PEGylation Protocol (include DST treatment)

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* This protocol based on the TJ Ha's Lab protocol. I added DSP treatment procedure.

A-1: Boil the slides in water in a microwave for 5-10 minutes
D-1: Take aminosilane out of a freezer. A couple of hrs at R/T in dark
B-1: Put coverslips in the reaction containers. Wash them with MilliQ water
B-2: Pour Acetone and sonicate for >20 mins
B-2: Take the container out. Dispose of acetone. Rinse with MilliQ water
B-2: Pour KOH and sonicate for >20 mins
A-2: Take out the boiled slides. Remove coverslips and epoxy completely by a razor blade
A-3: Scrub the slides with MeOH and tap water. Put them in the reaction container
A-4: Rinse the container with MilliQ water a few times. Pour 10% alconox and sonicate for 20mins
A-5: Take the container out and flush with tap water.
A-8: Rinse it with MilliQ water and fill it with acetone. Sonicate for 15 mins
A-9: Take the container out. Dispose of acetone.
A-8: Rinse it with MilliQ water and fill it with MilliQ water. Sonicate for 5 mins
A-9: Take the container out. Dispose of water. Rinse it with MilliQ water
A-8: Fill it with acetone. Sonicate for 15 mins
A-9: Take the container out. Dispose of acetone. Rinse and fill it with MilliQ water
A-10: Burn the slides thoroughly for half a min. Place them back in the reaction container fillled with water for rapid cooling.
B-3: Dispose of KOH and fill with MilliQ water
B-5: Burn with propane for ~1sec.(only for confocal microscopy experiment)
B-6: Put the coverslips back to the container, fill out with MeOH and sonicate for 10 min
A-11: Fill them with 1M KOH. Sonicate for 20 mins
A-12: Dispose of KOH and rins it with MiliQ water several times. Fill with MilliQ water
A-13: Burn the slides thoroughly for half a min. Place them back in the reaction container fillled with water for rapid cooling.
A-14: fill it with MeOH and sonicate for 15 min
let coverslips and slides sit in MeOH until aminosilanation. Dispose of MeOH right before aminosilanization.
D-2: Pour 100mL of MeOH into the reaction flask. Add 5mL of acetic acid
D-3: Add 1mL of aminopropylsilane and mix well
D-4: Pour the mixture in the containers (from part I) and incubate for 10 mins
D-5: For the next use, rinse and sonicate the used flask with MeOH and 1M KOH. Fill it with MilliQ.
D-6: For the next use, dehydrate aminopropyl silane in vacuo for > 15mins
E-1: Put water in the bottom of pipet tip boxes. Prepare centrifuger at 4degC.
E-2: Take PEG bottles from a freezer. Put them in dark at R/T
D-7: Sonicate the reaction containers for a min. Then incubate them for another 10 mins
E-3: Make a fresh buffer for PEGylation (100mM sodium bicarbonate : 10mL MilliQ water + 84mg sodium bicarbonate)
D-8: Seal the aminopropyl silane bottle under nitrogen. Keep in a freezer
D-9: Rinse coverslips with MeOH and MilliQ. Blow by nitrogen and put them in any clean boxes.
D-10: Do D-9 for slides. Put them in the prepared 'water-filled' boxes (from E-1)
D-11: Do D-5 for the reaction containers for the next use
E-4: Take out 1-2mg of biotin-PEG (for five slides). Put it in a 1.5mL eppendorf tube
E-5: Take out 40mg (for five slides) of mPEG (Laysan bio) and put it in the tube. (for 7 slides- 56mg)
E-6: For the next use, put PEG bottles in vacuo as D-6
E-7: Add 320uL of the buffer and mix gently by pipette. Spin it for 30sec at 10,000rpm at 4degC (for 7 slides, add 448ul)
E-8: Mix gentely (no bubbles). Drop 70uL of it on each slide
E-9: Place a coverslip very gently on the top of the slide (no bubbles)
E-10: Put the boxes in a dark and well-leveled place. Incubate for 2-3 hrs
E-11: After ~10mins, check and restore any misplaced coverslips
E-12: For the next use, treat PEG bottles as done in D-8. Keep in a desiccant bottle at -20C
C-0: (Optional) For futher suppression of nucleic acid adsorption, do C1~C7.
C-1: Take out DST bottle and put it in the dark at room temperature 30min before C-2
C-2: Make fresh 100mM sodium bicarbonate buffer (same as E-3)
C-3: Disassemble the pegylated slides and coverslips, rinse with plenty of MilliQ and dry completely by nitrogen.
C-4: Dissolve 3.4mg DST in 20ul DMSO and mix them well by pipette. (for five slides)
C-5: Add 330ul fresh 100mM sodium bicarbonate buffer. Mix them carefully w/ by pipette.
C-6: Do E8-E11, using the DST solution rather than the PEG solution and incubate for 1hr.
C-7: For the next use, treat DST bottles as done in D-6 and D-8. Keep in a desiccant bottle at 4C
E13: Disassemble them, rinse with plenty of MilliQ and dry completely by nitrogen.
E-14: Put the slides in corning tubes to avoid light. Store at -20degC