The Arima-HiC Kit and Service for Chromosome Spanning Assemblies

Introduction

The availability of high-quality reference genomes has had a profound impact on the understanding of genome function and species evolution. Recent years have seen a rapid expansion of long-read and long-range methods, including Hi-C[1,2] a NGS-based assay that preserves chromosome-range information during sample preparation.

Hi-C sequencing data uses the preserved chromosome-range information to transform contigs to chromosomes. Despite its utility, broad adoption of Hi-C has been plagued by labor-intensive complex protocols, prolonged workflow durations, inconsistent experimental results, excessive sequencing requirements, and expensive Bioinformatics analyses/services.

The Arima-HiC Kit overcomes these technical and economical limitations with the development of a highly simplified and robust protocol that streamlines Hi-C to a 6-hour, 8-step procedure, followed by library prep and NGS. The Arima-HiC sequencing data is then used to scaffold contigs to generate chromosome-spanning assemblies via open-source software such as SALSA[3,4] and 3D-DNA[5].

These key advancements have persuaded researchers, including the Vertebrate Genomes Project (Fig.1), to use Arima-HiC to generate high-quality assemblies of hundreds of species across plant and animal kingdoms.

Fast and Easy Workflow

The Arima-HiC workflow was optimized to enable first time Hi-C users to generate high-quality data with ease (Fig.2). The rapid 6-hour protocol limits prolonged exposure of chromatin to external agents, leading to significant enrichment of inter-contig signal.

The use of a unique combination of multiple 4-base cutting restriction enzymes (RE) for chromatin digestion results in greater spread (per-base uni-formity) of inter-contig signal to assemble all contigs regardless of gap-sizes.

Overall, the Arima-HiC Kit was designed to maximize the ease-of-use, with minimal total time and hands-on steps, compatibility with downstream library prep kits for Illumina NGS, 96-well plate compatible design, and support for a broad range of sample types and species (Table 1). Upon sequencing of the Arima-HiC libraries, open source Bioinformatics tools such as SALSA[3,4] and 3D-DNA[5] can be used to map Arima-HiC data to contigs to scaffold them into accurate chromosome-spanning assemblies. In addition to scaffolding, Arima-HiC data has also been used to polish individual bases of the contigs as Arima-HiC is sequenced on an Illumina NGS3 (Table 1).

Proven Performance

Rigorous external and internal testing resulted in an Arima-HiC Kit with robust performance. When key opinion leaders compared Arima-HiC data with Hi-C data generated by competitors, the Arima-HiC data manifested higher inter-contig signal (Fig.3A). Importantly, the competitor's Hi-C data manifested a rapid drop in inter-contig signal with increase in contig gap-sizes. Arima-HiC data, on the other hand, manifested 2-3 fold higher inter-contig signal regardless of the contig gap-size (Fig.3B).

Subsequent SALSA3,4 analyses of Arima-HiC data converted 4Mb Oxford Nanopore contigs to 125Mb NG50 chromosome-spanning human genome assemblies, with as little as 30X Arima-HiC sequencing depth3 (Fig.4). This is an extraordinary performance given that even the GRCH38 reference assembly generated by cost-prohibitive combination of multiple technologies had an NG50 of only ~140Mb, suggesting that the high quality of Arima-HiC libraries and sequencing data offers substantial technical and economic benefits to the user. These benefits can be leveraged via Arima-HiC kits or services (sample to assembly).

Additionally, computational estimations suggest that about one-third of the genome (estimated from chr1 of the human genome) is inaccessible when Hi-C is performed with a 4-base restriction enzyme (RE), resulting in potentially limited interaction signal for one-third of genomes. Arima-HiC overcomes this limitation by using a unique RE cocktail and generates near complete genome accessibility.
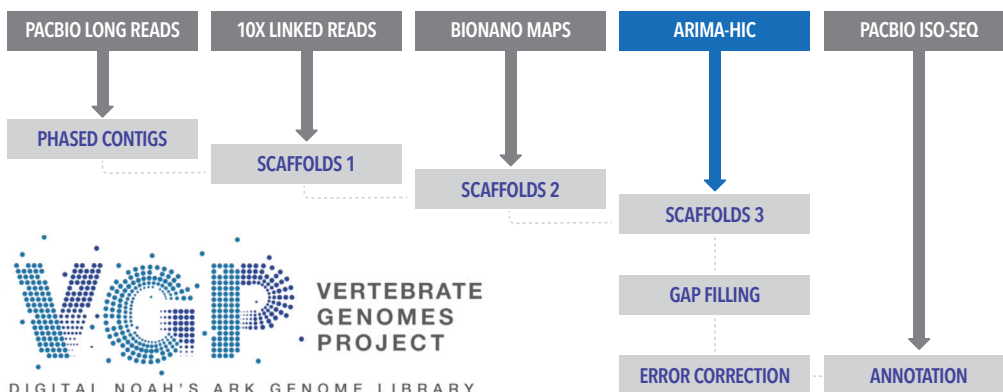
Highlights

