of chromosomes 15.



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# Angelman (UBE3A/D15S10) Region Probe

Cat. No. LPU 006

## UBE3A/D15S10: Red Fluorophore 15q Subtelomere Specific Control Probe (clone 154P1): Green Fluorophore

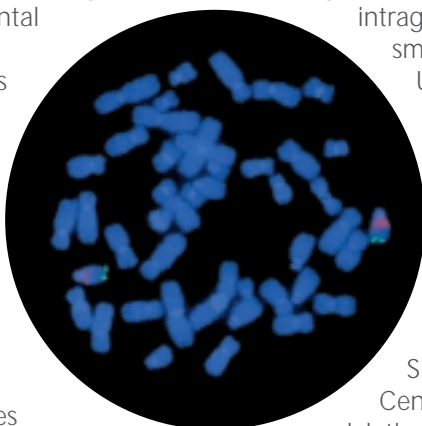
In 70% of patients with Prader-Willi (PWS) or Angelman syndrome (AS) a large interstitial deletion of 3–4 Mb at 15q11–13 is observed.<sup>1,2</sup> Mutations of the imprinting centre and uniparental disomy account for the remaining cases of PWS. However, 20% of AS show biparental inheritance and normal methylation suggesting the involvement of a single AS gene.

The UBE3A gene lies within the minimum AS critical region<sup>3</sup> approximately 400 Kb telomeric of SNRPN, it shows preferential expression of the maternal allele in the brain<sup>4</sup> and is mutated in 20-30% of AS patients with normal methylation and biparental contribution of 15q11–13. It is considered to be one of the AS genes.<sup>4</sup>

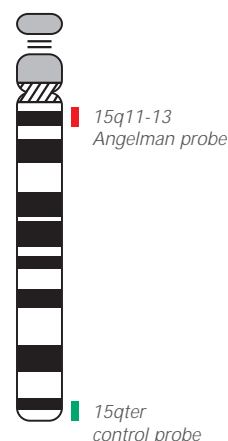
The Angelman Region probe is approximately 120 Kb of genomic

DNA and targets most of the UBE3A gene and includes the locus D15S10. This type of probe may be used to identify deletions of the AS region. It will not detect

intragenic deletions or small mutations of UBE3A. The probe may also be used to help determine the nature of a Prader-Willi syndrome deletion detected with the SNRPN/Imprinting Centre probe. Large deletions of 3–4 Mb at 15q11–13 will cause the deletion of both probe regions SNRPN/IC and UBE3A/D15S10. Smaller deletions, however, incorporating the IC and SNRPN, of approximately 200 Kb, will not cause deletion of the UBE3A/D15S10 probe. These deletions may indicate a much higher risk of recurrence and patients could be referred for further investigation.<sup>5</sup>



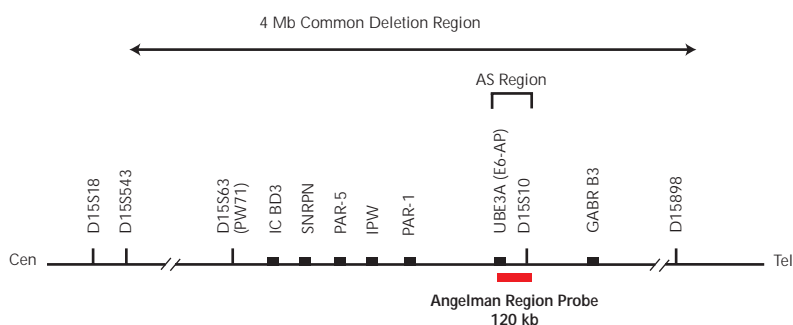
15



The Angelman Region Probe is directly labelled with a red fluorophore (Texas Red spectrum). The accompanying subtelomere specific control probe at 15qter (clone 154P1) is labelled with a green fluorophore (specific to the FITC spectrum).

