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Preparation of single-stranded DNA capture probes V.1

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MPI EVA Ancient DNA C...

Ancient DNA Core Unit
MPI EVA

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Abstract

This document describes the preparation of biotinylated single-stranded DNA capture probes for use in hybridization capture as described in Fu et al. 2013. It starts from amplified probe libraries carrying the following adapter sequences: ATAGGGATCGCACCAGCGTGT-variable-probe-sequence-CACTGCGGCTCCTCATCCACG. These probe libraries can be generated using custom-designed capture arrays such as one million feature arrays from Agilent Technologies (see supporting information in Fu et al. 2013; section 3.2.3.) or custom-synthesized oligonucleotide pools, for example from GenScript ('GenTitan™ Oligo Pools') or Integrated DNA Technologies ('oPools').

References

Fu, Q., Meyer, M., Gao, X., Stenzel, U., Burbano, H. A., Kelso, J., & Pääbo, S. (2013). DNA analysis of an early modern human from Tianyuan Cave, China. Proceedings of the National Academy of Sciences of the United States of America, 110, 2223-2227.

Note

This protocol describes the preparation of at least 80 µg of single-stranded probe using 96 parallel single primer extension reactions. The required template for probe production is a purified PCR products of a probe library adjusted to a concentration of 100 ng/µl. In the Ancient DNA Core Unit of the MPI-EVA, probe library templates are labeled with a probe name (e.g., AA213) followed by 'short amp'.

Materials

	Reagent/consumable	Supplier	Catalogue number
	Reagents		
	Herculase II Fusion DNA Polymerase Kit	Agilent	600679
	Oligonucleotide APL2 *	Sigma/Merck	-
	25 mM each dNTP	Thermo Fisher Scientific	R1122
	Water, Milli-Q	Merck	-
	SPRI beads (38% PEG)	self	
	Ethanol 70% (v/v)	self	
	EB buffer	self	-
	AAxx short amp library (100 ng/ µl)	self	-
	Consumables		
	50 ml Falcon tube, without stand	Greiner Bio-one	227261
	50 ml glass pipette	Greiner Bio-one	768180
	Eppendorf twin.tec PCR plate 96, semi-skirted	neoLab Migge	VB-0398
	15 ml Falcon tube, without stand	Greiner Bio-one	188261
	PCR cap strips	Eppendorf	0030124839
	Eppendorf safe-lock tubes, 1.5 ml	neoLab Migge	VB-0352

	Reagent/consumable	Supplier	Catalogue number
	Eppendorf safe-lock tubes, 2 ml	neoLab Migge	VB-0353

* Order oligonucleotide APL2 at 0.2 µmol synthesis scale (Sigma/Merck, HPLC purified). Dissolve in water at a concentration of 100 µM. Prepare a 10 µM working dilution in water. Sequence: 5'-biotin-CGTGGATGAGGAGCCGCAGTG-3'
† See document in the Appendix for the preparation of SPRI beads.
‡ See document in the Appendix for the preparation of EB buffer.

Equipment

- Thermal cycler for PCR strip tubes (e.g. Veriti 96-Well fast Thermal Cycler, cat. no. 4375305)
- Rotator (e.g. Phoenix Instrument, RS-TR05, cat. no. XK30.1)
- 50 ml magnetic separation rack for Falcon tubes (e.g. NEB, cat.no. S1507S)
- 1.5 ml magnetic separation rack (e.g. Bio-Rad, cat.no. 1614916)
- Serological pipette controller (e.g. battery-powered pipetting aid ROTILABO, cat. no. TC16.1)

Protocol

1. Prepare a master mix by combining the reagents listed in the table below in a 15 ml Falcon tube. Do not add the enzyme yet. Mix properly by vortexing and spin down. Add the polymerase, mix briefly by flipping the tube with a finger, and add 100 µl of the mix to each well of a 96-well PCR plate.

