READY, SET, GO!
GETTING STARTED WITH RNASCOPE

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Advanced Cell Diagnostics

TOPICS

- How Does RNAscope® Work?
- Getting Started with RNAscope in your Laboratory
- Tips and Tricks on Running the Assay
- Frequently Asked Questions
- Time for Q&A

RNASCOPE OVERVIEW

RNASCOPE WORKFLOW

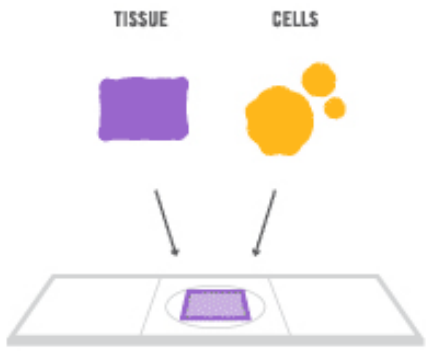
PERMEABILIZE
cells or tissue

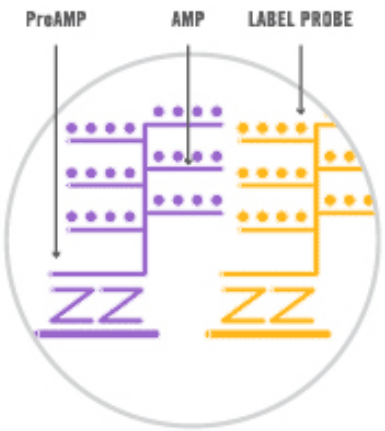
HYBRIDIZE
to target RNA

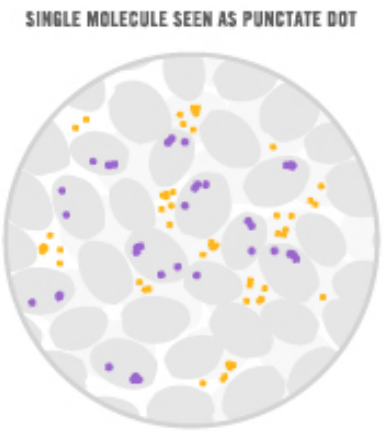
AMPLIFY
signal

VISUALIZE
with morphology

QUANTIFY
single-cell expression







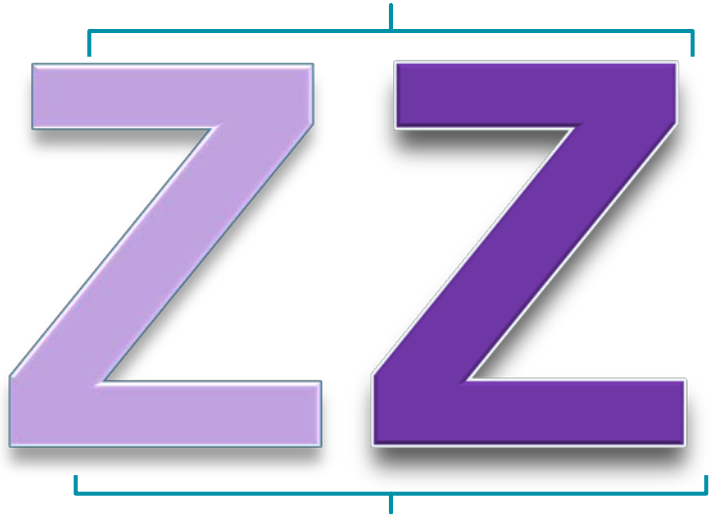
RNASCOPE® TECHNOLOGY: ZZ PROBE DESIGN

Preamplifer binding site: 28-bases

Upper region:
14-base tail sequence

Spacer

Lower region:
~8-25-base sequence



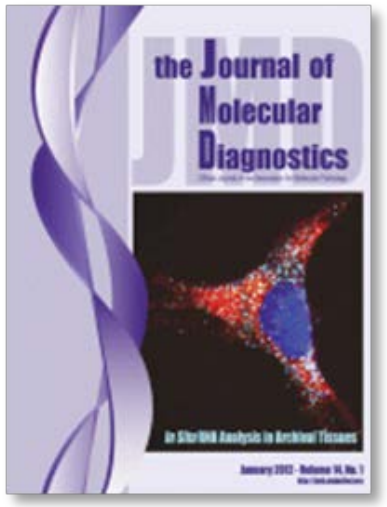
Target-specific binding site: ~50-base region

Target Probe Set:
Pool of 20 ZZ Oligo Pairs

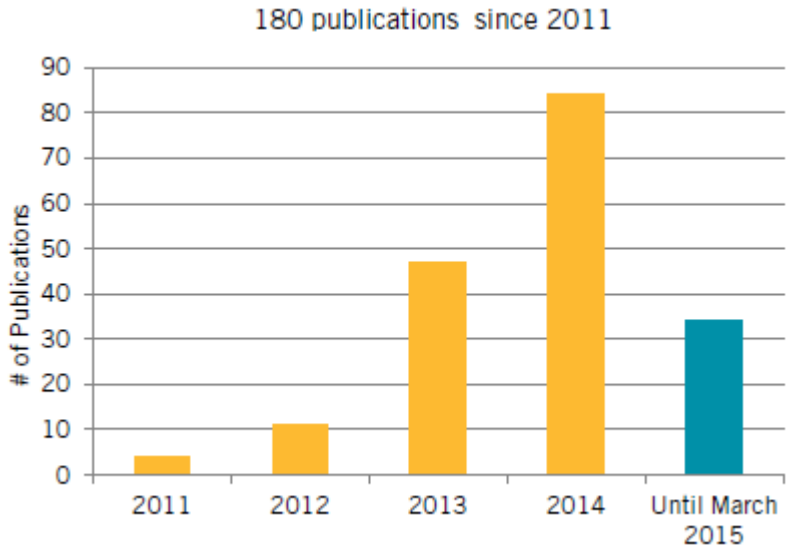
RNA Target Region
1KB
20 pairs of ~50 bases

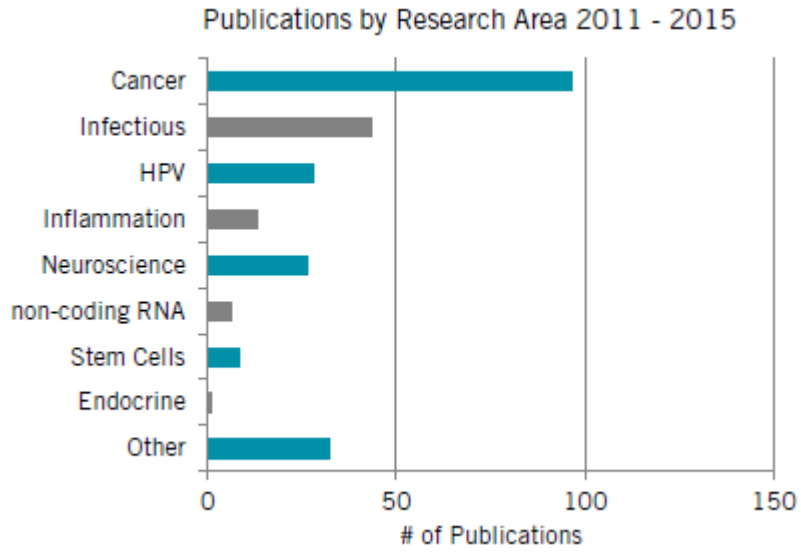
mRNA transcript detection: Highly specific & robust signal amplification

