**Xie Lab 2016/07/11**

**TELP library preparation protocol**

PolyC tailing reaction was done by setting up the following mixture in 0.2 ml PCR tubes (Axygen, PCR-02-L-C): 28 µl of end-repaired ChIP DNA and H2O, 1 µl of 10x EX buffer (Takara, supplied with RR006A), 1 µl of 1 mM dCTP (NEB, N0446S). The mixture was heated at 95°C for 1 min and snap-cooled down on ice (denature is optional when DNA ends were blunted already) followed by adding 1 µl of terminal transferase (TDT) (NEB, M0315S) and 35 min of incubation at 37 °C. After PolyC tailing, the reaction was brought to 75°C for 20 min to inactivate TDT.

**TDT reaction: 1 ~ 1.5 hrs**

|  |  |
| --- | --- |
| Reagents | Volume ( µl) |
| DNA (in H2O or QEB)  | 28  |
| 10x EX buffer | 1 |
| 1 mM dCTP | 1 |
|  |  |
| 95°C 1 min (in PCR machine), snap cool down on ice for 1 min |
|  |
| TDT enzyme | 1 |
|  |  |
| 37°C 35 min |
| 1 mM dATP (optional) | 1 |
| 37°C 5 min |
| 75°C 20 min |
|  |  |
| Bring to RT for next step |

In the same PCR tube, the following Extension Mix was added in each TDT reaction: 6.2 µl of H2O, 0.8 µl of KAPA2G Robust HS (KAPA, KK5515), 12 µl of 5x KAPA buffer A (KAPA, supplied with enzyme), 4.8 µl of 2.5mM dNTP (Takara, supplied with RR006A), 6 µl of 2 mM MP24\_G9 primer (Sigma-Aldrich). The extension program was as follows: 95°C (3 min), 16 cycles of 47°C (1 min), 68°C (2 min), followed by 72°C (10 min).

To remove excessive extension primer, 2 µl of Exonuclease I (NEB, M0293S) and 6 µl of Exo I buffer (NEB, supplied with enzyme) were added into the above Extension reaction and incubated for 1 hr at 37°C.

**Extension reaction: 2 ~ 2.5 hrs**

|  |  |
| --- | --- |
| Reagents | Volume ( µl) |
| TDT reaction (last step) | ~30  |
|  |
| Mix with the following mixture |
|  |
| H2O | 6.2 |
| 5x KAPA buffer A | 12 |
| 2.5 mM dNTP | 4.8 |
| 2 uM MP24\_G9 | 6 |
| KAPA 2G polymerase | 0.8 |
|  |  |
| Total of master mix | 29.8 |
| Total of reaction | ~60 |
|  |  |
| Run program in PCR machine:95°C 3 min, (47°C 1 min, 68°C 2 min) x16c, 72°C 10 min |
|  |  |
| Primer digestion:  |
|  |
| 10x Exo I buffer | 6 |
| Exonuclease I | 2 |
| 37°C 50min then 72°C 10min inactive |
| 4x B&W buffer | 22 |
|  |  |
| Total  | ~90 |

During Exo I digestion, 8 µl/sample of magnetic streptavidin C1 beads (Invitrogen, 650.01) was washed 2 times with 100 µl of 1x B&W buffer (10 mM Tris-HCl pH 8.0, 0.5 mM EDTA, 1 M NaCl) by brief vortex and standing on a magnetic rack (Millipore, 20-400) for 30 sec to separate beads and remove buffer. Beads were finally resuspended in 10 µl of 1x B&W buffer waiting for use in DNA binding step.

After Exo I treatment, 22 µl of 4x B&W buffer (40 mM Tris-HCl pH 8.0, 2 mM EDTA, 4 M NaCl) was added into the 68 µl of total reaction mix and all was transferred into a 1.5 ml Eppendorf Lobind tube (Eppendorf, 0030 108.051) containing the prewashed streptadvidin beads. Beads binding was carried out in a Thermomixer (Eppendorf, 5355 000.011), shaking at 1400 rpm (10 sec on, 10 sec off) at 23°C for 30 min. After binding, supernatant was removed by aspiration when the tube is on the magnetic rack. Beads were washed once with 100 µl of 1x B&W buffer, 3 times with 150 µl of EBT buffer (10 mM Tris-HCl pH 8.0, 0.02% triton-X100) as described above and resuspended in 8.4 µl of EB buffer (10 mM Tris-HCl pH 8.0) waiting for adapter ligation.

