# Illumina library construction Protocol

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## Abstract

This single-tube library construction protocol is for degraded DNA using adapters for the Illumina platform.

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## Before start

Calculate the DNA input and prepare the library adapters.

## Materials

- Bovine Serum Albumin by Contributed by users
- 25% Poly-Ethylene Glycol by Contributed by users
- NaCl by Contributed by users

PB binding buffer 19066 by Qiagen

- Monarch DNA Cleanup Columns (5ug) - 100 columns T1034L by New England Biolabs

Buffer PE 19065 by Qiagen

Buffer EB 19086 by Qiagen

0.2 mM dNTPs AM8200 by Thermo Fisher Scientific

AmpliTaq Gold DNA polymerase by Applied Biosystems

MgCl2 by Applied Biosystems

GeneAmp® 10X PCR Buffer II View by Applied Biosystems

SYBR Green by Thermo Fisher Scientific

- IS7 and IS8 primers following Meyer and Kircher 2010 by Contributed by users

AccuGene molecular biology water 51200 by Lonza

BSA-Molecular Biology Grade - 12 mg B9000S by New England Biolabs

Illumina InPE 1.0 forward by Contributed by users

custom made reverse primers by Contributed by users

## Protocol

End-repair

**Step 1.**

End-repair step following Carøe et al. 2017 with the addition of 1 µl "Reaction enhancer" consisting of 25% Poly-Ethylene Glycol (PEG4000); 2 µg/µL Bovine Serum Albumin (BSA) and 400 mM NaCl.

NOTES

**GigaScience Database** 02 Jun 2017

Carøe C, Gopalakrishnan S, Vinner L, Mak SST, Sindin MHS, Samaniego JA, et al. Single-tube library preparation for degraded DNA. Methods in ecology and evolution; 2017; in press.

Ligation

**Step 2.**

Ligation step following Carøe et al. 2017 with addition of 1 µl 10 µM adapters suited for the Illumina platform.

NOTES

**GigaScience Database** 02 Jun 2017

Carøe C, Gopalakrishnan S, Vinner L, Mak SST, Sindin MHS, Samaniego JA, et al. Single-tube library preparation for degraded DNA. Methods in ecology and evolution; 2017; in press.

Adpater Fill-in & library purification

**Step 3.**

Fill-in step of the adapter to complete library building and purification using 1:5 volume of PB binding buffer (Qiagen) and using Monarch DNA Cleanup Columns (New England Biolabs, Massachusetts, USA).

NOTES

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Adpater Fill-in & library purification

**Step 4.**

Wash with 750 µl buffer PE (Qiagen).

## Adpater Fill-in & library purification

**Step 5.**

Incubate for 5 minutes at 37 °C.

DURATION
00:05:00

## Adpater Fill-in & library purification

**Step 6.**

Elute in 40 µl buffer EB (Qiagen).

## Quantitative real-time PCR (qPCR)

**Step 7.**

To determine the number of cycles used in index PCR, perform a qPCR in a 20 µl reaction volume using 1:20 dilution of:

- purified library template
- 0.2 mM dNTPs (Invitrogen)
- 0.04 U/µl AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, California, USA)
- 2.5 mM MgCl2 (Applied Biosystems)
- 1X GeneAmp® 10X PCR Buffer II (Applied Biosystems)
- 1 µl SYBR Green (Invitrogen, Carlsbad, California, USA)
- 0.2 µM forward and reverse primers mixture (IS7 and IS8 primers following Meyer and Kircher 2010)
- 13.48 µl AccuGene molecular biology water (Lonza)

## Quantitative real-time PCR (qPCR)

**Step 8.**

qPCR cycling conditions:

| cycles | temprature | time |
|---|---|---|
| 1 | 95 °C | 10 min |
| | 95 °C | 30 sec |
| 40 | 60 °C | 60 sec |
| | 72 °C | 60 sec |

(using the Agilent MX3005 qPCR machine)

## PCR amplification

**Step 9.**

100 µl PCR reactions containing:

- 20 µl of purified library
- 0.2 mM dNTPs (Invitrogen)
- 0.1 U/µl AmpliTaq Gold DNA polymerase (Applied Biosystems)
- 2.5 mM MgCl2 (Applied Biosystems)
- 1X GeneAmp® 10X PCR Buffer II (Applied Biosystems)
- 0.4 mg/ml BSA (New England Biolabs Inc)
- 0.2 µM of each forward (Illumina InPE 1.0 forward)
- custom made reverse primers
- 51.2 µl AccuGene molecular biology water (Lonza, Basel, CH)

## PCR amplification

**Step 10.**

PCR cycling conditions:

| cycles | temprature | time |
|---|---|---|
| initial denaturation | 95 °C | 12 min |
| 13-21 | 95 °C | 30 sec |
| | 60 °C | 30 sec |
| | 72 °C | 40 sec |
| elongation step | 72 °C | 5 min |

## PCR amplification

**Step 11.**

Post-PCR, purify libraries with QiaQuick columns (Qiagen).

## PCR amplification

**Step 12.**

Incubate libraries for 10 min at 37 °C.

DURATION
00:01:00

## PCR amplification

**Step 13.**

Elute libraries with 30 µl buffer EB (Qiagen).

## Bead purification

**Step 14.**

Use small amounts of aliquots after purification for concentration quantification and fragment size estimation with the High-Sensitivity DNA Assay kit 2100 expert High Sensitivity DNA Assay in

Bioanalyzer 2100 (Agilent).

## Bead purification

**Step 15.**

Final purification using the AMpure XP system (Agentcourt, Beckman Counter, Indianapolis, USA) with 1.8X beads:library ratio, in order to remove any persisting primer dimers or other molecules with a fragment size of <100 bp.

## Quantification

**Step 16.**

Prior to sequencing submission, use small amounts of aliquots after purification concentration quantification and fragment size estimation with the High-Sensitivity DNA Assay kit 2100 expert High Sensitivity DNA Assay in Bioanalyzer 2100 (Agilent).

## Quantification

**Step 17.**

Pool libraries in equimolar concentrations for sequencing.

NOTES

**GigaScience Database** 02 Jun 2017

Meyer M, Kircher M. Illumina sequencing library preparation for highly multiplexed target capture and sequencing. Cold Spring Harb. Protoc. 2010;2010: db.prot5448.