gel 1.)

lanes 1,2,5,7,8,11,13:

- For all lanes except Strand BF/C in lane 7 extract 100uL from each tube, label with lane number and strand ID, and add to 900uL 1xTE buffer. For lane 7 extract 93.7 uL and add to 843.3uL of 1xTE.
- after lanes 3,4,6,9,10,12 reactions done load all lanes together like the following
- extract 2.5uL from each tube and add to 10uL of TE buffer to make a 12.5uL loading solution.
- load 10uL from each solution with 2uL loading dye into respective lanes
- <u>for lanes 3,4,6,9,10,12 loading see below the tables.</u>

Lanes 3 and 4: making substrate A

- add 100uL of Amp0001 (strandA) and 100uL of Amp0002 (StrandB) to 300uL of TE Buffer. To make 500uL of a 0.1uM StrandA and 0.2uM StrandB solution.
- Heat to 85 degrees celcius for 5 min, cool to RT in 20 min. heating in PCR 200uL max.
- Take 100uL of the solution out into another tube.
- LANE 4: to one of the 100uL solutions add 20uL of 1.5 uM Amp0005 (helperA) and 5uL of TE buffer to make a 125 uL solution of 0.24uM HelperA. This should have substrate A, byproduct A and any excess helperAs
- LANE 3: add 62.5uL of TE buffer to control.This should have substrate A and excess strandBs.
- Both these **125uL solutions** should have 0.08uM StrandA and 0.16uM StrandB. I.e Around **0.08uM of Substrate A, 0.08uM of byproduct A and 0.16 excess helper**

Lanes 9 and 10: making substrate B

- add 62.5uL of amp0003 (StrandC) and 100uL of amp0004 (strandD) to 337.5uL of TE buffer. To make a 500 uL solution of 0.1uM StrandC and 0.2uM StrandD.
- heat 200uL of this to 85 degrees celcius for 5 min, cool to RT in 20 min
- Take 100uL of the solution out into another tube
- LANE 10: add 15 uL of 2.0uM amp0006 (helperB) and 10uL of TE buffer to one of the 100uL solutions and to make a 125uL solution of 0.24uM Helper B. This should have substrate b, byproduct B and any excess helperBs.
- LANE 9: add 62.5uL of TE buffer to control. This should have substrate B and any excess StrandDs.
- Both these solutions should have 0.08uM StrandC and 0.16uM StrandD with 125uL to work with.

For future reference make working stocks of the too helpers the same so we dont have to recalculate for both lanes. and also find the final concs for ampsubstrate strands first then calculate the right amount of

Table. Reaction solutions

	Strands present	amount of DNA/ pmol	Concentration / uM
Lane 3 (312.5uL)	amp0001	25	0.08
	amp0002	50	0.16
lane 4 (312.5uL)	amp0001	25	0.08
	amp0002	50	0.16
	amp0005	75	0.24

		amount of DNA/ pmol	Concentration / uM
Lane 9 (312.5uL)	amp0003	25	0.08
	amp0004	50	0.16
lane 10 (312.5uL)	amp0003	25	0.08
	amp0004	50	0.16
	amp0006	75	0.24

Loading gels for lanes 3,4 9,10:

- lane 3: add 2.08uL of lane 3 reaction solution to 10.4uL TE buffer to form a 12.48uL solution.
- extract 2.48uL of this solution and add 2uL loading buffer and load nto lane 3
- lane 4: add 2.08uL of lane 4 reaction solution to 10.4 TE buffer to form a 12.48uL solution
- extract 2.48uL of this solution and add 2uL loading buffer and load into lane 4
- repeat for lanes 9 and 10.

- we have used 10uM of mg2+ while xuan has used 12 **m**M, for gel 2 should we use 10mM instead or remain consistent?

Setup remaining gel 1

lanes 6,12: making byproducts alone: Perform in PCR tube. Grab a new rack: label PCR1.byproducts.1

- For byproduct A in lane 6: add 32uL of 1.5uM HA (amp0005) into PCR rack well 1. Add 16uL of Mg2+ TE buffer to this to make 48uL of 1.0uM HA.

				(after heating) 20uL			

1.0 HA only (and then 0.5 HA + 0.5 A2	0.05HA + 0.05A2 = 0.05uM byproduct A		1uM HB (and then 0.5HB 0.5B2)	0.05uM byproduct B			

table PCR1.byproducts.1

- to well 1add 48uL of 1uM A2 (amp0002) to make a 96uL solution of 0.5uM A2 and 0.5uM HA.
- Transfer 4.8uL of well 1 0.5uM A2 and HA into 43.2 uL of TE Mg2+ in well 2. to make a 48uL 0.05uM A2 HA solution

- For byproduct B in LANE 12: add 25uL of 2uM HB (amp0006) to 25uL of Mg2+ TE buffer. Makes 50uL of 1uM amp0006.
  - then add 50uL of 1uM B2 (amp0004) to make a 100uL of 0.5uM StrandD 0.5uM Helper B solution. i.e 0.5uM Byproduct B solution
  - Split into 2 tubes: heat 1 50uL solution leave other. LABEL PLS
  - After heating, to both extract 2uL of this solution and add to 18uL of Mg2+ TE.(10x dilution) Making a 20uL of 0.05uM Byproduct B ready to load.

<u>Setup gel 2</u>

labelling required:

- rearrange working grid to: new solutions to be made are indicated by *

	1	2	3	4	5	6	7	8	9
a	A1 j1 0.5uM	a.)	*						
b	A2 1.0uM		*						
c	B1 0.8uM	B1 0.8uM	*						
d	B2 1.0uM	b.)	*						
e	HA 1.5uM		*						
f	HB 2.0uM		*						
g	Trigger 2.0uM		*						
h	just a prank								

i	Mg 2+ TE 100mM	Mg2+ TE 10mM	*	Mg2+ TE 100uM	Mg2+ TE 10uM	Mg2+ TE 10uM	Mg2+ TE 10uM		

table working1

need to label PCR racks for gel1: bottom one with PCR1.1 top one with PCR 1.2.1 (secound seet of lanes first solution) one below with PCR1.2.2 (second set of lanes, second solution) You will need 2 PCR racks for lanes 1-15 in gel 2. PCR2.1 will hold for lanes 1-7, PCR2.2 will hold for lanes 8-15

Making OG dendrimer working solutions

So i dont have to redo all the calculations below, we're going to make the same conc as we had for j1 working concs see table working1. All should be made up to 1mL.

- A1/AF (amp0031): add 5uL of 100uM amp0031 into 995uL TE (non Mg2+). put into A3 of Working grida
- A2/AG (amp-0032): add 10uL of 100uM amp0032 into 990uL TE (non Mg2+). put into B3 of working grid.
- B1/BF (amp0033): add 8uL 100uM amp0033 into 992uL non Mg2+ TE.. into C3
- B2/BF (amp0034): 10uL of amp0034 into 990uL non Mg2+ TE. Put in D3
- HA(amp0035): add 15uL of 100uM amp0035 into 985uL no Mg2+ TE.Into E3.
- HB(amp0036): add 20uL of amp0036 into 980uL of non Mg2+ TE. Put in F3.
- trigger(ap0037): add 20uL of amp0037 into 980 non Mg2+ TE. Put into G3.

Lanes 1,2: Into PCR2.1.1- j1 design a.) b.) seperately.

