Note: Bead washes are performed as follows: Magnetize beads, once beads are settled, remove supernatant and resuspend in 200uL of wash buffer* (no need to pipette up and down). Shake at 1200rpm for 30s before magnetizing again. Shaking is typically done at 1200rpm. This is the speed at which the beads typically remain in suspension. A higher shaking speed may be needed depending on the volume of beads or the buffer theyre in; use the speed at which the beads no longer remain settled after 30s.

*The wash volumes will change depending on the scale of the experiment. I use a minimum of 2x the volume of beads (i.e. 300uL beads would be washed with a minimum of 600uL of buffer; if using >500uL beads, up the total number of washes and use a 1mL wash volume.)

Prepare Biotinylated Protein G beads:

1. Reconstitute NHS-biotin. Allow 1mg of EZ-Link Sulfo-NHS-Biotin to equilibrate to RT (10-30 min).
2. Add 448 uL DMSO to 1mg vial for 5mM solution of NHS-Biotin.
3. Vortex for 10 seconds.
4. Bulk biotinylate Protein G beads:
    a. Put 1mL beads on magnetic rack and remove storage buffer. Save buffer in a separate tube.
    a. Wash beads with 1mL PBST
    b. Resuspend beads in 1mL PBST
    c. Add 20uL of 5mM Sulfo-NHS-Biotin, vortex for 10s immediately
    d. Rotate on Hula mixer for 30 min.
    e. Put on magnetic rack, remove 500uL and add 500uL 1M Tris pH 7.4
    f. Vortex 5s and rotate on Hula mixer for 30 min.
    g. Wash beads 2x with 1mL PBST
    h. Resuspend beads in original storage buffer.
        . Beads can be stored in 4C for same length of time as manufacturer specs
        i. Beads should be made at least two weeks in advance – some sort of voodoo going on where they need to age in the fridge for optimal oligo loading downstream.

Bind biotinylated and barcoded oligo to SA:

Original stock plate of oligos is at 100uM. Take 9uL, dilute in 81uL H20 for a 10uM plate. Use 20uL of the 10uM stock in reaction below. (20uL oligo, 5uL streptavidin, 75uL PBS 100uL reaction total))

1. Prepare the following reaction for each barcoded biotin oligo in a 96-well plate - 909nM final concentration: (2:1 ratio of biotin:SA)

Reagent	**Molarity (uM)**	**1X**
Barcoded biotin oligo	10	20 uL
Streptavidin (1mg/mL)	18.18	5 uL

PBS		75 uL

2. Shake at 1600 RPM for 30 min.
3. Dilute 1:4 in PBS (25uL SA oligos + 75uL PBS) to 227nM final concentration. Let diluted plate equilibrate in the fridge for ~3-5 days.

Store diluted plate at 4C, store 909nM stock plate at -20C.

QC Day 1:

This is done the day before the IP is started. The goal of this QC is to titrate for the amount of SA-oligos that should be loaded onto the beads to yield around 150-400 oligos per bead. The QC is also used to confirm that multiple key components within the larger SPIDR protocol are working as expected.

For SPIDR, 10uL of biotinylated protein G beads are used for each target in your sample. Each QC sample is prepared with 10uL of beads. To titrate, use the following volumes of SA-oligo at 227nM: 2.5uL, 5uL, and 7.5uL. The volumes below are for a 1X reaction with 10uL beads. I typically prep each of the three QC reactions in 1.5mL eppies. To account for batch variance between individual SA-oligos in the QC, I use a pool of ≥4 different SA-oligos for bead-loading.

