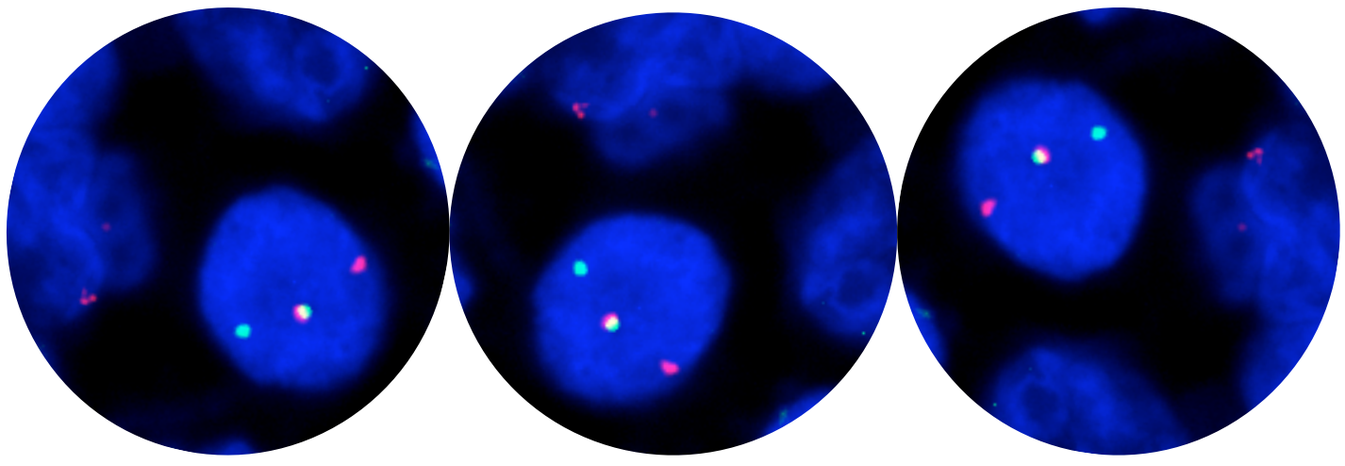
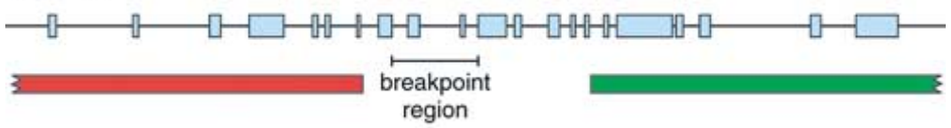
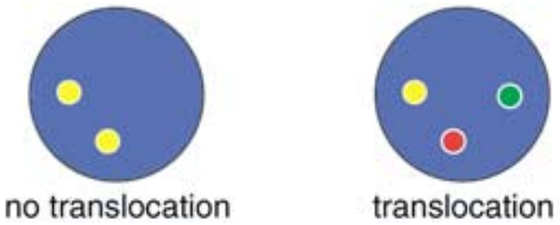
FISH

med Break Apart Rearrangment Prober







DakoCytomation

Split-Signal FISH DNA Probes for Lymphoma Research	Labeled Gene
BCL2 FISH DNA Probe, Split Signal	18q21
BCL3 FISH DNA Probe, Split Signal	19q13
BCL6 FISH DNA Probe, Split Signal	3q27
BCL10 FISH DNA Probe, Split Signal	1p22
CCND1 FISH DNA Probe, Split Signal	11q13
IGH FISH DNA Probe, Split Signal	14q32
IGK FISH DNA Probe, Split Signal	2p11
IGL FISH DNA Probe, Split Signal	22q11
MALT1 FISH DNA Probe, Split Signal	18q21
MYC FISH DNA Probe, Split Signal	8q24
PAX5 FISH DNA Probe, Split Signal	9p13

Vysis

MYC Dual Color, Break Apart Rearrangement Probe	8q24
ALK Dual Color, Break Apart Rearrangement Probe	2p23
IGH Dual Color, Break Apart Rearrangement Probe	14q32
SYT Dual Color, Break Apart Rearrangement Probe §	18q11.2
1p36/1q25 and 19q13/19p13 Dual-Color Probe Sets #	1p36 - 19q13 del

§ Synovial sarcoma # Oligodendroglioma

Pre-treatment

- Demasking
- Washing of slides in wash buffer for 2x5 min.at room temperature
- Dehydration in ethanol series (70%, 85%, 96%; 2 min. each)
- Air dry

- Application of *probe mixture* containing PNA oligos (or DNA) for blocking and fluorescent labelled DNA probes
- Adding coverslip and sealing with coverslip sealant