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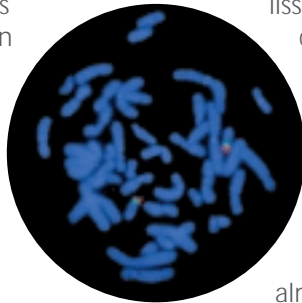
# Smith-Magenis/Miller-Dieker/ ILS Probe Combination

Cat. No. LPU 007

## Smith-Magenis: Green Fluorophore Miller-Dieker/Isolated Lissencephaly Sequence: Red Fluorophore

### Smith-Magenis syndrome (SMS)

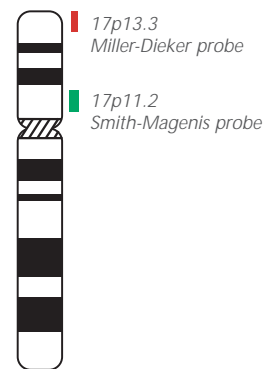
is a multiple congenital anomaly syndrome characterised by mental retardation, neuro-behavioral abnormalities, sleep disturbances, short stature, minor craniofacial and skeletal anomalies, congenital heart defects and renal anomalies.<sup>1,2</sup> It is one of the most frequently observed human microdeletion syndromes and is associated with an interstitial deletion of the chromosome band 17p11.2.<sup>1,2</sup> Molecular studies in SMS patients suggest a common deletion region spanning approximately 5 Mb.<sup>3</sup> Further investigation has shown that in more than 90% of patients the deletion is brought about by recombination between flanking repeat gene clusters surrounding this SMS critical region.<sup>4</sup> The FLI gene<sup>5</sup> maps within the critical region and has been shown to be deleted in SMS patients.<sup>6</sup>



### Miller-Dieker syndrome (MDS)

is a multiple malformation characterized by classical lissencephaly, a characteristic facial appearance and sometimes other birth defects.<sup>7</sup> It is associated with visible or submicroscopic rearrangements within chromosome band 17p13.3 in almost all cases.<sup>8</sup> Isolated lissencephaly sequence (ILS) consists of classical lissencephaly with no other major anomalies.<sup>9</sup> Submicroscopic deletions of chromosome 17p13.3 have been detected in almost 40% of these patients.<sup>8</sup> MDS is considered a contiguous gene deletion syndrome where deletion of physically contiguous genes leads to the complex phenotypic abnormalities seen in MDS. LIS1 is located at 17p13.3 and recognised as the causative gene responsible for the lissencephaly phenotype in both MDS and ILS.<sup>10,11</sup> A deletion of

17

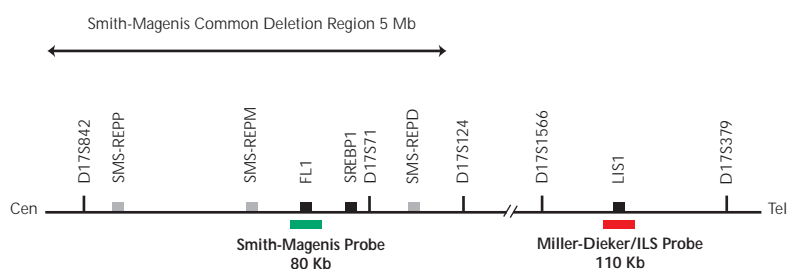


MDS patients always involves LIS1 together with telomeric loci in excess of 250 kb.<sup>10</sup>

The Smith-Magenis probe is 80 Kb and targets the entire FLI gene of 14 Kb including flanking proximal and distal regions. The Miller Dieker/ILS probe is 110 Kb and targets the entire LIS1 gene of 80 Kb. The probe mixture is dual labelled, with SMS labelled with a green fluorophore (FITC spectrum) and MDS in a red fluorophore (Texas Red spectrum).

