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Product Information

SEQPLEX™-I WGA Kit
Whole Genome Amplification, DNA Amplification

Catalog Number **SeqXi**
Storage Temperature -20 °C

TECHNICAL BULLETIN

Product Description

The SeqPlex™-i DNA Amplification Kit for whole genome amplification (WGA) is designed to facilitate Illumina next-generation sequencing (NGS) from extremely small quantities or from degraded/highly fragmented DNA. The SeqPlex™-i WGA kit allows amplification of such samples for direct input onto Illumina Next Generation Sequencing (NGS) flow cells. The SeqPlex™-I process is comprised of three steps: Pre-amplification/Library Synthesis, Amplification 1 and Amplification 2. See Process Workflow chart on page 2.

Materials and Reagents Required but Not Provided

- Thermal cycler & qPCR instrument
- Sample DNA
- Spectrophotometer, NanoDrop or similar
- 0.2 mL Thin-Walled PCR tubes, strip or plates
- Pipetters
- Pipette tips with aerosol barriers
- SYBR® Green I, (Pro. No. S9430) (optional)
- GenElute™ PCR Clean-up Kit, (Pro. No. NA1020) or HighPrep™ PCR Clean-up System (Pro. No. AC-6000)

Reagents Provided

Reagent	Reagent No.	24 RXN	96 RXN	384 RXN
10X Library Synthesis Solution for SeqXi	LP200	36 µL	144 µL	576 µL
10X Library Synthesis Buffer for SeqXi	LB200	36 µL	144 µL	576 µL
Library Preparation Enzyme for SeqXi	E0600	14.4 µL	57.6 µL	230.4 µL
2X Amplification 1 Mix for Seq-i	A8112	345.6 µL	1382.4 µL	5529.6 µL
Amplification 1 Polymerase for Seq-i	SP500	14.4 µL	57.6 µL	230.4 µL
5X Amplification 2 Mix for Seq-i	BA400	86.4 µL	345.6 µL	1382.4 µL
Amplification 2 Enzyme for Seq-i	BA500	12 µL	48 µL	192 µL
Dual Index Adapter Primers for Seq-i	AP100	48 µL	192 µL	768 µL
Water, Molecular Biology Reagent	W4502	5 mL	10 mL	20 mL

In the first step, Pre-amplification/Library Synthesis (Library Preparation Reagents), template DNA is replicated using primers comprised of semi-degenerate 3'- ends with universal 5'- ends. As polymerization proceeds, displaced single strands serve as new templates for additional primer annealing and extension producing random, overlapping replicons flanked by a universal primer (5') and primer complement (3') sequence.

In the second step, Amplified Library Synthesis (Amplification 1 Reagents), products from pre-amplification/library synthesis are amplified by single primer PCR via the universal end sequence. These amplification products typically range from 200 to 500+ base pairs. Amplicons from ChIP and/or degraded DNA, such as Formalin Fixed Paraffin Embedded (FFPE), are typically shorter and dependent upon length of the starting DNA.

In the third step, Sequencing Library Synthesis (Amplification 2 Reagents), single primer amplicons from amplification 1 are converted to dual Illumina primer PCR products ready for purification, quantification, and Illumina NGS. Amplification 1 amplicons are amplifiable with primers ending with 5'-…GCTCTTCCGATCT-3' such as:

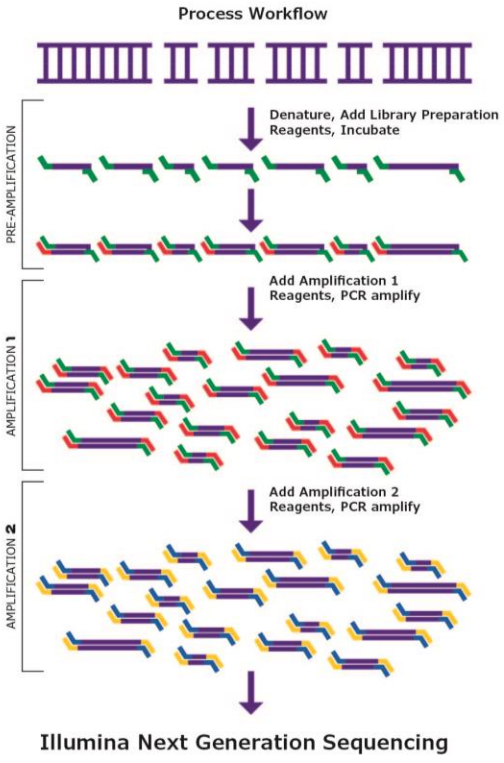
AATGATACGGCGACCACCGAGATCTACAC [i5] ACACTCTTTCCCTACACGACGCTCTTCCGATCT
CAAGCAGAAGACGGCATACGAGAT [i7] GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT

Note: Entering indexes i5 and i7 sequences into the Illumina sample sheet is machine specific. The table below shows examples of both index adapters that can be used in many combinations, in addition to the set included in the kit as AP100.