Co-denaturation & Hybridization

- Denaturation
- Overnight hybridisation

Stringent wash

- Washing of slide in stringent wash buffer

- Washing of slides in wash buffer for 2 min. at room temperature
- Dehydration in ethanol series (70%, 85%, 96%; 2 min. each)
- Air dry

Mounting & Reading

- Application of mounting medium containing blue counterstain and coverslip
- Reading of slide

Fluorescence In Situ Hybridization on Formalin-fixed and Paraffin-Embedded Tissue

Optimizing the Method

Bodil L. Petersen, MD, PhD, Mette C. Sørensen, Sanni Pedersen, and Marianne Rasmussen

Appl Immunohistochem Mol Morphol • Volume 12, Number 3, September 2004

Pre-treatment

- Demasking → 1. Enzyme / 2. HIER and Enzyme
- Washing of slides in wash buffer for 2x5 min.at room temperature
- Dehydration in ethanol series (70%, 85%, 96%; 2 min. each)
- Air dry

- Application of *probe mixture* containing PNA oligos (or DNA) for blocking and fluorescent labelled DNA probes
- Adding coverslip and sealing with coverslip sealant

Co-denaturation & Hybridization

- Denaturation → .. at 80°C - 85°C
- Overnight hybridisation → .. at 37°C - 45°C

Stringent wash

- Washing of slide in stringent wash buffer → Various at 65°C - 75°C

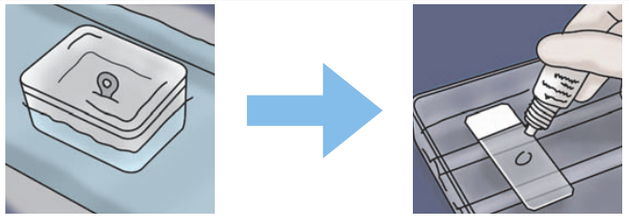
- Washing of slides in wash buffer for 2 min. at room temperature
- Dehydration in ethanol series (70%, 85%, 96%; 2 min. each)
- Air dry

Mounting & Reading

- Application of mounting medium containing blue counterstain and coverslip
- Reading of slide

TABLE 2. Influence of Conventional Enzymatic Pretreatment on the Efficiency of Hybridization (Using Fresh Tissue Sections From Tonsils Fixed for 48 Hours)

Enzymatic Pretreatment 37°C	Intensity of Hybridization
Pepsin 15 min	+++
Pepsin 30 min	+
Pepsin 60 min	+
Pepsin 90 min	+
Pepsin 24 timer	−
Protease 5 min	−
Proteinase K 5 min	−
Proteinase K 15 min	−
Proteinase K 30 min	−
Vysis Pretreatment Kit	++++
DakoCytomation Pretratment Kit	++++

Demasking	Hyb. Intensity	
	NBF 24 hrs	NBF 1 week
HIER 10′ -> Pepsin 10′	+++	++
HIER 10′ -> Pepsin 20′	+++	++
HIER 25′ -> Pepsin 10′	+++	++(+)
HIER 25′ -> Pepsin 25′	++++ *	+++

* Morfology not optimal !

TABLE 3. Influence of Formamide in the Washing Buffer on the Efficiency of Hybridization (Hybridization Performed With Enzymatic Pretreatment Alone on Fresh Tissue Sections Fixed for 48 Hours)

	NP-40	50% Formamide	70% Formamide
Centromeric probes	+++	+++	+++

NP–40: 0,3% NP–40 i 2x SSC

TABLE 4. Influence of the Temperature of the Washing Buffer on the Efficiency of Hybridization (Hybridization Performed With Enzymatic Pretreatment Alone)

	75°C, 3 min	80°C, 5 min
Centromeric probes	+	++++
LSI probes	++	+

Pre-treatment

- Demasking using DakoCytomation HIER/pepsin protocol
- Washing of slides in wash buffer for 2x5 min.at room temperature
- Dehydration in ethanol series (70%, 85%, 96%; 2 min. each)
- Air dry

- Application of *probe mixture* containing PNA oligos (or DNA) for blocking and fluorescent labelled DNA probes
- Adding coverslip and sealing with coverslip sealant

Co-denaturation & Hybridization

- Denaturation for 5 min.at 82˚C
- Overnight hybridisation at 45˚C or 37°C for Vysis probes