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# Williams-Beuren Syndrome Region Probe

Cat. No. LPU 011

**Williams-Beuren Probe: Red Fluorophore**  
**7 (D7Z1)  $\alpha$ -satellite Control Probe: Green Fluorophore**

Williams-Beuren Syndrome (WBS) is a developmental disorder caused by a microdeletion of 7q11.23.<sup>1</sup>

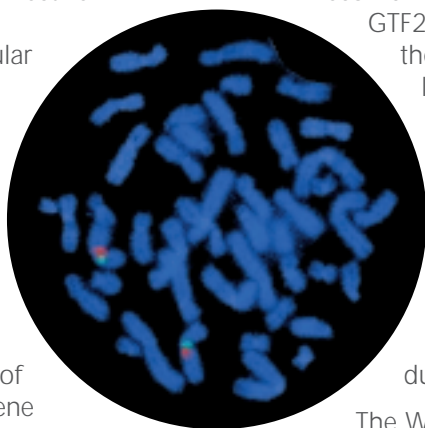
Patients display connective tissue problems, typically supravalvular aortic stenosis (SVAS), growth retardation, renal anomalies, transient hypercalcaemia, hyperacusis and mental retardation.<sup>2</sup>

Haploinsufficiency of the elastin (ELN) gene has been identified as responsible for SVAS<sup>3,4</sup> however, of the remaining 15 genes identified within the WBS deletion none of the remaining clinical features have been conclusively attributed to any one of these genes.

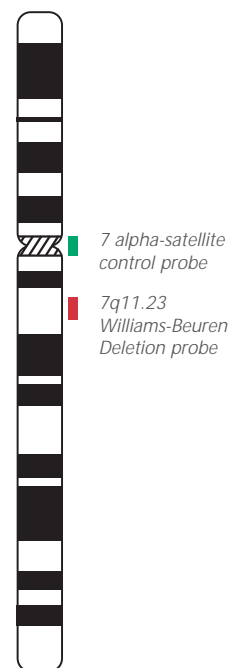
The most recent work maps the WBS deletion region to be 1.6 Mb<sup>5</sup> and flanked by highly homologous duplicons of 320–500 Kb within

which the common breakpoints cluster. The common WBS deletion results from non-homologous recombination between the GTF2I/NCF1 locus and the GTF2IP1/NCF1P1 locus or rare intrachromosomal exchange between the centromeric GTF2IP2/NCF1 and the telomeric GT2IP2/NCF1P2 duplicons.

The Williams-Beuren deletion probe is approximately 450 Kb and consists of three non-overlapping clones which cover much of the deletion region. The probe is directly labelled with a red fluorophore (Texas Red spectrum) and accompanied by the 7 centromere  $\alpha$ -satellite control probe directly labelled with a green fluorophore (FITC spectrum).

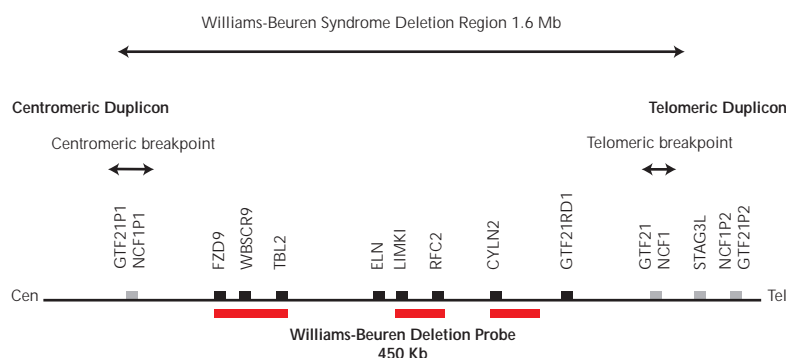


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5. Peoples R *et al* (2000) *Am J Hum Genet* **66**: 47-68



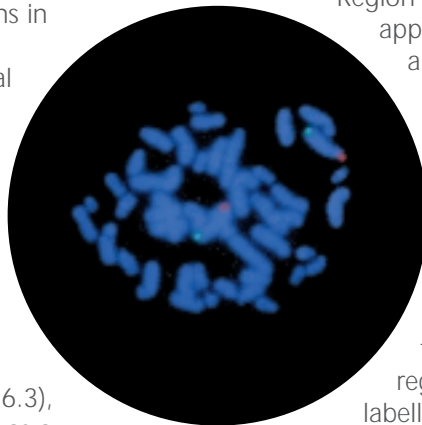
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# Wolf-Hirschhorn Critical Region (WHSCR) Probe