	Reagent	Volume (µl) per reaction	Volume (µl) in master mix (100 rxn)	Final concentration in reaction
	Water	65.50	6,550	
	5x Herculase II reaction buffer	20	2,000	1x
	10 µM APL2	10	1,000	1 µM
	25 mM each dNTP	1	100	0.25 mM
	Herculase II fusion polymerase	1.50	150	N/A
	Template: AAxx short amp 100 ng/µl	2	200	2 ng/µl

	Reagent	Volume (µl) per reaction	Volume (µl) in master mix (100 rxn)	Final concentration in reaction
	sum	*100*	10,000	

2. Seal the plate with PCR cap strips and centrifuge briefly. Place the plate in a thermal cycler and incubate under the following conditions:

	Step	Temperature (°C)	Time
	Initial denaturation	95	2 min
	Per cycle denaturation	95	30 s
	per cycle annealing	60	30 s
	per cycle extension	72	30 s
	Go to step 2, 19 times		
	Final extension	72	5 min
	Hold	8	forever

3. Transfer 20 ml of SPRI bead suspension (38% PEG) to a 50 ml Falcon tube. Add the PCR products from the plate to the Falcon tube and mix on a rotator for 60 min at room temperature.

4. Centrifuge the Falcon tube briefly and place it on a magnetic rack. Let it stand for about 1.5 hours.

5. Remove and discard the supernatant using a serological pipette. To wash the beads, leave the tube on the magnetic rack and add 50 ml of 70% Ethanol without disturbing the bead pellet. Let stand for approx. 2 min and remove the Ethanol with a pipette. Repeat this step for a total number of two wash steps.

6. Remove any residual Ethanol with a pipette and allow the beads to air dry for 1 - 1.5 hours.

Note

[Note]
Make sure the bead pellet is not completely drying out (no formation of cracks should be visible).

7. Remove the Falcon tube from the magnetic rack and place it horizontally on the bench. Add 300 µl EB buffer directly on top of the bead pellets. Do not move the Falcon tube. Wait for 5 min, lift the tube, close it and briefly centrifuge.

8. Resuspend the bead pellet in the bottom of the tube using a pipette. Transfer the bead suspension to a fresh 2.0 ml Eppendorf tube (tube #1).

9. Add another 50 µl EB buffer to the wall of the Falcon tube to rinse residual beads from the tube wall. Repeat this step.

10. If necessary, briefly centrifuge the Falcon tube to collect the liquid at the bottom. Resuspend beads with a pipette and transfer the suspension to the 2.0 ml Eppendorf tube (tube #1).

11. Briefly vortex tube #1 and let it stand for 5 min. Briefly centrifuge tube #1.

12. Place tube #1 into a magnetic rack and let it sit for 5 min.

13. Transfer the supernatant to a fresh 2.0 ml tube (tube #2).

14. Add 50 µl EB buffer to the the bead pellet in tube #1 and resuspend the pellet by vortexing. Let tube #1 stand for 2 min and centrifuge it briefly.

14. Place tube #1 into a magnetic rack and let it sit for 5 min.

15. Transfer the supernatant to tube #2 already containing the eluate from step 13. Briefly centrifuge tube #2 if necessary.

16. Place tube #2 in a magnetic rack and let it sit for 5 min to pellet any residual beads.

17. Transfer the final eluate to a fresh 1.5 ml tube (tube #3).

18. Measure the concentration of the final probe solution using the NanoDrop device at OD 33.

19. Dilute the probes in EB buffer to the concentration specified in the hybridization capture protocol. Required concentrations may vary depending on the characteristics of the probe set (e.g., mitochondrial vs. nuclear targets).

Note

[Note]
Since the final probe solution might be slightly foamy, it is recommended to add 2 µl of the eluate to the measuring pedestal of the NanoDrop device.

[Note]
The concentration of your final probe solution is expected to be greater than 200 ng/µl.

Note

[Labeling]
Label the final probe tubes with ssAAxx probe name, APL2, the concentration, the date and your initials.

12. Store the probe at -20°C until use.

Appendix

Document

NAME

SPRI beads, variable PEG concentration

CREATED BY
Ancient DNA Core Unit

Preview

Document

NAME

EB buffer

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Preview