Proven Performance

- Obtain best-in-class inter-contig Hi-C signal across sample types
- Innovative multi-restriction enzyme chemistry improves overall genome coverage
- Generate uniform "spread" of inter-contig HiC signal to scaffold contigs regardless of gap sizes

Fast and User-Friendly Workflow

- Rapid time to Hi-C libraries with minimal hands-on touchpoints, 6-hour prep time (1 hour hands-on time)
- Democratize analysis workflow by using well-documented open source tools such as SALSA (https://github.com/machinegun/SAL-SA) for chromosome-spanning assemblies

Assured Quality

- Arima-HiC is validated in a wide-range of sample preservation and transportation conditions through its selection by the Genome10K consortium to generate high-quality assemblies of 260 species
- Assured library quality to ensure sequencing and assembly success with quantitative and predictive and QC steps
- Obtain both long-range sequence and chromatin conformation information with one assay

PACBIO LONG READS → 10X LINKED READS → BIONANO MAPS → **ARIMA-HIC** → PACBIO ISO-SEQ

PHASED CONTIGS

SCAFFOLDS 1

SCAFFOLDS 2

SCAFFOLDS 3

GAP FILLING

ERROR CORRECTION   ANNOTATION

VERTEBRATE GENOMES PROJECT

DIGITAL NOAH'S ARK GENOME LIBRARY

Figure 1: Arima Genomics is a technology partner of the VGP Phase 1. Long-read PacBio sequencing is performed to generate the initial contigs, followed by long-range scaffolding approaches to assemble contigs into chromosomes.

(Image courtesy of Vertebrate Genomes Project)

Demonstrated Utility

The ease-of-use, consistency, and proven performances of Arima-HiC workflow, and, the open-source Bioinformatics strategy have made the Arima-HiC kits and services as a highly popular choice for generating chromosome-spanning assemblies. Indeed, Arima-HiC has been selected by the Genome10K consortium to generate high-quality assemblies and base-polishing of >260 vertebrate species[6].

An additional benefit is that the same Arima-HiC data generated for genome scaffolding can then be used to investigate the 3D conformation of the genome. Chromatin conformation information is useful for many studies including cell development, gene regulation, and pathogen resistance.

Additional Details

Please refer to the *Genome Conformation* and *Low Input Application Notes*, available by contacting **info@arimagenomics.com.**

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Table 1: Arima-HiC Specifications

Total Time	6 hours
Hands-on Time	1 hour
Number of Steps	8
Automation Capability	Single–tube, 96-well plate compatible
Restriction Enzymes (RE)	RE cutting at GATC and GANTC
Per-base Genome Uniformity "Spread" (fraction of genome with avg. seq. depth)	~90%, as estimated from Human genome. Similar performance expected in all species.
Sample Types	Seeds, Tissue, blood, cell lines, whole insects
Sample Storage Conditions	Fresh/frozen, Cross-linked, Ethanol
Input Quantity	Amount of sample manifesting ~1ug DNA, low input protocols also available
Species	Plants, Invertebrates, Vertebrates
Library Prep Compatibility	KAPA Hyperprep, Swift Accel NGS 2S, Illumina TruSeq, NEBNext Ultra II, others
NGS Compatibility	Illumina NGS paired end reads
Library Complexity	1 reaction provides libraries complex enough for 600M reads
Sequencing Depth (X), recommended	Arima-HiC: 15-60X, depending on the contig NG50
Data Analysis	SALSA tool (Ref 3) and other open source tools recommended
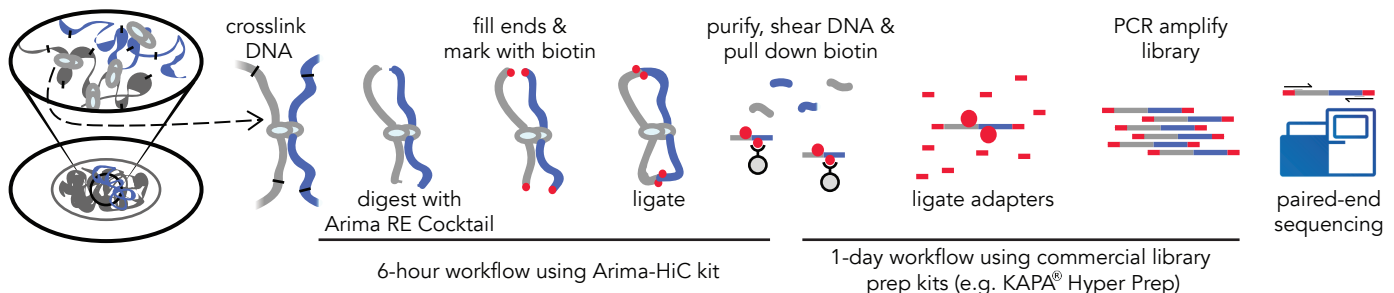
References

