End Repair and A-Tailing

Start Library construction with 50ul of ChIP material containing 6-8ng DNA per well in 96 well plate
Prepare Master Mix (MM) as below and add 10ul to each well
Mix well via pipet and spin plate
Incubate in thermocycler with lid pre-heated to 85°C
End repair @ 20°C for 30 min ---> A-tailing @ 65°C for 30 min ---> 4°C ∞

	1x	4 x	8 x	12 x	16 x	20 x	24 x	32 x	40 x
Fragmented, ds DNA	50	50	50	50	50	50	50	50	50
End Repair and A tailing buffer	7	42	70	98	126	154	182	238	294
End Repair and A tailing Enzyme	3	18	30	42	54	66	78	102	126
total MM/rxn	10	10	10	10	10	10	10	10	10

total vol is 60ul

Adapter Ligation

Add 45ul of MM to each well then add 5ul of adapters
Mix well via pipet and spin

	1x	4 x	8 x	12 x	16 x	20 x	24 x	32 x	40 x
Starting vol	60	60	60	60	60	60	60	60	60
PCR grade H2O	5	30	50	70	90	110	130	170	210
Ligation Buffer	30	180	300	420	540	660	780	1020	1260
DNA Ligase	10	60	100	140	180	220	260	340	420
Adapter Stock **(1uM)**	5	5	5	5	5	5	5	5	5
total MM/rxn	45	45	45	45	45	45	45	45	45

Total vol is 110ul

Post Adapter Ligation Clean Up

Add 88ul (0.8x) SPRI beads to each well and mix thoroughly
Incubate at room temperature (RT) for 2 min and return to magnet until supernatant is clear
Remove supernatant and perform 2x 200ul washes with freshly prepared 75% ethanol
Remove ethanol, spin briefly and remove residual ethanol with a P-10
Remove plate from magnet and allow beads to dry for 2-3 minutes @ RT
Add 25ul of Elution Buffer (10mM Tris) to each well and mix throroughly via pipet
Incubate at RT for 2 minutes, return to magnet and remove 20ul cleared eluate to a clean well for amplification

AMPLIFICATION

* Start with 20 uL of adapter ligated DNA

	1x	4 x	8 x	12 x	16 x	20 x	24 x	32 x	40 x
Adapter ligated library	20	20	20	20	20	20	20	20	20
Kappa HiFi HotStart Read Mix 2x	25	150	250	350	450	550	650	850	1050
F/R primers **(12.5uM)**	5	30	50	70	90	110	130	170	210
total MM/rxn	30	30	30	30	30	30	30	30	30

Total final vol/rxn 50ul

Mix well via pipet and spin

Amplification for Eppendorf Thermocyclers

98°C----[98°C---60°C----72°C]-----72°C-----4°C

45s---[15s----30s—30s]--1.0 min-- ∞

[------ 10 cycles-------]

Manual Dual-SPRI Cleanup Protocol_0.60x/0.80x

Add 50ul of EB to amplified sample and mix well (vol total 100 uL)

Add 60uL of SPRI beads to each well (.60x)

Mix sample well, incubate 2’ @ RT and place on magnet until solution clears

Remove supernatant to a clean well (this is material that you want)

Add 20uL of SPRI beads to each well (0.60x plus 0.20x equals 0.8x)

Mix well, incubate @ RT for 2’ and place on magnet until solution clears

Remove supernatant (you may want to save supt. just in case)

