

M E T A
S Y S T E M S

XCyte lab manual

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1 Fluorescence *in situ* hybridization

The technique of fluorescence *in situ* hybridization (FISH) is based on the reassociation of complementary DNA single strands. The probe is made of specific DNA pieces, whose nucleotides are labeled with fluorescent molecules. Denatured DNA from a certain sample forms the target. Complementary sequences of probe and target DNA are then allowed to reanneal. The fluorescence signal corresponding to the specific part of the double stranded DNA is detected by fluorescence microscopy.

Painting probes are used for a special type of FISH application. Chromosome or chromosomal region specific painting probes give prominence to a whole chromosome or a chromosomal region. The chromatin of chromosome preparations forms the target. This technique simplifies the analysis of numerical and structural chromosomal aberrations.

1.1 mFISH

The multicolor fluorescence *in situ* hybridization (mFISH) uses various fluorescence dyes to detect different painting probes at the same time. This offers the simultaneous presentation of all 24 different human chromosomes with a single hybridization in particular.

The detection of at least 24 different chromosome painting probes is achieved with five varicolored fluorochromes. Each paint is labeled with one of this five fluorochromes or a unique combination of them (combinatorial labeling). The separation of different excitation and emission spectra is guaranteed by appropriate filter sets (→ Chapter 2).

The resulting unequivocal color signature for each chromosome allows the analysis of hidden or complex chromosome aberrations or to describe the composition of marker chromosomes.

1.2 mBAND

The mBAND probes comprise region specific probes labeled with different fluorochromes or combinations of these. The partial overlap of adjacent banding probes results in a multitude of unique color ratios along the chromosome. Color ratio analysis allows to resolve the chromosome into a selectable number of bands of

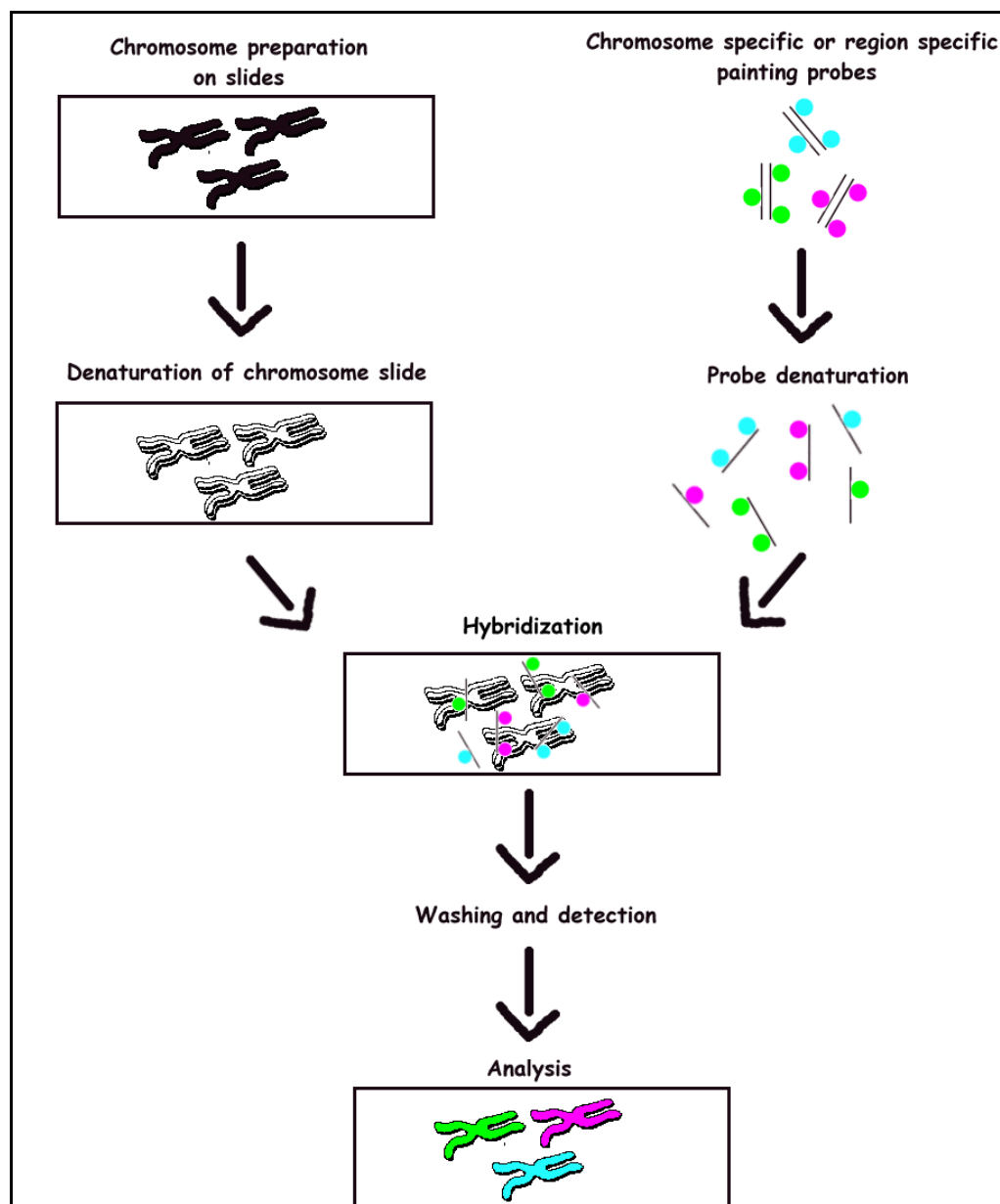
similar ratios. This quantitative color ratio analysis effectively multiplies the resolution of the region specific probes.