The examples below are ready to be added to the adapter sequences above as is, no need to reverse and take inverse of the i7 indexes.

Examples of i5 indexes	Examples of i7 indexes
D501 - TATAGCCT	D701 - CGAGTAAT
D502 - ATAGAGGC	D702 - TCTCCGGA
D503 - CCTATCCT	D703 - AATGAGCG
D504 - GGCTCTGA	D704 - GGAATCTC
D505 - AGGCGAAG	D705 - TTCTGAAT

A 20 µL Amplification 2 reaction will produce >100 ng of amplified double-stranded DNA when starting with 100 pg to 5 ng of high-quality DNA. Higher input quantities and higher quality DNA template generally result in increased yields. For damaged DNA, such as from FFPE, 1-50 ng input DNA is recommended. Reaction volumes can be scaled up or down to accommodate preparation of desired quantities of final product. Pre-amplification/Library synthesis reactions may be scaled to as low as 5 µL. Sufficient reagent has been supplied for the number of indicated kit reactions, at Pre-amplification/Library Synthesis, Amplification 1 and Amplification 2 volumes of 15, 30 and 20 µL respectively. Scaling up will reduce the overall number of reactions that the kit can accommodate. Be sure to use the same library synthesis volumes for all samples to be compared.



Precautions and Disclaimer
The SeqPlex™-i DNA Amplification Kit for whole genome amplification (WGA) is for R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
All components should be stored at -20 °C. When thawed for use, components should be kept on ice. Dissolve any precipitate in these solutions by briefly heating at 37 °C, with thorough mixing. Stability of the E0600, SP500 and BA500 will be affected if stored above -20 °C or allowed to remain for long periods at temperatures over 4 °C.

Procedure
The following procedure has been used successfully to amplify and sequence from small quantities, such as single cell and/or degraded/fragmented DNA, such as that from ChIP-isolated or FFPE tissues.

Note: Final yield after amplification varies significantly depending upon the quality of starting DNA. In most cases, >100 ng can be expected. If larger quantities are needed, reactions can be scaled up to accommodate this need, but will limit the overall reactions in the kit.

This procedure was developed using the specific reagents provided with, or recommended for use with, this kit. Substitutions may result in suboptimal results.

Pre-amplification/Library Synthesis

1. Most DNA has some level of fragmentation, such as DNA from many ChIP or FFPE samples, which are ready for input. Most DNA samples do not require additional fragmentation before inputting into this method**. If needed,** DNA may be fragmented using sonication, cavetation, enzymatic or thermal fragmentation.

2. Thaw the **10X Library Synthesis Solution for SeqXi (LP200), 10X Library Synthesis Buffer for SeqXi (LB200)** and **water (W4502)**. Mix thoroughly before use.

3. Combine 100 pg to 10 ng of high quality DNA, 1-50 ng FFPE DNA or a single cell with **LP200** at the following single-reaction scale.

   Combine as follows:
   1.5 µL of **LP200**
   X µL DNA (for example: 1 ng)
   Y µL water (W4502)

   12.9 µL Total reaction volume

Caution—Experienced WGA and WTA users:
Several components found in the Sigma-Aldrich SeqPlex RNA Amplification kit (SEQR), Sigma-Aldrich SeqPlex Enhanced DNA Amplification kit (SEQXE), GenomePlex WGA kits, Transplex® WTA1 kit and Complete Whole Transcriptome Amplification Kit (WTA2) are similarly named. Though generally analogous in function, **they are not interchangeable.**

4. Mix thoroughly, centrifuge briefly, and incubate in a thermal cycler programmed for:

   95 °C for 2 minutes
   16 °C for 5 minutes

5. After the sample has cooled to 16 °C for several minutes, add 1.5 µL of **10X Library Synthesis Buffer for SeqXi (LB200)** and 0.6 µL **of Library Preparation Enzyme for SeqXi (E0600)** to the sample. Cap tube and mix thoroughly. Centrifuge briefly and immediately proceed to next step. (For multiple reactions, a master mix comprised of **LB200** and **EO600** may be prepared. Add 2.1 µL of the master mix to each sample).