Wash beads x2 with 150ul of freshly prepared 75% EtOH

Spin plate return to magnet and remove all traces of EtOH with pipet

Dry DNA/SPRI pellet for 2’ then resuspend in 40ul of elution buffer

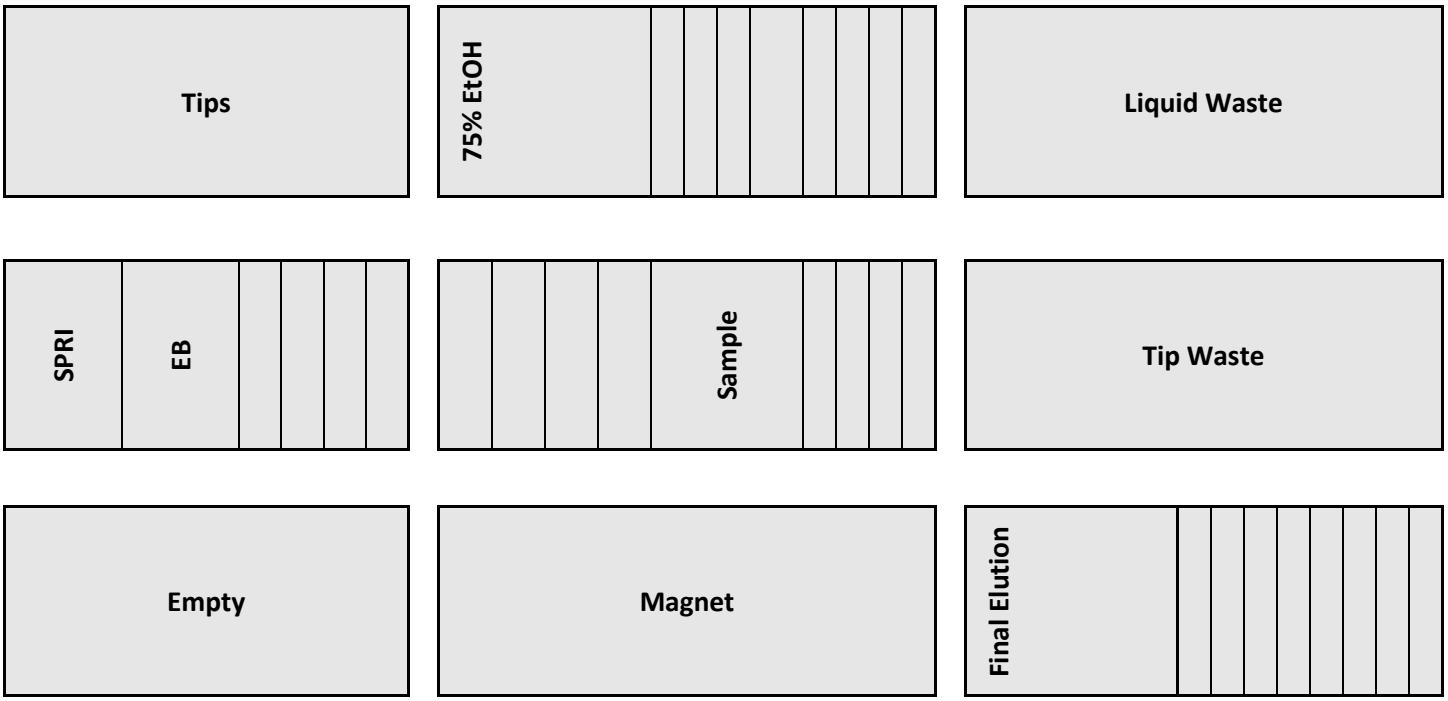
Quantify and run on BioAnalyzer

Enrichment Cleanup: Dual-SPRI Cleanup Protocol_0.55x/0.8x for Bravo

a. Stamp the following reagents into the appropriate plate and place them on the deck of the Bravo according to the diagram below.

.60x/.80x Dual SPRI Enrichment Cleanup		
Reagent	**uL Aspirated**	**uL to Stamp**
SPRI	60/20	95
75% EtOH (x2)	150 x2	400 (Deepwell)
EB	50/40	110

Bravo 0.60x/0.80x Dual SPRI Clean Up
Deck Layout



Reagents for Library Construction:

- KAPA Hyper Library Prep Kit — Roche (vendor cat. 7962363001)
- KAPA Hyper Library Prep Kit — SQM (KAPAHYPRPREPKT) 96 reactions w amplification
- Agencourt AMPure XP SPRI beads — Beckman (vendor cat. #A63881)
- Agencourt AMPure XP SPRI beads — SQM (vendor cat. AMPUREXP) 60 mL
- Elution Buffer — Qiagen (vendor cat. #19086) 10mM Tris
- Indexing F&R Primers — IDT (vendor cat. #042710ONEILL-INDEXING)
- Indexing F&R Primers — SQM (vendor cat. #INDEXPRMR500) 500ul at 25 uM

Adapter considerations

- Visit KAPA's website for adapter concentration calculators as well as other excellent technical data sheets
- Version 2.3 changes the percentage of ethanol in washes from 80% to 75%. The yield may be higher w/ 80% ethanol washes but the selection is a bit better at 75% ethanol.

Reagents	Broad Stock Room ID	Working concentration	Final conc.
P7 Adapter Plate (15uM)	INDEXADAPTPL	**1uM**	**0.05uM**
Primers, Indexing F&R (500ul@25um)	INDEXPRMR500	**12.5uM**	**1.25uM**