- From PCR'd solutions of A4 remove 5uL and add to 5uL of TE Mg2+ and that should give 10uL of 0.04 uM Substrate A. Label with volume and conc ON SIDE PLS.
- Do the same for PCR solution B10: take out 5uL and add to 5uL of TE Mg2+ giving 10uL of 0.04 uM substrate A. label.
- add 2uL of loading bufferto both.
-

Lane 3,4: OG a.) b.) seperately Get another PCR rack label PCR2.1.2

- a.) add 100uL of 0.5uM amp0031 (AF) and 100uL of 1.0uM amp0032 (AG) to 300uL Mg2+ TE buffer.
    -
- b.) add 62.5uL of amp 0033 (BF) and 100uL of amp0034 (BG) to 337.5uL of Mg2+ TE. This makes 500uL solution of 0.1uM 0.2uM amp0033 amp0034 respectively
    - *<u>in 2 tubes, extract 200uL into each,</u>*

Table PCR2.1.2

- Heat rack 2.1.2 to 85 celcius for 5 minutes, cool to 25 in 20 minutes.
    - a.)

        - Transfer this to new eppendorf tube and add 40uL of 1.5uM amp0035 (HA) and 10uL Mg2+ TE. This makes a 250uL solution of 0.08 to 0.16 to 0.24 uM solution. Label as a.) heat HA
    - b.)
        - transfer both 200uL into an eppendorf = 400uL of 0.1uM substrate B. Then add 60uL of 2.0uM ampm0036 (HB) and 40uL of Mg2+ TE to make 500uL of 0.08uM substrate B 0.08 byproduct B and 0.16uM HB. Label as b.) heat HB
    - LANE3: add 2.5uL of a.) heat HA, into 7.5uL of Mg2+ TE. Forms a 10uL of 0.02uM Substrate A 0.04uM HA into TUBE3 of PCR2.1.1.
    - LANE4: add 2.5uL of b.) heat HB, into 7.5uL of Mg2+ TE in TUBE4 of PCR2.1.1.

1	2	3	4	5	6	7	
Substrate A j1 @ 0.04	SubstrateB j1 @0.04	SubstrateA OG @0.02	SubstrateB OG @0.02	9uL a+b j1			

table PCR2.1.1

Lane 5,8,10,12,14: Substrates A+B j1 together solution a+b.)

- Grab a PCR rack label PCR2.a+b.1
- add volumes in a 1 to 2 ratio. 32uL of a4.) to 64uL of b10.) This should make a **96uL solution of 0.026uM Substrate A, 0.053 Substrate B, 0.053 Helper A, 0.106 Helper B (nb: 3x dilution)**
- Split into 2 PCR tubes: i.e extract 48uL into another PCR tube. 1 tube for a+b.) 20min delay another for a a+b.)7 day delay LABEL PLS.
- Grab another PCR rack label PCR2.a+b.2

8	9	10	11	12	13	14	15
9uL a+b j1	-	9uL a+b j1	-	9uL a+b j1	-	9uL a+b j1	-

table PCR2.2.1

- to a+b.) 20min delay: Split into 5 tubes: for lanes 5,8,10,12,14 respectively. lanes 8 10 12 and 14 should be in **PCR2.2.1** and lane 5 in the other with lanes 1-7 **into PCR2.1.1**. Extract 9uL of solution and add to each tube. Each tube should have 9uL of **0.026uM Substrate A, 0.053 Substrate B, 0.053 Helper A, 0.106 Helper B (nb: 3x dilution). Leave spaces for lanes 9,11,13 and 15. (do friday)**

LANES 6,9,11,13,15: a+b.) OG:

- to prepare working stock of a+b.) OG: add 240uL a.)heatHA to 480uL b.)heatHB in a new eppendorf tube. Makes a 3x dilution to 720uL of 0.026uM SubA , 0.053uM subB, 0.053uM HA and 0.107uM HB.
- LANE6: into PCR2.1.1 tube 6, add 2.5uL of a+b.) OG to2.5uL of mg2+ TE. (2x dilution in 5uL)

- LANE9,11,13,15: do the same to all making 5uL.

Now we can line up PCR2 with the triggers for each!

Lanes 7,8,10,12,14: preparing the j1 trigger. Make enough volume here to last.

- Get another PCR rack label PCR2.2.2 (second gel, second set of lanes, solution set 2), label across the bottom j1 triggers
- ACTUALLY DONT DO THE TABLES. extra pipette...up to u...

8	9	10	11	12	13	14	15
1x	-	0.5x	-	0.1x	-	0.01x	-

- table PCR2.2.2
- In eppendorf tubes: you will need 5 eppendorfs
    - make 20x dilution of 2uM trigger from working solution by taking 5uL of trigger uM adding it to 95uM Mg2+ TE buffer to make a 100uL solution of 0.1uM trigger. label
    - make a 3.84x dilution (13:37 dilution) by taking 39uL of trigger and adding it to 111uL of TE MG2+ buffer to make a 150uL solution of 0.026uM trigger **(1x trigger j1)**
- make a 2x dilution by taking 50uL of 0.026 uM trigger and add it to 50uL of Mg2+ TE buffer to make a 100uL 0.013 uM triger **(0.5x trigger j1)**
    - Make a 5x dilution by taking 20uL of 0.013uM trigger and adding it to 80uL of Mg2+ TE buffer. this makes a 100uL solution of 0.0026uM trigger **(0.1x trigger j1)**
    - 10x dilution. Take 5uL of 0.0026 uM trigger and add it to 45uL of Mg2+ TE buffer. this makes a 50uL solution of 0.00026 uM trigger solution **(0.01x trigger j1)**
    - add 10uL of trigger into PCR2.2.2 then load 9uL of that trigger into correct wells of PCR2.2.1

LANES9,11,13,15: Prepare the OG trigger. make enough volume here to last. Label rack as OG triggers.

- will need 5 eppendorf tubes.
    - make 20x dilution of 2uM trigger from working solution by taking 5uL of trigger uM adding it to 95uM Mg2+ TE buffer to make a 100uL solution of 0.1uM trigger. label
    - make a 3.84x dilution (13:37 dilution) by taking 39uL of trigger and adding it to 111uL of TE MG2+ buffer to make a 150uL solution of 0.026uM trigger **(1x trigger j1)**
- make a 2x dilution by taking 50uL of 0.026 uM trigger and add it to 50uL of Mg2+ TE buffer to make a 100uL 0.013 uM triger **(0.5x trigger j1)**
    - Make a 5x dilution by taking 20uL of 0.013uM trigger and adding it to 80uL of Mg2+ TE buffer. this makes a 100uL solution of 0.0026uM trigger **(0.1x trigger j1)**

    - 10x dilution. Take 5uL of 0.0026 uM trigger and add it to 45uL of Mg2+ TE buffer. this makes a 50uL solution of 0.00026 uM trigger solution **(0.01x trigger j1)**
- add 5uL of the correct of conc of trigger into wells of PCR2.2.1 when ready.

For friday:

- have to make new a.) + b.) solutions for 20 minute runs.
- a.) add 100uL of Amp0001 (strandA) and 100uL of Amp0002 (StrandB) to 300uL of TE Buffer. To make 500uL of a 0.1uM StrandA and 0.2uM StrandB solution.
- b.) add 62.5uL of amp0003 (StrandC) and 100uL of amp0004 (strandD) to 337.5uL of TE buffer. To make a 500 uL solution of 0.1uM StrandC and 0.2uM StrandD.
- PCR 200uL of each solution.
- add right amount of TE and helper
-

PAGE PURIFY? to see if adding helper here increases yield of substrate A? (and same for substrate B?)