RNASCOPE PUBLICATION AND LITERATURE REFERENCES



Wang, F. et al.2012





The New England Journal of Medicine

nature

Cell

Science AAAS

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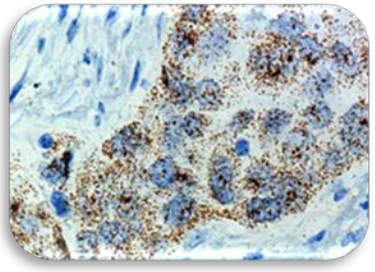
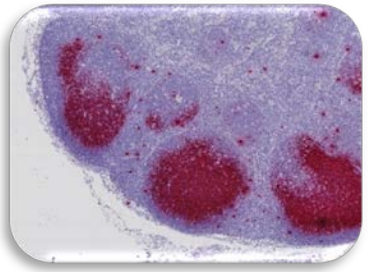
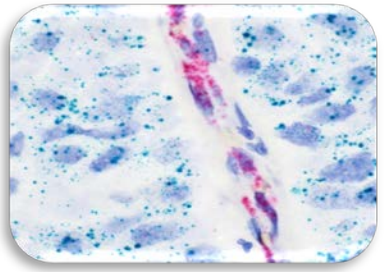
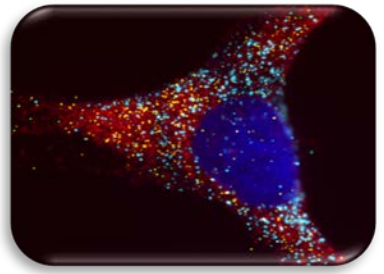
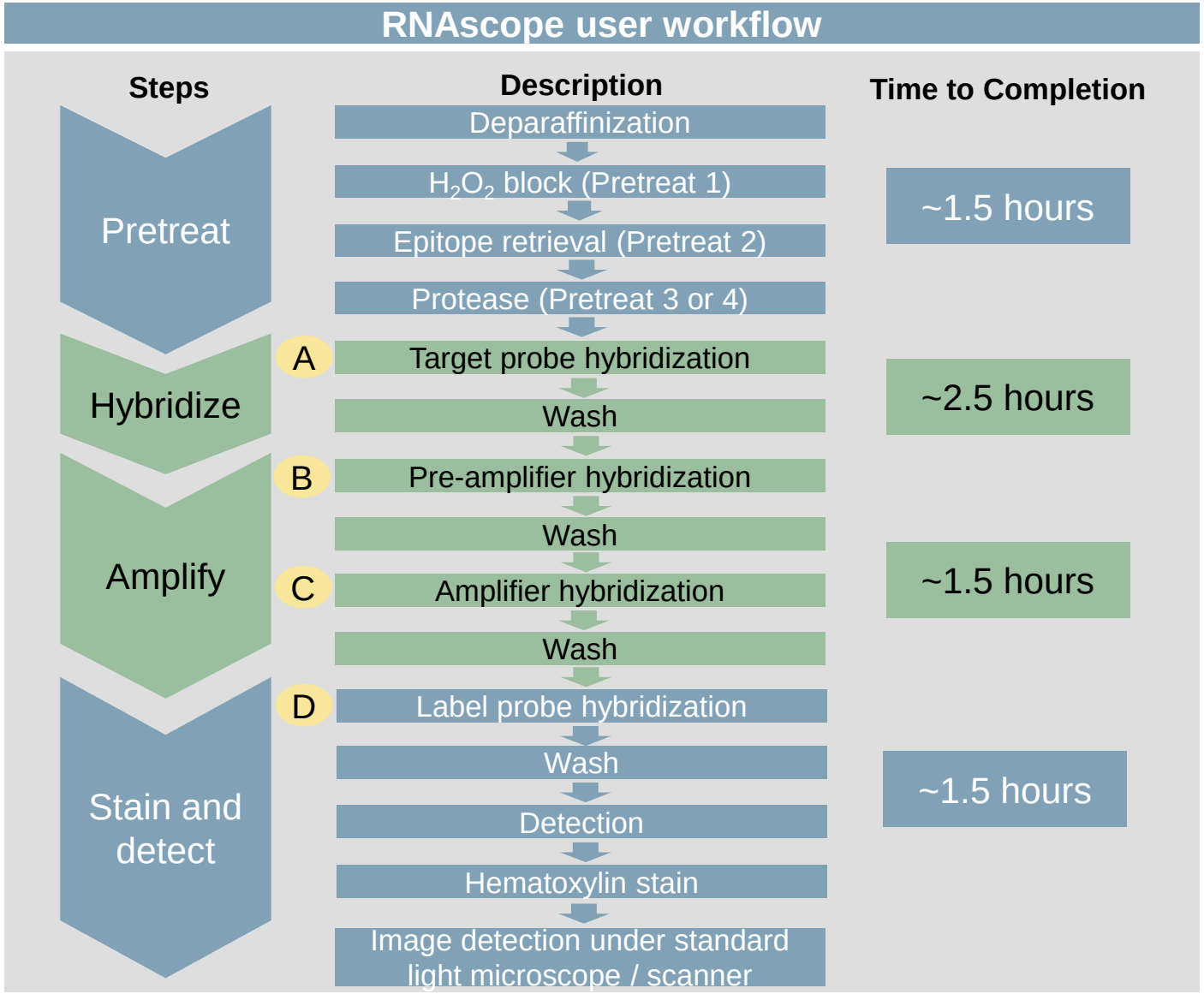
RNASCOPE WORKFLOW

RNASCOPE ASSAY SELECTION

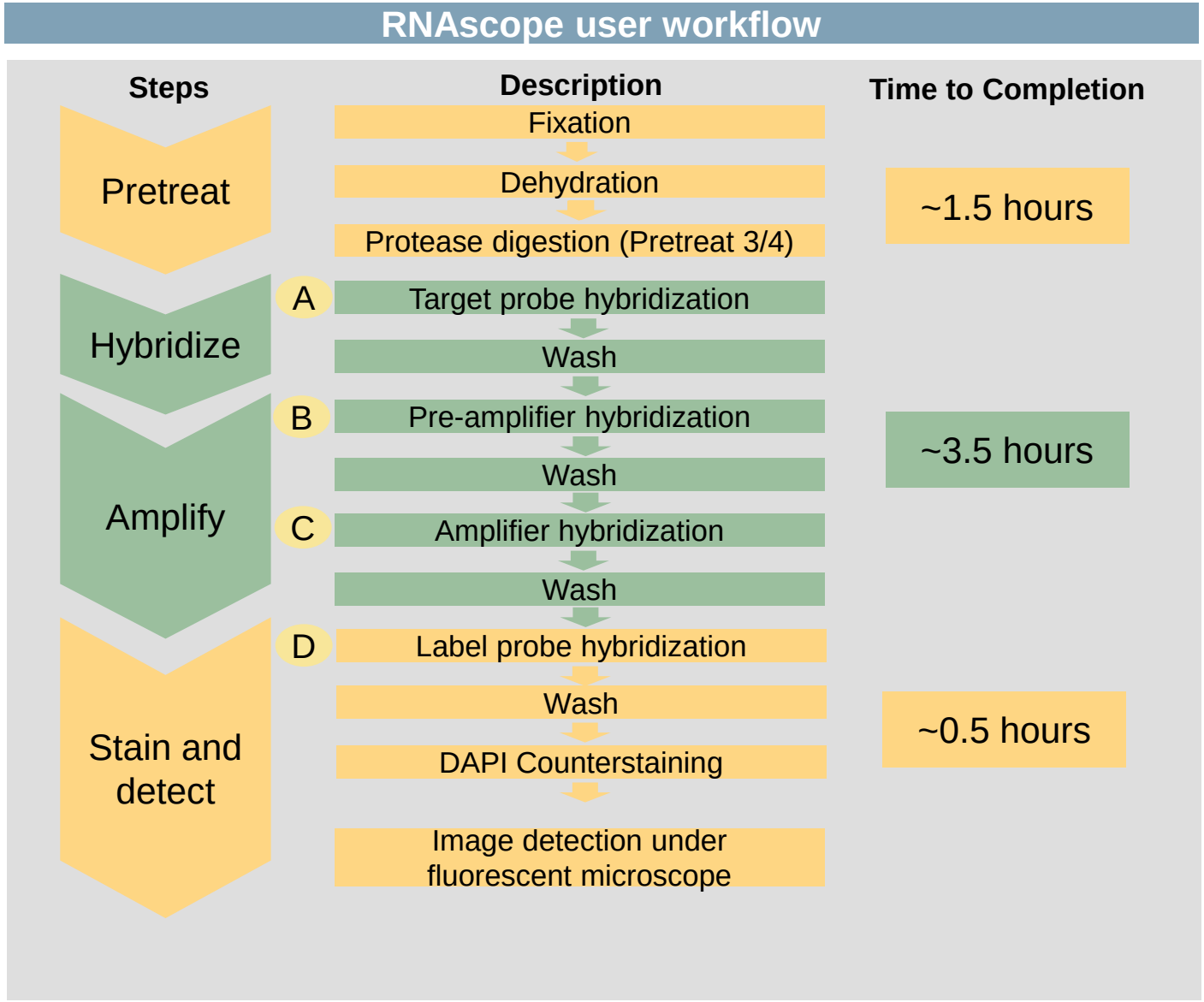
RNAscope Assays	RNAscope 2.0 HD BROWN	RNAscope 2.0 HD RED	RNAscope 2-plex	RNAscope Multiplex – Fluoroscence
Assay type	Chromogenic	Chromogenic	Chromogenic	Fluorescent
Dye Used	Diaminobenzene (DAB)-HRP	Fast Red -ALP	HRP-Green, Fast Red -ALP	FITC, Cy3, Cy5,
Channel	Channel 1	Channel 1	Channel 1, 2	Channel 1, 2, 3
Probes channel	C1 Probes	C1 Probes	C1, C2 Probes	C1, C2, C3 Probes

RNASCOPE WORKFLOW: CHROMOGENIC ASSAY



TIP : Detection protocols will vary based on the chromogenic assay used
Download manuals: http://www.acdbio.com/technical-support/downloads

RNASCOPE WORKFLOW: FLUORESCENT ASSAY



TIP : Pretreatment conditions will vary based on sample type

Download manuals: http://www.acdbio.com/technical-support/downloads

ONE DAY OR TWO DAY ASSAY?

