Protocol

# Whole-Exome Enrichment with the Illumina TruSeq Exome Enrichment Platform

Rui Chen, Hogune Im, and Michael Snyder[1]

*Department of Genetics, Stanford University School of Medicine, Stanford, California 94305*

Multiple platforms are available for whole-exome enrichment and sequencing (WES). This protocol is based on the Illumina TruSeq Exome Enrichment platform, which captures ~62 Mb of the human exonic regions using 95-base DNA probes. In addition to covering the RefSeq and Ensembl coding sequences, the enriched sequences also include ~28 Mb of RefSeq untranslated regions (UTR). The protocol can be performed at the benchside without the need for automation, and the resulting library can be used for targeted next-generation sequencing on an Illumina HiSeq 2000 sequencer.



## MATERIALS

**It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.**

### Reagents

Agarose gels, precast (E-Gel EX, 2% [Life Technologies G4020–02; 20 gels])
Agencourt AMPure XP magnetic bead-based purification system (Beckman Coulter A63881; 60 mL)
Bioanalyzer High-Sensitivity DNA Kit (Agilent 5067-4626)
dATP (100 mM; New England BioLabs N0440S)
End-It DNA End-Repair Kit (Epicentre ER0720)
Ethanol, molecular biology grade (Sigma-Aldrich E7023)
*Prepare a solution of 70% ethanol.*
Genomic DNA, human (high-quality, nondegraded, with an average fragment size of 40 kb or greater and an A260/A280 of 1.8–2.0)
Klenow fragment (3′ → 5′ exo-) with NEB Buffer #2 (New England BioLabs M0212s)
LigaFast Rapid DNA Ligation System (Promega M8221)
MinElute PCR Purification Kit (QIAGEN 28006)
Nuclease-free water (UltraPure Distilled Water [Life Technologies 10977-015] or equivalent)
PE Adapter Oligo Mix (Illumina 1001782)
PE oligonucleotides
PE-1.0: 5′-AAT GAT ACG GCG ACC ACC GAG ATC TAC ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC* T-3′ (25 μM)

[1]Correspondence: mpsnyder@stanford.edu


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PE-2.0: 5′-CAA GCA GAA GAC GGC ATA CGA GAT CGG TCT CGG CAT TCC TGC TGA ACC GCT CTT CCG ATC* T-3′ (25 μM)
*The asterisk symbolizes the addition of phosphorothioate bond between the last two deoxyribonucleotides to prevent degradation.*

Phusion High-Fidelity PCR Master Mix (New England BioLabs F-531L; 500 reactions)
TE Buffer (Life Technologies 12090-015)
TruSeq Exome Enrichment Kit (Illumina FC-121-1096 for 96 reactions)

## Equipment

2100 Bioanalyzer (Agilent)
Adhesive film (MicroAmp Clear [Life Technologies 4306311] or equivalent)
AFA microTUBES with snap caps (Covaris 520045)
DNA LoBind tubes, 1.5-mL (Eppendorf 022431021 or equivalent)
Heat blocks at 37°C and 70°C
Magnetic plate (Dynal DynaMag-96 Side Magnet [Life Technologies 12331D] or equivalent)
Magnetic stand (Dynal DynaMag-2 [Life Technologies 12321D] or equivalent)
Microcentrifuge (Fisher Scientific AccuSpin Micro 17 or equivalent)
Mini LabRoller (Labnet H5500 or equivalent)
PCR plates, 96-well (Agilent 410088 or equivalent)
PCR plates, skirtless 96-well (300-μL) (E&K Scientific 480096 or equivalent)
PCR tubes, 0.2-mL (sterile)
Pipette tips (sterile, nuclease-free with aerosol barrier)
Plate centrifuge (Thermo Scientific Sorvall Legend XTR or equivalent)
Storage plate, 96-deepwell (Thermo Scientific; Fisher AB-0859) (optional; see Step 26)
Thermal cycler (DNA Engine Tetrad 2 [BioRad PTC-0240G])
Ultra-sonicator (Covaris S-series Single Tube Sample Preparation System, Model S2)
*Set up the ultrasonicator following the manufacturer's instructions. Make sure the tank is filled with fresh deionized $H_2O$ chilled to 2°C–5°C and degassed for at least 30 min before use.*