mBAND takes multicolor analysis to a higher level of precision and detects intrachromosomal rearrangements.

1.3 The hybridization procedure

First you need a chromosome preparation on slide from the case of interest and, of course, the painting probe.

The FISH procedure as such is composed of only four steps: the denaturation of probe and target, the hybridization, the washing and detection and than the analysis.



The mFISH and mBAND procedure differs not from that of 'simple' FISH.

- Denaturation of probe and target:
The denaturation of DNA double strands could be induced by increasing temperature or pH of environmental solutions or by organic solvents. A combination of organic solvent and increased temperature is used for the denaturation of the probe and the target in general. This method is used for the probe denaturation.
For denaturation of the chromosomes we recommend a treatment with a basic solution to increase the pH, which is carried out in sodium hydroxide (0.07N NaOH) at room temperature.
- Hybridization:
The reannealing of probe and target occurs in the presence of hybridization buffer. The hybridization is carried out in a humidified chamber for 2-4 days at 37°C.
- Washing and detection:
The post hybridization washing is necessary to remove the remaining hybridization buffer and to undo unspecific probe binding.
Afterwards the indirect labeled probes are detected with an additional fluorescence dye. Finally, the counterstain is applied.
- Analysis:
The fluorescence signals are detected by fluorescence microscopy. Images have to be captured for every fluorescent dyes with different single band pass filter sets. All six color channels (for the five different fluorochromes and the DAPI counter stain) are superposed by the *isis* software. Image processing leads to karyotyps which than could be analyzed. The analysis is supported by several software features.

1.4 The MetaSystems XCyte mFISH and mBAND kits

The 24XCyte kit contains 24 different chromosome painting probes specific for the 24 different human chromosomes. Each paint is labeled with 1 of 5 different fluorochromes or a unique combination of them.

The XCyte mBAND kit contains a mix of regionspecific partial painting probes specific for one chromosome. Each probe is labeled with one of five different fluorochromes or a combination of them, respectively.

The XCyte painting probes are supplied ready to use. They are already dissolved in hybridization buffer.

The 24XCyte mFISH kit and the XCyte mBAND kits are available in two different pack sizes: 60 μ l and 120 μ l. The number of tests depends on the size of the hybridized area. 60 μ l of probe cocktail is sufficient for 5 hybridizations using a 24x24mm² coverslip or 8 hybridizations using an 18x18mm² coverslip.

The *B*-tect detection kit is available either for 10 and 20 applications. One application means one slide independent on the hybridized area.

The hybridization protocol and the corresponding label scheme are enclosed with each kit. The front page of the 24XCyte pack insert is given here exemplary (for labeling scheme see chapter 6.1). The hybridization procedure is the same for all XCyte kits.

XCyting colors of *MetaSystems*

24XCyte – *MetaSystems*' 24 color kit

B-tect – *MetaSystems*' Biotin detection kit

The kit contains 24 different chromosome painting probes specific for the 24 different human chromosomes. Each paint is labeled with 1 of 5 different fluorochromes or a unique combination of them. The details of the labeling scheme are given below.

CONTENTS:

24XCyte **D-0125-060-MC**

■ ■ ■ ■ ■ 60µl probe cocktail store at **-20°C**

Specification: The excitation/emission spectra of the fluorochromes are equivalent to FITC, Spectrum Orange™, TexasRed® and DEAC (Diethylamino-coumarin). A further labeling was carried out using Biotin, which has to be detected by Streptavidin-Cy™5.

B-tect **D-0901-060-NI**

□ □ □ □ □ 2x 1000µl blocking reagent store at **-20°C**

■ ■ ■ ■ ■ 20µl detection 1+3 store at **-20°C**

■ ■ ■ ■ ■ 10µl detection 2 store at **-20°C**

■ ■ ■ ■ ■ 200µl DAPI/antifade store at **4°C**

Specification: Cy™5 detection kit for Biotin labeled probes. Including DAPI-counterstain and antifade.

