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# Santa Cruz Reaction (SCR): Adapter-Splint Hybridization

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**We use this protocol and it's working**

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## Abstract

This protocol describes the preparation and hybridization of P5 and P7 adapter-splint complexes for Santa Cruz Reaction library construction. Following this protocol produces 12µM hybridized P5 and P7 adapter-splint complexes for SCR library build.

This protocol is based on: Kapp, J. D., Green, R. E., & Shapiro, B. (2021). A Fast and Efficient Single-stranded Genomic Library Preparation Method Optimized for Ancient DNA. Journal of Heredity, 112(3), 241-249. https://doi.org/10.1093/jhered/esab012

**Always cite the original paper** (Kapp et al. 2021) when using this protocol, in addition to citing this protocols.io protocol.

## Protocol materials

- 1M Tris-HCl, pH 8.0 **Invitrogen - Thermo Fisher Catalog #**15568025
- UltraPure™ 0.5M EDTA pH 8.0 **Thermo Fisher Scientific Catalog #**15575020
- T4 RNA Ligase Reaction Buffer - 3.0 ml **New England Biolabs Catalog #**B0216L

## Before start

- UV-treat aliquots (ddH2O, etc.) for 10 minutes before use.
- Well mix all the reagents 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 & Oligonucleotide Resuspension

1 **Prepare TE buffer in which to resuspend adapters**

- Store TE buffer at room temperature

Reagents:

1M Tris-HCl, pH 8.0 **Invitrogen - Thermo Fisher Catalog #**15568025

UltraPure™ 0.5M EDTA pH 8.0 **Thermo Fisher Scientific Catalog #**15575020

**Table 1** TE buffer preparation

| | Component | Final conc. (mM) | Volume (uL) |
|---|---|---|---|
| | 0.5M EDTA, pH 8.0 | 1 | 8 |
| | 1M Tris-HCl, pH 8.0 | 10 | 40 |
| | $H_2O$ | - | 3,952 |
| | Total | - | 4,000 |

**Note:**

- Final TE buffer volume depends on required amount per oligo for 100mM (rounded to 4ml for 4 tubes).

2 **Oligo Sequences & Resuspension**

- Note: All oligonucleotides are ordered with HPLC purification conducted by IDT.
- Note: It is recommended by Kapp et al. (2021) to order two separate batches of each splint oligonucleotide. See oligonucleotide quality control recommendations in section 11 of Kapp et al. (2021) Supplementary Material.
- The oligonucleotides are usually delivered as a dry film in a 2 mL tube.

2.1 Resuspend all oligos to 100µM using TE buffer. Make sure all the oligos are well mixed by vortexing and mixing by pipetting. Use Table 2 as a template.

Note

- Before resuspending the oligos, centrifuge the tubes for 30s at maximum speed. This ensures the dry film of oligos is at the bottom of the tube, as sometimes these can come loose and might fall out of the tube when you open it.
- The volume TE buffer used for resuspension depends on the molar or ng amount of oligo in the tube - this information is usually provided on the tube itself or the accompanying paperwork.

- **Table 2** Oligonucleotides sequences (IDT Format, 5′ → 3′) and the volume of TE required to reach a final concentration of 100 µM.

| | Oligo | Sequence | Size (bp) | Oligo amount | TE vol (µL) for 100µM final conc. |
|---|---|---|---|---|---|
| | P5 adapter | /5AmMC12/ACACTCTTTCCCTACACGACGCTCTTCCGATCT | 33 | | |
| | P5 splint | /5AmMC6/NNNNNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT/3AmMO/ | 40 | | |
| | P7 adapter | /5Phos/AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC/3AmMO/ | 34 | | |
| | P7 splint | /5AmMC12/GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNNNN/3AmMO/ | 41 | | |

## Adapter-Splint Hybridization Recipe

3 **P5 Adapter-Splint Hybridization: 12µM (50µL)**

Reagent: 10X

T4 RNA Ligase Reaction Buffer - 3.0 ml **New England Biolabs Catalog #**B0216L

**Table 3** P5 adapter-splint recipe.

| | Reagent | Volume (µL) |
|---|---|---|
| | $H_2O$ | 30.6 |
| | 10X T4 RNA ligase buffer | 5 |
| | 100µM P5 adapter | 6 |
| | 100µM P5 splint | 8.4 |
| | Total | 50 |

4 **P7 Adapter-Splint Hybridization: 12µM (50µL)**

Reagent: 10X

T4 RNA Ligase Reaction Buffer - 3.0 ml **New England Biolabs Catalog #**B0216L

**Table 4** P7 adapter-splint recipe.

| | Reagent | Volume (µL) |
|---|---|---|
| | $H_2O$ | 30.6 |
| | 10X T4 RNA ligase buffer | 5 |
| | 100µM P7 adapter | 6 |
| | 100µM P7 splint | 8.4 |
| | Total | 50 |

## Adapter-Splint Hybridization Reaction

5 Add components from the **Adapter Hybridization Recipe** to separate 0.2mL PCR tubes (one for P5, one for P7). Ensure proper mixing by pipetting or flicking the tube and spinning down in a minicentrifuge.

6 Perform hybridization in a thermocycler with heated lid ( 105 °C ):

Adapter-splint hybridization reaction conditions.

| | Temperature | Time |
|---|---|---|
| | 95°C | 1 minute |
| | Ramp down to 10°C | 0.1°C per second |
| | 10°C | Hold indefinitely |

7 Store hybridized P5 (12 µM) and P7 (12 µM) adapter stock solutions at -20 °C.

## Protocol references

Kapp, J. D., Green, R. E., & Shapiro, B. (2021). A Fast and Efficient Single-stranded Genomic Library Preparation Method Optimized for Ancient DNA. Journal of Heredity, 112(3), 241-249. https://doi.org/10.1093/jhered/esab012