Vacuum manifold (QIAvac 24 Plus; QIAGEN 19413)
Vortex (Fisher Scientific 02-215-370 or equivalent)



## METHOD

*The general scheme of DNA preparation for hybridization-based whole-exome capture and sequencing is diagrammed in Figure 1. The following protocol is based on the original method provided by Illumina (TruSeq Enrichment Guide, Revision H), with minor modifications based on our experience. A detailed performance evaluation of this method as well as a comparison to alternative platforms can be found in our previous publication (Clark et al. 2011); see Related Information.*

### Fragmenting Genomic DNA

1. In a 1.5-mL LoBind tube, dilute 3 μg of human genomic DNA in TE buffer to a total volume of 120 μL. Transfer the diluted DNA to an AFA microTUBE.
2. Load the microTUBE into the tube holder of the ultrasonicator and shear the DNA using the following settings: mode, frequency sweeping; duty cycle, 10%; intensity, 5; cycles per burst, 200; duration, 60 sec × 6 cycles; temperature, 4°C–7°C.
3. Transfer the sheared DNA to a fresh 1.5-mL LoBind tube. Check the shearing by running 2 μL of the sample on a 2% E-Gel EX gel.
   *The average DNA size should be ~150–200 bp.*
   *It is safe to stop here and store the sample at −20°C.*

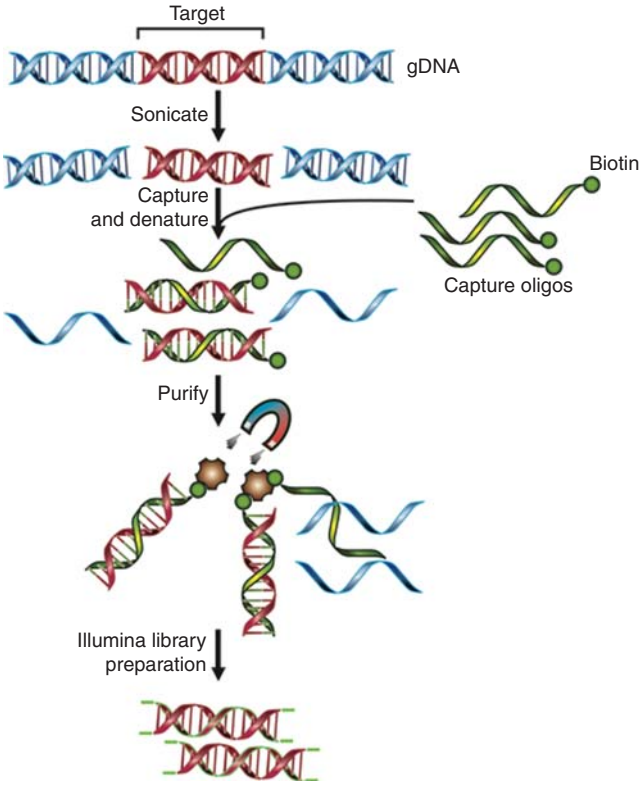


**FIGURE 1.** Common scheme for hybridization-based whole-exome enrichment methods. In summary, genomic DNA is fragmented, denatured, and hybridized with capture oligos during library preparation for high-throughput sequencing. The captured sequences are then enriched with streptavidin-conjugated paramagnetic beads and further amplified before being subjected to Illumina sequencing. Diagrammed DNA sequences in red, on-target regions; sequences in blue, off-target regions; single-stranded oligos in green, capture probes labeled with biotin; brown particles, streptavidin-conjugated paramagnetic beads.