Tween™ is a trademark of ICI America, Inc.; Spectrum Orange™ is a trademark of Vysis, Inc.; Cy™ is a trademark of Amersham Pharmacia Biotech Limited, Inc.; Texas Red® is a registered trademark of Molecular Probes, Inc.

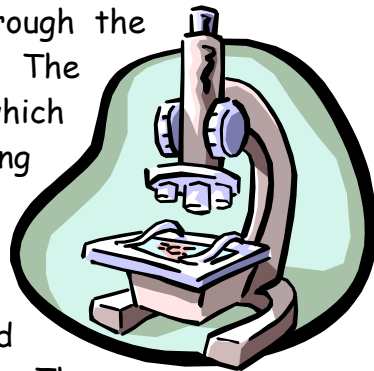
2 Fluorescence microscopy

Fluorescence is a photochemical process which takes place in a special type of molecules the so-called 'fluorophores' or 'fluorochromes'. When a fluorophore absorbs light, it is shifted into an excited state. A part of the absorbed energy may be lost by internal structural changes and interactions with other molecules. Returning in its ground state, the fluorophore emits light. Due to the energy loss the wavelength of the emitted light is higher compared to the absorbed light. This difference in wavelength is called 'Stokes shift'. Each fluorochrome has its own characteristic excitation and emission spectrum.

The fluorophore is excited repeatedly during illumination. Nevertheless, photochemical reactions may result in the irreversible destruction of the fluorophore, which then cannot be excited any longer (photo bleaching or fading).

The basic feature for the sensitivity of the fluorescence techniques is the Stokes Shift, it allows to discriminate between the emitted light and the absorbed light by a beam splitter.

In fluorescence microscopy the slide preparation is illuminated through the objective. The light source is a mercury vapor arc burner in general, which emits ultraviolet, visible and infrared light. The appropriate excitation wavelength for a particular fluorochrome is isolated using an excitation filter. The light passes the excitation filter and is focused through the objective onto the slide preparation. The emitted light passes the emission filter, which transmits light within a bandwidth according to the emission spectrum of the fluorochrome. The dichromatic beam splitter inserted between excitation filter and object reflects the short-waved excitation light onto the slide preparation. The longer-waved fluorescence light emitted by the object passes the beam splitter nearly completely and reaches the emission filter. This is necessary to create a dark background so that the fluorescence signal can be easily seen.



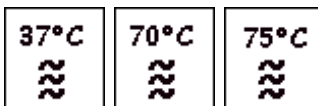
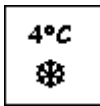

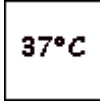




The filter set including beam splitter, excitation and emission filter must correspond to the excitation and emissions characteristics of the given fluorochrome. For multicolor fluorescence applications special small banded filter sets are necessary to separate adjoining

colors exactly. The excitation and emission maximum of each fluorescence dye used in the XCyte mFISH and mBAND kits are given in the table below.

Fluorochrome	Emission/ excitation maximum	Color of fluorescence signal	Color of excitation light
DAPI	455nm/345nm	light-blue	violet
DEAC	480nm/426nm	blue/turquoise	blue
FITC	530nm/502nm	green	turquoise
Spectrum Orange™	588nm/559nm	yellow	green
Texas Red®	615nm/595nm	red	yellow/orange
Cy™5	670nm/649nm	near IR (not visible)	red

3 Equipment and Reagents

3.1 Lab equipment

- Water bath at 37°C, 70°C and 75°C 
- Refrigerator (4°C) 
- Freezer (-20°C) 
- Incubator 37°C 
- Microcentrifuge 
- Tubes 500µl 
- Coplin jars for 70ml or 100ml 
- Humidified chamber 
- Variable micropipettes: 1µl - 20µl, 50µl - 100µl, 500µl - 1ml
- pH meter or pH indicator sticks
- Thermometer
- Timer
- Gloves
- Coverslips 18x18mm² or 22x22mm² or 24x24mm² and 24x60mm²
- Rubber Cement, e.g. Fixogum (Marabu, Germany)
- Chromosome preparation on slides

3.2 Reagents required

The following reagents are required for the hybridization procedure. They are not included in the 24XCyte mFISH kit or the XCyte mBAND kit or the B-tect detection kit.