<u>Setup gel 3</u>

labelling required:
need to label PCR racks for gel1: bottom one with PCR1.1 top one with PCR 1.2.1 (secound seet of lanes first solution) one below with PCR1.2.2 (second set of lanes, second solution) You will need 2 PCR racks for lanes 1-15 in gel 2. PCR2.1 will hold for lanes 1-7, PCR2.2 will hold for lanes 8-15

Lanes 1,2: Into PCR2.1.1

- From PCR'd solutions of A4 remove 5uL and add to 5uL of TE Mg2+ and that should give 10uL of 0.04 uM Substrate A. Label with volume and conc ON SIDE PLS.
- Do the same for PCR solution B10: take out 5uL and add to 5uL of TE Mg2+ giving 10uL of 0.04 uM substrate A. label.
- add 2uL of loading bufferto both. (leave this for 15/09)
-

Lane 3,4:

- do friday

1	2	3	4	5	6	7	
Substrate A @ 0.04	SubstrateB @0.04			9uL a+b			

table PCR2.1.1

Lane 5,8,10,12,14: Substrates A+B together solution a+b.)

- Grab a PCR rack label PCR2.a+b.1

- add volumes in a 1 to 2 ratio. 32uL of a4.) to 64uL of b10.) This should make a **96uL solution of 0.026uM Substrate A, 0.053 Substrate B, 0.053 Helper A, 0.106 Helper B (nb: 3x dilution)**
- Split into 2 PCR tubes: i.e extract 48uL into another PCR tube. 1 tube for a+b.) 1 day delay another for a a+b.)7 day delay LABEL PLS.

Remake j1 i1 and OG stocks:
For each:

- a.) 10uL AF to 15uL AG stocks (100uM) and 975uL Mg2+ TE to make 1000uL 1uM AF and 1.5uM AG. Heat everything in PCR tubes 20min 85 degrees and then cool.
    - Split into 200uL tube and 800uL tube: a.) and a.) + HA respectively.
        - to 800uL a.) + HA add 20uL of amp00X5 (HA) and 180uL Mg2+ TE. This makes 1000uL 0.8uM AF, 1.2uM AG and 2.0uM HA. (0.8 ComplexA, 0.4 Byproduct A and 1.6HA)
- b.) add 20uL BF to 30uL BG stocks to 1950uL Mg2+ TE to make 2000uL 1uM BF and 1.5uM BG. Heat everything in pCR tubes 20min 85 degrees and then cool.
    - Split into 400uL Tube and 1600uL tube: b.) and b.) + HB respectively.
        - to 1600uL tube add 40uL of amp00X6 and add 360uL Mg2+ TE to make 2000uL 0.8 BF, 1.2 BG and 2.0 HB.
- Assuming everything reacts: 0.8uM complexA/B, 0.4ByproductA/B, 1.6uM HA/HB.
- Incubate after adding HA/HB for 20 min.

Make a+b.)

- a + b.) 60uL of a.) + HA to 120uL b.) + HB. This makes 0.26uM A, 0.53uM B, 0.13 byproduct A, 0.26uM Byproduct B, 0.53uM HA and 1.06uM HB. Use 0.53uM as a reference point.
    - so around 10x dilution for loading solution.

Remake control strands:

- make 1uM solutions 1000uL solutions of everything:
    - add 10uL of that stock to 990uL Mg2+ TE buffer. Label with strand.

Gel 4

Lanes					
AF					
AG					
AF+AG					
AF+AG+HA					
BF					

BG					
BF+BG					
BF+BG+HB					
AF					
AG					
AF+AG					
AF+AG+HA					
BF					
BF+BG					
BF+BG+HB					

Gel 5

Gel 3 and 4 messed up :(try same thing but with OG sequences.

Lanes	Substrates	Predicted Structures			
1	Ladder	25 bp ladder			
2	AF	AF			
3	messup	messup			
4	AG	AG			
5	HA	HA			
6	AF+AG	Substrate A + excess AG			
7	AG+HA	byproduct A + excess HA			
8	AF+AG+HA	Substrate A + Byproduct A+ excess HA.			
9	BF	BF			

10	BG	BG			
11	HB	HB			
12	BF+BG	Substrate B+ excess BG			
13	BG+HB	Byproduct B + excess HB			
14	BF+BG+HB	Substrate B + Byproduct B			
15					

Re-prepare stocks (again)-make 100uL of 2uM solution for AF AG HA BF BG HB

- fill 2 of 6 PCR tubes with 98uL of Mg2+ TE buffer. tubes 2,3 and tube 5,6 get 19.6uL of Mg2+TE buffer only.
- Add 2uL of 100uM solution of AF into tube 1, only 0.4uL of AG into tube 2 and 0.4uL of 100uM HA into tube 3, repeat for B. label PCR stocks 2.0uM.
- mix well

Get two PCR tube strips and label as FINAL 1-15
Lanes 2-4:

- add 9.75uL Mg2+ TE buffer to tubes 2-4
    - add 0.25uL 2.0uM AF into 2, 0.25uL AG into 3 and 0.25uL HA into 4
- mix well

Lanes 8-10:

- add 9.75uL Mg2+ TE buffer to tubes 8-10
    - add 0.25uL 2.0uM AF into 2, 0.25uL AG into 3 and 0.25uL HA into 4
- mix well.

Prep reactions for lanes 5-7 and 11-13:

- **IN TUBE 1**: Add 50uL of 2uM AF, 28.5uL of Mg2+ TE and 1.5uL of 100uM AG (well D1)to make 80uL of 1.25uM AF, 1.875uM AG solution.
- **IN TUBE 3**: add 15uL 2.0uM AG, 4.6uL Mg2+TE and 0.4uL of HA to make 20uL of 1.5uM AG, 2.0uM HA solution.
- **IN TUBE 4**: Add 50uL of 2uM BF, 1.5uL of 100uM BG (well D1) and 28.5uL of Mg2+ TE to make 80uL of 1.25uM BF, 1.875uM BG solution.
- **IN TUBE 6**: add 15uL 2.0uM BG, 4.6uL Mg2+TE and 0.4uL of HB to make 20uL of 1.5uM AG, 2.0uM HB solution.
- label on both tubes and rack.

- Heat to 85 degrees celcius for 5 min, cool to 25 in 20 minutes.

- **IN TUBE 2**: then extract 16uL of TUBE 1, add 4uL Mg2+ TE to make 20uL of 1uM AF and 1.5uM AG. (or 1uM Substrate A and 0.5uM excess AG.)
- in the remaining 64uL **of TUBE 1**: add 14.4uL Mg2+ TE and 1.6uL 100uM HA (well D4) to restore 80uL of 1uM AF, 1.5uM AG, and 2.0uM HA.
- **IN TUBE 5**: extract 16uL of TUBE 4, add 4uL Mg2+ TE to make 20uL of 1uM BF and 1.5uM BG. (or 1uM Substrate B and 0.5uM excess BG.)
- in the remaining 64uL **of TUBE 4**: add 14.4uL Mg2+ TE and 1.6uL 100uM HB (well D5) to restore 80uL of 1uM BF, 1.5uM BG, and 2.0uM HB.

- leave to incubate for 20 minutes. time. label.

AF+AG+HA (80)	AF+AG (20)	AG+HA (80)	BF+BG+HB	BF+BG	BG+HB
Substrate A 0.63uM Byproduct A 0.5uM Helper A **1.1uM** (weird complex AF-AG-HA thingymjig) 0.36uM	Substrate A **1 uM** Excess AG 0.499uM sneaky AG dimer 0.0061uM	Byproduct A **1.5uM** Excess HA 0.5uM	Substrate B **1uM** Byproduct B 0.5uM Helper B 0.5uM	Substrate B **1uM** Excess BG 0.5uM	Byproduct B **1.5uM** Excess Helper B 0.5uM
1	2	3	4	5	6

Table. with nupack predicted concentrations @26 degrees, max complex size set to 3.