1. Make mastermix of 50% PBST + 50% 2X B&W buffer (200uL mastermix per starting 10uL beads)
2. Magnetize biotinylated Protein G beads, remove buffer, and wash desired amount of beads in 1mL PBST. (I wash all 30uL of beads in one tube.)
3. Remove PBST and resuspend beads in mastermix buffer (200uL mastermix per starting 10uL beads)
4. Aliquot 200uL of bead mastermix to each reaction tube.
5. Add 2.5uL of 227nM SA-oligo to tube #1, 5uL to tube #2, and 7.5uL to tube #3.
6. Shake at RT 1200 RPM for 30 min.
7. Quick spin for 30s at 100g.
8. Wash beads 2x with 200uL M2.
9. Wash beads 2x with 200uL PBST.
10. Resuspend beads in 11.2uL M2+H2O.
11. Add 2.4uL of 4.5uM Terminal Barcode (ODD or EVEN will depend on the overhang of your oligo) and 6.4uL 2X Instant Sticky Master Mix (ISMM).
12. Incubate at RT for 5 min, shaking at 1400 RPM.
13. Quench with 60uL M2-EDTA.
14. Magnetize and wash beads 2x with 200uL M2.
15. Resuspend beads in 210uL H2O.
16. Take 21uL (10% of starting volume of beads) and PCR amplify with 12.5uM 2PUNI-PC50/2PBC pool for 10 cycles.

	1 Rxn	
2Puni-PC 50 (12.5uM)	2 uL	
2PBC Indexed primer	2uL	
2x Q5 MM	25 uL	
Sample	21 uL	
	50uL	

Temp (C)	Time (s)	Cycles
98	60	1 cycle
98	15	10 cycles
69	15	
72	90	
72	2min	1 cycle

Add SA-oligos to biotinylated protein G beads

For each target in your sample, you'll need 10uL of biotinylated protein G beads. The volumes below are for a 1X reaction with 10uL beads. This can be done in an eppie or in deep well plates, if preparing multiple beads with multiple types of oligos. Prep an additional 20uL of beads (2 reactions or 2 wells) to be processed as QC samples prior to antibody loading. Although repetitive, this step is necessary because the oligos can be erratic.

1. Make mastermix of 50% PBST + 50% 2X B&W buffer (200uL mastermix per starting 10uL beads)
2. Magnetize biotinylated Protein G beads, remove buffer, and wash desired amount of beads in 1mL PBST.
3. Remove PBST and resuspend beads in mastermix buffer (200uL mastermix per starting 10uL beads)
4. Aliquot 200uL of bead mastermix to each well. (Note: two of these wells are for one more QC step)
5. Add 2.5uL* of 227nM SA-oligo to each well
    i. *This number has to be empirically predetermined (via QC the day prior) to achieve the appropriate starting oligos/bead (~200 ideally). This is due to batch variance.
6. Shake at RT 1200 RPM for 30 min.
7. Quick spin for 30s at 100g.
8. Magnetize beads and wash each well 2x with 200uL M2 and 2x with 200uL PBST.
9. Transfer the contents of the QC wells into two eppies.
10. Resuspend the other beads in 200uL PBST and keep on ice up until the antibody is loaded.

Oligo/bead QC (eppies):

1. Resuspend beads in 11.2uL M2+H2O.
2. Add 2.4uL of 4.5uM Terminal Barcode (ODD or EVEN will depend on the overhang of your oligo) and 6.4uL 2X Instant Sticky Master Mix (ISMM).
3. Incubate at RT for 5 min, shaking at 1400 RPM.
4. Quench with 60uL M2-EDTA.
5. Magnetize and wash beads 2x with 200uL M2.
6. Resuspend beads in 210uL H2O.
7. Take 21uL and PCR amplify with 6.25uM 2PUNI-PC50/2PBC pool for 10 cycles.

	1 Rxn	
2Puni-PC50 (12.5uM)	2 uL	
2PBC Indexed primer	2uL	

Temp (C)	Time (s)	Cycles
98	60	1 cycle
98	15	10 cycles
69	15	
72	90	
72	2min	1 cycle

2x Q5 MM	25 uL	
Sample	21 uL	
	50uL	

Clean up the reaction with 1.2x volume SPRI beads. Elute in 13uL water.
Quantify after PCR by qubit/bioA, calculate number of starting molecules per bead. Ideal number is ~200 oligos per bead. Try to keep the starting range of oligos/bead between ~150-400.

