ancient dsDNA UDG Library_Pinhasi_Lab

Category	Experimental Procedures
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Version	4

Labels:

Preparation | samples | blanks | UV tubes

1. **Choose Index tags (p5 and p7) for each sample before going to the lab**
2. **UV-treat siliconised pcr tubes, 1.5 ml tubes (3 sets for two clean-ups, 1 set for library), PE and PB as needed for 30 min.**
3. **Before starting check the availability of the next reagents: Adapter mix | indexing primers (p7 and p5) | quiagen minielute**
4. **Bring the samples to room temperature (max 10 samples per person per day).**
5. **Include a blank using the blank from the DNA extraction protocol**
6. **Include a blank for using water as template**

UDG Treatment

1. **Prepare the UDG MasterMix adding 1 extra reaction:**

UDG Treatment MasterMix

Reagent	1X	??X
Buffer Tango 10 x	6 ul	
dNTP's (10 mM each)	0.6 µl	
ATP (100 mM)	0.6 µl	
USER (1 U/ul)	3.6 ul	
ddH2O	16.4 uL	
Total	27.2 µl	

2. **Mix the mastermix by pipetting and spin down**
3. **Transfer 27.2µl of mastermix to PCR tubes**
4. **Add to each tube 25µL of DNA extract and pipette-mix**
5. **Incubate for 3 hours at 37ºC** **This is a modification from the partial UDG protocol from Pinhasi Lab**
6. **Spin down**

UDG inhibition

1. Add to each reaction 3.6 ul of UGI (2U/ul)

2.Mix the mastermix by pipeting

3.Incubate for 30 min at **37ºC**

4. Spin down

Blunt-End Repair

1. **add to each sample from the previous UDG treatment:**

Blunt-End Repair Mix

Reagent	**1X**	**9X**
T4 PNK (10 U/ul)	**3 µl**	
T4 Polymerase	**1.2 µl**	
UDG treated Sample	**55.8 µl**	
Total	**60 µl**	

2. Mix by pipetting and spin down

3. Incubate 15 min at 25ºC | 5 min at 12ºC

4. Spin down.

Sample Clean Up

1. Get the Qiagen Min-Elute purification kit: columns (fridge), PB, PE and EBT buffers. Label each column

2. Add 350µL of PB binding buffer to each Qiagen column and then add the 70µL of each end-repaired sample

3. Centrifuge 1 minute at maximum speed (13.000 rpm) and discard the flowthroug

4. Add 750µL of PE washing buffer to the Qiagen column and centrifuge again for 1 minute at 13.000 rpm

5. Dry spin again to remove all ethanol (included in the PE buffer), 1 minute, 13.000 rpm

6. Place the spin column in a new 1.5mL tube

7. Add 20ul of EB elution buffer

8. Incubate for 5 min

9. Centrifuge 1 minute at maximum speed (13.000 rpm)

10. Discard column and keep the elute in the fridge until ligation.

Adapter Ligation

1. Prepare the next mastermix adding 1 extra reaction.

Adapter ligation mix

Reagent	1X	9X
ddH2O	**10 µl**	
T4 DNA ligase buffer (10x)	**4 µ**	
PEG-4000 (50%)	**4 µl**	
Adapter mix (100uM each)	**1 µl**	
Add directly to the sample not master mix **T4 DNA ligase (5U/ul)**	**1 µl**	0
Total	**20 µl**	

2. Mix the mastermix by pipetting and spin down
3. Transfer **19µl** of **Ligation mastermix** to the 1.5 ml tubes containing the sample from clean up step (in the firdge) for a total reaction volume of 40 µl
4. Add **1ul** of **T4 DNA ligase** (5U/ul) to each sample.
5. Mix the mastermix by pipetting and spin down
5. **Incubate for 30 min at 22°C**
6. Spin down

