ancient dsDNA Partial_UDG Library_Pinhasi_Lab_MV

Category	Experimental Procedures
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Version	6

Labels:

Preparation

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 and add PCR blank as water 25 ul

UDG Treatment

1. **Prepare the UDG MasterMix adding 0.5 extra reaction:**

UDG Treatment MasterMix

Reagent from Freezer, thaw on bench except for USER	**1X**	6.5x	10.5x
Buffer Tango 10 x	**6 ul**	39 ul	63 ul
dNTP's (10 mM each)	**0.6 µl**	3.9 ul	6.3 ul
ATP (10 mM)	**6 µl**	39 ul	63 ul
USER (1 U/ul) - KEEP ON ICE BLOCK, MIX GENTLY	**3.6 ul**	23.4 ul	37.8 ul
ddH2O	**11 uL**	71.5 ul	115.5 ul
Total	**27.2 µl**	176.8 ul	285.6 ul

2. Mix the mastermix by pipetting and spin down
3. Transfer **27.2µl of mastermix t**o PCR tubes
4. Add to each tube 25µL of DNA extract and pipette-mix
5. **Incubate for 30 mins at 37ºC**
6. Spin down

UDG inhibition

1. Add to each reaction **3.6 ul of UGI (2U/ul)**
2. Mix the mastermix by pipetting

3 . **Incubate for 30 min at 37ºC**

4. Spin down

Blunt-End Repair

1. **Add to each sample from the previous UDG treatment, add 0.5 as extra:**

Blunt-End Repair Mix

Reagent	**1X**	7.5X	11.5x
T4 PNK (10 U/ul) MIX GENTLY	**3 µl**	22.5 ul	34.5 ul
T4 Polymerase (5 U/ul) MIX GENTLY	**1.2 µl**	9 ul	13.8 ul
UDG treated Sample	**55.8 µl**	418.5 ul	641.7 ul
Total	**60 µl**	450 ul	690 ul

2. Add **4.2 ul of mastermix** to each sample

3. Mix by pipetting and spin down

4. **Incubate 15 min at 25ºC (thermomix or hood) | 5 min at 12ºC (thermomix or fridge in a bag)**

5. Spin down

Sample Clean Up

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

2. Add **300µL 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 flowthrough

4. Add **700µL 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 **ROTATE COLUMN**

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 (12.000 rpm)

10. Use **10ul pipette to remove elute** around ring and pass through filter

11. Centrifuge 1 minute at maximum speed (12.000 rpm)

12. 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	12x
ddH2O	**10 µl**	90 ul	120 ul
T4 DNA ligase buffer (10x)	**4 µ**	36 ul	48 ul

PEG-4000 (50%)	**4 µl**	36 ul	48 ul
Adapter mix (100uM each)	**1 µl**	9 ul	12 ul
VORTEX			
T4 DNA ligase (5U/ul)	**1 µl**	9 ul	12 ul
Total	**20 µl**	180 ul	240 ul

2. Mix the mastermix by pipetting and spin down
3. Transfer **20 µl of Ligation mastermix** to the 1.5 ml tubes containing the sample from clean up step (in the fridge) for a total reaction volume of 40 µl
4. **Incubate for 30 min at 22°C**
5. Spin down

Sample Clean Up

1. Get the Qiagen Min-Elute purification kit: columns (fridge), PB, PE and EB 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 flowthrough
4. Add **700µL 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 **ROTATE COLUMN**
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. Use **10ul pipette to remove elute** around ring and pass through filter
11. Centrifuge 1 minute at maximum speed (13.000 rpm)
12. 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**	8X	11x
ddH2O	**13.5 µl**	108 ul	148.5 ul
Thermopol reaction buffer (10x)	**4 µl**	32 ul	44 ul
dNTPs (10mM each)	**1 µl**	8 ul	11 ul
Bst polymerase, large fragment (8 U/ul)	**1.5 µl**	12 ul	16.5 ul
Total	**20 µl**	160 ul	220 ul

2. Mix the mastermix by pipetting and spin down
3. Transfer **20µl of adapter Fill-in mastermix** to the PCR tubes
4. **Transfer to each tube 20µl of the last clean up** step (in the fridge) 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	1 X	premix = 23 samples x 4 split-ins	Split-in sub-master (4)
Q5U Hot Start High-Fidelity DNA Polymerase (includes 10x Rxn Buffer and dNTPs 10mM)	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		

1. Make mastermix
2. Prepare 1.5 ml tubes for each sample and blanks; label tubes
3. Add **150 ul of mastermix** to the tube of each sample
4. To each tube, add **8.4 ul of each** of the previously chosen index primers (p5 and p7)
5. Add **10 ul of DNA template**
4. Pipette **50 ul** from the 1.5 ml tubes into 4 tube strips for PCR reactions
5. Go to the **GENERAL LAB** and don't come back after PCR step; amplicons can contaminate aDNA lab

GENERAL LAB PCR

Step	Temperature	Time
Denaturation - Initial	98 °C	5 mins
Denaturation - Cycle	98 °C	10 secs
Annealing - Cycle	60 °C	30 secs
Primer Extension - Cycle	65 °C	60 secs
Primer Extension - Final	65 °C	5 min
Reaction Termination	4 °C	Infinity

1. Run PCR program Q5U as outlined above
2. Set to 8 cycles (can adjust based on template concentration)
3. Move amplicons to fridge (stopping point) or continue to clean-up step

Sample Clean Up

1. Get the Qiagen Min-Elute purification kit: columns (fridge), PB, PE and EB buffers; label each column
2. Use a pipette to transfer/pool the split in reactions in a 5 ml tube and add 500 ul PB
3. Pipette **600 ul** of mixture onto column and spin for 1 minute at maximum speed
4. Add **700 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
7. Store in fridge or quantify immediately

Quantification

Quantify library and extraction by Qubit; library by fragment analyzer.
Store in freezer

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

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***