Bind antibody to Protein G beads:

1. Remove PBST from beads.
2. Bind each antibody to 10uL SA-oligo-BioProtG beads in 200uL PBST (1X reaction):
    a. Use 2.5ug of each antibody.
3. Shake plate at 1200 RPM for 30 min at RT.
    a. At this time, start lysate prep. (Note: lysate prep can be done in advance and frozen at -80C.)
4. Wash 2x with 200uL **PBST + 2mM biotin.**
5. Resuspend beads in 200uL **PBST + 2mM biotin** and shake for 10 min at RT. Place on ice until lysate is ready. This makes sure that any excess oligo hopping is quenched when pooling.
6. Pool beads into a single 2mL LoBind tube (5mL tube is preferred, if available).
7. Wash 2x with 1mL PBST **+ 2mM biotin.**

Lyse cells:

Prepare Lysis Buffer (1X, for 10M cells. Have also successfully used this vol for 5M cells and 15M cells):

Stock Component	**Volume**	**Final Concentration**
RIPA buffer	1000uL	
50X Promega protease inhibitor cocktail (PIC)	20uL	1X
Turbo DNAse	5uL	
100x Mn/Ca mix	10uL	1X
Promega RNasein	5uL	
Total	>1mL	

Lyse:

1. Lyse 10M crosslinked cells in 1mL of Lysis Buffer. Incubate for 10 min on ice.
2. Sonicate with 18% power (3-4W) at 4C for 3 min (0.7sec on, 3.3sec off). (Sonicator is in the Hobert lab, 8th floor. Sonicate on ice to keep temperature down).
3. Heat at 37C for 10min to allow for increased DNAse activity, shaking 1200 RPM.
4. Add 40uL of 0.25M EDTA/EGTA to quench the DNAse.

RNAse I Fragmentation:

1. Split lysate into 2x500uL aliquots.
2. Add 1uL of RNase If to each aliquot (note, this step will need to optimized for different cellular inputs. Ideal fragmentation size is ~300-400nt on average).
3. Incubate lysate for 10 min at 37C.
4. Make 2mL of quench buffer (1mL RIPA + 20uL PIC + 10uL RNasein) and put on ice.
5. Add 500uL of ice-cold quench buffer to each lysate aliquot and immediately place on ice for 3 min.
6. Spin down at 4C at 15000g for 2 min to clarify lysate.
7. Move supernatant into new tubes. If using more than 200uL of beads, add additional quench buffer to scale with bead volume such that an additional 100uL beads = 1 additional mL of lysis buffer. So, for example, an experiment with 300 beads would have a total final volume of 3mL. Keep cold for remainder of the protocol unless specified.

Check input lysate:

1. Clean up 50uL of lysate by Zymo RNA clean and concentrator kit, elute in 44uL.
2. Do an additional TURBO DNAse treatment to get rid of residual chromatin (in-lysate DNase is insufficient to remove all of it). To 44uL RNA, add 5uL 10X TURBO buffer and 1uL TURBO DNAse. Incubate 10 min at 37C 1200 rpm.
3. Clean up with Zymo RNA cleanup kit. Elute in 25uL.
4. Run on BioA to check for RNA size distribution (avg 300-400nt)
5. Save RNA for possible RNA-seq downstream.

Pooled IP:

1. Magnetize pooled SA-oligo Protein G beads and remove buffer.
2. Add **100uL** of **100mM biotin** to lysate for each 1mL of lysate (for 10mM final biotin in the IP).
    a. NOTE: Biotin at this concentration requires the addition of NaOH to go into solution. Manufacturer instructions make it way too basic – just add the NaOH dropwise until it goes into solution. Add some to RIPA and pH to ensure that the biotin solution doesn't overwhelm the buffer.
3. Immediately add lysate to beads and incubate at 4C, rotating overnight.
4. After binding to beads, collect flow-through and save on ice.

    a. Flash freeze FT for further use (MS, RNA-seq).
5. Wash beads 2x with RIPA buffer (2x volume of beads used) and shake for 30s at RT, 1200RPM.
    a. Note for all wash steps: if beads are still stuck to the wall after the first shake, shake for an additional 30s-1min until mixed into the wash buffer. Avoid vortexing.
6. Wash 2x with High Salt buffer
7. Wash 2x with CLAP-Tween buffer

RNA end repair:

Decided to omit the biotin for this step in case of alkaline hydrolysis. Biotin solution is ~pH 11.

