Vysis TelVysion DNA Probe FISH Procedure

The TelVysion probes consist of DNA sequences homologous to specific telomeric regions, mixed with blocking DNA to minimize hybridization to other chromosomes. The TelVysion probes are directly labeled with either SpectrumOrange or SpectrumGreen fluorophores. The labeled probes are hybridized to target DNA in specimens that are fixed and mounted to slides (in situ hybridization). Following a series of wash steps, results can be viewed with a fluorescence microscope.

The unique telomeric TelVysion probes are specific for a single human chromosome arm. The TelVysion probe contains a locus estimated to be within 300 kb of the end of the chromosome. Evidence for telomere localization is derived from half-YAC*, telomere-associated repeat (TAR; subtelomeric repeat) or sequence data.


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**Legend:** This diagram represents the positional relationship of DNA sequences contained within the telomere regions of human chromosomes (not to scale). The TelVysion probe hybridizes to the region containing the unique telomere DNA sequences as indicated by the black boxes. Information contained within this diagram has been provided courtesy of D. Ledbetter, Ph.D. and C. Lese, Ph.D. University of Chicago, Chicago, IL.

TelVysion probes are specific to the p and/or q arm of each of the human chromosomes. When hybridized and visualized, the TelVysion probes may elucidate specific chromosome changes, such as deletions or translocations of the specific telomere regions.

Vysis TelVysion probes are designed to detect the chromosome specific subtelomeric regions on the human chromosomes.

1. Cloning of human Telomere containing fragments in yeast by complementation of the yeast Telomere function is referred to a half-YAC cloning. 

**General Purpose Reagents**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Vysis Order No./Abbott Order No.</th>
<th>Package Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>20X SSC</td>
<td>32-804850/02J10-032</td>
<td>500g</td>
</tr>
<tr>
<td>DAPI II Counterstain</td>
<td>32-804831/06J50-001</td>
<td>500 µL x 2</td>
</tr>
<tr>
<td>NP-40</td>
<td>32-804816/07J05-001</td>
<td>1000 µL x 2</td>
</tr>
<tr>
<td>Propidium Iodide Counterstain</td>
<td>32-804829/07J06-001</td>
<td>500 µL x 2</td>
</tr>
<tr>
<td>LSI/WCP Hybridization Buffer</td>
<td>32-804826/06J67-001</td>
<td>150 µL x 2</td>
</tr>
<tr>
<td>100% Ethanol (EtOH)</td>
<td>NA/60J67-001</td>
<td>500 µL x 2</td>
</tr>
<tr>
<td>12N HCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1N NaOH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>formamide, ultrapure grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>purified water (distilled or deionized)</td>
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</tbody>
</table>

**Warning & Precautions:** Fluorophores are readily photobleached by exposure to light. To limit this degradation, handle all solutions and slides containing fluorophores in reduced light.

**FISH Procedure**

The following procedure has been validated for performance on cultured peripheral blood lymphocytes and is used to determine the probe quality.

The user is responsible for validating the procedure for their specific application.

**Preparing the Reagents**

**NOTE:** Where indicated, measure the pH of these solutions at ambient temperature. Use a pH meter with a glass electrode unless otherwise noted.

**20X SSC solution**

Mix thoroughly 132g 20X SSC in 400 mL purified H₂O. Measure pH and adjust to pH 5.3 with HCl. Add purified H₂O to bring final volume to 500 mL. Store at ambient temperature. Discard stock solution after 6 months, or sooner if solution appears cloudy or contaminated.

**2X SSC solution / 0.3% NP-40 wash solution**

Mix thoroughly 100 mL 20X SSC (pH 5.3) with 850 mL purified H₂O. Add 3 mL NP-40 and mix thoroughly until NP-40 is completely dissolved. Measure pH and adjust to pH 7.0-7.5 with NaOH. Add purified H₂O to bring final volume to 1 liter. Store at ambient temperature. Discard stock solution after 6 months, or sooner if solution appears cloudy or contaminated.

**0.4X SSC / 0.3% NP-40 wash solution**

Mix thoroughly 20 mL 20X SSC (pH 5.3) with 950 mL purified H₂O. Add 3 mL of NP-40 and mix thoroughly until NP-40 is completely dissolved. Measure pH and adjust to pH 7.0-7.5 with NaOH. Add purified H₂O to bring final volume of the solution to 1 liter. Store at ambient temperature. Discard stock solution after 6 months, or sooner if solution appears cloudy or contaminated.

**2X SSC / 0.1% NP-40 wash solution**

Mix thoroughly 100 mL 20X SSC (pH 5.3) with 850 mL purified H₂O. Add 1 mL NP-40 and mix thoroughly until NP-40 is completely dissolved. Measure pH and adjust to pH 7.0-7.5 with NaOH. Add purified H₂O to bring final volume to 1 liter. Store at ambient temperature. Discard stock solution after 6 months, or sooner if solution appears cloudy or contaminated.

**Denaturation Solution (70% formamide / 2X SSC)**

Mix thoroughly 49 mL formamide, 7 mL 20X SSC (pH 5.3) and 14 mL purified H₂O in a glass Coplin jar. Measure pH using pH indicator strips to verify pH is 7.0-8.0. Between uses, store covered at 2-8°C. Discard after 7 days.

**Ethanol Solutions (70%, 85%, 100%)**

Prepare v/v dilutions of 100% ethanol with purified H₂O to make stock solutions of 75% and 80% ethanol. Between uses, store covered at ambient temperature.

Discard stock solutions after 6 months.

For working solutions, pour 70 mL 100% EtOH into one of three jars; 70 mL 85% EtOH into another, and 70 mL 70% EtOH into the last. Use at ambient temperature. Discard after 7 days or if excessive dilution or evaporation has occurred.