   Combine as follows:
   1.5 µL of **LB200**
   0.6 µL of **EO600**
   12.9 µL from previous step
   ---
   15 µL Total reaction volume

6. Place reaction(s) in a thermal cycler and incubate as follows:

   16 °C for 10 minutes
   25 °C for 10 minutes
   37 °C for 10 minutes
   16 °C for 10 minutes
   25 °C for 10 minutes
   37 °C for 10 minutes
   42 °C for 5 minutes
   95 °C for 1 minutes
   4 °C Hold

7. Remove reaction(s) from thermal cycler and centrifuge briefly. Amplification 1 may be started immediately or store Pre-Amplification product at -20 °C for up to three days.

Amplification 1

Caution—Experienced WGA and WTA users:

- **SeqPlex™-I uses a 2X Amplification Mix for Amplification 1**
- **SYBR Green I (S9340) is recommended to monitor the amplification**
- **Annealing/Extension temperature is 70 °C**
- **A 10-min 70 °C post-amp 1 hold is required**

1. Add the following reagents to the 15 µL of Pre-amplification/Library Synthesis product from Step 7. (For multiple reactions, a master mix comprised of reagents listed below may be prepared. Add 15/15.6 µL -/+ SYBR of the master mix to each reaction):

   Combine as follows:
   14.4 µL **2X Amplification 1 Mix for Seq-i (A8112)**
   0.6 µL **Amplification 1 Polymerase for Seq-i (SP500)**
   0.6 µL SYBR Green I (S9430) diluted 1/1000* (recommended)
   15.0 µL **Pre-amp/Lib. Synthesis from Step 7**
   - µL Instrument Specific Reference Dye (optional, if needed add to final total)

   30/30.6 µL (-/+ SYBR) Total reaction volume

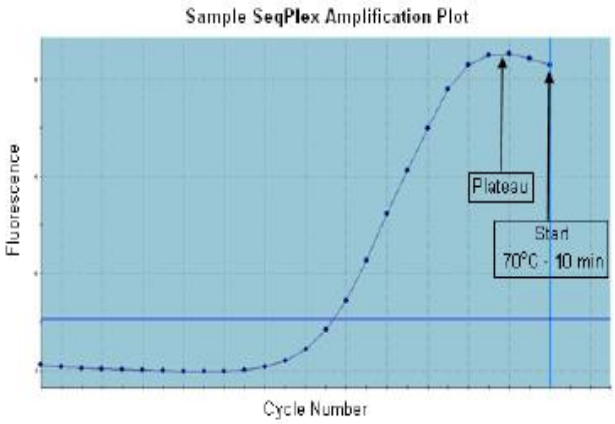
   * - For the best representation, real-time PCR with addition of freshly diluted SYBR Green I to the amplification reaction is strongly recommended to enable monitoring of the reaction progress. **SYBR Green I (S9430) must be diluted 1,000-fold (1/1000) in water and 0.6 µL used per 30 µL of amplification 1 reaction for a total of 30.6 µL to avoid inhibiting the amplification reaction.** SYBR Green I formulations other than S9430 have not been tested and are not recommended. Optimal results are achieved by not proceeding past the amplification "plateau". Proceeding past 1-2 cycles after "plateau", as indicated on **Figure 1,** will not negate the reactions. The optimal number of amplification cycles varies with starting DNA template quantity and quality.

   If amplification is performed without adding SYBR Green I, 17-22 cycles will usually give good results with 0.1-1.0 ng of high-quality DNA. Low quality DNA may require higher input quantities and/or more cycles. If input amounts are near 10 pg or lower, as many as 29 cycles may be required to reach amplification plateau.

   Note: If more than 29 cycles are required to achieve plateau, subsequent NGS results may be unsatisfactory. Consult the Troubleshooting Guide.

DNA Input Amount and Typical # of Cycles to Reach Plateau Reference	
Input Quantity	# of Cycles
10 pg	25
100 pg	22
1 ng	17
10 ng	14

Figure 1



2. Cap tube(s) and mix thoroughly. Centrifuge briefly and cycle in a real-time thermal cycler as follows:

   Initial Denaturation:
   94 °C for 2 minutes
   Cycle until plateau: (<25)
   94 °C Denature for 15 seconds
   70 °C Anneal/Extend for 5 minutes (read fluorescence)
   After cycling:
   70 °C for 10 minutes
   4 °C Hold

3. After cycling is complete, remove reaction(s) and centrifuge briefly. Amplification 2 may be started immediately or store Amplification 1 product at -20 °C for up to three days.