After 20 mins: in new PCR strip below the above
- to new TUBE 1: add 21uL MG2+ TE
- to new TUBE 2: add 19uL Mg2+ TE
- to new TUBE 3: add 29uL Mg2+ TE
- to new TUBE 4: add 19uL Mg2+ TE
- to new TUBE 5: add 19uL Mg2+ TE
- to new TUBE 6: add 29uL mg2+ TE

- to new TUBE 1: add 1uL old tube 1 (substrateA+HA)
- repeat for all.

AF+AG+HA (22uL)	AF+AG (20)	AG+HA (30)	BF+BG+HB (20uL)	BF+BG (20uL)	BG+HB (30uL)
Substrate A 0.03uM	Substrate A **0.05 uM**	Byproduct A **0.05 uM**	Substrate B **0.05 uM**	Substrate B **0.05 uM**	Byproduct B **0.05 uM**

Byproduct A 0.03uM Helper A **0.05uM** (weird complex AF-AG-HA thingymjig) 0.02uM	Excess AG 0.025uM sneaky AG dimer ~0.00 uM	Excess HA 0.016uM	Byproduct B 0.025 uM Helper B 0.025 uM	Excess BG 0.025 uM	Excess Helper B 0.016 uM
1	2	3	4	5	6

Table. reactions diluted to PAGE runnable solutions.

Lanes 5-7 and 11-13:

- 10uL of TUBE1 into lane 7 tube.
- 10uL of TUBE2 into lane 5 tube.
- 10uL of TUBE3 into lane 6 tube.

- 10uL of TUBE4 into lane 13 tube.
- 10uL of TUBE5 into lane 11 tube.
- 10uL of TUBE6 into lane 12 tube.

Load 2uL loading buffer into all FINAL tubes. Pipette 10uL into Gel 5. GLHF.

Gel 6 - OG

lane #	Lane code	structures	length (bp)	Expected banding	purpose	Future uses
1	Ladder	25 bp ladder				
2	AF	AF (0.04)				
3	AF	AF (0.06)				
4	AF	AF (0.08)				
5	AG	AG (0.06)				
6	HA	HA (0.06)				
7	AF+AG	Substrate A + excess AG				
8	AG+HA	byproduct A + excess HA				
9	AF+AG+HA	Substrate A +				

		Byproduct A+ excess HA.				
10	BF	BF (0.06)				
11	BG	BG (0.06)				
12	HB	HB (0.06)				
13	BF+BG	Substrate B+ excess BG				
14	BG+HB	Byproduct B + excess HB				
15	BF+BG+HB	Substrate B + Byproduct B				

Re-prepare stocks (again)-make 100uL of 2uM solution for AF AG HA BF BG HB

- fill 2 of 6 PCR tubes with 98uL of Mg2+ TE buffer. tubes 2,3 and tube 5,6 get 39.2uL of Mg2+TE buffer only.
- Add 2uL of 100uM solution of AF into tube 1, only 0.8uL of AG into tube 2 and 0.8uL of 100uM HA into tube 3, repeat for B. label PCR stocks 2.0uM.
- mix well

I'm adding a 4x dilution step here in the middle, may make some of the ssDNA more likely to show up.
Get another PCR strip label stock 0.5uM: numbers 1-6 *make 40uL of 0.5uM solution for AF AG HA BF BG HB*

- Add 30uL of Mg2+ TE buffer into each of the 6 new tubes.
- mix by pipetting up and down, then extract 10uL from each and add to respective new diluted tube.
- mix well using vortex then centrifuge.
- heat on PCR for X min and cool for 20min.

Get two PCR tube strips and label as FINAL 1-15 *make 10uL of **0.06uM** solution for AF AG HA BF BG HB*

Lanes 3, 5, 6:

- add 8.8 uL Mg2+ TE buffer to tubes 3,5,6
    - add 1.2uL 0.5uM AF into 3, 1.2 uL 0.5uM AG into 5 and 1.2 uL 0.5uM HA into 6
- mix well

Lanes 10-12:

- add 8.8 uL Mg2+ TE buffer to tubes 10,11,12
    - add 1.2uL 0.5uM AF into 10, 1.2 uL 0.5uM AG into 11 and 1.2 uL 0.5uM HA into 12
- mix well

AF variation to see if dilution is the problem at all- 10uL 0.04uM and 0.08uM solutions of AF

lane 2: label 0.04AF

- add 9.2uL Mg2+ TE buffer to tube 2
    - add 0.8uL 0.5uM AF into 2.
- mix well

Lane 4: label 0.08AF

- add 8.4uL Mg2+ TE buffer to tube 4
    - add 1.6uL 0.05uM AF into 4

Prep reactions for lanes 7-9 and 13-15:

- **IN TUBE 1**: Add 50uL of 2uM AF, 28.5uL of Mg2+ TE and 1.5uL of 100uM AG (well D1)to make 80uL of 1.25uM AF, 1.875uM AG solution.
- **IN TUBE 3**: add 15uL 2.0uM AG, 4.6uL Mg2+TE and 0.4uL of HA to make 20uL of 1.5uM AG, 2.0uM HA solution.
- **IN TUBE 4**: Add 50uL of 2uM BF, 1.5uL of 100uM BG (well D1) and 28.5uL of Mg2+ TE to make 80uL of 1.25uM BF, 1.875uM BG solution.
- **IN TUBE 6**: add 15uL 2.0uM BG, 4.6uL Mg2+TE and 0.4uL of HB to make 20uL of 1.5uM AG, 2.0uM HB solution.
- label on both tubes and rack.

- Heat to 85 degrees celcius for 5 min, cool to 25 in 20 minutes.
- lightly mix.

- **IN TUBE 2**: then extract 16uL of TUBE 1, add 4uL Mg2+ TE to make 20uL of 1uM AF and 1.5uM AG. (or 1uM Substrate A and 0.5uM excess AG.)
- in the remaining 64uL **of TUBE 1**: add 14.4uL Mg2+ TE and 1.6uL 100uM HA (well D4) to restore 80uL of 1uM AF, 1.5uM AG, and 2.0uM HA.
- **IN TUBE 5**: extract 16uL of TUBE 4, add 4uL Mg2+ TE to make 20uL of 1uM BF and 1.5uM BG. (or 1uM Substrate B and 0.5uM excess BG.)
- in the remaining 64uL **of TUBE 4**: add 14.4uL Mg2+ TE and 1.6uL 100uM HB (well D5) to restore 80uL of 1uM BF, 1.5uM BG, and 2.0uM HB.

- leave to incubate for 20 minutes. time. label.\

AF+AG+HA (80)	AF+AG (20)	AG+HA (80)	BF+BG+HB	BF+BG	BG+HB
Substrate A **0.63uM** Byproduct A 0.5uM Helper A 1.1uM (weird complex AF-AG-HA thingymjig) 0.36uM	Substrate A **1 uM** Excess AG 0.499uM sneaky AG dimer 0.0061uM	Byproduct A 1.5uM Excess HA 0.5uM (diluting **1.0** to 0.05uM)	Substrate B 1uM Byproduct B 0.5uM Helper B 0.5uM (diluting **0.8uM** to 0.05uM)	Substrate B 1uM Excess BG 0.5uM (diluting **0.8uM** to 0.05uM)	Byproduct B **1.5uM** Excess Helper B 0.5uM (diluting **0.8** to 0.05uM)
1 (changed)	2 (nonchange)	3 (changed to between the two 1.0uM)	4 (changed)	5 (changed)	6 (changed)

Table. with nupack predicted concentrations @26 degrees, max complex size set to 3. WE HAVE CHANGED THE REF DILUTION POINTS from gel 5. make sure with Shelley if its ok to change some but keep others the same)

After 20 mins: in new PCR strip below the above

- to new TUBE 1: add 19uL MG2+ TE
- to new TUBE 2: add 23.5uL Mg2+ TE
- to new TUBE 3: add 23.5uL Mg2+ TE
- to new TUBE 4: add 37uLuL Mg2+ TE (make tube 4 alot bigger in vol than the others, see if this makes a difference)
- to new TUBE 5: add 37uL Mg2+ TE
- to new TUBE 6: add 18uL mg2+ TE

- to new TUBE 1: add 2uL old tube 1 (substrateA+HA)
- to new TUBE 2: add 1.5uL old tube 2
- to new TUBE 3: add 1.5uL old tube 3
- to new TUBE 4: add 3uL old tube 4
- to new TUBE 5: add 3uL old tube 5
- to new TUBE 6: add 2uL old tube 6

MIX WELL.

