Online workshop
"In situ hybridization: principles and practice"

Questions & Answers of key topics by Dr. Murat Yaylaoglu

1. How stable is the Taq if re-thawed multiple times?

   dNTPs are more sensitive to thawing.

2. Is it possible to prepare a master mix and store it for longer until further use and then just add the primers later to the mix?

   Fundamentally yes, test it.

3. How do you know what is the expression level of your (unknown) gene - or are you trying to have a guess prior to assessing the cDNA amount to be used?

   Ncbi gene (expression)

4. Any specific requirements concerning the tube materials? low bonding, wall-thickness etc.?

   Match it to your equipment

5. If we do not have a PCR machine with no controllable temperatures across the block (Biometra or equivalent) what shall I Do?

   Use a mid point like 55C. Change other parameters like MgCL.

6. Should I use a positive control when I am doing the PCRs? What should be the + control?

   Negative control is critical. Your positive is the first run you manage to get to work, maybe Martin can share a positive control with you.

7. Should I be concerned with too much primer-dimers?

   Gradient should solve this problem but fundamentally column purification solves this problem.

8. What to do if on the gel there are more than 1 band- new primers or just cut the gel and proceed to gel purification … which Is better?

   It may be an isoform, but gel purification is a good solution, gradient PCR may solve this problem.

9. If on Temperature gradient PCR all the bands look the same what to do?

   That is a good result, column purify and use the band as a template if it's the right size.

10. Instead of a clear band I have on my gel smear bands. What this means and how to fix it?

    Your cDNA might be bad, if gradient PCR does not solve it, design new primers.

11. What is the shelf-life of the purification columns?

    More than what is written on the box unless you live in a moist country its forever. Solutions may go bad.

12. What about different Taq polymerase. What is your experience with polymerase kits and what is your experience with different types of Taq?

    Different Taqs can be an issue.

13. What are the other samples (except tissue slides) that can be used for RNA ISH? We are working with cell cultures from bone marrow, whole blood, fibroblasts and amniocytes so I am thinking of a way to combine this new method with the already known ones

    ISH and IHC and various other methods combine beautifully. You need to plan which to do first.
    You can do ISH on all cells.

14. How expensive is the procedure and where we can cut cost?

    Unfortunately, Tyramide amplified ISH is expensive, you can switch to radioactive ISH.

15. After the incubation with my RNA probe o/n my slides are bone dry? Why?

    You need to control moisture or decrease the hybridization time or add probe multiple times.