Cat. No. LPU 009

## WHSCR: Red Fluorophore 4q Subtelomere Specific Control Probe (clone dj963K6): Green Fluorophore

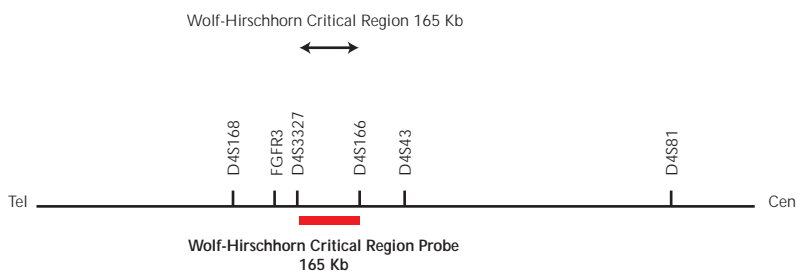
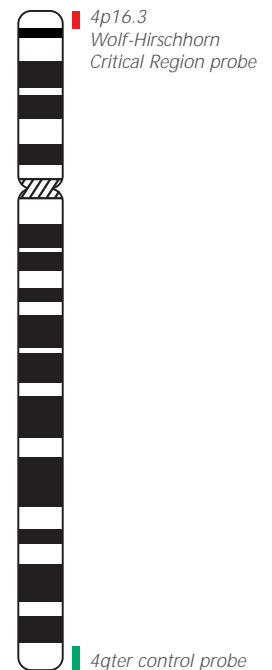
Wolf-Hirschhorn syndrome is a multiple malformation syndrome characterised by severe growth deficiency, severe to profound mental retardation with the onset of convulsions in early infancy, microcephaly, sacral dimples and characteristic facies ('Greek helmet appearance').<sup>1</sup> The phenotype results from the partial deletion of the short arm of chromosome 4 (4p16.3), originally observed as a large cytogenetically visible terminal deletion of 2 Mb. Molecular analyses of patients with small terminal and interstitial deletions have allowed for the definition of the Wolf-Hirschhorn



Critical Region which is 165 Kb and lies between D4S166 and D4S3327.<sup>2</sup>

The Wolf-Hirschhorn Critical Region Probe is approximately 165 Kb and targets the entire critical region. This type of probe may be used to identify Wolf-Hirschhorn deletions with the minimal involvement of the 165 Kb critical region. It is directly labelled with a red fluorophore (Texas Red spectrum). The accompanying subtelomere specific control probe at 4qter (clone dj963k6<sup>3</sup>) is labelled with a green fluorophore (FITC spectrum).

4



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# Aquarius® Microdeletion Syndrome Probe Range Summary

Product Description	Probe Locus	Probe Locus Identification	Control Locus	No. Tests	Cat. No.
DiGeorge/VCFS TUPLE1 Region Probe	22q11.2	TUPLE1	22qter	10	LPU 004
DiGeorge/VCFS N25 (D22S75) Region Probe	22q11.2	N25-D22S75	22qter	10	LPU 010
Prader-Willi/Angelman (SNRPN) Region Probe	15q11-13	SNRPN/IC	15qter	10	LPU 005
Angelman (UBE3A/D15S10) Region Probe	15q11-13	UBE3A/D15S10	15qter	10	LPU 006
Smith-Magenis/Miller-Dieker probe	17p11.2 17p13.3	FL1 LIS1	N/A	10	LPU 007
Williams-Beuren Region Probe	7q11.23	WSCR	7 cen/D7Z1	10	LPU 011
Wolf-Hirschhorn Region Probe	4p16.3	WHSCR/D4S166-D4S3327	4qter	10	LPU 009

Cytocell's products should be used in accordance with the Instructions For Use.

## Ordering Information

For more information on Cytocell's products or to place an order, please contact your authorised local distributor. For distributor details, visit [www.cytocell.com](http://www.cytocell.com)



**Cytocell Technologies Ltd.**  
6-7 Technopark  
Newmarket Road  
Cambridge CB5 8PB  
United Kingdom  
T: +44 (0) 1223 467064  
F: +44 (0) 1223 360732  
E: [probes@cytocell.com](mailto:probes@cytocell.com)  
W: [www.cytocell.com](http://www.cytocell.com)

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