AF+AG+HA	AF+AG	AG+HA	BF+BG+HB	BF+BG	BG+HB

(21uL)	(25uL)	(25uL)	(40uL)	(40uL)	(20uL)
Substrate A **0.06uM** Byproduct A 0.047uM Helper A 0.1047uM (weird complex AF-AG-HA thingymjig) 0.034uM	Substrate A **0.06 uM** Excess AG 0.047uM sneaky AG dimer 0.0061uM	Byproduct A 0.09uM Excess HA 0.03uM (diluting **1.0** to **0.06**uM)	Substrate B 0.075uM Byproduct B 0.0375uM Helper B 0.0375uM (diluting **0.8uM** to 0.06uM)	Substrate B 0.075uM Excess BG 0.0.0375uM (diluting **0.8uM** to 0.06uM)	Byproduct B 0.15uM Excess Helper B 0.05uM (diluting **0.6** to 0.06uM)
1 (changed)	2 (nonchange)	3 (changed to between the two 1.0uM)	4 (changed)	5 (changed)	6 (changed)
lane 9	lane 7	lane 8	15	13	14

Table. reactions diluted to PAGE runnable solutions.

in final lanes 7-9, 13-15:

- 10uL of TUBE 1 into lane 9
- 10uL of TUBE 2 into lane 7
- 10uL of TUBE 3 into lane 8
- 10uL of TUBE 4 into lane 15
- 10uL of TUBE 6 into lane 13
- 10uL of TUBE 7 into lane 14.

Gel 7-i2 doing the same thing just with different strands so if Gel 6 fails and gel 7 fine, then its not got anything to do with the concs.

lane #	Lane code	structures	length (bp)	Expected banding	purpose	Future uses
1	Ladder	25 bp ladder				
2	AF	AF (0.06)				
3	AG	AG (0.06)				
4	HA	HA (0.06)				
5	AF+AG	Substrate A + excess AG				
6	AG+HA	byproduct A + excess HA				
7	AF+AG+HA	Substrate A + Byproduct A+ excess HA.				
8	BF	BF (0.06uM)				
9	BG	BG (0.06)				
10	HB	HB (0.06)				
11	BF+BG	Substrate B+ excess BG				
12	BG+HB	Byproduct B + excess HB				
13	BF+BG+HB	Substrate B + Byproduct B				
14						
15						

Re-prepare stocks (again)-make 100uL of 2uM solution for AF AG HA BF BG HB

- fill 2 of 6 PCR tubes with 98uL of Mg2+ TE buffer. tubes 2,3 and tube 5,6 get 39.2uL of Mg2+TE buffer only.

- Add 2uL of 100uM solution of AF into tube 1, only 0.8uL of AG into tube 2 and 0.8uL of 100uM HA into tube 3, repeat for B. label PCR stocks 2.0uM.
- mix well

I'm adding a 4x dilution step here in the middle, may make some of the ssDNA more likely to show up.
Get another PCR strip label stock 0.5uM: numbers 1-6 *make 40uL of 0.5uM solution for AF AG HA BF BG HB*

- Add 30uL of Mg2+ TE buffer into each of the 6 new tubes.
- mix by pipetting up and down, then extract 10uL from each and add to respective new diluted tube.
- mix well using vortex then centrifuge.

Get two PCR tube strips and label as FINAL 1-15 *make 10uL of **0.06uM** solution for AF AG HA BF BG HB*

Lanes 2,3,4:
- add 8.8 uL Mg2+ TE buffer to tubes 2,3,4.
    - add 1.2uL 0.5uM AF into 2, 1.2 uL 0.5uM AG into 3 and 1.2 uL 0.5uM HA into 4
- mix well

Lanes 8,9,10:
- add 8.8 uL Mg2+ TE buffer to tubes 8,9,10
    - add 1.2uL 0.5uM AF into 8, 1.2 uL 0.5uM AG into 9 and 1.2 uL 0.5uM HA into 10

- mix well

Prep reactions for lanes 5,6,7 and 11,12,13:
- **IN TUBE 1**: Add 50uL of 2uM AF, 28.5uL of Mg2+ TE and 1.5uL of 100uM AG (well D1)to make 80uL of 1.25uM AF, 1.875uM AG solution.
- **IN TUBE 3**: add 15uL 2.0uM AG, 4.6uL Mg2+TE and 0.4uL of HA to make 20uL of 1.5uM AG, 2.0uM HA solution.
- **IN TUBE 4**: Add 50uL of 2uM BF, 1.5uL of 100uM BG (well D1) and 28.5uL of Mg2+ TE to make 80uL of 1.25uM BF, 1.875uM BG solution.
- **IN TUBE 6**: add 15uL 2.0uM BG, 4.6uL Mg2+TE and 0.4uL of HB to make 20uL of 1.5uM AG, 2.0uM HB solution.
- label on both tubes and rack.

- Heat to 85 degrees celcius for 5 min, cool to 25 in 20 minutes.
- lightly mix.

- **IN TUBE 2**: then extract 16uL of TUBE 1, add 4uL Mg2+ TE to make 20uL of 1uM AF and 1.5uM AG. (or 1uM Substrate A and 0.5uM excess AG.)

- in the remaining 64uL **of TUBE 1**: add 14.4uL Mg2+ TE and 1.6uL 100uM HA (well D4) to restore 80uL of 1uM AF, 1.5uM AG, and 2.0uM HA.
- **IN TUBE 5**: extract 16uL of TUBE 4, add 4uL Mg2+ TE to make 20uL of 1uM BF and 1.5uM BG. (or 1uM Substrate B and 0.5uM excess BG.)
- in the remaining 64uL **of TUBE 4**: add 14.4uL Mg2+ TE and 1.6uL 100uM HB (well D5) to restore 80uL of 1uM BF, 1.5uM BG, and 2.0uM HB.

- leave to incubate for 20 minutes. time. label.\

AF+AG+HA (80)	AF+AG (20)	AG+HA (80)	BF+BG+HB	BF+BG	BG+HB
Substrate A **0.63uM** Byproduct A 0.5uM Helper A 1.1uM (weird complex AF-AG-HA thingymjig) 0.36uM	Substrate A **1 uM** Excess AG 0.499uM sneaky AG dimer 0.0061uM	Byproduct A 1.5uM Excess HA 0.5uM (diluting **1.0** to 0.05uM)	Substrate B 1uM Byproduct B 0.5uM Helper B 0.5uM (diluting **0.8uM** to 0.05uM)	Substrate B 1uM Excess BG 0.5uM (diluting **0.8uM** to 0.05uM)	Byproduct B **1.5uM** Excess Helper B 0.5uM (diluting **0.8** to 0.05uM)
1 (changed)	2 (nonchange)	3 (changed to between the two 1.0uM)	4 (changed)	5 (changed)	6 (changed)

Table. with nupack predicted concentrations @26 degrees, max complex size set to 3. WE HAVE CHANGED THE REF DILUTION POINTS from gel 5. make sure with Shelley if its ok to change some but keep others the same)

After 20 mins: in new PCR strip below the above
- to new TUBE 1: add 19uL MG2+ TE
- to new TUBE 2: add 23.5uL Mg2+ TE
- to new TUBE 3: add 23.5uL Mg2+ TE
- to new TUBE 4: add 37uLuL Mg2+ TE (<u>make tube 4 alot bigger in vol than the others, see if this makes a difference)</u>
- to new TUBE 5: add 37uL Mg2+ TE
- to new TUBE 6: add 18uL mg2+ TE

- to new TUBE 1: add 2uL old tube 1 (substrateA+HA)
- to new TUBE 2: add 1.5uL old tube 2
- to new TUBE 3: add 1.5uL old tube 3
- to new TUBE 4: add 3uL old tube 4
- to new TUBE 5: add 3uL old tube 5
- to new TUBE 6: add 2uL old tube 6

MIX WELL.