- **Aqua dest.**
- **Ethanol (100%)**
- **1N NaOH** Dissolve 40g Sodium hydroxide pellets ($M=40.00\text{g/mol}$) per liter of final volume in distilled water.
- **1xPBS** Phosphate Buffered Saline Solution
- **Tween20**
Polyoxyethylenesorbitan-monolaurate
Syrup (e.g. Sigma P-1379)
Tween™ is a trademark of ICI America, Inc.
- **20xSSC** 3.0M NaCl und 0.3M Sodium Citrate:
Add 175.2g NaCL and 88.3g Na citrate per liter of final volume to distilled water.
- **Pepsin** Pepsin stock solution: Dissolve 1g pepsin (Sigma, P-7012) in 50ml sterile distilled H₂O, store in 500µl aliquots at -20°C
- **1N HCl**
- **Formaldehyde (37%)**
- **1M MgCl₂** 9,52g MgCl₂ ($M=95,21\text{g/mol}$) or 20,33g MgCl₂·H₂O ($M=203,30\text{g/mol}$)
add water up to a final volume of 100ml



4 Preparation

4.1 Chromosome preparation on slides

Metaphase spreads are prepared according to the conventional cytogenetical methods. Check slides for spreading and morphology under phase contrast before hybridization.

Careful chromosome slide preparation is essential of a successful hybridization. Slides should not be older than two weeks. We recommend preparing the slides one day prior to hybridization. For long term storage keep slides at -20°C .

Remaining cytoplasmic proteins of the cells may impair the hybridization. For that reason we recommend a protein digesting pretreatment prior to hybridization (\rightarrow Chapter 4.4).

4.2 Stock solutions

We recommend to prepare stock solutions for the pre and post hybridization washing steps. They can be used up to three month. Store in a dark place at room temperature.

200ml **0.07N NaOH**

14ml 1N NaOH

186ml Aqua dest.

500ml **0.1xSSC**, pH7.0-7.5

497.5ml Aqua dest.

2.5ml 20xSSC

200ml **1xSSC**, pH7.0-7.5

190ml Aqua dest.

10ml 20xSSC

500ml **2xSSC**, pH7.0-7.5

450ml Aqua dest.

50ml 2xSSC

500ml **4xSSCT** (4xSSC containing 0.05% Tween), pH7.0-7.5

400ml Aqua dest.

100ml 20xSSC

250 μl Tween20

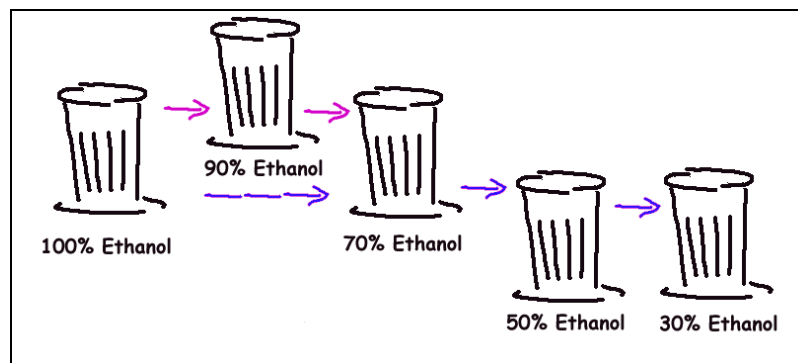
4.3 Ethanol series

The ethanol series are necessary for re- and dehydration of the slide preparation. The rehydration procedure ensures that the

chromosomes are prepared well for a following washing or incubation step. This kind of 'soaking' improves the effectiveness of agents solved in hydrous solution. On the other hand reactions of the preparation with e.g. buffer substances are stopped by the dehydration procedure. This is especially important if strong solvents are used or in case that the slide preparations will be stored for a longer time.

Two ethanol series are needed. One with 100%, 90% and 70% ethanol for the protein digestion pretreatment procedure (→ Chapter 4.4). And another one with 100%, 70%, 50% and 30% ethanol for the pretreatment and denaturation procedure of the chromosome slides (→ Chapter 4.5).

Prepare five coplin jars, one for each ethanol concentration. The 100% and the 70% ethanol are used in both series. These solutions are useable for four to six weeks. They are stored at room temperature. Use lids to protect the solutions from evaporation.



	100ml coplin jar	70ml coplin jar
100% Ethanol	100ml Ethanol (100%)	70ml Ethanol (100%)
90% Ethanol	90ml Ethanol (100%) 10ml Aqua dest.	63ml Ethanol (100%) 7ml Aqua dest.
70% Ethanol	70ml Ethanol (100%) 30ml Aqua dest.	49ml Ethanol (100%) 21ml Aqua dest.
50% Ethanol	50ml Ethanol (100%) 50ml Aqua dest.	35ml Ethanol (100%) 35ml Aqua dest.
30% Ethanol	30ml Ethanol (100%) 70ml Aqua dest.	21ml Ethanol (100%) 49ml Aqua dest.