1. Lieberman-Aiden E, et al "Comprehensive Mapping of Long-Range Interactions Reveals Folding Principles of the Human Genome" Science 326, 289-293 (2009)
2. Rao SP, et al "A 3D Map of the Human Genome at Kilobase Resolution Reveals Principles of Chromatin Looping" Cell 159, 1665-1680 (2014)
3. Ghurye J, et al "Integrating Hi-C links with assembly graphs for chromosome-scale assembly" BioRxiv, doi: http://dx.doi.org/10.1101/261149 (2018)
4. SALSA Bioinformatics Tool for Arima-HiC. https://github.com/machinegun/SALSA
5. Dudchenko O, et al "De novo assembly of the Aedes aegypti genome using Hi-C yields chromosome-length scaffolds" Science 356, 92-95 (2017)
6. Genome10K Press Release (1). https://www.businesswire.com/news/home/20180117006561/en
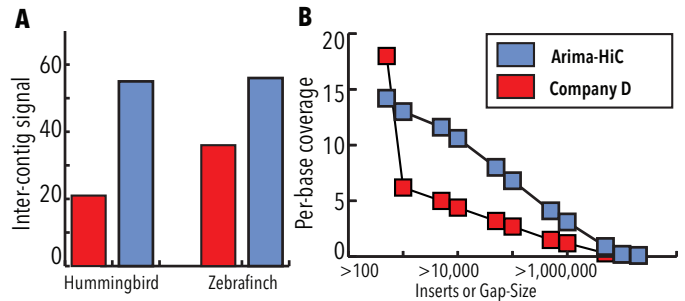
Figure 3: Arima-HiC data generates higher inter-contig signal strength and spread, critical features that enable chromosome-spanning assemblies at reduced sequencing. (A) Arima-HiC data and Competitor's Hi-C (data generated by Company D) is mapped to Hummingbird and Zebrafinch contigs generated by Pacific Biosciences Sequencers. Regardless of the species, Arima-HiC consistently generates higher inter-contig signal. (B) Hummingbird Hi-C datasets from Arima Genomics and Competition analyzed in the context of insert-sizes. That is, when Hi-C reads are categorized by insert-sizes, Arima-HiC maintains high signal (2-3 fold) & coverage regardless of insert-size. In the context of assembly, this "spread" of signal can enable contigs of all gap-sizes to be well-assembled, to generate accurate assemblies at reduced sequencing cost. To enable an unbiased analyses, these were performed with 2M Hi-C reads. Analyses performed by Arang Rhie (NIH). Shared with permission.
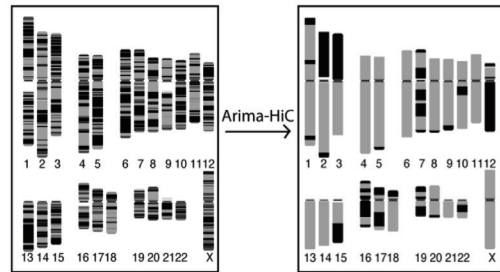
Figure 4: 125 Mb NG50 Human chromosome-spanning assemblies, generated by Arima-HiC data 4Mb contigs from Oxford Nanopore converted to 125Mb assemblies via Arima-HiC data, using SALSA open-source tool. Charge in color in the ideogram represents a contig gap or error. Post Arima-HiC assemblies have minimal color change, suggesting long (NG50 125Mb and chromosome-spanning) & accurate assemblies. Figure from Ref (3), shared with permission.


Figure 2: The Arima-HiC workflow results in ligated and biotinylated DNA that is prepared as a library and amplified using pre-validated library prep kits, and then subject to paired-end Illumina sequencing.

Dwarf Crocodile   Salamander   European Turtle Dove   Hummingbird   Common Yellowthroat   Italian Sparrow   Long-tailed Finch
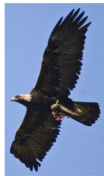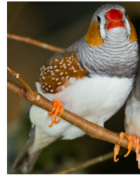
Kakapo   Golden Eagle   Blue-capped Cordonbleu   Zebra Finch   Chicken   Reedfish   Tire Track Eel

Greenland Shark   Mexican Tetra (Blind Cave Fish)   Japanese Pufferfish   Indian Glassy Fish   Blunt-snouted Clingfish   Climbing Perch   Atlantic Cod

Gilt-head Bream   Burbot   Zebrafish   Turquoise Killifish   Eurasian Red Squirrel   Pale Spear-Nosed Bat   Macaque

Canadian Lynx   Cow   Horse   Mouse   Horseshoe Crab   Ant   Mosquito
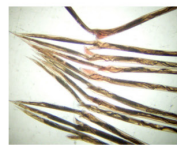
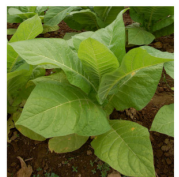Bumblebee   Drosophila melanogaster   Schmidtea mediterranea   Barber's Pole Worm   Starlet Sea Anemone   Wheat   Brachypodium
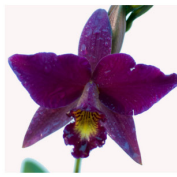
Tobacco   Orchid   Arabidopsis

*A Representation of the Plant and Animal Species
Being Analyzed via the Arima-HiC Workflow*