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Blunt-End-Single-Tube (BEST): Adapter Hybridization

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We use this protocol and it's working

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Abstract

This protocol describes the process of the hybridization of P5 and P7 adapters for the Blunt-End-Single-Tube (BEST) library build protocol. Following this protocol produces the P5+P7 adapter complex for use in the BEST library build.

This protocol is based on: Carøe, C., Gopalakrishnan, S., Vinner, L., T. Mak, S. S., S. Sinding, M. H., Samaniego, J. A., Wales, N., Sicheritz-Pontén, T., & P. Gilbert, M. T. (2018). Single-tube library preparation for degraded DNA. *Methods in Ecology and Evolution*, *9*(2), 410-419. https://doi.org/10.1111/2041-210X.12871

Always **cite the original paper** when using this protocol, in addition to citing this protocols.io protocol.

Protocol materials

- Molecular grade H20 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**W4502
- 5M NaCl **Invitrogen - Thermo Fisher Catalog #**AM9760G
- 1M Tris-HCl, pH 8.0 **Invitrogen - Thermo Fisher Catalog #**15568025
- EB buffer **Qiagen Catalog #**19086
- 0.5M EDTA **Invitrogen Catalog #**AM9260G
- TE Buffer, RNase Free **Invitrogen Catalog #**AM9858

Before start

- UV-treat aliquots (ddH2O, etc.) for 10 minutes before use.
- Mix all the reagents well, before use.
- Do not UV Eppendorf Tubes/PCR tubes
- Clean all surfaces with 5% bleach solution followed by 70% ethanol before and after use.
- Clean all equipment with 70% ethanol before and after use.

Buffer Preparation

1 **Prepare the following reagents:**

Adapters (sequences in table below):

- IS1 (stock: 500µM)
- IS2 (stock: 500µM)
- IS3 (stock: 500µM)

Other reagents:

- Molecular grade H20 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**W4502
- 5M NaCl **Invitrogen - Thermo Fisher Catalog #**AM9760G
- 1M Tris-HCl, pH 8.0 **Invitrogen - Thermo Fisher Catalog #**15568025 (identical to EB buffer **Qiagen Catalog #**19086)
- 0.5M EDTA **Invitrogen Catalog #**AM9260G
- TE Buffer, RNase Free **Invitrogen Catalog #**AM9858

Table 1 Adapter oligo sequences in 5'-3' direction. Sequences sourced from Carøe et al. (2018) Table S1.

	Oligo	Sequence
	IS1	5′OMedT*ACACTCTTTCCCTACACGACGCTCTTCCGATCT*A
	IS2	5′OMedT*GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT*A
	IS3	AGATCGGAAGAGC[C3spacer]

*=Phosphothioate linkage
All oligos can be synthesized at Biomers, Germany, HPLC purified, shipped dry, and resuspended in 10 mM Tris-HCl.

2 **Prepare the oligo hybridization buffer (10X)**

	Reagent	Volume	Final Concentration
	5M NaCl	50 µL	500 mM
	1M Tris-HCl, pH 8.0	5 µL	10 mM
	0.5M EDTA	1 µL	1 mM

	Reagent	Volume	Final Concentration
	ddH2O	444 µL	/
	Total	500 µL	

Preparation of double stranded adapter

3 **P5 Adapter (in PCR tube)**

	Reagent	Volume
	IS1 (500µM)	40 µL
	IS3 (500µM)	40 µL
	ddH2O	10 µL
	Oligo hybridization buffer	10 µL
	Total	100 µL

4 **P7 Adapter (in a different PCR tube to P5)**

	Reagent	Volume
	IS2 (500µM)	40 µL
	IS3 (500µM)	40 µL
	ddH2O	10 µL
	Oligo hybridization buffer	10 µL
	Total	**100 µL**

5 Vortex both tubes & spin down. Make sure they are well mixed.

6 Incubate in a thermal cycler with a heated lid (105°C) at 95°C for 10s, then ramp down to 12°C at a rate of 0.1°C per second (see table below).

	Temperature	Time
	95°C	10 sec
	Ramp down to 12°C	0.1°C per sec
	10°C	Hold

7 After cooldown, mix the two adapters in a 1.5 mL Eppendorf tube. This is your final stock tube, so label properly with name (e.g. BEST P5+P7 adapter mix), date, and concentration.
This gives a P5+P7 solution of **200 µM**.

8 Dilute with TE buffer to the desired working concentration, e.g., 20 µM, 10 µM, 5 µM, 3 µM, 2 µM.
The working concentration depends on the input DNA amount, which will be specified in the BEST library build protocol.

Protocol references

Carøe, C., Gopalakrishnan, S., Vinner, L., T. Mak, S. S., S. Sinding, M. H., Samaniego, J. A., Wales, N., Sicheritz-Pontén, T., & P. Gilbert, M. T. (2018). Single-tube library preparation for degraded DNA. *Methods in Ecology and Evolution*, *9*(2), 410-419. https://doi.org/10.1111/2041-210X.12871