1. Prepare the PNK mix:

Component	**Volume (for 1 sample)**
10X PNK Buffer	10uL
PNK enzyme	10uL
Superase*in	1uL
H2O	79uL
Total	100uL

2. Incubate sample with 100uL of PNK mix for 10 min shaking at 37C.
3. Wash 2x with High Salt buffer and 2x with CLAP-Tween buffer.

RNA adapter ligation:

1. Prepare Ligation Master Mix. Note that the order matters here! Add all components together except for the enzyme - vortex before adding the ligase and RNAse inhibitor **last** to ensure they don't crash out in the high PEG concentration. Because this is so viscous, make an extra 0.5-1x of master mix to account for pipetting loss.

Component	**Volume for 1X reaction**	**Volume for __X reaction**
H2O	39.5uL	
10X NEB T4 Ligase Buffer	10uL	
DMSO	5uL	

ATP (10mM)	1.5uL	
50% PEG 8000	30uL	
100mM biotin	*4uL*	
T4 RNA ligase*	8.33uL	
Superase*in	1.67uL	
Total	100uL	

2. Quick spin to pellet Protein G beads, remove buffer.
3. Add 1.5uL of RNA adapter **RT Adapter (20uM)** to beads.

871	RT Adapter	/5Phos/rArUrCrArGrCrACTTAGCG TCAG/3SpC3/

4. Add **100uL** of **Ligation MM** to beads using low-retention tips. Carefully mix. Immediately move tube to shaker (1400 RPM) and incubate for 1h 15min at RT. (Note: To save time, I typically start preparing the plate(s) I will need for split pool before this incubation ends.)
5. Wash beads 3x with CLAP-Tween buffer.

On-bead RT:

Decided to omit the biotin for this step in case of alkaline hydrolysis. Biotin solution is ~pH 11.

1. Prepare RT MM:

Component	**Volume for 1 reaction**
5X FS Buffer	20uL
0.1M DTT	5uL
10mM dNTP	10uL
Superase*in	5uL
H2O	60uL
~~*100mM biotin*~~	~~*4uL*~~
Total	100uL

2. Remove buffer from beads and add 90uL of RT MM to beads. Incubate at 42C for 2 min at 1300 rpm.
3. Add **1uL** of **5uM RT Primer NO biotin** and **10uL** of **Superscript III**. Incubate for an additional 20 min at 1300 rpm.

874	RT Primer NO biotin	/5Phos/ TGACTTG CTGACGCTAAGTGCTGAT

4. Wash 3x with CLAP-Tween buffer, shaking between washes (30s).
5. Remove buffer, resuspend beads in 100uL of the following mastermix. Incubate at 37C for 15 min to digest away free RT primer.

Component	**Volume for 1 reaction**
5X FS Buffer	20uL
Exo I	20uL
H2O	48uL
100mM biotin	10uL
Superase*in	2uL
Total	100uL

6. Wash 3x with CLAP-Tween buffer

Split Pool:

1. Make **dilute M2 + H2O + Superase*in + Biotin** mix. Volume required will depend on size of the experiment. Account for 11.2uL M2+H2O per well.

