OligoMix® is a versatile, innovative, custom product for genomics discoveries. We synthesize thousands of oligonucleotide sequences in massive parallel on a microarray chip and then cleave the oligos, releasing them into solution in a single microtube. Synthesis occurs via standard DMT chemistry assuring efficient stepwise yield and a high quality final product. The product is delivered as a pool in a single microtube – ready for use in your experiment.

Sequence Capture Applications
• Targeted Resequencing
• Genomic Variant Analysis
• Targeted Methylation Analysis

Synthetic Biology Applications
• Gene Synthesis

FISH Applications
• Oligopaint FISH Probes

Library Construction Applications
• CRISPR/Cas9 Guide RNA Library Preparation
• siRNA Library Preparation
• Construction of Protein Coding / Antibody Libraries
At less than 0.8¢ per base, OligoMix® is about 20 times more cost and time efficient than conventional oligos. Delivered in a single microtube, it enables inexpensive genome-scale experiments.

**CUSTOMIZABLE**

*User Defined Sequences* - Customers can specify each oligonucleotide sequence (lengths up to 150-mers). There is No Limit to the number of sequences we can synthesize.

*Synthesis Chemistry* - Our flexible synthesis technology allows the use of multiple synthesis chemistries: Choose DNA, RNA, modification chemistries, or even hybrids of these.

*Labeling and Functionalization* - We can synthesize oligonucleotides in OligoMix® containing labels, such as terminus phosphate, amino and thiol with linkers, biotin, FAM or other dyes. Individual sequences in one OligoMix® may contain different labels.

**RELIABLE**

Multiple QC steps are implemented at various stages of OligoMix® manufacturing. We examine hybridization on multiple built-in control sites to assess the quality of array synthesis. The cleaved OligoMix® is subjected to qRT-PCR assays designed for the control sequences.

**SIMPLE & FAST**

Download our excel spreadsheet order form, paste in your sequences and email back to us. Product can be delivered in 1-2 weeks.

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**Product Description**

<table>
<thead>
<tr>
<th><strong>mix of DNA oligonucleotide sequences</strong></th>
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**Number of Oligos**

<table>
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<tr>
<th>thousands of sequences or more per tube</th>
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</table>

**Oligo Form**

<table>
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<tr>
<th>single stranded (ss); desalted and ready for reaction</th>
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**Length**

<table>
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<th>up to 150 mers (inquire for longer oligos)</th>
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</thead>
</table>

**5’ or 3’ Terminus Modifications**

<table>
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<tr>
<th>phosphate, fluorescent dyes, biotin, linkers, and others</th>
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</table>

**Internal Modifications**

<table>
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<tr>
<th>modified DNA or RNA bases</th>
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**Yield**

<table>
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<tr>
<th>*tens of attomoles per sequence and a total of sub-fmols per OligoMix® tube</th>
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**Price**

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<tr>
<th>(see <a href="http://www.lcsciences.com/discovery/oligomix">www.lcsciences.com/discovery/oligomix</a>)</th>
</tr>
</thead>
</table>

**Delivery**

<table>
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<tr>
<th>14 days</th>
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*One fmole per sequence is the calculated amount of starting material for oligo synthesis. As with all standard oligonucleotide synthesis, the resulting final material will be of less quantity depending on the sequence length, composition, and other factors.*
Microfluidic Array Platform — *in situ* Synthesis

OligoMix® achieves high synthesis purity because it is produced via an advanced microarray synthesis technology (µParaflo®) that integrates a photo-generated acid (PGA) chemistry, digital photolithography (DLP), and advanced microfluidics to enable high throughput parallel synthesis of custom DNA microarrays. The PGA chemistry enables the use of standard oligo building blocks, and eliminates the need for any specially modified nucleotides which may exhibit lower coupling efficiency. DLP technology enables programmable synthesis of custom sequences and the µParaflo® microfluidic device contains the synthesis reactions each within a picoliter-scale reaction chamber, producing more uniform synthesis than reactions performed on the open surface of a slide.