4.4 Protein digesting pretreatment prior to hybridization

Careful chromosome slide preparation is essential of a successful hybridization. Slides should not be older than two weeks. We recommend preparing the slides one day prior to hybridization. For long term storage keep slides at -20°C .

Remaining cytoplasmic proteins of the cells may impair the hybridization. Pretreat the slides if necessary.

Solutions required:

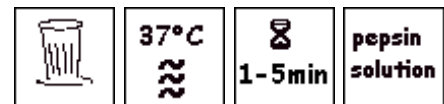
- 1N HCl
- 1xPBS
- 70%, 90%, 100% ethanol
- **Pepsin stock solution:** Dissolve 1g pepsin (Sigma, P-7012) in 50ml sterile distilled H_2O , store in $500\mu\text{l}$ aliquots at -20°C . The pepsin concentration refers to the given pepsin type. For different types of pepsin the concentration and the incubation time have to be checked out.
- **Postfixation solution:** 1% formaldehyde in 1xPBS + 50mM MgCl_2
60 μl 37% Formaldehyde
2ml 1xPBS
100 μl 1N MgCl_2
This solution can be used for three to five days. Store at 4°C .

Procedure:

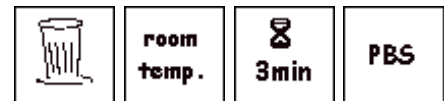
- Add 1ml 1N HCl to 99ml H₂O, preheat to 37°C

- Add 500µl of the pepsin solution, mix well

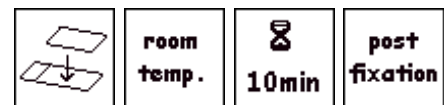
- Immediately immerse the slides into the pepsin solution and **incubate** 2min for amniocytes and lymphocytes or up to 5min for bone marrow



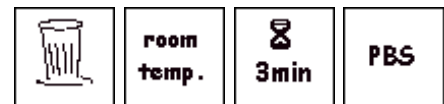
- **Wash** slides in 1xPBS for 3min



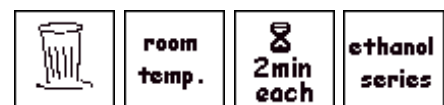
- Apply 100µl of the postfixation to each slide, overlay with a 24x60mm² coverslip and **incubate** for 10min at room temperature



- **Wash** slides in 1xPBS for 3min



- **Dehydrate** slides in 70%, 90%, 100% ethanol for 2min each



- Let air dry

- For longer storage (> 1 day) keep slides at -20°C

Note: If the protein digestion pretreatment is done prior to pre hybridization washing immediately, the ethanol series for dehydration and rehydration are not necessary. Transfer slide from 1xPBS into preheated 2xSSC directly (→ Chapter 4.5).

Note!

4.5 Pretreatment and denaturation of chromosome slides

Solutions required:

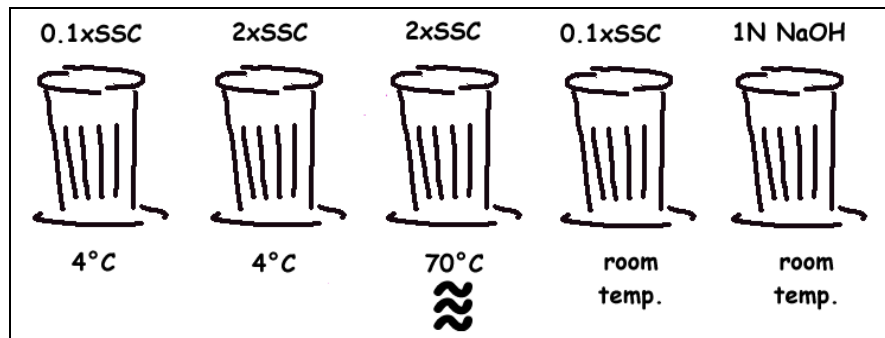
- 0.1xSSC, pH7.0-7.5
- 2xSSC, pH7.0-7.5
- NaOH 0.07mol/l
- Ethanol series: 100%, 70%, 50%, 30%

Prepare five coplin jars for the prehybridization washing steps: one with 0.07N NaOH, two with 0.1xSSC and another two with 2xSSC.

Check the pH value (at room temperature) of each solution before using it.

Put one coplin jar with 0.1xSSC and one with 2xSSC into the refrigerator. Preheat a coplin jar with 2xSSC to 70°C in a water bath. The remaining two coplin jars - one with 0.1xSSC and one with 1N NaOH - are stored at room temperature.

These solutions may be used for three to five days, except the preheated 2xSSC which has to be prepared fresh prior to each denaturation procedure.



Check the temperature of the preheated solution. Notice that the given temperature in the protocol specifies the temperature of the solution, which may be different from the temperature indication of the water bath.