Superase*in	50uL
H2O	935 uL
M2 Buffer	935 uL
100mM biotin (4mM final)	80uL
Total	2 mL

2. Prepare plate(s) of barcodes:
    a. Starting barcode will be determined by the overhang of the RT primer/oligo. Currently we always start with a round of ODD barcodes
    b. Number of rounds depends on barcode complexity needed and size of aliquots you want to take.
    c. Aliquot 2.4uL 4.5uM barcode into each well of a 96w PCR plate
3. In an example 24-well x 6 round split pool, starting with **Odd** and ending in **TermLigOdd*** the scheme could look like this:
   * In each well: 2.4uL 4.5uM barcode, 11.2uL M2+H2O, 6.4uL ligation master mix.
    a. Round 1: Odd A+B
    b. Round 2: Even A+B

c. Round 3: Odd C+D
d. Round 4: Even C+D
e. Round 5: Odd E+F
f. Round 6: TermLigOdd A+B

4. Prepare Barcode Ligation Mastermix. Volume required will depend on the scale of the experiment. Account for 6.4uL Barcode Ligation MM per well, plus dead volume as this is extremely viscous. Distribute Barcode Ligation MM into a 12-strip tube. Keep in a metal rack on ice.

Component	**Volume for 1 well**
Instant Sticky Master Mix	3.2uL
NEBNext Quick Ligation Buffer	2uL
1,2-propanediol	1.2uL
Total	6.4uL

5. Magnetize beads and resuspend in M2+H2O (+ Superase*in & biotin). Volume required will depend on the scale of the experiment. Total volume should be 11.2uL of beads in this M2+H2O mix per well – note that the beads will also take up volume! Try to minimize the amount of dead volume so that all beads are distributed across the wells. For example – even though 24 wells would mean 11.2uL*24 wells for a final volume of 268.8uL, you may end up only adding 260uL of buffer to accommodate the bead volume.
6. Pipette up and down to mix. Do your best to not make it frothy but some bubbles are unavoidable at this stage.
7. Distribute 11.2uL beads to each well of the barcode plate.
8. Using the multichannel, add 6.4uL of Barcode Ligation Master Mix to each well. Pipette to thoroughly mix (check bottom of plate to make sure beads are fully resuspended).
9. Incubate at RT for 5 min.
10. Using a multichannel, add 60uL of M2+EDTA to each well to quench ligation reaction.
11. Pool beads together.
12. Wash out wells with an additional 150uL M2+EDTA and add to bead pool. (Use the same 150uL for all wells)
13. Between rounds of split-pool:
    a. Wash 2x with 2 bead volumes CLAP-Tween
14. After final round of split-pool:
    a. Wash beads 2x with 1mL M2.
    b. Wash beads 3x with 1mL CLAP-Tween buffer.
15. Resuspend beads in 1mL CLAP-Tween buffer and separate beads into aliquots according to final complexity needed (use barcode correction calculator). Make one 500K bead aliquot for potential oligo QC library (can be sequenced to depth on a miSeq).
16. Remove buffer from each bead aliquot and resuspend in 32.4uL CLAP-Tween*
    a. *This is a compatible stopping point as all steps past this point are not sensitive to cDNA or oligo shedding. Store at 4C.

b. For the smaller QC aliquot, proceed to NLS boil and re-capture (no RNase/splint)

RNase H & on-bead splint:

Note that the split has to be previously annealed, as with the barcodes. See separate annealing protocol for details.

1. RNase digestion. Do not make MM, add components directly to beads in 32.4uL CLAP-Tween.

Component	**Volume for 1 sample**
10X RNAse H Buffer	4uL
RNAse H	2.4uL
RNAse Cocktail	1.2uL
Bead sample	32.4uL
Total	40uL

2. Incubate for 20 min at 37C 1200 rpm.
3. Add the following components directly to sample – DO NOT remove buffer after RNase step:

	1 Rxn	
Sample	40uL	
2x ISMM	40uL	
2PUNI-Splint (4.5uM) * with Spcr	1uL	
Total	80uL	

772	SplintTop-2Puni-Spcr	TACACGACGCTCTTCCGATCT NNNNNN/3SpC3/

939	SplintBot-2Puni-5phos-3spcr	/5Phos/AGA TCG GAA GAG CGT CGT GTA /3SpC3/

Anneal top and bottom splint together, make and keep at -20C. See annealing protocol below.