Synthesis quality requirements for more typical microarray applications such as gene expression profiling are less demanding because issues with synthesis quality or spot uniformity have been successfully moderated by image filtering methods or averaging of replicate spots to increase data confidence. However, in applications that involve cleaving the probes from the microarray chip (such as target capture for NGS) any and all impurities from synthesis end up in your capture probe mixture. There is no possibility to mitigate their effects through data manipulation so the requirement for synthesis quality is very high. LC Sciences’ microarray synthesized oligos (OligoMix®) have been compared to and demonstrated as effective as conventional column synthesized oligos for use in targeted sequencing applications¹.

Quality Control

We examine hybridization on multiple built-in control sites and analyze the uniformity and intensity of the hybridization spots, the cross chip spot uniformity, and the match (PM) and mismatch (MM) hybridization signal ratios to assess the quality of OligoMix® synthesis. Finally, the cleaved OligoMix® is subjected to qRT-PCR assays designed for the control sequences. Our QC criteria use standardized procedures and parameters, such as the signal intensity, the signal covariance (CV), and the PM/MM ratio.

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Sequence Capture Applications

Targeted Sequencing

Though next-generation DNA sequencing (NGS) provides very high levels of coverage, even on complex genomes, it is still advantageous to reduce the complexity of samples and sequence smaller targeted regions – in particular, when sample numbers are very high and the goal is detection of less prevalent mutations. Several academic and commercial groups have developed a variety of capture methods for enriching or selectively amplifying subsets of the genome for targeted sequencing.

The key performance parameters of these methods are capture specificity and sensitivity, the ability to multiplex many samples and capture large regions of interest, and of course, cost. Recently developed methods include: Solution Hybrid Selection (SHS)\(^2,23\), Molecular Inversion Probes (MIP)\(^2,24\), Selective Genomic Circularization (SGC)\(^6,25,26\) and Oligo-Selective Sequencing (OS-Seq)\(^1\). All of these methods have been demonstrated to be effective at selectively enriching desired regions of interest within a given genome.

- It has been demonstrated that the use of microarray synthesized oligos produces the required numbers and quality of oligos quite effectively at a far lower cost\(^27\).
- OligoMix\(^®\) has been demonstrated as an effective method of oligo synthesis for targeted sequencing in MIP\(^2\), SGC\(^6\), and OS-Seq\(^1\) targeted sequencing methods.
**Targeted Methylation Analysis**

As with most genomic analysis methods, CpG methylation analysis must be: quantitative, high-throughput, cost-effective, and both scalable and flexible with respect to coverage. Ideally, one would be able to efficiently investigate the methylation of large numbers of CpGs in large numbers of samples.

The standard method for measuring methylation involves treatment of DNA with sodium bisulfite which causes conversion of unmethylated cytosines (C) to uracils (U), whereas 5-methylcytosine (5mC) remains unchanged. The differences in reactivity of Cs and 5mCs to bisulfite can be distinguished by subsequent microarray or sequencing methods.

Both of these methods can benefit from prior targeted capture and amplification of suspected CpG regions in order to reduce the complexity of samples and focus the analysis on specific genomic segments. The use of oligonucleotides for targeted capture increases both sample throughput and coverage, while decreasing cost per sample. Using an OligoMix synthesis strategy vs. individual oligo synthesis further increases flexibility, scalability and cost efficiency of targeted methylation analysis methods. Recently, two new capture methods have been developed for targeted methylation analysis.

One challenge for these methodologies lies in the construction of capture probe panels. They must be customizable to different genomic targets, scalable to a very large sample size (1,000–100,000 samples), and inexpensive. The current procedures are labor intensive and costly, making it impractical for construction of very large panels or custom panels. As a parallel oligo synthesis technology capable of producing virtually unlimited numbers of oligos of lengths up to 100 nucleotides as a pool, OligoMix overcomes this barrier and represents a significantly more cost effective method for construction of probe panels than single-plex PCR.

- **Probes generated by OligoMix were compared to single-plex PCR constructed probes using ROC analysis and no significant difference in performance was observed.**

- **Oligo pools therefore represent an inexpensive means for constructing large and custom dU probe panels and greatly improve the flexibility of the assay with respect to coverage.**

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**Library-Free Bisulfite Padlock Probes (BSPPs)**

In the BSPP sequencing approach, padlock probes are annealed to bisulfite converted genomic DNA, captured targets are circularized then PCR amplified with bar-coded primers and directly sequenced.