Note: Start the probe denaturation during pretreatment and denaturation of the metaphase spreads. Time the procedure so that the prepared slide has just dried as the probe prehybridization is completed.

Note!

Procedure:


- **Rehydrate** slide: 100%, 70%, 50%, 30% ethanol, 1min each

	room temp.	⌚ 2min each	ethanol series
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- Transfer slide into 0.1xSSC at room temperature for 1min

	room temp.	⌚ 1min	0.1x SSC
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- **Incubate** slide for 30min in 2xSSC at 70°C


	70°C ⌚	⌚ 30min	2x SSC
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- Remove coplin jar from water bath, let **cool down** to 37°C (takes about 20min)


	room temp.	⌚ approx. 20min	2x SSC
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Start probe denaturation!


- Transfer slide to 0.1xSSC at room temperature, 1min

	room temp.	⌚ 1min	0.1x SSC
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- **Denature** slide in 0.07N NaOH at room temperature, 1min

	room temp.	⌚ 1min	1N NaOH
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- Put slide into 0.1xSSC, 4°C, 1min

	4°C ❄️	⌚ 1min	0.1x SSC
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- Put slide into 2xSSC, 4°C, 1min

	4°C ❄️	⌚ 1min	2x SSC
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- **Dehydrate** slide: 30%, 50%, 70%, 100% ethanol, 1min each

	room temp.	⌚ 1min each	ethanol series
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- Let air dry

4.6 Probe denaturation and hybridization

Preheat water bath and incubator.

Note: Start the probe denaturation during pretreatment and denaturation of the metaphase spreads. Time the procedure so that the prepared slide has just dried as the probe prehybridization is completed. Pipette the denatured and prehybridized probe onto the denatured chromosome preparation immediately.

We recommend to divide the probe cocktail into appropriate aliquots to avoid repeated freeze thaw cycles.

Note!

For research use only!

Warning: *Painting probes contain formamide. Handle carefully.*

Avoid contact with skin, wear gloves while handling the reagents

To prevent photo bleaching, handle all reagents and slides containing fluorochromes in reduced light!

Centrifuge all tubes prior to opening to assemble the contents at the bottom of the tube!

Procedure:

- Pipette the required volume of probe cocktail into a tube.

Probe cocktail (■ ■ ■ ■ ■) per hybridization:

Use 7 μ l for a 18x18mm² coverslip,
or 10 μ l for a 22x22mm² coverslip,
or 12 μ l for a 24x24mm² coverslip

- **Denature** the probe by incubating at 75°C for 5min

- Put on ice briefly

- **Incubate** at 37°C for 30min

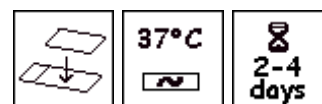
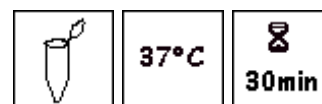
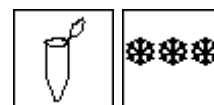
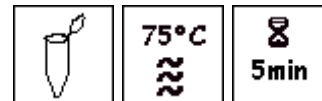
- **Spin** briefly to collect probe cocktail

- Pipette the denatured and prehybridized probe cocktail onto the denatured chromosome preparation

- Overlay with coverslip

- Seal with rubber cement

- **Incubate** 2-4 days in a humidified chamber at 37°C



4.7 Posthybridization washing steps and detection of the Biotin labeled probes with Cy5

Solutions required:

- 1xSSC, pH7.0-7.5, 75°C
- 4xSSCT = 4xSSC, pH7.0-7.5 containing 0.05% Tween™20, room temperature
- 1xPBS, room temperature

Prepare two coplin jars for the post hybridization washing steps: one with 1xSSC and one with 4xSSCT.

Check the pH value (at room temperature) of each solution before using it.

Dispose of solutions after each washing step.

Preheat the 1xSSC to 75°C in a water bath.

Check the temperature of the preheated solution. Notice that the given temperature in the protocol specifies the temperature of the solution, which may be different from the temperature indication of the water bath.

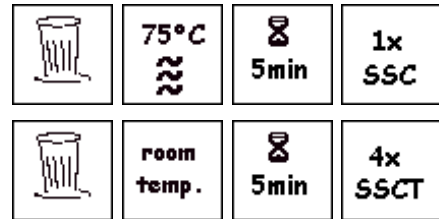
Thaw the *blocking reagent* (□□□□□).

During blocking step and detection slides should be incubated at 37°C in the humidified chamber.