1. Mix up and down 10 times.
2. Incubate at RT mixing for 1h, 1400 RPM.
    a. This is an additional o/n stopping point at 4C.
3. Magnetize beads and move supernatant to a separate tube, labeled by aliquot identity. Set aside. At this point, we expect that most cDNA is in the supernatant, while the biotinylated oligos are still on the beads.

NLS elution for oligo and RNA:

1. Resuspend each bead aliquot in 60uL NLS elution buffer.
2. Boil at 91C for 6 min, 1350 RPM.
3. Magnetize beads, save supernatant.
4. Wash with 60uL PBS + 0.1% NP-40, shake for 30s at 1200RPM, and pool wash with supernatant.
5. Pool supernatant with splint supernatant from previous steps.
6. Add 67uL of 4X B&W buffer to 200uL supernatant for 1X final concentration.

SA bead oligo capture:

1. Wash 40uL C1 streptavidin beads 2x in 400uL M2.
2. Bind supernatant to C1 beads for 30 min, 1200 RPM shaking.
3. Save supernatant (which contains cDNA) and move to PCR tubes or PCR plate.
4. Wash beads 3x in 200uL M2.
5. Remove M2 and resuspend in 21uL H2O. At this point, the oligos are on the beads and can be put directly into the PCR reaction.

RPM empty insert depletion:

1. Add 10uL of **10uM RPI_depletion_probe** biotinylated probe [/5Biosg/CTTCCGATCTATCAGCACTTAG/3SpC3/] to each cDNA aliquot (currently in PCR tubes). The volume here will be very close to PCR tube capacity, don't be alarmed.
2. Incubate in Thermocycler with following program:
    a. 98C - 1 min
    b. 80C - 10 min
    c. Drop to RT, hold.
3. Wash 40uL C1 beads 2x in 400uL M2.
4. Bind supernatant to C1 beads for 30 min, 1200 RPM shaking. [At this time start oligo PCR]
5. Move supernatant to a new tube and proceed to Silane clean cDNA.

Silane clean cDNA:

1. Wash 12uL silane beads in 50uL RLT buffer (Qiagen kit). Resuspend beads in 800uL RLT in 2mL tube.
2. Bind beads to supernatant (containing cDNA) 1 min. [beads + 800uL RLT + cDNA sup.]
3. Add 667.5uL 100% EtOH to sample, incubate for 2 min.
4. Wash 3x 1mL 80% EtOH. Let dry.
5. Resuspend in 23uL H2O. Incubate at RT 2 min, take 21uL eluate for cDNA PCR.

PCR amplify cDNA:

This will amplify only the cDNA that has received 2PUNI splint and Term barcode. Number of cycles will depend on bead input – always do 4 cycles of the 3 step PCR, any remaining additional steps can be done with the 2 step cycle. Note that 2PUni has no index – if you want to do dual indexing, we have a set of i5 indexed primers (named A1-A12). 2PBC primers contain i7 indexes and are named D1-D12.

	1 Rxn	
2Puni + 2PBC (12.5uM)	4 uL (2uL each)	
2x Q5 MM	25 uL	
Sample	21 uL	
	50uL	

Temp (C)	Time (s)	Cycles
98	60	1 cycle
98	15	4 cycles
69	15	
72	90	
98	15	8 cycles
72	90	
72	2min	

1. Cleanup by binding 1.2x volume of SPRI beads to supernatant and let sit for 5 min.
2. Wash 2x with 150 uL 80% EtOH (made fresh)
3. Elute in 20uL H2O.
4. Run libraries on E-Gel 2%EX agarose gels (15 min run). Gel cut libraries.
    a. Cut approximately ~40bp above RPM empty insert peak. (Note: don't be afraid to be liberal here. The empty insert is persistent and cDNA molecules with a short corresponding RNA sequence are unmappable.)
    b. Clean up with Zymo gel cleanup kit. Elute in 13uL.
5. Run on BioA to estimate the library's molarity and calculate the # of cycles needed to further amplify, if necessary. Note that a single gel cut is often insufficient to deplete the RPM empty insert.
6. Amplify cDNA library further with a 2-step PCR.
    a. Cycle further according to desired loading concentration of the library, based on the sequencing platform.
    b. Correct for additional library loss at this point. We can generally assume 50% of molecule loss from gel cut and SPRI, respectively (so +2 cycles to whatever you calculate).
7. SPRI cleanup and gel cut for a sequence-able library.