**Methylation by Target Amplification by Capture & Ligation (mTACL)**

Digested genomic DNA fragments are mixed with a dU probe panel and common primers, denatured and re-annealed. Captured region are ligated to the primers and reacted with bisulfite, to convert unmethylated cytosines to uracils. The product can be analyzed by microarray or sequencing to detect the presence of U at CpG sites.
Modern synthetic biology involves not only the construction of existing genes to elicit their functions, but also designed mutation and sequence shuffling to create new, functional gene constructs which perform as biomolecular machines. One limitation for gene synthesis is the cost of making the building blocks (oligonucleotides) that are assembled together to make genes. Multiplex, parallel DNA construction on a large scale requires pools of large numbers of short synthetic oligos.

Microarray technology provides a fast and economical means for massive parallel synthesis of oligos and the µParaflo® technology represents a significant advancement in microarray synthesis technology. Large numbers of DNA constructs of designed sequences can be synthesized and then simultaneously assembled by joining the short synthetic oligos with multiplex reactions. There are challenges associated with gene synthesis from complex oligo pools such as: synthesis errors, pool sequence complexity and low yield per sequence, but over the past decade several methods have been developed to overcome these.

Block-Based Gene Assembly\textsuperscript{10} - Combination of hybridization-based oligo selection and parallel amplification into a single process that allows simple and cost-efficient production of unlimited amounts of high-quality building materials directly from unpurified pools of microarray-synthesized oligos.

Megacloning\textsuperscript{9} - Oligos synthesized from microarrays are “read” by next-generation sequencing to identify those with desired sequences. The DNA is then sorted and retrieved selectively.

- In 2 methods, results were compared and demonstrated to be similar to column synthesized oligos\textsuperscript{10,11}.
- “The genes constructed from microarray-synthesized oligos using our block-based gene assembly protocol were found to be indistinguishable from genes assembled by the standard protocol from the high-quality column-synthesized oligos”\textsuperscript{10}
Fluorescence in situ hybridization (FISH) utilizes fluorescent probes to bind portions of DNA that have a high degree of sequence complementarity. This allows researchers to detect and localize specific DNA sequences on chromosomes or RNA targets in various cell and tissue types to determine the spatial-temporal patterns of gene expression within. In medicine, FISH can be used to diagnose or evaluate the progression of a disease, such as cancer, to identify a particular species or to perform various types of karyotyping.

Developing a FISH assay requires the use of oligonucleotide probe sets, like oligopaint probes, which are fluorescently labeled, single-stranded DNA oligonucleotides that can be used to visualize genomic regions ranging in size from tens of kilobases to many megabases. LC Sciences’ OligoMix offers a unique solution for researchers looking to generate oligopaint probes, as users are able create fully designed libraries of tens of thousands of specific, single-stranded oligonucleotide sequences for binding particular genomic regions. Several researchers have demonstrated the effectiveness of OligoMix in their FISH-assays and have provided model strategies for generating oligopaint probes through their work. The strategies they present are important because they provide an experimental model other individuals can emulate and apply to new areas of fluorescence hybridization.

The basic protocol for generation of the oligopaint FISH probes begins with a complex ssDNA library of thousands to hundreds of thousands of unique oligos; like the OligoMix libraries which are quickly and inexpensively generated using LC Sciences’ microfluidic array.

In one strategy, oligos can be designed with a pair of primer sequences (a forward primer and a reverse compliment of the reverse primer) that flank a genomic sequence bound on either side by sites for nicking endonucleases. Incorporating two nicking endonuclease sites allows for the production of strand specific probes. Amplification with a labeled F primer and digestion yields a probe targeting the reverse complement of the genomic sequence.

In a second strategy, circle-to-circle amplification (c2ca) is used instead of PCR, to generate oligopaint probes. In circle-to-circle amplification, targeted template strands are cyclized via ligation into circular template strands, which are subsequently synthesized into chain-like repeated copies of the circular template by an enzyme with high processivity and strand displacement capacity.

This amplification method is unique, because it overcomes some of the drawbacks of PCR like sequence-dependent amplification bias. In addition to this, c2ca is an isothermal process and therefore does not require quick temperature changes which impede the scalability of PCR reactions.

Because c2ca amplified oligos do not carry a direct label, a common binding site is used for a fluorophore-labelled ‘secondary’ oligonucleotide.