Denaturation procedure adapted from: Fritz et al, *Hum Genet* (1998)103:441-449; Rieder et al, *Leukemia* (1998)9:1473-1481

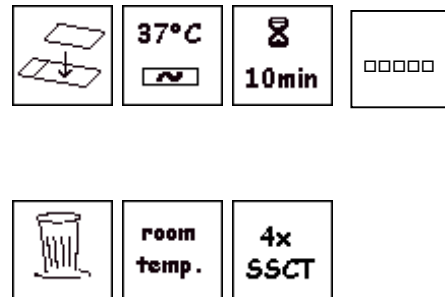
Procedure:

- Remove rubber cement and coverslips carefully
- **Wash** slides in preheated (75°C) 1xSSC, 5min
- **Incubate** slides for 5min in 4xSSCT



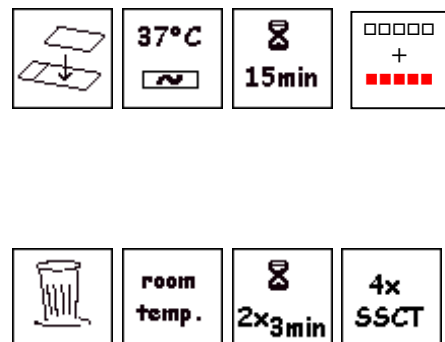
Blocking-step:

- Apply 50µl of *blocking reagent* (□□□□□) to each slide, overlay with a 24x60mm² coverslip and **incubate** 10min at 37°C
- Flip off coverslips, put slides into 4xSSCT and continue with the next step



Detection step 1:

- For each slide mix 50µl *blocking reagent* (□□□□□) with 1µl *detection 1+3* (■ ■ ■ ■ ■) reagent. Apply 50µl to each slide, overlay with a 24x60mm² coverslip and **incubate** 15min at 37°C
- **Wash** 2x 3min in 4xSSCT



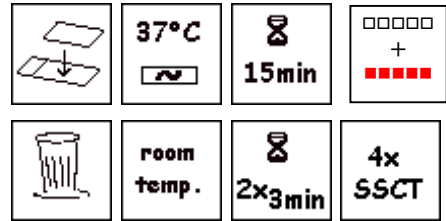
Detection step 2:

- For signal amplification, mix 50µl *blocking reagent* (□□□□□) with 1µl *detection 2* (■ ■ ■ ■ ■) reagent. Apply 50µl to each slide, overlay with a 24x60mm² coverslip and **incubate** 15min at 37°C
- **Wash** for 2x 3min in 4xSSCT



Detection step 3:

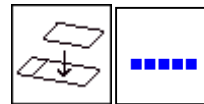
- See detection step 1
- **Wash** for 2x 3min in 4xSSCT



(Detection steps 2 and 3 are optional and are only necessary if the Cy5 fluorescence signal is weak and you want to amplify the signal)

Counterstaining:

- **Wash** for 3min in 1xPBS
- Drain fluid off and blow dry with a rubber ball or let air dry
- Apply 20 μ l of the *DAPI/antifade* (■■■■■)
- Overlay with a 24x60mm² coverslip



Slides are useable up to six month. Store slides at -20°C.

5 Troubleshooting

5.1 Weak or no signal

- *Chromosome slide is not adequately denatured:*
Ensure that washing solutions were made according to the protocol.
Check the temperature of the preheated solutions.
Check the pH value (at room temperature) of each solution before using it.
Ensure that denaturation time for slide in 0.07N NaOH is exactly one minute.
Ensure that washing solutions are not stored too long:
Prepare fresh. Store in a dark place at room temperature.
- *Probe cocktail is not adequately denatured:*
Ensure that the denature temperature is correct.
- *Incubation for hybridization is too short:*
Incubate 2-4 days for hybridization in humidified chamber:
Prevent from drying up. Check the incubation temperature.
- *Metaphase spreads contain cytoplasmic proteins:*
Apply protein digesting pretreatment prior to hybridization.
- *Chromosome slide is too old:*
Slides should not be older than two weeks. We recommend preparing slides one day before hybridization. For long term storage keep slides at -20°C.
- *Low Cy5 signal:*
Apply detection steps 2 and 3.
Avoid air bubbles under coverslip during incubation.
In case that DAPI/antifade is applied already onto the slide:
Remove coverslip carefully. Rinse slide with 70% ethanol.
Dehydrate slide in 70%, 90% and 100% ethanol for 2min each. Let air dry. Apply detection steps 2 and 3.
- *Low FITC signal:*
Check the pH value (at room temperature) of each solution before using it.
Apply protein digesting pretreatment prior to hybridization.

5.2 High slide background

- *Metaphase spreads contain cytoplasmic proteins:*
Apply protein digesting pretreatment prior to hybridization.

- *Post hybridization washing not adequate:*
Ensure that washing solutions were made according to the protocol.
Check the temperature of the preheated solutions.
Check the pH value (at room temperature) of each solution before using it.
Ensure that washing solutions are not stored too long:
Prepare fresh. Store in a dark place at room temperature.