<u>On-bead PCR to amplify oligo:</u>

1. PCR pre-amplification **<u>on-bead</u>**

    a. Use 2PUNI_PC50 and i7 indices (use the **same i7** as the one used for the cDNA amplification above) for 4 cycles.

	1 Rxn

Temp (C)	Time (s)	Cycles

2Puni + 2PBC (12.5uM)	4 uL (2uL each)
2x Q5 MM	25 uL
Sample	21 uL
	50uL

98	60	1 cycle
98	15	4 cycles
69	15	
72	90	
72	120	

1. Clean-up with 1.2x volume of SPRI beads* – bind to supernatant and let sit for 5 min.
2. Wash 2x with 150 uL 80% EtOH (made fresh).
3. Elute in 21uL H2O.

Post-amp PCR - use the **same i7 index** (here also called 2PBC) in the following nested PCR as the **pre-amplification PCR** for the remaining cycles. If you want to do dual indexing, use the same i5 index primers as in the cDNA PCR.

	1 Rxn	
2Puni + 2PBC (12.5uM)	4 uL (2uL each)	
2x Q5 MM	25 uL	
Sample	21 uL	
	50uL	

Temp (C)	Time (s)	Cycles
98	60	1 cycle
98	15	4 cycles
69	15	
72	90	
98	15	4 cycles
72	90	
72	2min	

1. Bind 1.2x vol. SPRI beads to supernatant and let sit for 5 min.
2. Wash 2x with 150 uL 80% EtOH (made fresh).
3. Elute in 20uL H2O.
4. Run on E-Gel 2%EX agarose gels gel and excise oligo band.
    a. Clean up with Zymo gel cleanup kit. Elute in 13uL.
5. Run on BioA or tapestation.

<u>Annealing Protocol</u>

- Use this to anneal the cDNA Splint and the barcodes used in split pool.
- 10X Annealing buffer: 100mM Tris-HCl pH 7.5, 2M LiCl (100uL 1M Tris, 250uL 8M LiCl, 650uL H2O)

<u>For 90uM annealed product:</u>

1. Combine 9uL 200uM Top Strand + 9uL 200uM Bottom Strand + 2uL 10X Annealing buffer
2. Incubate at 85C for 5 min (on thermocycler)
3. Ramp down at 0.1C/sec to RT.
4. Store annealed products at -20C.

Buffers

1. CLAP-Tween:
   50mM HEPES pH 7.5
   0.1% Tween

2. High Salt Wash Buffer
   50mM HEPES pH 7.5
   1M NaCl
   1% NP-40
   0.5% Na-Deoxycholate
   0.1% SDS

3. M2
   20mM Tris pH 7.5
   50mM NaCl
   0.2% Triton X-100
   0.2% Na-Deoxycholate
   0.2% NP-40

4. M2+EDTA
   M2 + 50mM EDTA (45mL M2 + 5mL 0.5M EDTA)

5. M2+H2O
   1:1 by volume M2:H2O

6. NLS Elution Buffer (N-Lauroylsarcosine sodium salt solution)
   20mM Tris pH 7.5
   10mM EDTA
   2.5mM TCEP
   2% NLS detergent

7. RIPA Buffer
   50mM HEPES pH 7.5
   100mM NaCl
   1% NP-40
   0.5% Na-Deoxycholate
   0.1% SDS

8. 0.25M EDTA/EGTA (store at 4C to prevent EGTA from precipitating)

9. 100x Mn/Ca mix
   250mM MnCl2
   50mM CaCl2

10. 1X PBST: 1X PBS + 0.1% Tween

11. 4X B&W Buffer
    20mM Tris pH 7.5
    2mM EDTA

4M NaCl

*The ratio may change depending on the SPRI beads used.