ONE DAY ASSAY

Sample preparation

Sample pretreatment

RNAscope assay

TWO DAY ASSAY

Sample preparation

DAY 1

Sample pretreatment

DAY 2

RNAscope assay

TIP : Review the User Manuals PART 1 and PART 2 for optional stopping points

GETTING STARTED WITH RNASCOPE IN YOUR LAB

GET STARTED BY FOLLOWING 3 EASY STEPS

1

Check that all required materials are available

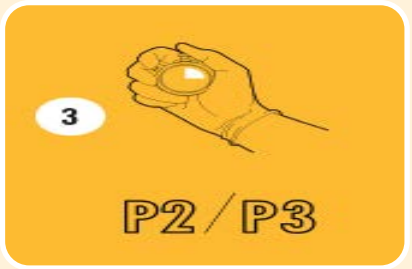
THE CHECKLIST

2

Always use control probes and slides

USE CONTROLS

3

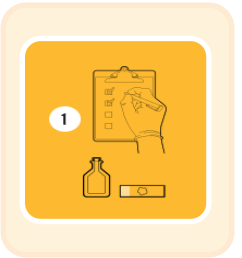


Optimize RNAscope assay conditions for your tissues for publication quality data

OPTIMIZE

TIP : Visit www. acdbio.com/go for more information on getting started

THE CHECKLIST: WHAT YOU NEED



☑	RNAscope target probes (single-plex and multiplex)
☑	Positive and Negative control probe
☑	Hot–Plate for pretreatment/ target retrieval step
☑	RNAscope reagent kit (Detection Kit, Pretreatment kits & Wash Buffer)
☑	HybEZ Hybridization system
☑	EZ Batch slide processing system or Tissue-Tek system
☑	Ecomount for 2.0 HD Red & 2-plex chromogenic assay
☑	RNAscope control slides
☑	Immedge hydrophobic barrier pen
☑	User supplied reagents (refer to user manual)
☑	Read the user manual (Part 1 – Sample Prep & Part2 – Detection Assay)

TIP : Visit www. acdbio.com/go for more information on getting started. Checklist is available on the website and in the manual

USING A HOT PLATE

Hotplate for retrieval/boiling

TIP : When using a hot plate for pre-treatment step – pay close attention to the TIME and boiling TEMPERATURE

RNASCOPE REAGENT KIT CONTENTS

OLD

NEW

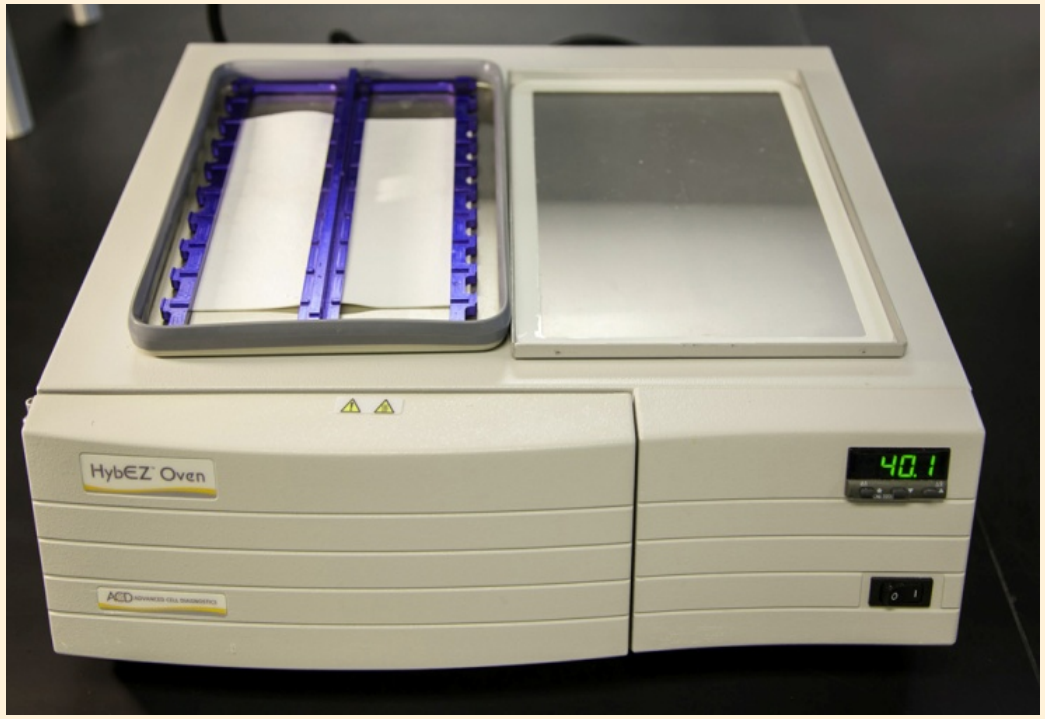
Contents of the reagent kit

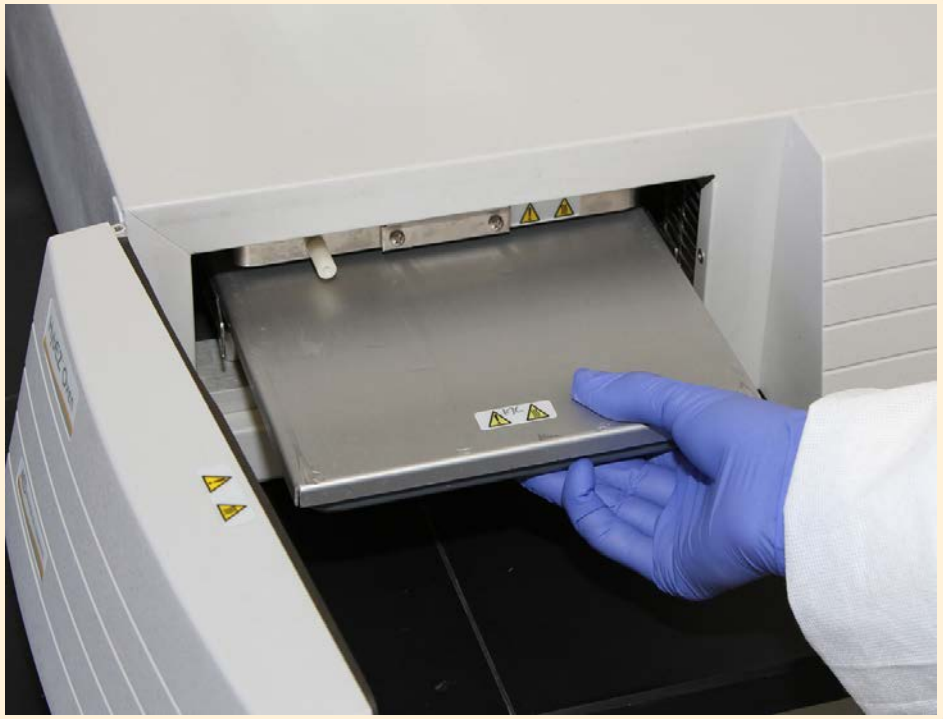
1. Pretreatment reagents
2. RNAscope detection kit
3. Wash buffer

TIP : Warm probes at 40 ºC for 10 minutes before use
TIP :Warm 50x wash buffer at 40 ºC for 20 minutes if you notice a precipitation

USING A HYBEZ HYBRIDIZATION OVEN

☑ *HyBEZ hybdrization system*



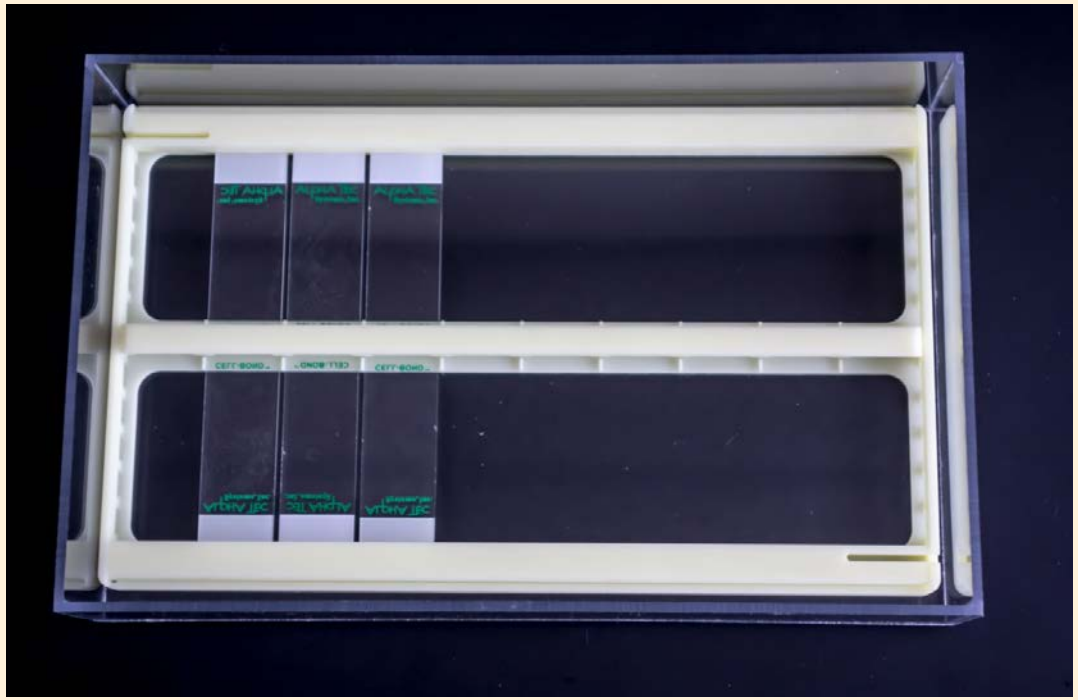


TIP: HybEZ oven is required as it provides both temperature and humidity control, necessary to obtain optimal RNAscope results