The strategies presented here provide a framework for generating oligopaint probes from OligoMix which can be subsequently be used in various FISH applications. By emulating the strategies detailed here, researchers can develop their own oligopaint libraries to apply to new areas of fluorescence hybridization.
Cloning complex libraries of reporter constructs with predefined nucleic acid sequences can be expensive and time-consuming. The use of synthetic oligonucleotides for sequence templates is a common practice when natural sources of the nucleic acid are not available, or a non-natural designed structure is desired. Traditionally, the oligos are synthesized individually, each on a separate solid support (column), and then individually engineered into the larger library. Because the synthesis and cloning steps are expensive and individual manipulation is labor intensive, this usually results in libraries with limited numbers of sequences (limited complexity).

OligoMix® enables us to synthesize thousands of designed sequences at once on a single microarray chip. By synthesizing the sequences in massive-parallel on a microfluidic chip, the overall cost and time required for synthesis is dramatically decreased and the cumbersome procedure of multiple transformation reactions for all unique constructs is avoided.

Production of cloned libraries with micro-array-based oligo synthesis provides a rapid, high-throughput, cost-effective approach for the generation of complex libraries of designed oligo sequences. The flexibility to create completed designed custom sequences means this approach can address an array of biological questions, such as short hairpin RNA (shRNA) or CRISPR/Cas9 libraries for high-throughput loss-of-function genetic screens and antibody or other protein-coding DNA libraries for diversity studies and directed evolution strategies.

Protein Coding / Antibody Library Preparation\textsuperscript{17,18}

Synthetic antibody libraries have proven to be effective tools for drug discovery and development through the generation of functional, high-affinity antibodies against a wide variety of antigens. The performance of a synthetic antibody library depends in large part on the diversity of the library, which must be designed based on thorough understanding of the antibody structure and function. Insights from structural and functional analyses of functional antibodies are used to design synthetic oligonucleotides that introduce chemically and spatially defined diversity into the CDR loops. The synthetic CDR repertoires are incorporated into phage-display vectors to produce phage-displayed antibody repertoires.

- Custom synthesis of tens of thousands of specific (non-degenerate) oligo sequences enables the production of protein coding libraries with fully designed library diversity.
- Specific sequences with any codon choices, deletions, insertions, and length variations are easily made.
- The limitations of random codon generation are eliminated without expensive cost.
Small Interfering RNA (siRNA) Library Preparation

RNAi for gene knock-down is a tool that has great promise. But beyond screening for basic identification of gene function, RNAi has the potential for therapeutic application via siRNA mediated gene silencing.

When considering siRNAs therapeutic potential, important issues that need to be addressed are siRNA specificity and analysis of any affect on off-target genes causing potential side-effects.

One approach is the use of an siRNA target library. A team led by researchers at the Karolinska Institute has developed a straightforward, efficient and cost effective method for generating an siRNA target library, by combining an siRNA target validation vector with OligoMix®.

CRISPR/Cas9 Guide RNA Library Preparation

In CRISPR-Cas9 mutation screens, guide RNAs targeting tens of thousands of sites within genes are cloned into viral vectors and delivered as a pool into target cells along with Cas9. By identifying guide RNAs that are enriched or depleted in cells which exhibit a desired phenotype, researchers can systematically identify genes that are required for that particular phenotype.

In a typical guide RNA library prep strategy, oligos are individually synthesized on a microarray chip. These are then PCR amplified to incorporate vector compatible restriction sites, cloned into plasmid vectors (as above), and then transduced into viral libraries. Library pools of guides are then used for screening purposes. Following isolation of genomic DNA from positively selected cells, amplification by PCR across the guide region is performed and the guide identified by sequencing.20,21

By scaling the guide RNA synthesis processes to massively parallel arrays, screening assays at unprecedented throughput are made possible. LC Sciences’ OligoMix® offers a unique solution for researchers looking to generate large guide RNA libraries, as users are able create fully designed libraries of thousands of specific, single-stranded oligonucleotide sequences for recognizing particular genomic regions.
OligoMix® References

Targeted Capture Applications


6. LC Sciences' customers - data unpublished

7. LC Sciences' internal development work - data unpublished

Synthetic Biology Applications


FISH Applications


Library Construction Applications


Other Oligo Synthesis References