AF+AG+HA (21uL)	AF+AG (25uL)	AG+HA (25uL)	BF+BG+HB (40uL)	BF+BG (40uL)	BG+HB (20uL)
Substrate A **0.06uM** Byproduct A 0.047uM Helper A 0.1047uM (weird complex AF-AG-HA thingymjig) 0.034uM	Substrate A **0.06 uM** Excess AG 0.047uM sneaky AG dimer 0.0061uM	Byproduct A 0.09uM Excess HA 0.03uM (diluting **1.0** to **0.06**uM)	Substrate B 0.075uM Byproduct B 0.0375uM Helper B 0.0375uM (diluting **0.8uM** to 0.06uM)	Substrate B 0.075uM Excess BG 0.0.0375uM (diluting **0.8uM** to 0.06uM)	Byproduct B 0.15uM Excess Helper B 0.05uM (diluting **0.6** to 0.06uM)
1 (changed)	2 (nonchange)	3 (changed to between the two 1.0uM)	4 (changed)	5 (changed)	6 (changed)
lane 7	lane 5	lane 6	13	11	12

Table. reactions diluted to PAGE runnable solutions.

in final lanes 5-7, 11-13:

- 10uL of TUBE 1 into lane 7
- 10uL of TUBE 2 into lane 5
- 10uL of TUBE 3 into lane 6
- 10uL of TUBE 4 into lane 13
- 10uL of TUBE 6 into lane 11
- 10uL of TUBE 7 into lane 12.

remember to 2x dilute the dna ladder. 5uL ladder with 5uL buffer.

Gel 8 - OG dendrimer trialout.

What we learnt from gel 6: Single strands good conc for sybr gold is 0.1uM.

- need a buffer shield lane to prevent the distortion seen in end lanes of gel 5.

lane #	Lane code	structures	length (bp)	Expected banding	purpose	Future uses
1	Ladder	25 bp ladder				
2	AF	AF (0.1)				
3	AG	AG (0.1)				

4	AF+AG	Substrate A + excess AG				
5	AF+AG+HA	Substrate A + Byproduct A+ excess HA.				
6	BF	BF (0.06uM)				
7	BG	BG (0.06)				
8	BF+BG	Substrate B+ excess BG				
9	BF+BG+HB	Substrate B + Byproduct B				
10	AF+AG + BF+BG	Substrate A + Substrate B			no dendrimer	
11	AF+AG + BF+BG + target.	Substrate A + Substrate B + Target			no dendrimer	
12	AF+AG+HA + BF+BG+HB	Substrate A + HA Substrate B + HB			no dendrimer	
13	AF+AG+HA + BF+BG+HB + target	Substrate A + HA Substrate B + HB Target			DENDRIMER pretty pls	
14	target (same conc)	target			control	
15	buffer shield	-	should be nothing...		prevent lane 14	

					band distortion	

Lanes 2,3 6,7: extract from PCR 0.5uM stocks (fingers crossed these solutions still good)

- get 2 PCR tube racks for final loading solutions. label
- mix the PCR 0.5uM stocks, quick centrifuge
    - to tube LANES 2,3,6,7: add 8uL Mg2+ TE buffer.
        - to LANE 2: add 2uL 0.5uM AF.
        - to LANE 3: add 2uL 0.5uM AG.
        - to LANE 6: add 2uL 0.5uM BF.
        - to LANE 7: add 2uL 0.5uM BG.
    - mix well

Lanes : if we can use tuesdays stocks EVEN BETTER:

AF+AG+HA (80)	AF+AG (20)	AG+HA (20)	BF+BG+HB (80uL)	BF+BG (20)	BG+HB (20)
Substrate A **0.63uM** Byproduct A 0.5uM Helper A 1.1uM (weird complex AF-AG-HA thingymjig) 0.36uM	Substrate A **1 uM** Excess AG 0.499uM sneaky AG dimer 0.0061uM	Byproduct A 1.5uM Excess HA 0.5uM (diluting **1.0** to 0.05uM)	Substrate B 1uM Byproduct B 0.5uM Helper B 0.5uM (diluting **0.8uM** to 0.05uM)	Substrate B 1uM Excess BG 0.5uM (diluting **0.8uM** to 0.05uM)	Byproduct B **1.5uM** Excess Helper B 0.5uM (diluting **0.8** to 0.05uM)
1 (changed)	2 (nonchange)	3 (changed to between the two 1.0uM)	4 (changed)	5 (changed)	6 (changed)
80-2 = **78 uL**	20-1.5 = **18.5 uL**	20-1.5 = **18.5 uL**	80-3 = **77 uL**	20-3 = **17 uL**	20 - 2 = **17 uL**

Table OG1. now with revised volumes after gel 6.

Lanes 4,5,8,9: in PCR strip (dilute) label on both rack and tubes pls. label 1-6 corresponding to same as before. (there will be empty ones since we're not doing byproduct controls and direclty loading into final loading for some solutions.)

- to new TUBE 1: add 19uL MG2+ TE
- to new TUBE 2: add 23.5uL Mg2+ TE
- do nothing to TUBE 4,5

- to new TUBE 1: add 2uL old tube 1 (substrateA+HA)
- to new TUBE 2: add 1.5uL old tube 2

Mix well, rest for 5 min.

- for LANE 4: transfer 10uL of new TUBE2 into lane 4 final.
- for LANE 5: transfer 10uL of new TUBE1 into lane 5 final.
- for LANE 8: dont do anything to dilute strip TUBE 5. Add DIRECTLY INTO FINALS = 10uL of dilute gel-6 TUBE5 solution.
- for LANE 9: dont do anything to dilute strip TUBE 4. add DIRECTLY INTO FINALS = 10uL of dilute gel-6 TUBE4 solution.

For LANE 1: add 1.5 uL DNA ladder and 4.5 uL Mg2+ TE buffer into lane 1 final.

Making trigger concs
Get target PCR rack and put into Gel-8 holder and label: trigger stuff.

- 20x dilution to 5uM trigger: add 47.5uL of Mg2+ TE buffer, then 2.5uL of 100uM trigger. makes 50uL
- mix well.
- 5x dilution from 5uM to 1uM trigger: 20uL of Mg2+ TE buffer, then 5uL of 5uM trigger. makes 25uL
- mix well
- 10x dilution from 1uM to 0.1uM trigger: add 18uL mg2+ TE buffer and 2uL of 1uM trigger.

Lane 14: get new PCR rack called ‘new undiluted PCR rack and placed in gel 8 holder.’