ACCESSORIES FOR WASHING STEPS

- ***Tissue Tek washing tray***

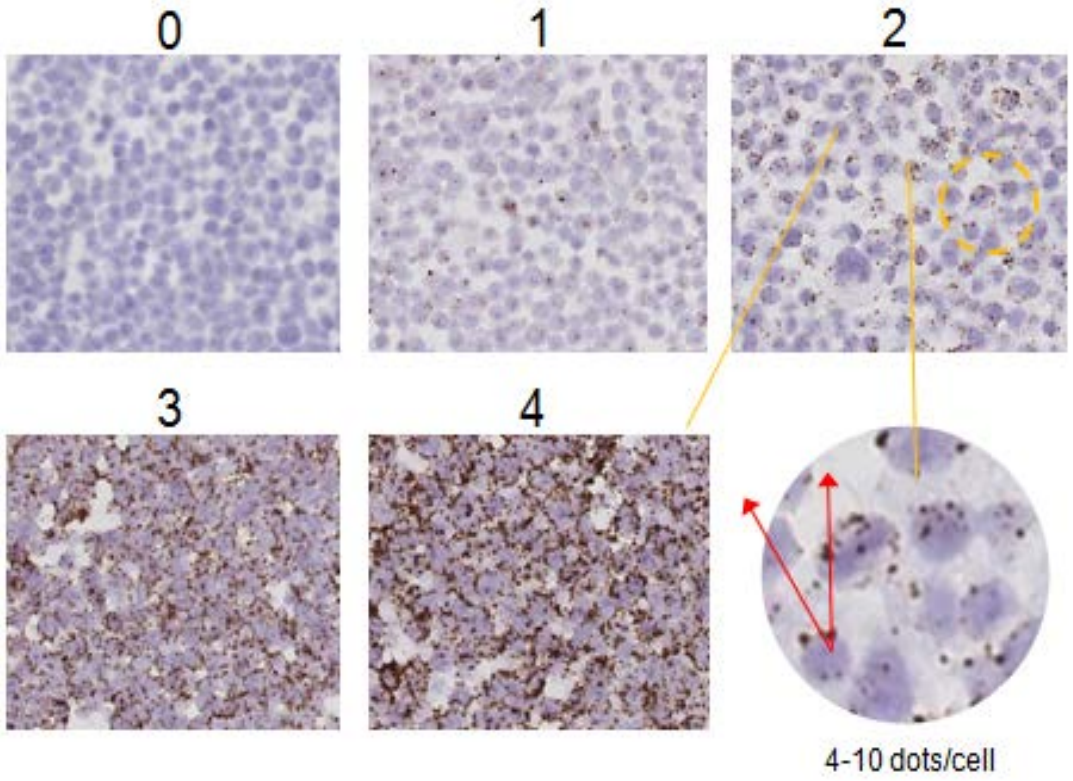
- ***EZ Batch for slide processing***



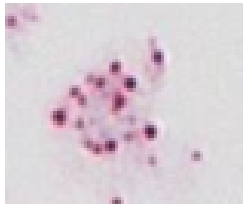
TIP : ACD EZ Batch slide processing tray is easy and convenient for loading multiple slides for washing steps.

QUALIFY YOUR SAMPLES USING CONTROLS

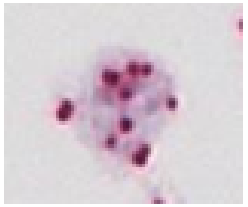
RNASCOPE® SEMI- QUANTITATIVE SCORING



Score	Criteria
0	No staining or <1 dot/ 10 cells*
1	1-3 dots/cell
2	4-9 dots/cell. None or very few dot clusters
3	10-15 dots/cell and <10% dots are in clusters
4	>15 dots/cell and >10% dots are in clusters

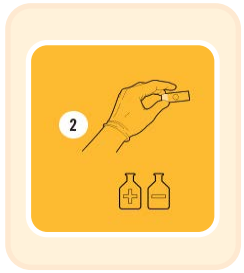


Score = 3



Score = 3

QUALIFY YOUR SAMPLES USING CONTROLS

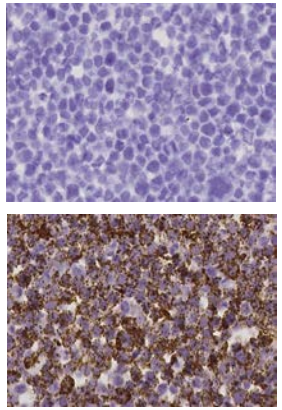


Control slides e.g. Hela

Negative control probe, e.g. DapB

Positive control probe, e.g. PPIB

QC check
PPIB > 2
DapB < 1

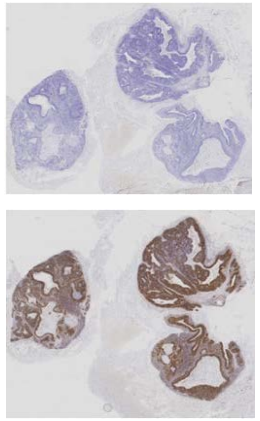


Sample

Negative control probe, e.g. DapB

Positive control probe, e.g. PPIB

QC check
PPIB > 2
DapB < 1



PASS

Run your target probes

FAIL

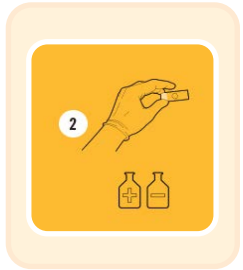
- *Verify technique*
- *Check RNA quality with new samples*
- *Perform Assay optimization*

Technique check

Sample/RNA quality check

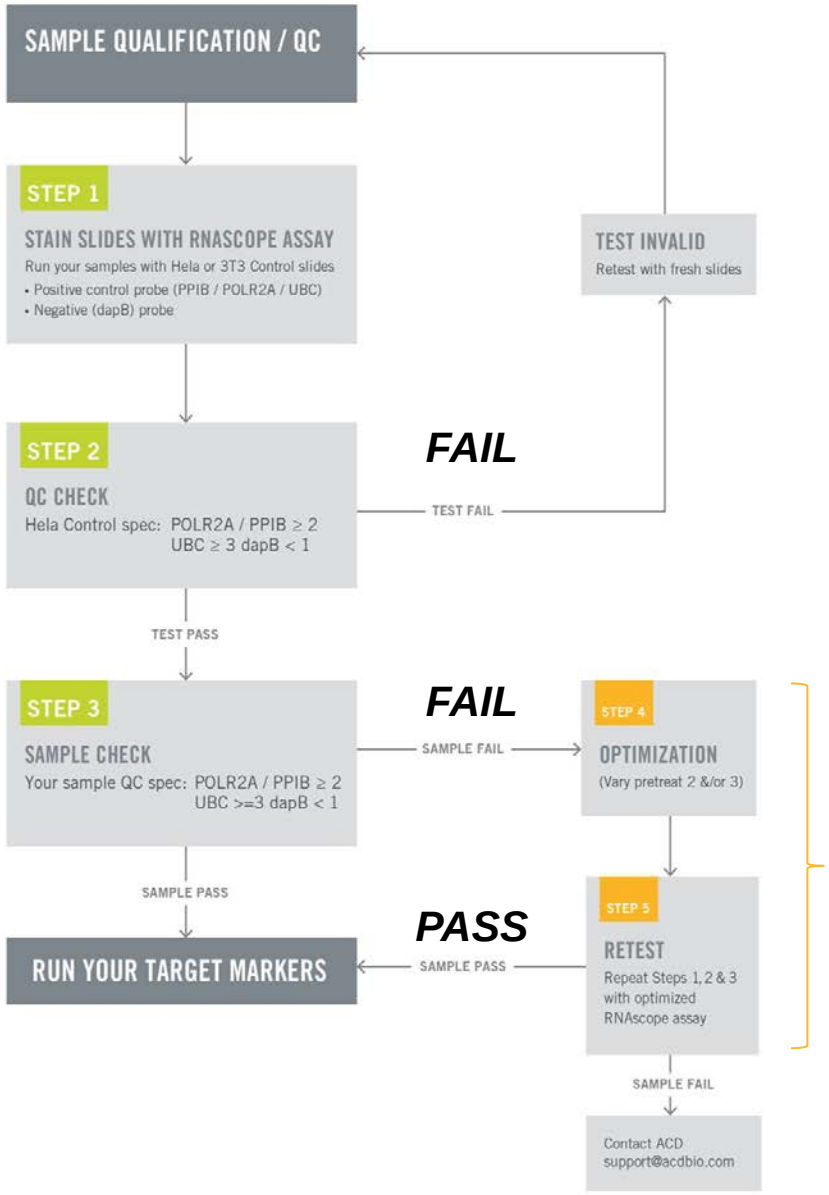
TIP : Always start with standard conditions

OPTIMIZE YOUR ASSAY



Technique check

**Sample/
RNA quality check**

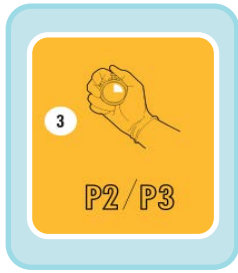


OPTIMIZE YOUR ASSAY

TIP : Refer to the Troubleshooting Guide

OPTIMIZE YOUR ASSAY

OPTIMIZE YOUR SAMPLE IN 3 EASY STEPS

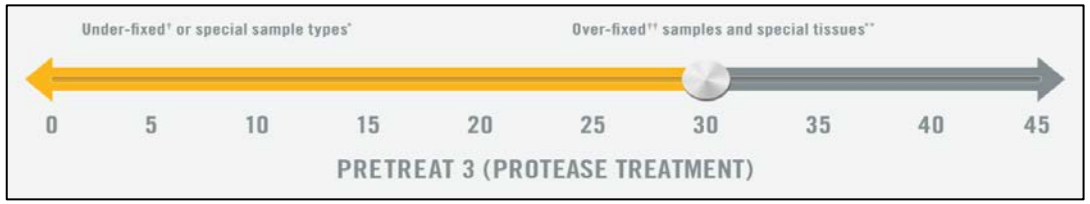


STEP 1
START WITH STANDARD CONDITIONS

Observe Staining Pattern -
High background, over-digested? = ***underfixed***
No signal/weak signal, under-digested? = ***overfixed***

