Gel 13

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	BF+BG+HB	Substrate B + Byproduct B				
15	Trugger					

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 (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 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 14

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			no dendrimer	

		B + Target				
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	

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)

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- 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. 0.5uL ladder with 6uL buffer.