Attaching the SNAP tag ligand, benzylguanine (BG), to oligos

This protocol is for linking the benzylguanine NHS ester (SNAP tag ligand or BG-NHS) to an amine modified oligonucleotide. This chemical group will later be linked to motors with the SNAP tag to produce an oligo-linked motor for hybridization attachment with 12hb origami structures with complementary handles.

Protocol

1) Using a needle, add non-aqueous DMSO to the vial with the dry SNAP tag ligand BG-NHS to a final concentration of 20 mM (~207ul per 2mg bottle). Make sure everything is at room temperature when doing this to reduce condensation.
2) Resuspend amine-functionalized oligo in water to a final concentration of 2 mM.
   - Take a small sample (~1 ul) and dilute original stock of amino-oligo 1:3000 for gel sample ("before")
3) Mix and incubate at room temperature for 30 minutes:
   - 4 ul of 2 mM NH2-Oligo in aqueous soln
   - 8 ul of 200 mM HEPES pH 8.5.
   - 12 ul of 20 mM BG-NHS in DMSO
4) **Additional Notes:**
   - Due to the increased rate of degradation of BG-NHS due to hydrolysis, we typically use a full bottle (scaling a single reaction upon performing multiple different sequence reactions in parallel) the same day it is dissolved. If necessary it is stored for 4 months at -20C and discarded afterward.
   - Typically after a couple of minutes a white precipitate is observed in the reaction mix but this has not significantly affected the end product. The oligos can be submitted for UV spectroscopy to measure concentrations and assess yield throughout.
5) Dilute reacted oligo 1:500 for gel sample ("after")
   - A small gel shift indicate successful BG-oligo linkage (equivalent to ~1bp)
6) Run 5 ul of "before" and "after" oligos on a 20% TBE gel
   - gel should be run at 200V for 65 minutes
   - stain gel with SYBR gold in water for 30 minutes rocking at room temperature.
   - rinse gel in water for a few minutes rocking at room temperature
   - image gel using appropriate techniques for sybr gold: http://probes.invitrogen.com/media/pis/mp11494.pdf
   - Estimate or use ImageJ to quantify percentage of oligo that was successfully linked to the ligand
7) Store final stock at -20 C until purified

Purifying oligos with Bio-Rad Spin Columns from excess label

For Bio-Rad spin columns information see http://www.bio-rad.com/prd/en/US/LSR/SKU/732-6221/Micro-Bio-Spin-6-Columns and http://www.bio-rad.com/webroot/web/pdf/lsr/literature/LIT-507G.pdf). *Use 4 columns for each 24 ul of oligo. Do not load more than 30 ul onto the first column. This is done because each time the BG-oligo is spun through the column, some extra buffer elutes, leading to a final volume of ~75 ul.*

Protocol

1) Prepare 4 columns and remove excess packing buffer with the following protocol (this is steps 1-3 on the instruction sheet)
   - Invert column several times to resuspend gel buffer mixture.
   - Snap off the bottom tip and place in a 2mL collection tube. Remove cap and watch for column draining. If column does not train, push cap back on and remove again.
   - Centrifuge for 2 min at 1000g and 4C. Discard packaging buffer.
   - Exchange the column buffer with the desired buffer for the ultimate protein labeling by adding 500ul of buffer to gel, and centrifuging for 1 min at 1000g and 4C. Discard buffer and repeat 3X more.
2) To purify the BG-oligo from excess free BG-NHS, apply 24ul of the first sample to each column sequentially. Centrifuge for 1min at 1000g at 4C.
   - **DO NOT** use each column more than once
   - Use UV spectrophotometer to measure final TOTAL oligo concentration
3) The sample should go through 4 columns to remove excess BG-NHS.
4) Store at -20C.