6 Analysis procedure

In order to get meaningful results great care must be taken to assure correct image acquisition. The fluorescence illumination of the microscope should be carefully adjusted to achieve a uniformly illuminated field. Use the automatic integration control.

Image capture:

- Select the experiment type for mFISH
- Capture all six color channels

Image processing:

- Background correction
- Define region (if necessary)
- Automatic upper and lower threshold
- Correct pixel shift (if necessary)



To include all color channels make sure that the spectrum symbol is selected before you apply these commands. This is most important in order to preserve the correct fluorescent ratio of the raw image.

Karyotype chromosomes:

- Adjust the object threshold
- Separate the chromosomes
- Enter karyotype view
- Automatic classification

Analysis

- Single color gallery
- Binary display
- False colors

For detailed information see Appendix or *isis*-Manual:
Chapter 7: Color Karyotyping Module and
Chapter 8: mFISH/mBAND Module

6.1 mFISH analysis

The *isis*-mFISH system classifies the chromosomes on basis of their specific fluorochrome combination according to the 24XCyte labeling scheme. Essential for a successful classification is a careful slide preparation, a regular hybridization and a correct image acquisition.

Select the classifier *LABEL*. In most cases this classifier gives a meaningful result. But depending on lab conditions it may be necessary to modify the classifier.

With the color classifier training you define the correlation between the fluorochrome combination for each chromosome and an assigned false color (see Appendix or *isis*-Manual, Chapter 8.4.1).

Once you have generated a color classifier it can be used for the analysis of all normal or aberrant metaphases, which have been hybridized under comparable conditions.

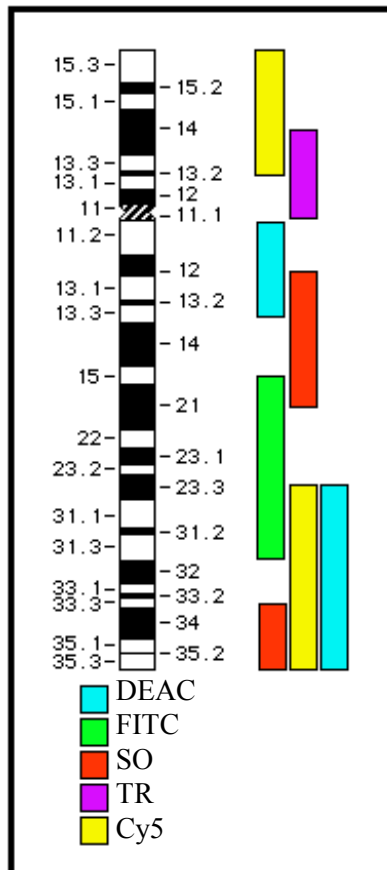
24XCyte - labeling scheme

#	DEAC Ex 426nm/ Em 480nm	FITC Ex 502nm/ Em 530nm	Spectrum Orange™ Ex 559nm/ Em 588nm	Texas Red® Ex 595nm/ Em 615nm	Cy™5 Ex 649nm/ Em 670nm
1					■
2	■				
3				■	
4		■			
5			■		
6		■			■
7	■				■
8				■	■
9			■		■
10	■	■			
11		■		■	
12		■	■		
13	■			■	
14	■		■		
15			■	■	
16	■	■			■
17		■		■	■
18		■	■		■
19	■			■	■
20	■		■		■
21			■	■	■
22	■	■		■	
X	■	■	■		
Y	■		■	■	

6.2 mBAND analysis

The mBAND probes comprise region specific probes labeled with different fluorochromes or fluorochrome combinations. The XCyte5 labeling scheme for chromosome 5 is given below for illustration.

XCyte5 - labeling scheme



This particular chromosome paints exhibit a gradual intensity decrease from their center towards their ends. The overlaps of the intensity profile of adjacent probes result in color ratio variations along the chromosome that are quantitated by the isis software. Pseudo color assignment yields a definable number of color band per chromosome. This quantitative color ratio analysis effectively multiplies the resolution of the region specific probes.

When you are using the isis system for mBAND the first time, you have to train the mBAND color classifier, respectively on the different XCyte mBAND kits and the hybridization conditions you have employed.

With the color classifier training you define the correlation between the fluorochrome ratio for each chromosome band and an assigned false color. Thus, the training step must be performed on a normal chromosome (see Appendix or *isis-Manual*, Chapter 8.4.1).

Once you have generated a color classifier it can be used for the analysis of all normal or aberrant metaphases, which have been hybridized under comparable conditions. Essential for a successful classification is a careful slide preparation, a regular hybridization and a correct image acquisition.