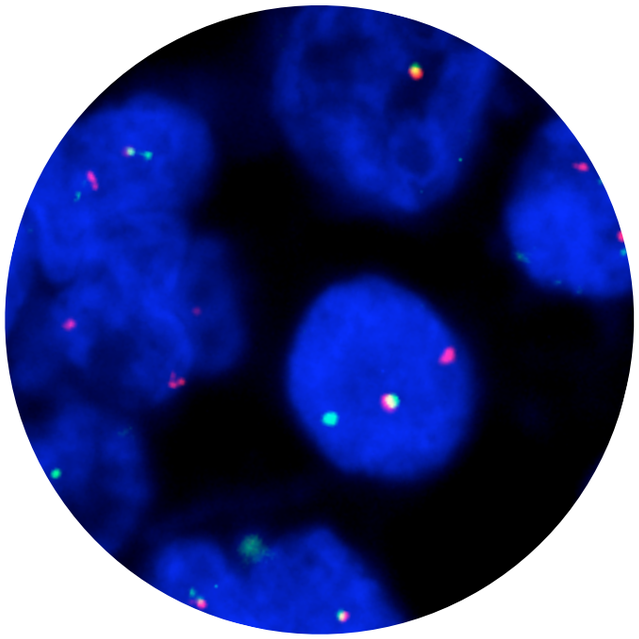
Stringent wash

- Washing of slide in stringent wash buffer for 10 min. at 65°C

- Washing of slides in wash buffer for 2 min. at room temperature
- Dehydration in ethanol series (70%, 85%, 96%; 2 min. each)
- Air dry

Mounting & Reading

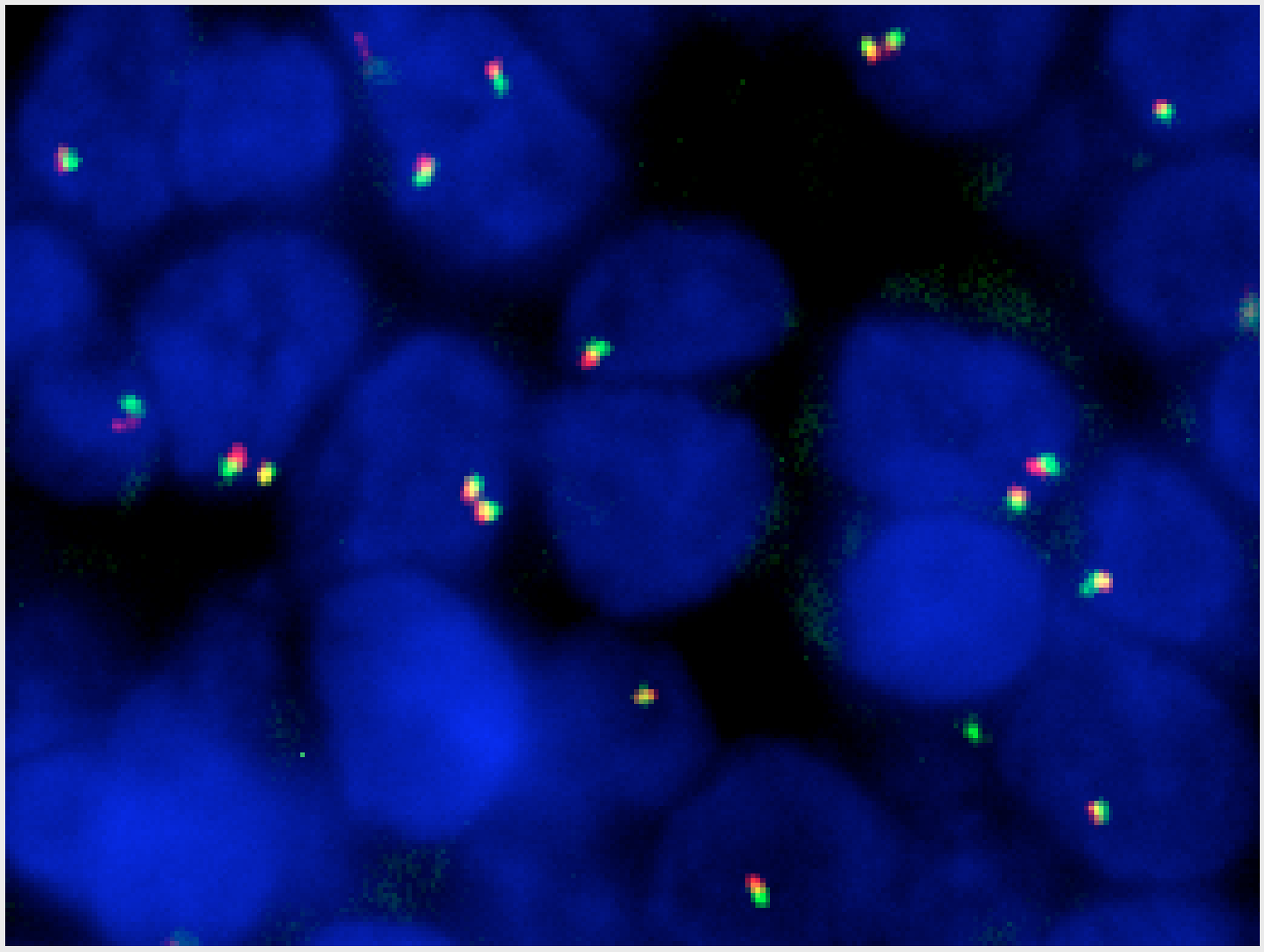
- Application of mounting medium containing blue counterstain and coverslip
- Reading of slide