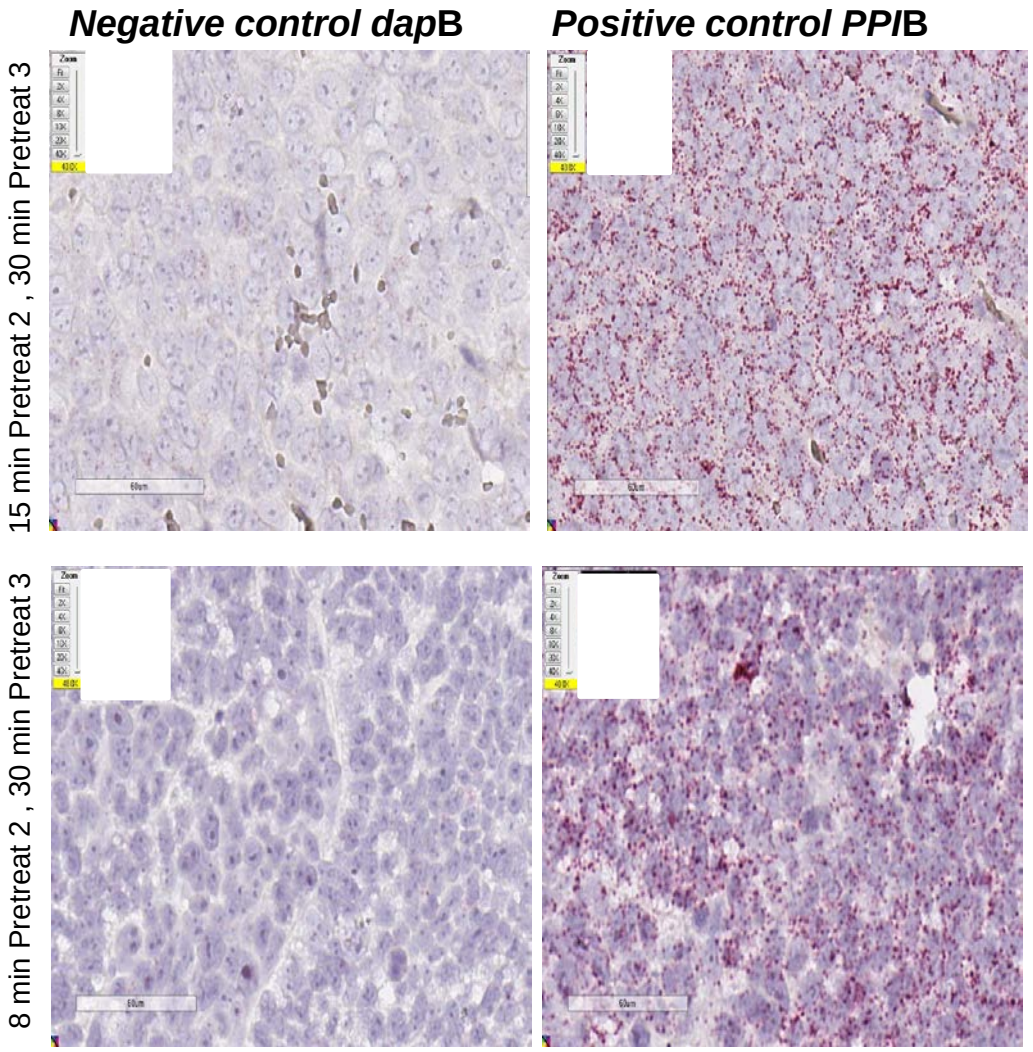
STEP 2
ADJUST PRETREATMENT 2, BOILING TIME

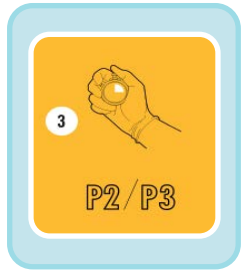
STEP 3
ADJUST PRETREATMENT 3/4, PROTEASE TIME*



TIP: For cultured cells, protease is diluted 1:15 in 1X PBS
* For fresh frozen samples, only protease pretreatment is required and is performed at room temperature

TROUBLESHOOTING: **OVERDIGESTION**





Sample: FFPE Xenograft

Assay: RNAscope 2.0 HD Red

Issue: Destroyed morphology, ghost nuclei, high nuclear background, weak hematoxylin staining

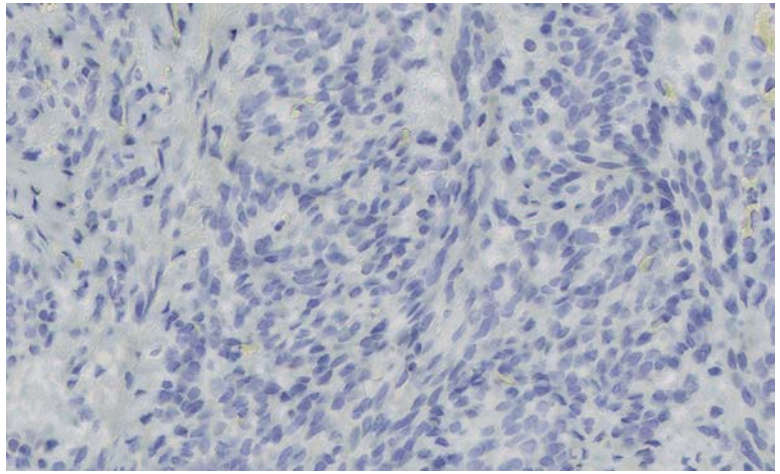
Optimization: Decrease pretreatment 2 conditions.

Result: Strong staining for positive controls with no/negligible background, intact morphology, strong hematoxylin staining

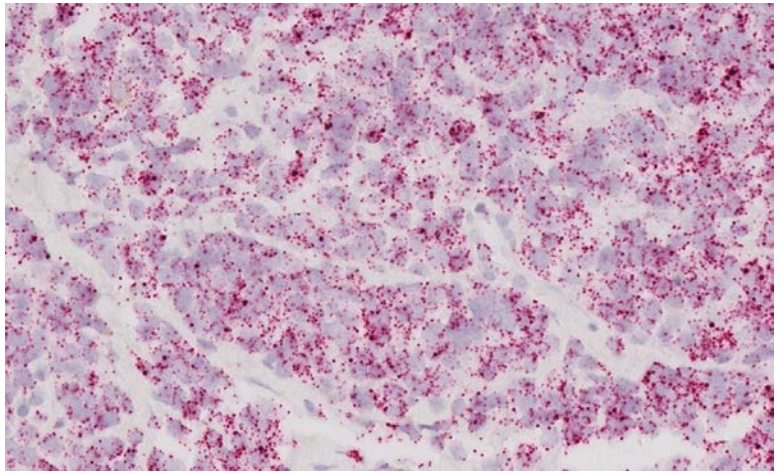
TIP: Visit http://www.acdbio.com/technical-support/downloads/rnascope-ish-guide-troubleshooting/

EXAMPLE OF SUCCESFUL RNASCOPE RESULTS

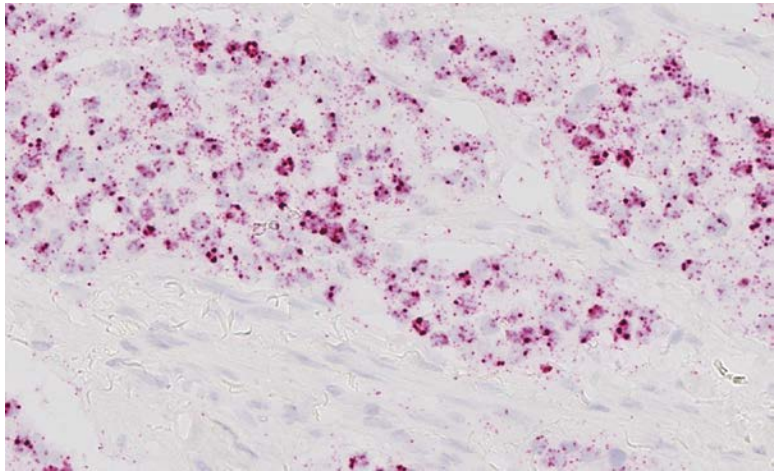
Negative control, DapB



Positive control, PPIB



Target probe



RNAscope 2.0 HD Red

Human breast cancer tissue

TROUBLESHOOTING TIPS

FACTORS AFFECTING RNASCOPE ASSAY PERFORMANCE

☒	Fixation conditions are not optimal
☒	RNA is degraded
☒	Hybridization conditions not optimal
☒	Samples drying during assay