## Purifying DNA with Agencourt AMPure XP Magnetic Beads

4. Equilibrate the Agencourt AMPure XP reagent for at least 30 min to room temperature and mix well by vortexing.
5. Add 216 µL of homogenous AMPure XP reagent to the sheared DNA sample (~120 µL) and mix well by vortexing. Incubate the mixture on the Mini LabRoller for 5 min at room temperature.
6. Place the tube in the magnetic stand. Wait for the solution to clear (~2 min) and carefully discard the cleared solution without touching the beads.
7. Wash the beads twice with 500 µL of 70% ethanol for 1 min per wash.
8. Dry the beads for 5 min in a heat block at 37°C.
9. Add 34 µL of nuclease-free water to the beads and mix thoroughly by vortexing. Incubate the mixture on the Mini LabRoller for 5 min at room temperature.
10. Place the tube back into the magnetic stand and wait for the solution to clear (~2 min). Transfer the supernatant containing the eluted DNA to a fresh 1.5-mL LoBind tube.

*It is safe to stop here and store the sample at −20°C.*

## Preparing the Adaptor-Ligated DNA Library

### *Repairing DNA Ends*

11. For each sample of sheared DNA, prepare the following reaction in a 1.5-mL LoBind tube using the reagents provided in the End-It DNA End-Repair Kit.


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| Reagent | Amount to add |
|---|---|
| DNA sample from Step 10 | 34 µL |
| 10× End-Repair Buffer | 5 µL |
| 2.5 mM dNTP mix | 5 µL |
| 10 mM ATP | 5 µL |
| End repair-enzyme mix | 1 µL |
| Total volume | 50 µL |

12. Incubate the reaction for 45 min at room temperature, and then inactivate the enzymes for 10 min at 70°C.
13. Purify the end-repaired DNA using one MinElute column according to the manufacturer's instructions. Briefly, add 250 µL of Buffer PB to the reaction, mix well, load the mixture onto a MinElute column and filter on the QIAvac 24 Plus vacuum manifold, wash the column with 750 µL of Buffer PE, centrifuge the column at maximum speed (17,000*g*) for 1 min, and elute the DNA in 32 µL of Buffer EB.

### *Adding an Adenine Base to End-Repaired Fragments*

14. For each sample, prepare the following reaction in a 0.2-mL PCR tube.

| Reagent | Amount to add |
|---|---|
| DNA sample from Step 13 | 32 µL |
| NEB Buffer #2 | 5 µL |
| 1 mM dATP | 10 µL |
| Klenow fragment (3′ → 5′ exo-; 5 U/µL) | 3 uL |
| Total volume | 50 µL |

15. Incubate the reaction in a thermal cycler for 30 min at 37°C.
16. Purify the DNA using a MinElute column as described in Step 13, but elute the purified DNA in 19 µL of Buffer EB.

### *Ligating the Paired-End Adaptor*

17. For each sample, prepare the following reaction in a 1.5-mL LoBind tube.

| Reagent | Amount to add |
|---|---|
| DNA sample from Step 16 | 19 µL |
| 2× Rapid Ligation Buffer (LigaFast) | 25 µL |
| PE Adaptor Oligo Mix (15 µM) | 1 µL |
| T4 DNA ligase (3 U/µL) (LigaFast) | 5 µL |
| Total volume | 50 µL |

18. Incubate the reaction at room temperature for 15 min.
19. Purify the ligated DNA using a MinElute column as described in Step 13, but elute the purified DNA in 23 µL of Buffer EB.