- add Mg2+ TE buffer then _ 0.1uM trigger to make some 0.06uM trigger
-

Reactions for lanes 10,11,12,13
In the OG1 gel 6 undiluted HEAT pcr rack of 1-6 label with date (26/09/17), then mix well again: use above lane 14 rack ‘new undiluted PCR rack and put in gel 8 holder.’ (label undiluted, then label indv)

- extract 30uL of TUBE4 and add to TUBE1 (of new rack in gel 8 holder), add 15uL of TUBE1. This makes 45uL of AF, AG, HA, BF, BG, HB. (3x dilution for TUBE1, 1.5x dilution for TUBE4)
- extract 12uL of TUBE5 and add to TUBE2 (of new rack in gel 8 holder0, add 6uL of TUBE2. This makes 18uL of AF, AG, BF, BG. (3x dilution of TUBE2, 1.5x dilution of TUBE 5.)

mix well

- add 20uL of TUBE1, then 0.16uL Mg2+ TE and then 0.84uL of 5uM trigger into TUBE3.
- add 6.66uL of TUBE2, then 2.13 Mg2+ TE and then 2.2uL of 1u trigger into TUBE4.
- **cap tightly, mix well, cap tightly.**
- leave and let it *hopefully* do its magic for 30 minutes.
- make 0.2uM by: _____

AF+AG+HA BF+BG+HB	AF+AG BF+BG (15)	AF+AG+HA BF+BG+HB+	AF+AG BF+BG+		TRIGGER

(45)		TRIGGER (21)	TRIGGER (11)		
Substrate A **0.21uM** Byproduct A 0.16uM Helper A 0.36uM (weird complex AF-AG-HA thingymjig) 0.12uM Substrate B 0.66uM Byproduct B 0.33uM Helper B 0.33uM	Substrate A **0.33 uM** Excess AG 0.16uM sneaky AG dimer 0.002uM Substrate B 0.66uM Excess BG 0.33uM	Substrate A **0.20uM** Byproduct A 0.158uM Helper A 0.34uM (weird complex AF-AG-HA thingymjig) 0.11uM Substrate B 0.63uM Byproduct B 0.317uM Helper B 0.317uM TRIGGER **0.20uM**	Substrate A **0.2 uM** Excess AG 0.097uM sneaky AG dimer 0.001uM Substrate B 0.4uM Excess BG 0.2uM TRIGGER **0.2 uM**		Trigger 0.2uM
1 (do a 3.5x dil)	2 (5.5x dil)	3 (do a 3.33x dil)	4 (do a 3.33x dil)		

Table. OG gel-8.

Setup some gels while you wait or /lunch/breathe/let-cortisol-return-to-normal-levels.

After 30 mins: in new PCR strip below the above:

- in new TUBE1: add 15uL Mg2+ TE buffer
- in new TUBE2: add 18uL Mg2+ TE buffer
- in new TUBE3: add 17.5uL Mg2+ TE buffer
- in new TUBE4: add 17/5uL Mg2+ TE buffer

- in new TUBE1: add 6uL of old TUBE1. makes 21uL
- in new TUBE2: add 4uL of old TUBE2. makes 22uL
- in new TUBE3: add 7.5uL of old TUBE3. Makes 25uL
- in new TUBE4: add 7.5uL of old TUBE4. makes 25uL

MIX WELL.

AF+AG+HA BF+BG+HB (45)	AF+AG BF+BG (15)	AF+AG+HA BF+BG+HB+ TRIGGER (21)	AF+AG BF+BG+ TRIGGER (11)		TRIGGER
Substrate A **0.21uM** Byproduct A 0.16uM Helper A	Substrate A **0.33 uM** Excess AG 0.16uM	Substrate A **0.20uM** Byproduct A 0.158uM Helper A	Substrate A **0.2 uM** Excess AG 0.097uM		Trigger 0.2uM

0.36uM (weird complex AF-AG-HA thingymjig) 0.12uM Substrate B 0.66uM Byproduct B 0.33uM Helper B 0.33uM	sneaky AG dimer 0.002uM Substrate B 0.66uM Excess BG 0.33uM	0.34uM (weird complex AF-AG-HA thingymjig) 0.11uM Substrate B 0.63uM Byproduct B 0.317uM Helper B 0.317uM TRIGGER **0.20uM**	sneaky AG dimer 0.001uM Substrate B 0.4uM Excess BG 0.2uM TRIGGER **0.2 uM**		
1	2	3	4	5	6
lane 12	lane 10	lane 13	lane 11		lane 14

TableOG gel-8 reactions diluted to PAGE runnable solutions. (NEED TO DIVIDE EVERYTHING by relevant dilution factors DO THIS AFTER 28/09 lab session, even better during while waiting for gel)

in final lanes 5-7, 11-13:

- 10uL of TUBE 1 into lane 12
- 10uL of TUBE 2 into lane 10
- 10uL of TUBE 3 into lane 13
- 10uL of TUBE 4 into lane 11
- 10uL of TUBE 6 into lane 14

This dendrimer gel will most likely fail so we will plan on how to account for this, one way:

- PAGE PURIFY the substrate A's and substrate B's. Make the HA HBs then add them in solution like they did in the same concs.

<u>Gel 9 - i2 dendrimer trialout.</u>

Ok so gel 8 seems pretty successful (on the surface), we will visualise the sample via AFM to confirm. If dendrimer formation inefficient, we will PAGE purify Substrate A and B and perform same gel.

- " AFM imaging
- Assembly products were diluted in 1× TAE/Mg2+ buffer and deposited onto freshly cleaved mica (SPI, USA) for 3 min.
- Samples were then rinsed with deionized water and dried with nitrogen. AFM imaging experiments were performed in air in tapping mode on a Dimension 3100 AFM (Veeco, USA).
- Silicon probes Tap300Al-G (BudgetSensors, Bulgaria) with resonant frequency 300 kHz, force constant 40 N/m and tip radius 10 nm were used in the experiments.
- The image background was flattened by Nanoscope IIIa software. " Xuan Hsing 2014

NB for gel 8 we put AFAG and BF BG wrong way around. Other things learnt from gel 8 include:

- need less ladder, try 1.0uL this time in 5uL buffer.
- **29 minutes on 200V pushes fastest band nearly to the bottom**, a rough indicator is lighter loading dye band touching bottom green rubber contact.

lane #	Lane code	structures	length (bp)	Expected banding	purpose	Future uses
1	Ladder	25 bp ladder				
2	AF	AF (0.1)				
3	AG	AG (0.1)				
4	AF+AG	Substrate A + excess AG				
5	AF+AG+HA	Substrate A + Byproduct A+ excess HA.				
6	BF	BF (0.01uM)				
7	BG	BG (0.01)				
8	BF+BG	Substrate B+ excess BG				
9	BF+BG+HB	Substrate B + Byproduct B				
10	AF+AG + BF+BG	Substrate A + Substrate B			no dendrimer	
11	AF+AG + BF+BG + target.	Substrate A + Substrate B + Target			no dendrimer	

12	AF+AG+HA + BF+BG+HB	Substrate A + HA Substrate B + HB			no dendrimer	
13	AF+AG+HA + BF+BG+HB + target	Substrate A + HA Substrate B + HB Target			DENDRIMER pretty pls	
14	target (same conc)	target			control	
15	buffer shield	-	should be nothing...		prevent lane 14 band distortion	

Lanes 2,3 6,7: extract from PCR 0.5uM stocks from gel 7 holder. (fingers crossed these solutions still good)

- get 2 PCR tube racks for final loading solutions. label
- mix the PCR 0.5uM stocks, quick centrifuge
    - to tube LANES 2,3,6,7: add 8uL Mg2+ TE buffer.
        - to LANE 2: add 2uL 0.5uM AF.
        - to LANE 3: add 2uL 0.5uM AG.
        - to LANE 6: add 2uL 0.5uM BF.
        - to LANE 7: add 2uL 0.5uM BG.
    - mix well

Lanes 10-14: if we can use Tuesday's stocks EVEN BETTER:

AF+AG+HA (80)	AF+AG (20)	AG+HA (20)	BF+BG+HB (80uL)	BF+BG (20)	BG+HB (20)
Substrate A **0.63uM** Byproduct A 0.5uM Helper A 1.1uM (weird complex AF-AG-HA thingymjig) 0.36uM	Substrate A **1 uM** Excess AG 0.499uM sneaky AG dimer 0.0061uM	Byproduct A 1.5uM Excess HA 0.5uM (diluting **1.0** to 0.05uM)	Substrate B 1uM Byproduct B 0.5uM Helper B 0.5uM (diluting **0.8uM** to 0.05uM)	Substrate B 1uM Excess BG 0.5uM (diluting **0.8uM** to 0.05uM)	Byproduct B **1.5uM** Excess Helper B 0.5uM (diluting **0.8** to 0.05uM)
1 (changed)	2 (nonchange)	3 (changed to	4 (changed)	5 (changed)	6 (changed)

		between the two 1.0uM)			
80-2 = **78 uL**	20-1.5 = **18.5 uL**	20-1.5 = **18.5 uL**	80-3 = **77 uL**	20-3 = **17 uL**	20 - 2 = **17 uL**

Table i2-1. now with revised volumes after gel 7.