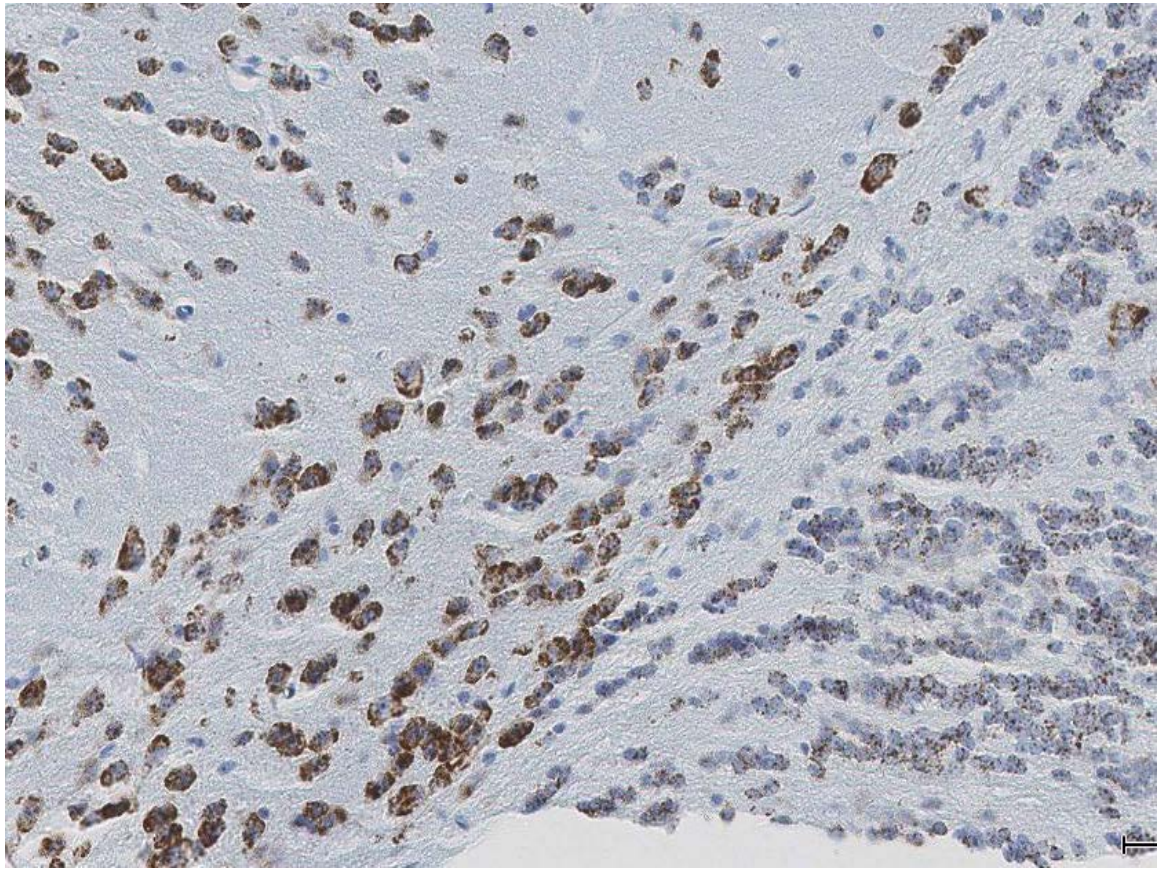
↓ THE SOLUTIONS

☑	Fix samples as recommended. E.g., for FFPE use 10% NBF RT, 16-32 hrs
☑	Acquire new samples and assess RNA quality
☑	Use the HybEZ hybridization oven only
☑	Use Immedge pen and add adequate reagents to avoid drying

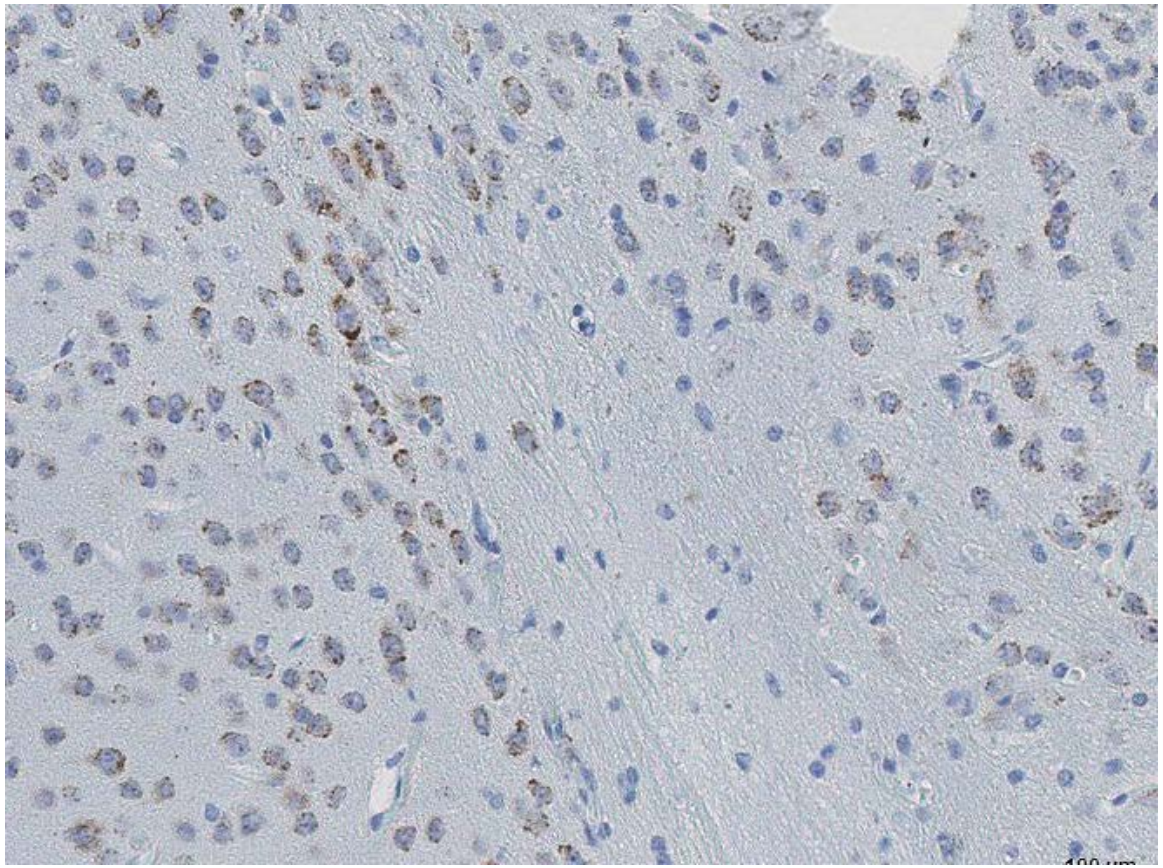
NBF: Neutral Buffered Formalin

IMPACT OF FIXATION CONDITIONS

24 hours fixation/**Optimal**



3 weeks fixation/**Over fixed**



Synaptophysin

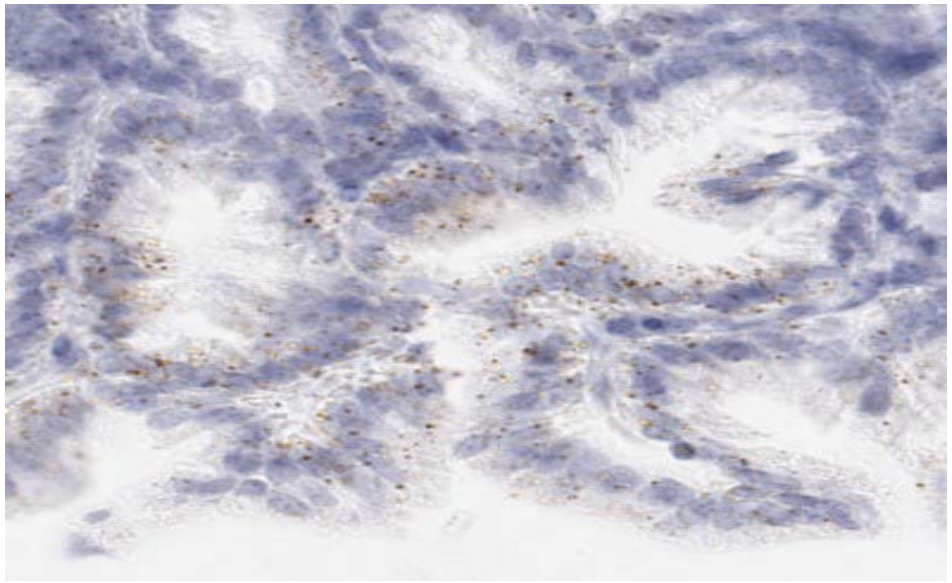
Sample: FFPE brain sample

Assay: RNAscope 2.0 Brown

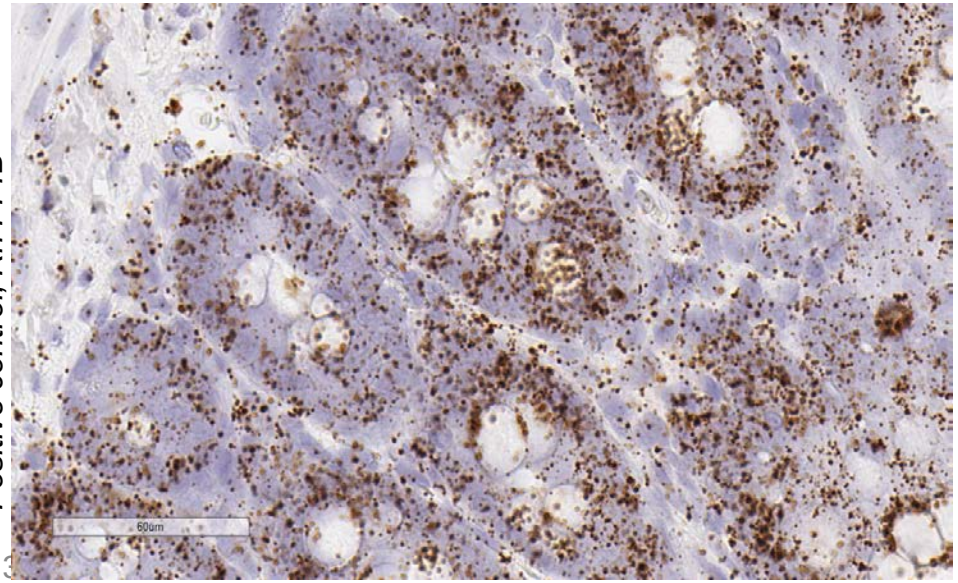
TIP: Sample fixation has a great effect on the success of your assay
Solution: Increase pretreatment for better target accessibility