### *Amplifying the Adaptor-Ligated Library*

20. For each sample, prepare the following reaction in a 0.2-mL PCR tube.

| Reagent | Amount to add |
|---|---|
| Adaptor-ligated library DNA from Step 19 | 23 µL |
| Phusion high-fidelity PCR master mix (2×) | 25 µL |
| PE-1.0 (25 µM) | 1 µL |
| PE-2.0 (25 µM) | 1 µL |
| Total volume | 50 µL |

21. Place the tubes in a thermal cycler and amplify the library DNA using the following program: 30 sec at 98°C, 12 cycles of (10 sec at 98°C, 30 sec at 65°C, 30 sec at 72°C), 5 min at 72°C.
22. Purify the amplified sample library using one MinElute column as described in Step 13, but elute the DNA in 31 µL of nuclease-free water.
23. Check the quality of the amplified preenrichment library by diluting the DNA 1:50 and running 1 µL on a high-sensitivity DNA chip using a 2100 Bioanalyzer following the manufacturer's instructions. Record the measured concentration of the library.

*The amplified library should have a peak size of ~350 bp.*

*It is safe to stop here and store the sample at −20°C.*

## Hybridizing the Library

### *First Hybridization*

24. For each library, dilute 500 ng of the amplified DNA from Step 22 to 40 µL with nuclease-free water. In a 96-well PCR plate, prepare the first hybridization reaction using the reagents provided in the TruSeq Exome Enrichment Kit as follows. Mix well by pipetting up and down 10 times.

| Reagent | Amount to add |
|---|---|
| Amplified preenrichment library DNA (500 ng) | 40 µL |
| Capture Target Oligos | 10 µL |
| Capture Target Buffer 1 | 50 µL |
| Total volume | 100 µL |

25. Seal the plate with adhesive film and centrifuge briefly to collect the reaction at the bottom of the well. Incubate the plate in a thermal cycler using the following program: 10 min at 95°C, 18 cycles of (1 min at 93°C, decreasing 2°C per cycle), 20 h at 58°C.

### *First Wash*

*Reagents for Steps 28–35 are provided in the TruSeq Exome Enrichment Kit.*

26. Briefly centrifuge the plate after incubation. Transfer the entire contents of each well to a fresh 1.5-mL LoBind tube (or to the corresponding wells of a deepwell storage plate, if processing multiple samples).
27. Resuspend the Streptavidin Magnetic Beads by vortexing. Add 250 µL of bead suspension to each sample and mix by pipetting up and down 10 times. Incubate for 30 min at room temperature. Briefly centrifuge the tube, separate the beads on a magnetic stand, and discard the supernatant when it becomes clear.
28. Vortex the tube of Wash Solution 1 until no crystal structures are visible. Add 200 µL of Wash Solution 1 to the beads and resuspend by pipetting up and down 10 times. Briefly centrifuge the tube, separate the beads on a magnetic stand, and discard the supernatant when it becomes clear.
29. Vortex the tube of Wash Solution 2 tube for 5 sec. Add 200 µL of Wash Solution 2 to the beads and resuspend by pipetting up and down 10 times. Briefly centrifuge the tube, separate the beads on a magnetic stand, and discard the supernatant when it becomes clear.
30. Add 200 µL of Wash Solution 2 to the beads and resuspend by pipetting up and down 10 times. Transfer the resuspended beads to one well of a 300-µL 96-well PCR plate. Seal the plate and incubate on a thermal cycler for 30 min at 42°C with the heated lid set to 100°C. Briefly centrifuge the plate, separate the beads on a magnetic plate, and discard the supernatant when it becomes clear. Work as fast as possible during this step to maintain temperature.
31. In the same PCR plate, repeat Step 30 with 200 µL of Wash Solution 2.
32. Add 200 µL of Wash Solution 3 to the beads and resuspend by pipetting up and down 10 times. Transfer the resuspended beads to a new 1.5-mL LoBind tube. Briefly centrifuge the tube, separate the beads on a magnetic stand, and discard the supernatant when it becomes clear.

33. In the same tube, repeat Step 32 using 200 µL of Wash Solution 3.
34. For each library, prepare the elution premix by mixing 28.5 µL of Elute Target Buffer 1 with 1.5 µL of 2 N NaOH in a separate tube. Add 30 µL of elution premix to the beads, resuspend by pipetting up and down 20 times, close the cap and let the mixture stand at room temperature for 5 min.
35. Briefly centrifuge the tube and separate the beads on a magnetic stand. When the supernatant becomes clear, transfer 29 µL of supernatant to a fresh 1.5-mL LoBind tube containing 5 µL of Elute Target Buffer 2. Mix the eluted library by vortexing the tube for 5 sec and centrifuge briefly.