Sample Clean Up

1. Get the Qiagen Min-Elute purification kit: columns (fridge), PB, PE and EBT buffers. Label each column
2. Add **200µL** of **PB** binding buffer to each Qiagen column and then add the **50µL** of **each ligated sample**
3. Centrifuge 1 minute at maximum speed (13.000 rpm) and discard the flowthroug
4. Add **750µL** of **PE** washing buffer to the Qiagen column and centrifuge again for 1 minute at 13.000 rpm
5. Dry spin again to remove all ethanol (included in the PE buffer), 1 minute, 13.000 rpm
6. Place the spin column in a new 1.5mL tube
7. Add **20ul** of **EB** elution buffer
8. Incubate for 5 min
9. Centrifuge 1 minute at maximum speed (13.000 rpm)
10. Discard column and keep the elute in the fridge until Fill-in.

Adapter Fill-in

1. Prepare the next mastermix adding 1 extra reaction.

Adapter Fill-in MasterMix

Reagent	1X	9X

Reagent	1X	9X
ddH2O	**13.5 µl**	**121.5**
Thermopol reaction buffer (10x)	**4 µl**	**36**
dNTPs (10mM each)	**1 µl**	**9**
Bst polymerase, large fragment (8 U/ul)	**1.5 µl**	**13.5**
Total	**20 µl**	

2. Mix the mastermix by pipetting and spin down
3. Transfer 20**µl** of **adaprer Fill-in mastermix** to the PCR tubes
4. Transfer to each tube 20**µl** of the last clean up step (in the firdge) or a total reaction volume of 40 µl
5. Mix the mastermix by pipetting and spin down
6. **Incubate for 30 min at 37°C | 20 min at 80°C | hold 4°C**
7. Spin down
8. keep the reaction in the fridge

Indexing PCR

1. Prepare the next mastermix adding 1 extra reaction.

Reagent	1X	premix = 23samples x 4 split-ins	Split-in sub-master (4)
Pfu Turbo Cx Poly	1	92	
10x Rxn buffer	5	460	
dNTPs (10mM)	1	92	
H20	29	2668	
Add this only to the split-in sub-masters			150 ul of premix
P7 (5uM)	2	-	8.4
P5 (5uM)	2	-	8,4
Template	10	-	40
Total	50		

2. Add 150 ul of premix to the PCR tube of each sample containing the last step of filling for a total volume of 190 ul
3. Add 8.4 ul of each of the previously chossen index primers(p5 and p7).
4. split into 4 PCR reactions each containing 50 ul
5. Go to the GENERAL LAB. (Do not dear to run the PCR in the clean lab).

6. Run the PCR Pfu_indexing programme in ThermoCycler: 95/2 minutes; 95/30s; 12 cycles-95/30s, 58/30s, 72/1min; 72/10min; 4/infinity.

Sample Clean Up

1. Get the Qiagen Min-Elute purification kit: columns (fridge), PB, PE and EBT buffers. Label each column
2. Pool the split in reactions in a 5 ml tube and add 500 ul x 4 PB.
3. Pipette 600 ul of mixture onto column and spin for 1 minute at maximum speed. Repeat 4x
4. Add 750 ul PE and spin for 1 minute at maximum speed
5. Remove flow-through and Dry spin again to remove all ethanol (included in the PE buffer), 1 minute, 13.000 rpm
6. Move columns to new 1.5 tubes and add **30** ul EB buffer, wait for 5 minute, spin for 1 minute at maximum speed.

Quantification

Quantyfy by qubit and Fragment analyzer.

if needed do a bead clean up using 1.5X to 1.8X beads to remove remaining adaptor dimers.

Observations

Dont use any other polimerase besides PFU as it can pass the uracils generated by damage. otherwise espect a reduction in endogenous content.

Attachments

FileID	Name
4538	Pinhasi_lab_Adaptors_19.11.20--p7 and p5 Indexes.xlsx
4806	Swarts_LAB_combs_pinhasi_indexes.xlsx
4807	Partial_UDG_aDNA_3_Double_Stranded_Library_Prep_Protocol_UCD.docx.pdf
4809	Example of UDG ds Library protocol at MPI.pdf

*This procedure was originally created by **Miguel Vallebueno***