TROUBLESHOOTING: **UNDER FIXATION**

Positive control, Rn PPIB



Positive control, Rn PPIB



Sample: Flash Frozen followed by FFPE sample preparation (fixation), Rat intestines

Assay: RNAscope 2.0 HD Brown

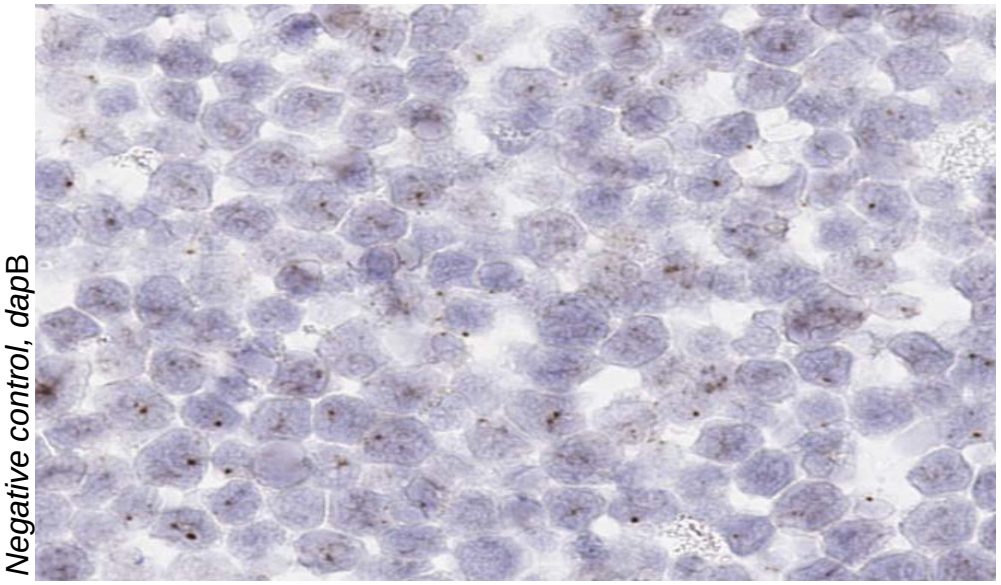
Issue: Weak staining, destroyed morphology, FFPE sample is under fixed

Optimization: Fixation according to recommended guidelines for FFPE samples

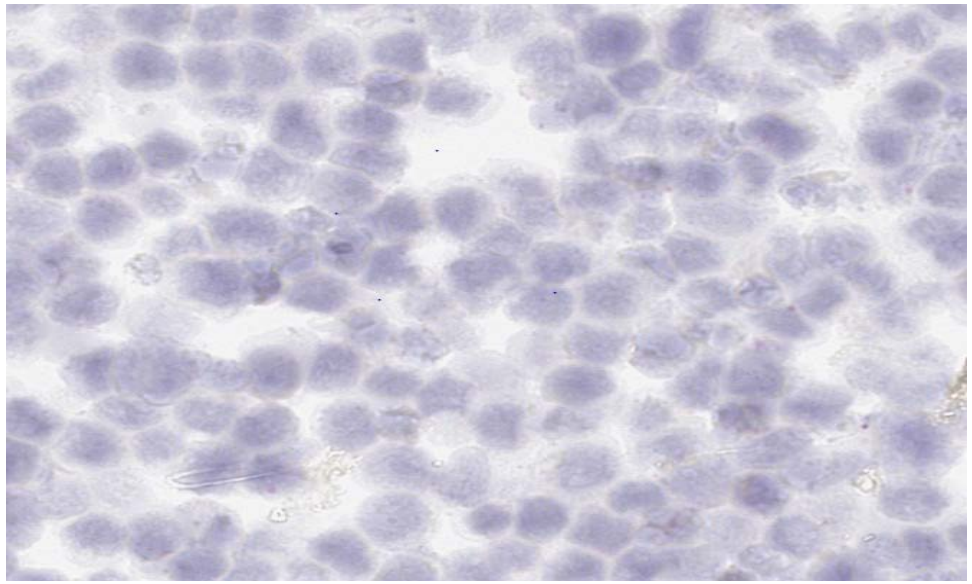
Result: Strong staining for positive control, PPIB, intact morphology

TIP : Refer to the Troubleshooting Guide
http://www.acdbio.com/technical-support/downloads/rnascope-ish-guide-troubleshooting/

TROUBLESHOOTING: **ASSAY WORKFLOW**



Negative control, dapB



Negative control, dapB

Sample: FFPE Hela pellet

Assay: RNAscope 2.0 HD Brown

Issue: Tissue dried out, high background

Optimization: Do not allow drying between amplification steps. Use the Immedge hydrophobic barrier pen

Result: Clean background

TIP: Refer to the Troubleshooting Guide; http://www.acdbio.com/technical-support/downloads/rnascope-ish-guide-troubleshooting/

REFER TO SAMPLE PRETREATMENT GUIDELINE

Tissue Pretreatment Guidelines

Follow the recommended pretreatment conditions based on your tissue type for:

- Any new or previously untested FFPE tissues
- Samples prepared suboptimally

Guidelines for Optimal Tissue Pretreatment

- ☐ Test representative samples with positive and negative control probes. [Controls should be: Positive = uniform signal; negative = blank].
- ☐ Fix sample in **FRESH** 10% **NBF** for **16–32 HOURS** at **ROOM TEMPERATURE**.

  NOTE: Do not fix at 4°C. DO NOT fix for < 16 hrs or >32 hrs. Refer to Table 1 for under/over-fixed tissue pretreatment guidelines.
- ☐ Vary **PRETREAT 2** and/or **PRETREAT 3 TIME** based on your tissue type (see Table 2).

  NOTE: Certain Xenografts and Cell Pellets, require very mild pretreatment (**PRETREAT 2** for 8 min, **PRETREAT 3** for 15 min).

Table 1. Tissue Pretreatment Guidelines

Reagent	Mild	Standard	Extended
Pretreat 2	15 min	15 min	30 min
Pretreat 3	15 min	30 min	30 min

Table 2. Tissue Pretreatment Table

Species	Tissue type	Pathology	Pretreat Condition
Mouse / Rat	Intestine	Normal	Standard
	Intestine	Tumor	Standard
	Embryo	Normal	Standard
	Brain	Normal	Standard
	Spleen	Normal	Mild
	Eye/Retina	Normal	Standard
	Liver	Normal	Extended
	Kidney	Normal	Standard
Human	Breast	Tumor	Standard
	Colon	Tumor	Standard
	Colon	Normal	Standard
	Lung	Tumor	Standard
	Lung	Normal	Standard
	Prostate	Tumor	Standard
	Prostate	Normal	Standard
	Lymph node	Tumor	Mild
	Lymph node	Normal	Mild
	Tonsil	Normal	Mild
	Pancreas	Normal	Standard
	Cervical	Cancer	Standard
Human	Cervical	Normal	Standard
	Cervical dysplasia	Abnormal	Standard
	Brain	Tumor	Standard
	Brain	Normal	Standard
	Head	Cancer	Standard
	Neck	Cancer	Standard
	Liver	Cancer	Standard
	Kidney	Normal	Standard
	Skin	Normal	Standard
	Melanoma	Tumor	Standard
	Nevus	Benign	Standard
	Placenta	Normal	Standard
	Skin (TMA*)	Normal	Standard
	Breast (TMA)	Normal	Standard
	Melanoma (TMA)	Normal	Standard
	Nevus (TMA)	Benign	Standard
	Stomach (TMA)	Normal	Standard
	Stomach (TMA)	Tumor	Standard
	Cell pellets**	—	Mild
	HeLa cells† (ACD control)	—	Standard

* Tissue Microarray
** Fixed with 10% NBF
† Fixed with 10% Formaldehyde/PBS

For information about species or tissue type not listed here, contact support at support@acdbio.com.

TIP : Refer to the user manual for tissue specific pretreatment guidelines

FREQUENTLY ASKED QUESTIONS

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- ## RNAscope assay compatibility with different tissues

  RNAscope manual assay can be used with FFPE, fresh-frozen, fixed-frozen and cultured cells. RNAscope automated assays are primarily supported with the FFPE tissue. Please refer to the User Manual Selection Guide: http://www.acdbio.com/technical-support/downloads

- ## Key differences between RNAscope ISH assay and IHC

  No cooling is required during Epitope retrieval, users should directly put the slides in water at room temperature, dehydrate and proceed to Pretreatment 3 step as per the manual Part 1

TIP: Visit www.acdbio.com/support for additional FAQs

GUIDELINES TO FOLLOW WITH RNASCOPE ASSAY

- ✓ Read the manual and perform RNAscope exactly
- ✓ Utilize optional stopping points
- ✓ Flicking/tapping the slides for adequate drying of slides
- ✓ Storage in desiccant (FFPE) for RNA integrity
- ✓ Always use fresh reagents
- ✓ Warm probes and wash buffer at 40°C, precipitation occurs during storage
- ✓ Remember to optimize pretreatment conditions, when you switch tissues

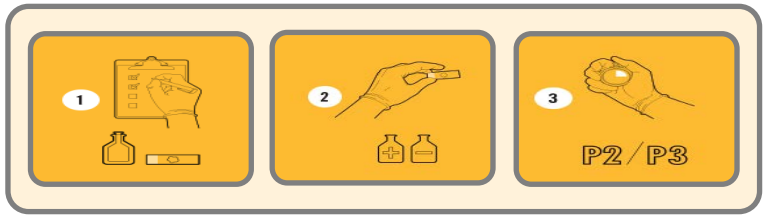
TIP: Refer to the Troubleshooting Guide
http://www.acdbio.com/technical-support/downloads/rnascope-ish-guide-troubleshooting/

