Gel 10

lane #	Lane code	structures	length (bp)	Expected banding	purpose	Future uses
1	Ladder					
2	Virus					
3	lock					
4	target					
5	Lock+target (1a) (non purified)					
6	Lock+target (purified)					
7	LockC + targetC + virusA					
8	LockC + targetC +virusB					
9	LockC + targetC + virusC					
10	LockC + targetC + virusD					
11	LockD + targetD (1b) + virusA					

12	LockD + targetD + virusB					
13	LockD + targetD + virusC					
14	Buffer					

Make 100uL 0.1uM solutions of everything

- mix all working solutions well. Grab a PCR tube row label dilute 1234a1 and 1234b1 @ 0.1uM
- add 95uL Mg2+ TE buffer to 1,2,3,4 of a1 and b1.
- add 5uL of each respective 2uM solution amp00X1,2,3,4 into the respective dilute tubes. This makes 100uL of 0.1uM every oligo
- mix well and centrifuge

Lanes 2,3,4,8: Adaptor1.0tubeless

- Get two tube rows, label as final on the tubes.
- transfer over:
    - LANE2: 10uL dilute TUBE3a1
    - LANE3: 10uL dilute TUBE2a1
    - LANE4: 10uL dilute TUBE1a1
    - LANE8: 10uL dilute TUBE4a1

Lane 6: Adaptor1.0tubeless

- In new PCR tube row label as HEAT: label Tube1-4a 1-4b
    - in tube 1a add:
        - 50uL of 2uM TUBE1a1, 30uL of **5uM** (second row) TUBE2a1, and 20uL Mg2+ TE.
        - this makes 100uL of what NUPACK claims to be 1uM adaptor and 0.5uM excess lock.
        -

Lane 13: Adaptor1.0halftubes

-
    - in tube 1b add:
        - 50uL of 2uM TUBE1b1, 30uL of **5uM** (second row) TUBE2b1, and 20uL Mg2+ TE.
        - this makes 100uL of what NUPACK claims to be 1uM adaptor and 0.5uM excess lock.
        - Heat in PCR and cool.

- Dilute the adaptor solutions as appropriate.

- load into LANE6:

- load into LANE13:

Lanes 9,10,11,15: Adaptor1.0halftubes
- Get two tube rows, label as final on the tubes.
- transfer over:
    - LANE9: 10uL dilute TUBE3b1
    - LANE10: 10uL dilute TUBE2b1
    - LANE11: 10uL dilute TUBE1b1
    - LANE15: 10uL dilute TUBE4b1

Gel 11 replicate of 1 with analgous NUPACK base pair sets

Replicate but with second trial of analogous DNA strands. If we produce identical results to gel1, we demonstrated increased reliability of NUPACK code we designed to prevent formation of offtargets in adaptor synthesis.

lane #	Lane code	structures	length (bp)	Expected banding	purpose	Future uses
1	Ladder					
2	Virus					
3	lock					
4	target					
5	Lock+target (1a) (non purified)					

6	Lock+target (purified)					
7	LockA + targetA + virusA					
8	LockA + targetA + virusB					
9	LockA + targetA + virusC					
10	LockA + targetA + virusD					
11	LockB + targetB (1b) + virusA					
12	LockB + targetB + virusB					
13	LockB + targetB + virusC					
14	LockB + targetB + virusD					
15	Buffer					

and OFC same gel structure for other 2 versions also extracted product of same lanes.
Make 100uL 2.0uM solutions of everything

In a new PCR tubes labeled 1234a2, 1234b2: add 98uL of Mg2+ TE buffer to each.

- TUBE1a2: transfer 2uL of A6
- TUBE2a2: transfer 2uL of A7
- TUBE3a2: transfer 2uL of A8

- TUBE4a2: transfer 2uL of A9

- TUBE1b2; transfer 2uL of B6
- TUBE2b2: transfer 2uL of B7
- TUBE3b2: transfer 2uL of B8
- TUBE4b2: transfer 2uL of B9
- mix well centrifuge

Make up 5uM stocks of MOAR lock strand (Amp00X2/)

In new PCR tube row below above row label under TUBE2 as 5uM lock. Add 5uL of well A7 into 95uL of Mg2+ TE. Add 5uL of well B7 into 95uL Mg2+ TE.