**Beads binding: 1 ~ 1.5 hrs** (When starting DNA >2 ng, this step can be replaced by minelute column purification)

|  |  |
| --- | --- |
| Reagents | Volume ( µl) |
| Streptavidin C1 beads slurry  | 8  |
| Remove buffer, wash with 100 µl of 1x B&W 2~3 times, resuspend beads in 10 µl of 1x B&W buffer |
|  |
| Mix the following: |
| Briefly washed beads | 10 |
| digested Extension reaction | 90 |
| Shaking at 1400 rpm (10'' on 10'' off), 23°C for 15 ~30 min  |
| Washing (1x B&W) | 100 (x1) |
| Washing (EBT) | 150 (x3) |
| Resuspending buffer (EB) | 8.4 |
|  |  |
| Ready for next ligation step |

10 µM of DNA adapter was prepared ahead of ligation by mixing 5 µl of 100 µM oligo P1\_TALK\_A\_p, 5 µl of 100 µM oligo P1\_TALK\_B, 5 µl of annealing buffer (100 mM Tris-HCl pH 7.0, 100 mM NaCl) and 35 µl of H2O. The mixture was subjected to heating at 95°C for 3 min and gradual cooling down to 37°C in 1 hr.

Adapter and beads captured DNA were ligated together in a 20 µl of reaction mix, containing 1 µl of Quick ligase (NEB, M2200L), 10 µl of 2x Quick ligation buffer (NEB, supplied with enzyme), 8.4 µl of beads suspension and 0.6 µl of 10 mM adapter. Ligation was carried out in 4°C fridge over night (15 hr) on a rotating wheel to prevent beads deposition.

On next day, ligation suspension was brought to room temperature and stood on table for 10 min. Then supernatant was removed. Beads were further washed once with 100 µl of 1x B&W buffer, 3 times with 150 µl of EBT buffer. DNA was eluted in 40 µl of H2O in a thermomixer setting at 72°C, shaking at 1400 rpm (10 sec on, 10 sec off) for 30 min. After elution, beads suspension was briefly spun at 100 g for 10 sec. DNA in supernatant was collected by aspiration on the magnet and then subjected directly to PCR amplification.

**Ligation: 0.5 hr --> overnight --> 1 hr** (When beads are not used, elution step need to be replaced by column purification)

|  |  |
| --- | --- |
| Reagents | Volume ( µl) |
| Set up the following mix on ice: |
|  |  |
| DNA & beads in EB buffer | 8.4 |
| 10 uM Adapter "NL9" (annealed) | 0.6 |
| 2x quick ligation buffer | 10 |
| Quick T4 ligase | 1 |
|  |  |
| Total | 20 |
| Rotate at 4°C overnight (14 ~20 hrs)Bring to RT for 10 min |
|  |
| Carry out washing and elution: |
| Washing buffer (1x B&W) | 100 (x1) |
| Washing buffer (EBT) | 150 (x3) |
|  |  |
|  Elution (H2O +0.02% triton) | 30 |
| Shaking at 1400 rpm (10'' on 10'' off), 72°C for 30 min |
|  |  |
| Transfer supernatant to clean tube. It is ready for PCR |

**One Step PCR: 2 ~ 2.5 hrs**

|  |  |
| --- | --- |
| Reagents | Volume ( µl) |
| DNA (in H2O)  | 30 |
| 10x EX Taq buffer | 5 |
| 2.5 mM dNTP mix | 5 |
| 20 µM P1\_FL | 2 |
| 20 µM Index7 | 2 |
| EX Taq HS polymerase | 0.5 |
| ddH2O | 5.5 |
| Total | 50 |
| Run program in PCR machine:95°C 3', ( 95°C 30'', 58°C 30'', 70°C 1') x14 ~19c, 72°C 3' |
|  |
| Purify with minelute column, elute in 25 µl of EB |

Purify PCR products to remove possible dimers. (200bp-800bp)