Lanes 4,5,8,9: in PCR strip (dilute) label on both rack and tubes pls. label 1-6 corresponding to same as before. (there will be empty ones since we're not doing byproduct controls and direclty loading into final loading for some solutions.)

- to new TUBE 1: add 19uL MG2+ TE
- to new TUBE 2: add 23.5uL Mg2+ TE
- do nothing to TUBE 4,5

- to new TUBE 1: add 2uL old tube 1 (substrateA+HA)
- to new TUBE 2: add 1.5uL old tube 2

Mix well, rest for 5 min.

- for LANE 4: transfer 10uL of new TUBE2 into lane 4 final.
- for LANE 5: transfer 10uL of new TUBE1 into lane 5 final.
- for LANE 8: dont do anything to dilute strip TUBE 5. Add DIRECTLY INTO FINALS = 10uL of dilute gel-6 TUBE5 solution.
- for LANE 9: dont do anything to dilute strip TUBE 4. add DIRECTLY INTO FINALS = 10uL of dilute gel-6 TUBE4 solution.

For LANE 1: add 1.0 uL DNA ladder and 4.5 uL Mg2+ TE buffer into lane 1 final.

Making trigger concs

Get target PCR rack and put into Gel-8 holder and label: trigger stuff.

- 20x dilution to 5uM trigger: add 47.5uL of Mg2+ TE buffer, then 2.5uL of 100uM trigger. makes 50uL
- mix well.
- 5x dilution from 5uM to 1uM trigger: 20uL of Mg2+ TE buffer, then 5uL of 5uM trigger. makes 25uL
- mix well
- 10x dilution from 1uM to 0.1uM trigger: add 18uL mg2+ TE buffer and 2uL of 1uM trigger.

Lane 14: get new PCR rack called 'new undiluted PCR rack and placed in gel 8 holder.'

- add 10uL 0.1uM trigger into LANE14 final tube.
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Reactions for lanes 10,11,12,13

In the i2-1 gel 7 undiluted HEAT pcr rack of 1-6 label with date (26/09/17), then mix well again: get NEW PCR TUBES 'new undiluted PCR rack and put in gel 9 holder.' (label undiluted, then label indv)

- extract 30uL of TUBE4 and add to TUBE1 (of new rack in gel 9 holder), add 15uL of TUBE1. This makes 45uL of AF, AG, HA, BF, BG, HB. (3x dilution for TUBE1, 1.5x dilution for TUBE4)
- extract 12uL of TUBE5 and add to TUBE2 (of new rack in gel 8 holder0, add 6uL of TUBE2. This makes 18uL of AF, AG, BF, BG. (3x dilution of TUBE2, 1.5x dilution of TUBE 5.)

mix well

- add 20uL of TUBE1, then 0.16uL Mg2+ TE and then 0.84uL of 5uM trigger into TUBE3.
- add 6.66uL of TUBE2, then 2.13 Mg2+ TE and then 2.2uL of 1u trigger into TUBE4.
- **cap tightly, mix well, cap tightly.**
- leave and let it *hopefully* do its magic for 30 minutes.
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AF+AG+HA BF+BG+HB (45)	AF+AG BF+BG (15)	AF+AG+HA BF+BG+HB+ TRIGGER (21)	AF+AG BF+BG+ TRIGGER (11)		
Substrate A **0.21uM** Byproduct A 0.16uM Helper A 0.36uM (weird complex AF-AG-HA thingymjig) 0.12uM Substrate B 0.66uM Byproduct B 0.33uM Helper B 0.33uM	Substrate A **0.33 uM** Excess AG 0.16uM sneaky AG dimer 0.002uM Substrate B 0.66uM Excess BG 0.33uM	Substrate A **0.20uM** Byproduct A 0.158uM Helper A 0.34uM (weird complex AF-AG-HA thingymjig) 0.11uM Substrate B 0.63uM Byproduct B 0.317uM Helper B 0.317uM TRIGGER **0.20uM**	Substrate A **0.2 uM** Excess AG 0.097uM sneaky AG dimer 0.001uM Substrate B 0.4uM Excess BG 0.2uM TRIGGER **0.2 uM**		
1 (do a 3.5x dil)	2 (5.5x dil)	3 (do a 3.33x dil)	4 (do a 3.33x dil)		

Table. OG gel-8.

Setup some gels while you wait or /lunch/breathe/let-cortisol-return-to-normal-levels.

After 30 mins: in new PCR strip below the above:

- in new TUBE1: add 15uL Mg2+ TE buffer
- in new TUBE2: add 18uL Mg2+ TE buffer
- in new TUBE3: add 17.5uL Mg2+ TE buffer
- in new TUBE4: add 17/5uL Mg2+ TE buffer

- in new TUBE1: add 6uL of old TUBE1. makes 21uL
- in new TUBE2: add 4uL of old TUBE2. makes 22uL
- in new TUBE3: add 7.5uL of old TUBE3. Makes 25uL
- in new TUBE4: add 7.5uL of old TUBE4. makes 25uL

MIX WELL.

AF+AG+HA BF+BG+HB (45)	AF+AG BF+BG (15)	AF+AG+HA BF+BG+HB+ TRIGGER (21)	AF+AG BF+BG+ TRIGGER (11)		TRIG GER
Substrate A **0.21uM** Byproduct A 0.16uM Helper A 0.36uM (weird complex AF-AG-HA thingymjig) 0.12uM Substrate B 0.66uM Byproduct B 0.33uM Helper B 0.33uM	Substrate A **0.33 uM** Excess AG 0.16uM sneaky AG dimer 0.002uM Substrate B 0.66uM Excess BG 0.33uM	Substrate A **0.20uM** Byproduct A 0.158uM Helper A 0.34uM (weird complex AF-AG-HA thingymjig) 0.11uM Substrate B 0.63uM Byproduct B 0.317uM Helper B 0.317uM TRIGGER **0.20uM**	Substrate A **0.2 uM** Excess AG 0.097uM sneaky AG dimer 0.001uM Substrate B 0.4uM Excess BG 0.2uM TRIGGER **0.2 uM**		Trigge r 0.2uM
1	2	3	4	5	6
lane 12	lane 10	lane 13	lane 11		lane 14

TableOG gel-8 reactions diluted to PAGE runnable solutions. (NEED TO DIVIDE EVERYTHING by relevant dilution factors DO THIS AFTER 28/09 lab session, even better during while waiting for gel)

in final lanes 5-7, 11-13:

- 10uL of TUBE 1 into lane 12
- 10uL of TUBE 2 into lane 10
- 10uL of TUBE 3 into lane 13
- 10uL of TUBE 4 into lane 11
- 10uL of TUBE 6 into lane 14