Histology FISH Accessory Kit, Code No. K5599

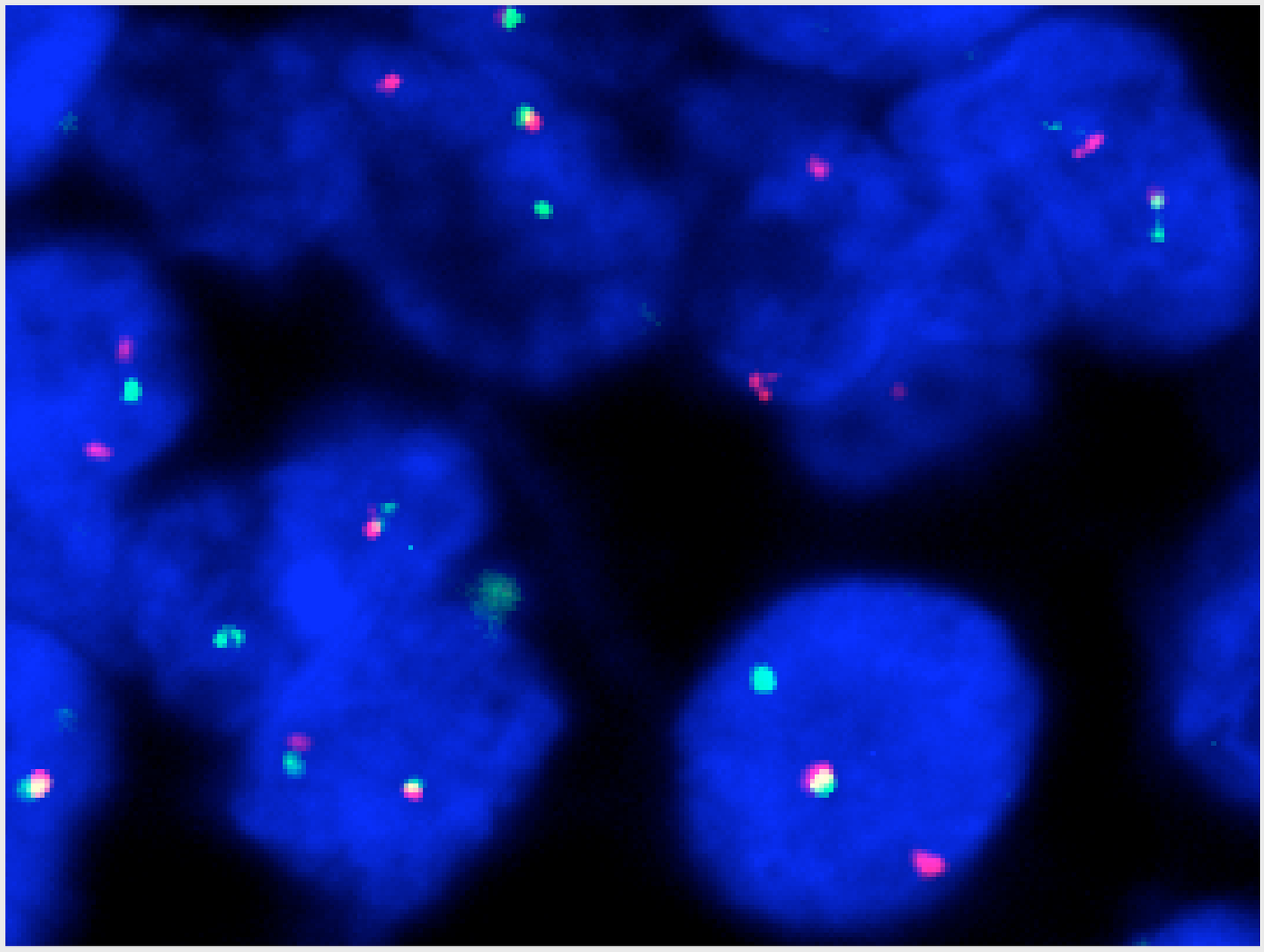
- **Vial 1:** Pre-Treatment Solution (x 20), 75 mL
- **Vial 2:** Pepsin, ready-to-use, 5 mL
- **Vial 3:** Wash Buffer (x 20), 500 mL
- **Vial 4:** Stringency Buffer (x 20), 150 mL
- **Vial 5:** Fluorescence Mounting Medium with blue counterstain, ready-to-use, 0.3 mL
- **Item 6:** Coverslip Sealant, ready-to-use, 1 tube