Amplification 2

Caution—Experienced WGA and WTA users:

- **SeqPlex™-I uses a 5X Amplification Mix for Amplification 2**
- **SYBR Green I (S9340) is recommended to monitor the amplification**
- **Annealing/Extension temperature is 70 °C**
- **A 10-minute 70 °C post-amp 2 hold is required**

1. Add the following reagents to a new tube or prepare a master mix for multiple reactions (Add 18 µL of the master mix to each tube). You will use 2 µL of the Amplification 1 product from Step 3 per reaction:

Note: The dual index adapter primers (AP100) provided in this kit will only work for one set of samples. ***If pooling samples for sequencing is required, the user must provide additional index primer sets***. Using index primers at a concentration of 2 µM each is recommended. Lower primer concentration results in higher single stranded hetero duplexes while higher concentrations produce more fully duplex product. Higher concentrations also produce more adapter dimers. See example index primer sequences page 2.

Combine as follows:

3.6 µL **5X Amplification 2 Mix for Seq-i (BA400)**
0.5 µL **Amplification 2 Enzyme for Seq-i (SP500)**
2.0 µL **Dual Index Adapter Primers for Seq-i (AP100)**
11.5 µL **Water, Molecular Biology Reagent (W4502)**
0.4 µL SYBR Green I (S9430) diluted 1/1000*
2.0 µL **Amplification 1 product from Step 3**
- µL Instrument Specific Reference Dye (optional, if needed add to final total)

20.0 µL Total reaction volume

* - For the best representation, real-time PCR with addition of freshly diluted SYBR Green I to the amplification reaction is strongly recommended to enable monitoring of the reaction progress. **SYBR Green I (S9430) must be diluted 1,000-fold (1/1000) in water and 0.4 µL used per 20 µL of amplification 2 reaction to avoid inhibiting the amplification reaction.** SYBR Green I formulations other than S9430 have not been tested and are not recommended.

Optimal results are achieved by not proceeding past the amplification "plateau". Proceeding past 1-2 cycles will not negate the reactions, but best representation is achieved around "plateau". The optimal number of amplification cycles varies with starting DNA template quantity and quality.

If amplification is performed without adding SYBR Green I, 8-12 cycles will usually give good results. Low quality DNA may require higher input quantities and/or more cycles as observed in the amplification 1 cycling.

2. Cap tube(s) and mix thoroughly. Centrifuge briefly and cycle in a real-time thermal cycler as follows:

Initial Incubation/Denaturation:
37 °C for 5 minutes
94 °C for 2 minutes

Two Cycles:
94 °C for 30 seconds
60 °C for 5 minutes
70 °C for 1 minute

Cycle until plateau: (<12)
94 °C Denature for 15 seconds
70 °C Anneal/Extend for 1 minute (read fluorescence)

After cycling:
70 °C for 10 minutes
4 °C Hold

Note: The extended incubation at 70 °C after cycling is essential for maximal duplex synthesis.

3. After cycling is complete, remove reaction(s) and centrifuge briefly. Reactions can be purified now or stored at -20 °C for up to three days.

Purification

The final Amplification 2 reaction(s) can be purified using the GenElute PCR Clean-Up Kit (NA1020). Follow the Technical Bulletin for the kit. Elute in 50 µL nuclease-free water, instead of the Elution Solution provided in the kit. Alternatively, the reactions can be purified with magnetic beads using the HighPrep™ PCR Clean-up System (product number AC-6000). In order to minimize sequencing of primer dimers, use a clean up method that removes fragments less than 100 bp in length. The purified reaction(s) can be used immediately or stored at -20 °C for several weeks.

Yield Determination

The amplification product is generally a mixture of single- and double- stranded amplicons. Yield will vary depending on the quality of starting DNA. Concentration can be measured by:

Traditional A260 absorbance can be used to measure concentration. One A_{260} unit is equivalent to 50 ng/µL dsDNA.

Fluorescent dyes such as PicoGreen®. Duplex-specific dyes may underestimate the actual DNA yield.

Quantitative PCR-based library quantification methods. Recommended, as quantification is not specific to strandedness.

Optional: Amplification quality may be assessed by capillary electrophoresis. Typically, 1 µL of crude or purified amplification product is sufficient for capillary chips, such as those for Agilent's Bioanalyzer or TapeStation. The amplification product is generally a mixture of single and double stranded amplicons.

Sequencing

The SeqPlex-i DNA final product is now ready to enter Illumina Next-Generation Sequencing (NGS). The first nine bases of each read should be disregarded, these bases were a result from the pre-amplification/library synthesis primers used in that section of the kit.