**Procedural Notes:** Prior to use, thaw reagents at ambient temperature, vortex, then centrifuge each tube 2-3 seconds using a standard bench-top microcentrifuge.

Measure the temperatures of the solutions inside the Coplin jar; use of a calibrated thermometer is required.

**NOTE:** When performing a hybridization that contains TelVysion probes, follow the TelVysion protocol.

**Preparing the Specimen Target**

**NOTE:** Bring Coplin jars containing the denaturation solution to ambient temperature. Place jars in a 74±1°C water bath approximately 30 minutes prior to use to bring the solution to temperature.

1. Mark hybridization areas with a diamond tipped scribe on the underside of the specimen slide.
2. Ensure that the temperature of the denaturation solution is 73±1°C.
3. Immerses the slides in the denaturation solution for 5 minutes.

**NOTE:** Immerses no more than four slides in the Coplin jar simultaneously.

4. Dehydrate slides for 1 minute in 70% EtOH, followed by 1 minute in 85% EtOH, and 1 minute in 100% EtOH.

**NOTE:** Keep the slides in 100% EtOH until you are ready to dry all slides and apply the probe mixture.

**Preparing the Probe Mixture**

1. Add the following, for each target area, to a microcentrifuge tube at ambient temperature:
   - 7 µL LSI/WCP Hybridization Buffer
   - 1 µL probe
   - 2 µL purified H₂O

**NOTE:** For probes labeled with different fluorophores, up to three may be added at 1µL each. The total volume of probe and H₂O should not exceed 3 µL. H₂O is not necessary if mixing two probes.

2. Centrifuge tube for 1-3 seconds.
3. Vortex and then centrifuge again.
4. Place tube in a 73±1°C water bath for 5 minutes.
5. Remove tube from water bath.
6. Place tube on a 45-50°C slide warmer until ready to apply probe to target DNA.

**NOTE:** If slides are ready when probe is denatured, you can apply probe immediately to target DNA.

**Hybridizing the Probe to the Specimen Target**

**NOTE:** The total time that the slide is on the warmer should not exceed 2 minutes.

**NOTE:** Prepare a humidified box by placing a paper towel moistened with water on the side of an airtight container. Place in 37°C incubator.
1. Remove the slides from the 100% EtOH.
2. Dry slides by touching the bottom edge of the slides to a blotter and wiping the underside of the slides dry with a paper towel.
3. Place slides on a 45-50°C slide warmer to evaporate remaining EtOH or air dry the slides.
4. Apply 10 µL of probe mixture to one target area and immediately apply coverslip. Repeat for additional target areas.
5. Seal coverslip with rubber cement.
6. Place slides in a prewarmed humidified box and place box in a 37°C incubator for 6-16 hours. To produce an assay with sufficient signal, start with a 12-16 hour hybridization for most TelVysion probes.

**Removing the Slides from EtOH**

**NOTE:**

For paraffin-embedded sections or cytology specimens containing cells of epithelial origin substitute 2X SSC/0.3% NP-40 wash solution for the 0.4X SSC/0.3% NP-40 wash solution. A room temperature wash is not needed for this specimen type:

Pour 70 mL of 0.4X SSC/0.3% NP-40 into a Coplin jar. Place jar in a 74±°C water bath at least 30 minutes prior to use. Use 1 day, then discard.

Pour 70 mL of 2X SSC/0.1% NP-40 into a Coplin jar. Use at ambient temperature. Use 1 day, then discard.

**NOTE:**

To maintain the proper temperature in 0.4X SSC/0.3% NP-40, wash four slides simultaneously. If you have less than four slides, add blank slides that are at ambient temperature to bring the total to four.

Start timing when the fourth slide is immersed.
1. Remove coverslip from one slide and immediately immerse the slide in the 0.4X SSC/0.3% NP-40. Agitate slides for 1-3 seconds. Repeat with other slides.
2. Remove slides after 2 minutes ± 30 seconds.

**NOTE:** Ensure the temperature of the wash solution is 73±°C before washing another four slides.
3. Immerse slides in 2X SSC/0.1% NP-40. Agitate slides for 1-3 seconds. Remove slides after 5 seconds to 1 minute.

**Using this Vysis filter. . . Allows simultaneous excitation and emission of . . .**

- DAPI/Orange: DAPI and SpectrumOrange fluorophores
- DAPI/Green: DAPI and SpectrumGreen fluorophores
- DAPI/Orange/Green: DAPI, SpectrumOrange and SpectrumGreen fluorophores
- Orange: SpectrumOrange fluorophores
- Green: SpectrumGreen fluorophores

**Storage**

Store hybridized slides, with coverslip and counterstain, at -20°C in the dark.

**Using Codenaturation**

Codenaturation is a process that simplifies fluorescence in situ hybridization (FISH) by combining denaturation of probe mixture and specimen into a single step. Typically, codenaturations are performed by placing the specimen slides with probe mix and coverslips applied and sealed, on the surface of the Vysis HyBrite or ThermoBrite Denaturation/Hybridization Systems at the denaturation temperature.

Published conditions for codenaturation specify a broad range of temperatures and times, reflecting the need to optimize conditions for specific applications and specimen types. The parameters are described in the user guides for Vysis HYBrite or ThermoBrite Denaturation/Hybridization Systems and are intended to provide a set of starting parameters. Further optimization may be required depending on the specimen. The appearance of a hybridization using codenaturation may vary from a hybridization where the specimen target is denatured and dehydrated before the probe is applied.

ThermoBrite is a trademark of Iris Sample Processing, Inc.