	FISH PROTOCOL	ºC	Time	Machinery / tools
FIRST DAY - MORNING	*Go and find ice. Set incubator at 37ºC with a humid chamber (wet paper with 2xSSC) inside it. Make sure that there are enough plastic (i.e. parafilm) coverslips.*			
PREVIOUS (facultative)	Rinse slides with **EtOH 95%** 10 min, air-dry.	RT		Hellendhal jars
RNAse TREATMENT (100 µg/ml)	For 4 slides: **8 µl Rnase** (10 mg/ml) + **792 µl 2xSSC**. Vortex.	Ice		Vortex Pipette
	Incubate slides with **200 µl RNAse** with plastics (i.e. parafilm) in humid chamber.	37	1 h	Incubator Humid chamber Vortex Pipette
WASHING	In **2xSSC** with gentle agitation, remove plastic coverslips with forceps (or allow the to fall off).	RT	5 min x 3	3D-Shaker Hellendhal jars
Prior to pepsin TREATMENT	Treat slides with **HCl 0.01N.**	RT	2 min	Hellendhal jars Pipette
	Dilute the pepsin stock (10 mg/ml) 1:200 in 0.01N HCl.	Ice		Centrifuge Pipette
Pepsin TREATMENT (0.05 mg/ml)	Incubate slides with 100 **µl pepsin 0.05 mg/ml**, with plastic coverslips (in h. ch.)	37	15 min	Pipette
WASHING	Remove plastic coverslips. Wash with ultrapure water (sterilised).	RT	2 min	Hellendhal jars
	2xSSC	RT	5 min x 2	3D-Shaker Hellendhal jars
FIXATION **(facultative depending on the materials, but usually recommended)**	Fix slides in neutral **formaldehyde 3.7%.**	RT	10 min	Hood
DEHYDRATION	In ethanol series: **EtOH at 50%, 70% i 100%** (at -20ºC).	RT	2 min each	3D-Shaker Hellendhal jars
DRYING	Air drying.	RT	>1h 30min	

Everything in red is either optional or alternative.

	FISH PROTOCOL	ºC	Time	Machinery / utiles
FIRST DAY - AFTERNOON	*Go and find ice. Prepare baths, one at 75ºC and one at 55ºC.* *Preheat humid chamber at 37ºC.*			
PROBE MIXTURE PREPARATION	For 30 µl per slide mix (x4 slides): -formamide: 15 µl (60 µl) -50% dextran sulfate: 6 µl (24 µl) -20xSSC: 3 µl (12 µl) -**labelled rDNA probe 1** : 50 to 100 ng per slide -**labelled rDNA probe 2**: 50 to 100 ng per slide - water: up to 30 µl (up to 120 µl)	Ice		Eppendorfs Hood Pipette
PROBE MIXTURE DENATURATION	Denature probe mixture.	75	10 min	Bath 75
	Cool on ice bath, rapidly. Vortex and spin.	Ice		Ice Vortex Centrifuge
	Incubate probe mixture. Spin again.	Ice	5 min	Centrifuge
BEGINNING HYBRIDISATION PROCESS	Apply 30 µl per slide of the denatured probe per slide. Cover with plastic coverslips. Put slides into closable and preheated boxes (at 75ºC) with thin bottom (cleant previously with ethanol), and these in the bath.	75	10 min	Bath 75 Box for bath Pipette
CONTINUING HYBRIDISATION PROCESS	Transfer boxes CAREFULLY to the other bath (at 55ºC).	55	5 min	Bath 55 Boxes for bath
HYBRIDISATION PROCESS	Transfer slides CAREFULLY to a preheated (37ºC) humid chamber (wet paper with 2xSSC). Seal the chamber with parafilm.	37	>18 h	Incubator Humid chamber Parafilm

Everything in red is either optional or alternative.

	FISH PROTOCOL	ºC	Time	Machinery / tools
SECOND DAY - MORNING				
WASHING	**2xSSC**	42	5 min x 2	Bath with linear shaking Hellendhal jars
ASTRINGENT WASHING	**0.1xSSC**	42	5 min x 2	Bath with linear shaking Hellendhal jars
ALTERNATIVE ASTRINGENT WASHING	**50% formamide** in 1xSSC	42	10 min	Bath with linear shaking Hellendhal jars
WASHING	**2xSSC**	42 cool to 20	5 min x 2	Bath with linear shaking Hellendhal jars
WASHING	**2xSSC**	RT	5 min	Hellendhal jars
WASHING	**4xSSCT**	RT	7 min	Hellendhal jars
RINSE	Briefly rinse in **1xPBS** and dry	RT		Hellendhal jars
DRYING	Allow to dry briefly (not too weet, not too dry)	RT		
MOUNTING	Mounting in **Vectashield** (**12 µl** per slide). Allow Vectashield to expand (in the dark).	RT, dark	5-10 min	Big coverslips (24 x 40 mm) Pipette
STORAGE	In appropriate folders (in the dark).	4	for ever	Fridge Folders

Everything in red is either optional or alternative.