Troubleshooting Guide

Observation	Cause	Recommended Solution
No product was detected after amplification.	Incorrect Annealing/Extension temperature or time	Perform Amp 1 reaction again with 70^{o}C Annealing/Extension for 5 minutes each cycle.
	Too few PCR cycles during Amplification	Perform reaction again with more cycles (up to 29) and monitor amplification with SYBR Green in a real-time thermal cycler
	Starting DNA was insufficient or too severely degraded	Perform reaction again with more starting DNA
	DNA may be single stranded	Use a non-duplex-dependent endpoint method (such as qPCR) to detect DNA amplification
No amplification curve was seen during real-time PCR (qPCR) monitoring	SYBR Green was not added	SYBR Green I, Catalog No. S9430, is not included with SeqPlex-i reagents, but must be added to monitor real-time PCR.
	A reference dye specific to the qPCR instrument may be required	Add instrument specific reference dye. If adding reference dye is not possible, excess cycles should be completed to ensure complete cycling.
Poor representation after NGS	Input DNA not fragmented enough	Verify size and fragment if necessary prior to starting the SeqPlex procedure.
	Not enough cycles during SeqPlex Amplification	Monitor amplification cycling and cycle until plateau is reached for both Amp1 and Amp 2. If monitoring completely fails, a default 29 cycles may be used for Amp 1 and 12 cycles for Amp 2.
NGS IVC abnormalities	IVC plots show identical sequence (primer) for the first few nucleotides in multiple reads	Optimum instrument cluster calling may be achieved by normalizing the run to a lane that does not contain SeqPlex DNA.

SeqXi Experienced User Protocol

Pre-Amplification/Library Synthesis

Reagent	Step 1	Step 2	Step 3	Step 4
DNA	1µL to 10µL	Place samples at 95°C for 2 min, cool to 16°C for 5 min Add reagents in step 3 to samples.		PCR 16 °C—10 min 25 °C—10 min } 2 cycles 37 °C—10 min 42 °C—5 min 95 °C—1 min 4 °C—hold
10X LSS (LP200)	1.5µL			
W4502	10.4µL to 1.4µL			
10X LSB (LB200)			1.5µL	
LPE (EO600)			0.6µL	
Total	**12.9µL**		**15µL**	

Amplification 1

Reagent	Step 1	Step 2
2X Amplification 1 Mix for Seq-i (A8112)	14.4µL	PCR 94 °C—2 min 94 °C—15 sec } < 25 cycles (**monitor**) 70 °C—5 min 70 °C—10 min 4 °C—hold
Amplification 1 Polymerase for Seq-i (SP500)	0.6µL	
SYBR Green (diluted 1:1000) *(S9430, not included in kit, but recommended for monitoring cycling)*	(0.6µL)	
Pre-Amplification/Library Synthesis product	15µL	
Total	**30.0 (30.6)µL**	

Amplification 2

Reagent	Step 1	Step 2
5X Amplification 2 Mix for Seq-i (BA400)	3.6µL	PCR 37 °C—5 min 94 °C—2 min 94 °C—30 sec 60 °C—5 min } 2 cycles 70 °C—1 min 94 °C—15 sec } < 12 cycles (**monitor**) 70 °C—1 min 70 °C—10 min 4 °C—hold
Amplification 2 Enzyme for Seq-i (BA500)	0.5µL	
Dual Index Adapter Primers for Seq-i (AP100)	2µL	
Water (W4502)	11.5 or 11.9µL	
SYBR Green (diluted 1:1000) *(S9430, not included in kit, but recommended for monitoring cycling)*	(0.4µL)	
Amplification 1 Reaction (undiluted)	2µL	
Total	**20µL**	

Frequently Asked Questions

1. **Is SeqPlex™-I DNA compatible with microarrays and qPCR?** Yes, SeqPlex-i DNA may be used in these applications like genomic DNA or existing GenomePlex products.

2. **Are there advantages to SeqPlex™-I over GenomePlex?** SeqPlex-i Pre-Amplification primers have been designed to target more frequently than existing GenomePlex WGA primers and therefore may provide the advantage of superior genome coverage in some regions.

3. **Will reducing cycles during amplification improve representation?** No, you need to reach “plateau” for optimum representation. Proceeding past 1-2 cycles will not negate the reactions, but best representation is achieved around “plateau”. Insufficient cycling leads to a significant reduction in representation/coverage.

4. **Will SeqPlex™-I DNA require special NGS sequencing protocols?** No, SeqPlex-i DNA fits directly into NGS workflows, just like ChIP DNA. Sequencing instrument operators should be notified of running SeqPlex DNA and to expect a slight signal from any remaining primers in the IVC plots.

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SYBR is a registered trademark of Molecular Probes, Inc.

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