SUMMARY

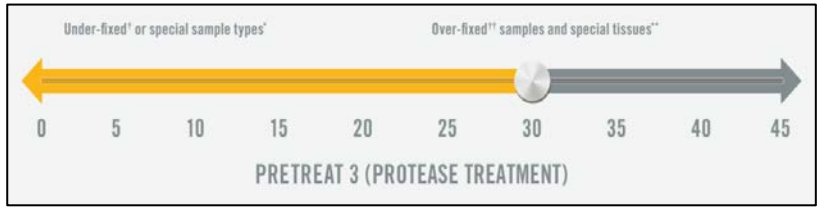
1. Reviewed RNAscope technology

   - ZZ probe design and the workflow for manual assays

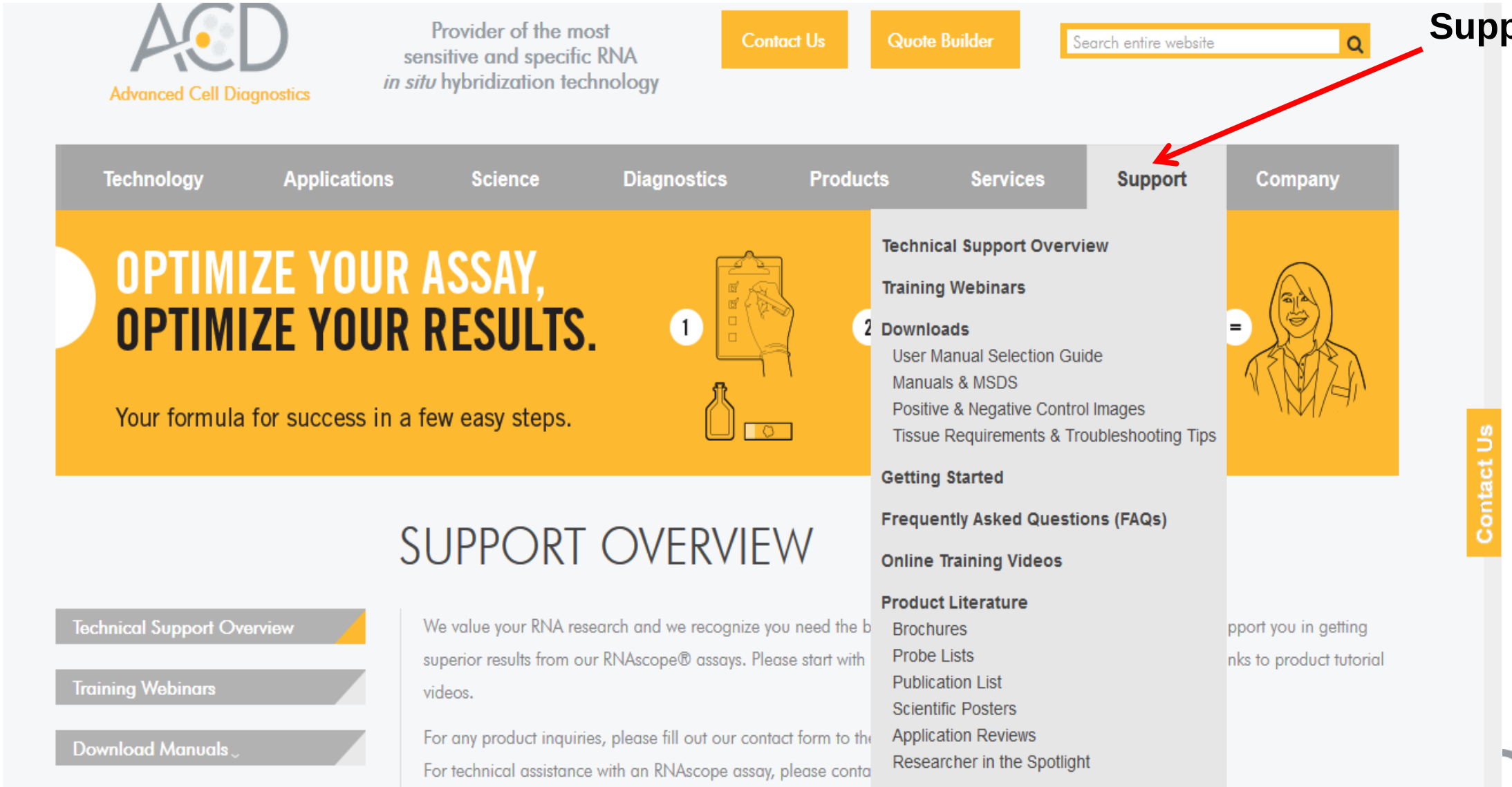
2. Tips for getting started with RNAscope in your lab
   - 3 easy steps to getting started in your lab



3. Tips for success with RNAscope assay, when you switch tissues
   - Pretreatment 2 and 3 optimization



VISIT THE SUPPORT PAGE TO LEARN MORE



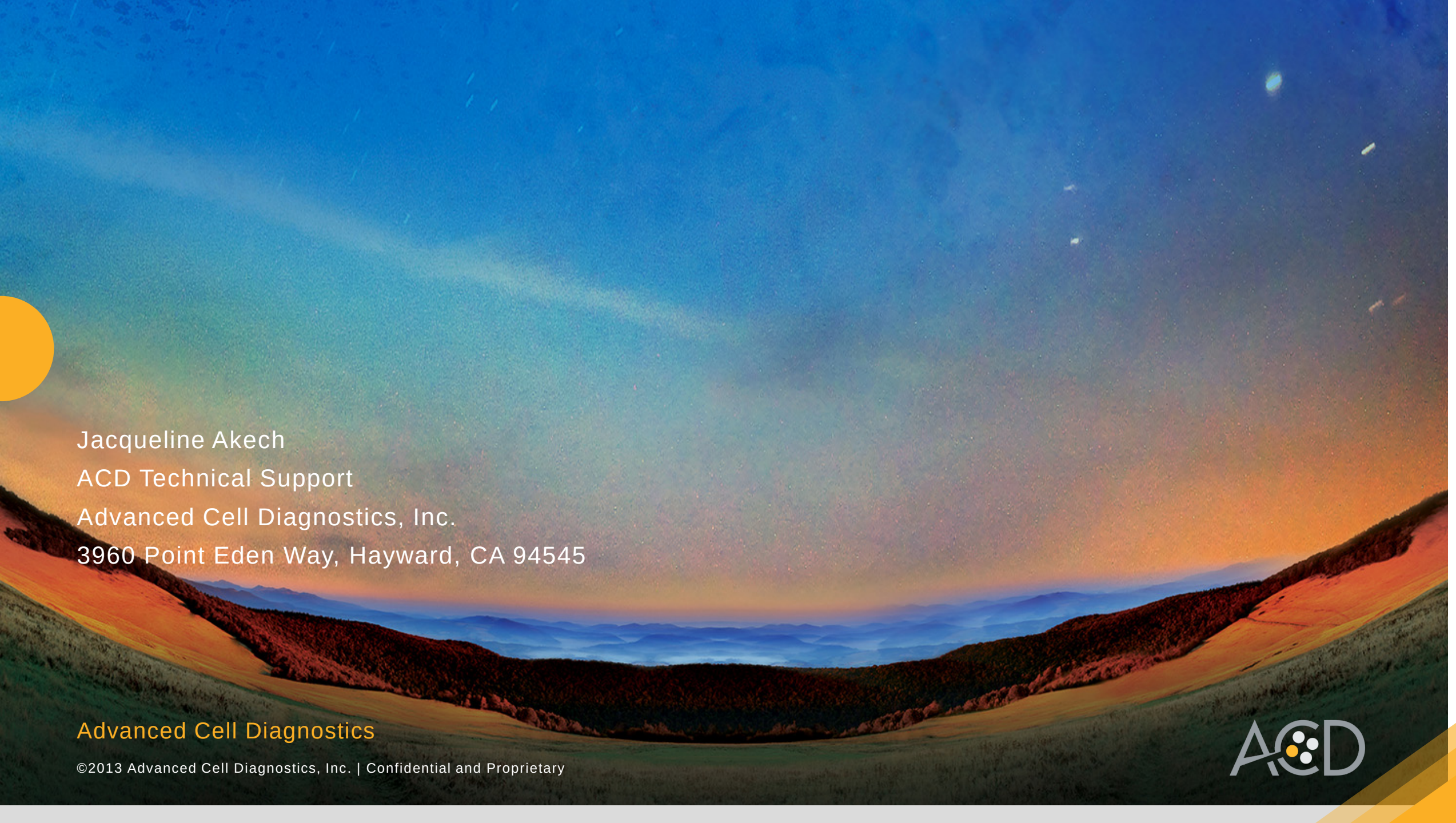
TIP: Visit www.acdbio.com/technical-support/support-overview

CONTACT ACD SUPPORT

- Support via email –support@acdbio.com
- Support via phone-1-877-376-3636, option 3
  - Time 8:00am-6:00pm PST
- Support Resources available on website www.acdbio.com

Manuals	Getting Started	FAQs	Videos	Product Literature
Download manuals, technical notes and MSDS.	Simple tips & tricks for you to get the best RNAscope result from day1.	Browse through our product frequently asked questions or add one of your own.	View our product and workflow videos on our Video page.	Find RNAscope publication lists, gene lists and download product brochures.
Go →	Go →	Go →	Go →	Go →

QUESTIONS?



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