BCL2 (18q21)



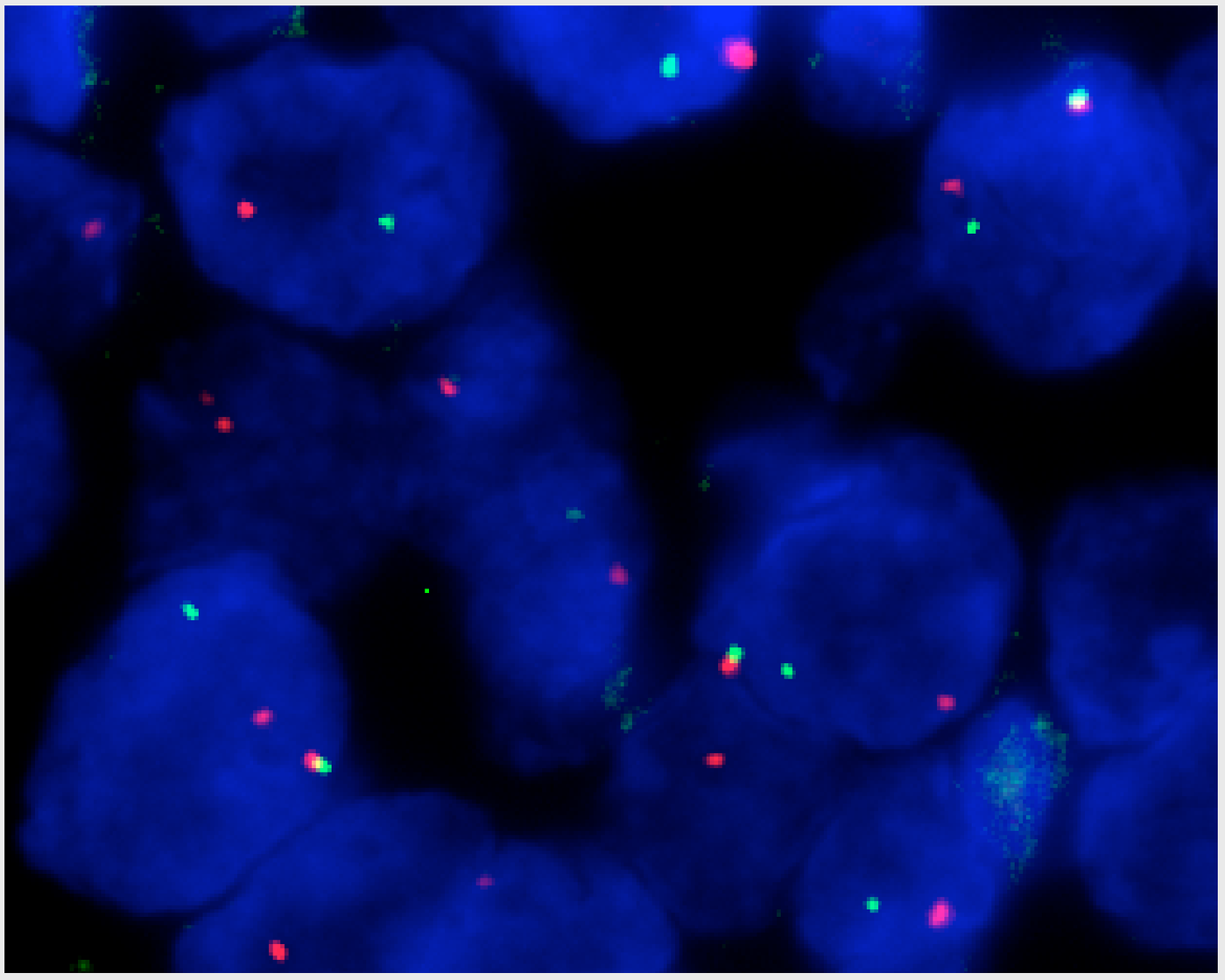
Tonsil

BCL2 (18q21)



Follicular lymphoma

SYT (18q11.2)



Synovial Sarcoma