Make 100uL 0.1uM solutions of everything

- mix all working solutions well. Grab a PCR tube row label dilute 1234a2 and 1234b2 @ 0.1uM
- add 95uL Mg2+ TE buffer to 1,2,3,4 of a1 and b1.
- add 5uL of each respective 2uM solution amp00X1,2,3,4 into the respective dilute tubes. This makes 100uL of 0.1uM every oligo
- mix well and centrifuge

Lanes 2,3,4,8: Adaptor1.0tubeless

- Get two tube rows, label as final on the tubes.
- transfer over:
    - LANE2: 10uL dilute TUBE3a2
    - LANE3: 10uL dilute TUBE2a2
    - LANE4: 10uL dilute TUBE1a2
    - LANE8: 10uL dilute TUBE4a2

Lane 6: Adaptor1.0tubeless

- In new PCR tube row label as HEAT: label Tube1-4 (a1) 1-4 (b1)
    - in tube 1a add:
        - 50uL of 2uM TUBE1a2, 30uL of **5uM** (second row) TUBE2a2, and 20uL Mg2+ TE.
        - this makes 100uL of what NUPACK claims to be 1uM adaptor and 0.5uM excess lock.
        -

Lane 13: Adaptor1.0halftubes

-
    - in tube 1b add:
        - 50uL of 2uM TUBE1b2, 30uL of **5uM** (second row) TUBE2b2, and 20uL Mg2+ TE.
        - this makes 100uL of what NUPACK claims to be 1uM adaptor and 0.5uM excess lock.
        - Heat in PCR and cool.

- Dilute the adaptor solutions as appropriate.

- load into LANE6:

- load into LANE13:

Lanes 9,10,11,15: Adaptor1.0halftubes
- Get two tube rows, label as final on the tubes.
- transfer over:
    - LANE9: 10uL dilute TUBE3b2
    - LANE10: 10uL dilute TUBE2b2
    - LANE11: 10uL dilute TUBE1b2
    - LANE15: 10uL dilute TUBE4b2

gel 12 - demonstrating adaptor specificity. (for adaptors of gel1 from PAGE purification)

lane #	Lane code	structures	length (bp)	Expected banding	purpose	Future uses

1	Buffer					
2	Virus					
3	lock					
4						
5	LockA + targetA (a) (mismatch)					
6	LockA + targetA (a) (match)					
7	LockB + targetB (b) (mismatch)					
8	LockB + targetB (b) (match)					
9	LockC + targetC (mismatch)					
10	LockC + targetC (match)					
11	LockD + targetD (mismatch)					
12	LockD + targetD (match)					

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13	Lock+target+ fuel					
14	Lock+target + fuel + matching virus					
15	Buffer					

Samples used were leftover samples from Gels 10 and 11.
if this doesn't work best. Remake in nupack with longer detector domain.

Make 100uL 0.1uM solutions of everything from adaptor-a1, use this as control strands for everything.

- mix all working solutions well. Grab a PCR tube row label dilute 1234a1 @ 0.1uM
- add 95uL Mg2+ TE buffer to 1,2,3,4 of a1.
- add 5uL of each respective 2uM solution amp00X1,2,3,4 into the respective dilute tubes. This makes 100uL of 0.1uM every oligo
- mix well and centrifuge

- dilute 0.8uL of ladder into 5.2uL Mg2+ TE buffer.

Lanes 1,2,3,4:
- LANE2: add 10uL of TUBE3 dilute (0.1uM)
- LANE3: add 10uL of TUBE2 dilute
- LANE4: add 10uL of TUBE1 dilute.
- LANE1: add 5uL of dilute ladder.

Lane 5: HEAT b1 is/should be at 2.0uM target, 2.5uM Lock. I.e NUPACK predicts around ~2.0uM adaptor (lock+target.)

Dilute 2.0uM unpurified adaptor to 100ul of 0.05uM.
- label TUBE as dilute. add 97.5uL Mg2+ TE. Then transfer over .25uL of 2.0 uM unpurified adaptor (a1)
- LANE5: add 10uL of this diluted 0.05uM unpurified adaptor solution.

Lanes 7-14: add 1x trigger to each (trigger near full conversion w/o need for fuel provided prescence of a sufficiently favourably bound external sequence.)

1x is where amount of viral trigger = amount of adaptor (irl viral trigger much lower, which is why we have the fuel see gels 5 onwards :))

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