

NARG recommendations for Real Time PCR probe synthesis

It is much easier to perform the synthesis with either BHQ1-cpg or TAMRA-cpg synthesis columns. The BHQ1 synthesis and purification procedures can be the same as for your regular oligonucleotides. Nucleoside phosphoramidites, not PAC-protected, but either dG^{dmf} or dG^{ibu} may be used. The most important point is to pay attention to the 6-FAM reporter-coupling step. Use enough coupling time to assure maximum coupling efficiency for the reporter.

Synthesis using quencher cpg column:

- Dissolve 6-FAM and place bottle directly on instrument. If you have to transfer the solution and are worried about water vapor contaminating the solution, add molecular sieves.
- Prime lines
- Set to default 0.2 μ mol protocol and set so that last DMT does not automatically come off at the end of the synthesis. This is so you can make a visual assessment of the synthesis, by observing the DMT removal prior to FAM coupling, later.
- Start synthesis cycles of the oligo
- Observe initial acetonitrile (ACN) wash step. There may be some color coming from the CPG depending upon the type of column that you are using. This is normal.
- Observe initial 5'DMT removal from CPG. This should be bright orange.

FAM coupling (6-FAM at 100 μ M):

- Extend wait step to 10 minutes
- Start cycle and observe 5'DMT removal. This should be bright orange.
- Be sure that, after coupling of FAM, there is Oxidation and a final rinse of ACN.
- Note: When synthesis, labeling and washing are done, dry the column by passing Helium (Argon, Nitrogen) through it. When you stop the gas flow and the CPG moves inside the column like powder, it is dry. This makes the cleavage process much cleaner and more efficient.

Cleavage and Deprotection

- Use concentrated NH₄OH for BHQ-1 probes. BHQ1 probes can be cleaved and deprotected in ammonia identical to any regular oligonucleotide.
- Use "TAMRA Cocktail" only for TAMRA probes.

TAMRA Cocktail (there are other formulations for TAMRA Cocktail)

1 part tert-butylamine
1 part Methanol
2 parts H₂O

- Mix tert-butylamine with H₂O to dilute the reactive tert-butylamine.
- Add Methanol to this solution.

- It is exothermic, hence the diluting of the tert-butylamine, so don't be alarmed if you feel warmth emanating from the beaker!
- Place solution in a closed container, such as a glass scintillation vial, and put in refrigerator if you are not using right away. This solution lasts months.

Two procedures for Cleavage by Syringe

TWO SYRINGES:

- Use two 1 mL syringes that fit very snugly onto the column to avoid leakage or, worse, exploding onto you. If you do not have a very snug fit, get a clean razor blade and clip the tip of the syringe by about 2-3 mm.
- Bring up 1 mL of cleavage solution and place on one side of the column. Place the empty syringe on the opposite end.
- Gently push the solution through the column until the column is saturated from frit to frit.
- Let it sit for 10 minutes.
- Push 0.1-0.2 mL through every 10 minutes for BHQ and every 15 minutes for TAMRA.
- You can use more cocktail to get residual oligo off the walls of column and after emptying oligo into a plastic tube for deprotection, the syringe. Be careful to not contaminate cleavage solution by getting the syringe or column in contact with it.

ONE SYRINGE:

- Place a 1 mL syringe, filled with 1 mL of deprotection solution, on one end of the column, snugly.
- Place on top of plastic tube in which you will deprotect oligo, so that the other end of the column from the syringe end, hangs into the tube like the tip of a funnel.
- Tape column onto tube so that so there is a tiny opening to vent.
- Put the tube-column-syringe contraption in a tube rack in a fume-hood.
- Gently push the solution through the column until the column is saturated from frit to frit.
- Let it sit for 10 minutes.
- Push 0.1-0.2 mL through every 10 minutes for BHQ and every 15 minutes for TAMRA.
- You can use more cocktail to get residual oligo off the walls of column. There will be no need to rinse out the syringe to get residual oligo, because it was never in contact with the oligo.
- This method was developed to save materials, save time, avoid being shot in the face with deprotection cocktail, and is generally less messy.

Deprotection:

- Tightly cap the tubes. Make sure washer is seated in the lid. If it isn't, there will not be a tightseal and a risk of the gas from the volatile liquid blowing the lid off.
- Place on heat block set to 60°C

BHQ-1: 2-2.5 hours

TAMRA: Overnight (~16 hours)

- Remove from heat and place upright in ice bucket or freezer so that solution freezes. It is safe to remove the lids, now.
- Immediately place in unheated Savant-type dryer (centrifuge with vacuum). Be careful that no heat is applied, especially for TAMRA. It really doesn't speed the drying process very much to apply heat and it increases the chance of the oligo blasting out of the tube, because this is volatile liquid.
- Before freezing completely, the sample is cold enough to safely remove the lid and take out a small amount to dilute in water and run on Anion-exchange HPLC (and Reversed-phase HPLC, too, if you wish) for synthesis analysis.
- Once samples have been lyophilized, they are stable to send. Protect from light because they are photosensitive.