*It is safe to stop here and store the sample at −20°C.*

### *Second Hybridization and Wash*

*Reagents for the following section are provided in the TruSeq Exome Enrichment Kit.*

36. In a 96-well PCR plate, prepare the second hybridization reaction as follows. Mix well by pipetting up and down 10 times.

| Reagent | Amount to add |
|---|---|
| Eluted library DNA from Step 35 | 30 µL |
| Nuclease-free water | 10 µL |
| Capture Target Oligos | 10 µL |
| Capture Target Buffer 1 | 50 µL |
| Total volume | 100 µL |

37. Seal the plate and centrifuge briefly to collect the reaction at the bottom of the well. Incubate the plate in a thermal cycler using the following program: 10 min at 95°C, 18 cycles of 1 min at 93°C, decreasing 2°C per cycle, and 20 h at 58°C.
38. Repeat Steps 26–35 to perform the second wash and elution.

*The final volume of each eluted library is 34 µL.*

*It is safe to stop here and store the sample at −20°C.*

## Performing Post-Capture Amplification

39. In a 96-well PCR plate, prepare the PCR amplification reaction as follows using the reagents provided in the TruSeq Exome Enrichment Kit. Mix well by pipetting up and down 10 times.

| Reagent | Amount to add |
|---|---|
| Eluted library DNA from Step 38 | 10 µL |
| Nuclease-free water | 10 µL |
| PCR Master Mix | 25 µL |
| PCR Primer Cocktail | 5 µL |
| Total volume | 50 µL |

40. Seal the plate and centrifuge briefly to collect the reaction at the bottom of the well. Incubate the plate in a thermal cycler using the following program: 30 sec at 98°C, 18 cycles of (10 sec at 98°C, 30 sec at 60°C, 30 sec at 72°C), 5 min at 72°C.
41. Purify the amplified library with AMPure XP beads as described in Steps 4–10, with the following modifications: Add 90 µL of homogenous AMPure XP reagent to the reaction, and elute the purified DNA in 30 µL of QIAGEN Buffer EB.
42. Check the quality of the final library as follows.
    i. Run 1 µL on a high-sensitivity DNA chip using the Agilent 2100 Bioanalyzer following the manufacturer's instructions.

*The amplified library should have a peak size of ~350 bp.*

ii. Record the measured concentration of the library for reference when pooling multiple libraries.

*We recommend pooling libraries based on their molar concentration as determined by the Bioanalyzer.*

*The library is now ready for paired-end sequencing on an Illumina HiSeq 2000 sequencer. Alternatively, the library can be placed at −80°C for long-term storage.*

## RELATED INFORMATION

Multiple platforms are available for WES, particularly for use with Illumina next-generation sequencers. In our previous publication (Clark et al. 2011), we compared the performance of three of the most popular hybridization-based whole exome capture and sequencing platforms for the human exome, including the Illumina TruSeq system described here; protocols for the two other platforms are provided in **Whole-Exome Enrichment with the Agilent SureSelect Human All Exon Platform** (Chen et al. 2015a) and **Whole-Exome Enrichment with the Roche Nimblegen SeqCap EZ Exome Library SR Platform** (Chen et al. 2015b). In summary, the performance of each platform was generally on par with the others, with specific, but minor, pros and cons. Researchers would be wise to choose among the platforms based on their specific experimental needs (e.g., genomic regions of interest, coverage vs. depth, etc.). The size of the targeted regions for each platform is flexible: By designing custom probes with Agilent, Roche, or Illumina, each protocol is suitable for enrichment of a lower number of custom targets for Illumina sequencing.

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