

# Luminex® FlexMAP™ Microspheres

## Product Information Sheet

Distributed by MiraiBio

800-624-6176

### Summary and Intended Use

FlexMAP™ Microspheres are xMAP® Microspheres modified with unique oligonucleotide anti-tags. Each FlexMAP Microsphere set (each set has a slightly different dye combination to generate a unique color identification) carries a different anti-tag. The anti-tags serve as functional groups for the capture of molecules containing complementary tag sequences (ordered separately). FlexMAP Microspheres are primarily used in multiplexed hybridization assays where the desired targets contain, in addition to the assay-specific content sequences, oligonucleotide tags complementary in sequence to the anti-tags attached to the microspheres. The tag attached to each assay-specific sequence interacts with its complementary anti-tag on the microspheres such that the reaction products may be sorted using the Luminex 100™ System. The anti-tag/tag interactions are isothermal and are designed to produce minimal cross-hybridization (“cross-talk”). Use of this product is intended to eliminate the separate step to conjugate content-specific capture probes to xMAP Microspheres and to allow solution phase reaction kinetics. FlexMAP Microspheres are intended for further manufacture in assays using xMAP technology. For related xMAP instructions and protocols refer to the system manual provided with your Luminex instrumentation. A sample protocol for a FlexMAP Microsphere-based application is provided with this Product Information Sheet.

Your specific application will require incorporation of the complementary tag sequences into your assay-specific oligonucleotides in order for the completed reactants to be captured onto the anti-tag-coupled Microspheres. To ensure optimal assay characteristics, you can use the TAG IT™ Oligo Design Software ([www.luminexcorp.com/products/cat\\_reagents.shtml](http://www.luminexcorp.com/products/cat_reagents.shtml)) to assist your assay design. TAG-IT will suggest which bead sets to choose for your specific application and provide you with ordering information for the tag-modified assay-specific oligonucleotides. Alternatively, tag sequences may be assigned to assay-specific content sequences (See **Appendix A**). Normal cross-reactivity and secondary structure optimization is required with the appended sequences when not using TAG IT.

### Product Description

FlexMAP Microspheres are provided in Tris-EDTA, pH 8.0.

Property	Manufacturing Target
Microsphere Concentration	For L100-Uxxx-01, $2.5 \times 10^5$ microspheres/mL
Microsphere Concentration	For 14-00xxx, $6.25 \times 10^6$ microspheres/mL
Functional group	FlexMAP Anti-Tag

### Recommended Assay Conditions

Target concentrations of 5 – 50 fmol, FlexMAP hybridization time of 30 - 60 minutes at 37°C in 200mM salt or equivalent.

### Limitations

To ensure consistency, please consider the following limitations when performing your assays. Minimize exposure to light to maintain the integrity of the microspheres. The microspheres will also settle if left undisturbed requiring re-suspension prior to dispensing. Do not use this product with strong organic solvents.

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Manufacturer  
Luminex Corporation  
Austin, Texas 78727, USA  
[www.luminexcorp.com](http://www.luminexcorp.com)

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### Safety Precautions

Although this product is not known to contain hazardous or carcinogenic components at toxic levels, it may be harmful when inhaled, comes in contact with skin, or swallowed. There may be danger of cumulative effects. Keep away from food, drink, and animal feeding stuffs. If product comes in contact with skin, wash immediately with plenty of water. Wear suitable protective clothing. In case of accident or if you feel unwell, seek medical advice immediately and show this product label or container to your medical provider. Material Safety Data Sheet available upon request.

### Handling Instructions

Always ensure that microspheres are homogeneously suspended prior to dispensing. Remove from freezer and allow microsphere suspension to equilibrate to room temperature. Place on rotator and gently rotate for 1-2 minutes. Mix 5-10 times by gentle inversion and gently tap sample container bottom on bench top to minimize sample retention in the cap immediately prior to dispensing. If removed from the original container, take care to protect microspheres from light.

### Storage

Store frozen upon receipt for up to 6 months. Store at 2 - 8°C after thawing for up to 3 months. If removed from the original container, take care to protect microspheres from light.

### Product Numbering / Order Information

FlexMAP Microspheres are available at concentrations of  $2.5 \times 10^5$ /ml and  $6.25 \times 10^6$ /ml each in 1.0 mL aliquots for each of the 100 Microsphere sets. The product number on the container label relates to the software target region and anti-tag number. For example: L100-U001-01 represents Region 001, LUA Antitag 01; 14-00050 represents Region 050, LUA Antitag 50; etc.

### References

21 CFR Part 1910.1200, Hazard communication

### Trademarks

FlexMAP, xMAP, Luminex 100 and Luminex are all trademarks of Luminex Corporation. TAG-IT is a trademark of Tm Biosciences Corporation.



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### FlexMAP Sample Application – Coagulation Genotyping

The following is a general overview of a sample multiplexed genotyping assay performed on the FlexMAP platform by Tm Bioscience (Toronto, Ontario). The assay is designed to simultaneously detect six single nucleotide mutations in five genes associated with coagulation disorders – factor V, factor XIII, factor II, MTHFR 1298, MTHFR 677 and TFPI. The general assay is comprised of three general steps:

- Multiplex PCR using genomic DNA
- Multiplex ASPE using tagged primers and biotin-dCTP
- Hybridization on the FlexMAP platform

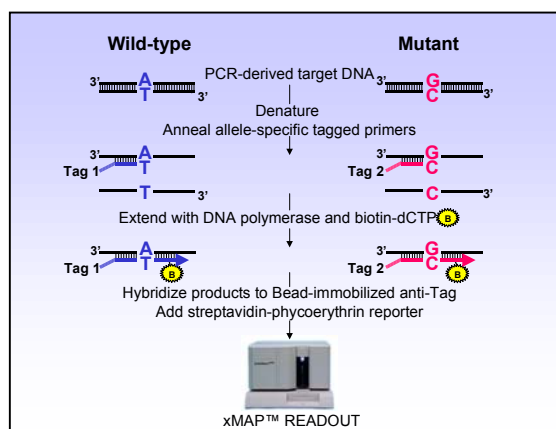
*Please Note: This application is presented for information only – your results may vary. This assay is for research use only and is not intended for diagnostic purposes. Nothing in this publication should be construed as an authorization or an implicit license to practice PCR under any patents held by Hoffman-LaRoche, Inc. or to practice or use ASPE.*

#### a) Multiplex PCR

Multiplex PCR using 12 primers was carried out under optimized multiplex conditions. For each multiplex PCR, 50 ng genomic DNA was amplified. Amplimers ranged in size from 97 to 154 bp.

#### b) Multiplex ASPE

Allele-specific primer extension (ASPE) chemistry is a simple, robust method for analyzing multiple genotypes in a single tube. The method employs a PCR-derived target DNA containing the variant together with two 5' universally-tagged allele-specific primers whose 3' ends define the alleles. A thermophilic DNA polymerase is used for primer extension and biotin-dCTP label incorporation. Because the two-tagged allele-specific primers overlap the mutant site in the target DNA, only the correctly hybridized primer(s) will be extended to generate labeled product(s). A non-complementary primer will not be extended or labeled due to the 3' mismatched base. The figure below outlines the main steps in a typical ASPE reaction. Following the multiplexed PCR amplification, PCR products are treated with shrimp alkaline phosphatase to inactivate any remaining nucleotides (especially dCTP) so that biotin-dCTP may be efficiently incorporated during ASPE. Samples should also be treated with EXO I to degrade any remaining PCR primers which may interfere with the tagged ASPE primers and extension reactions.



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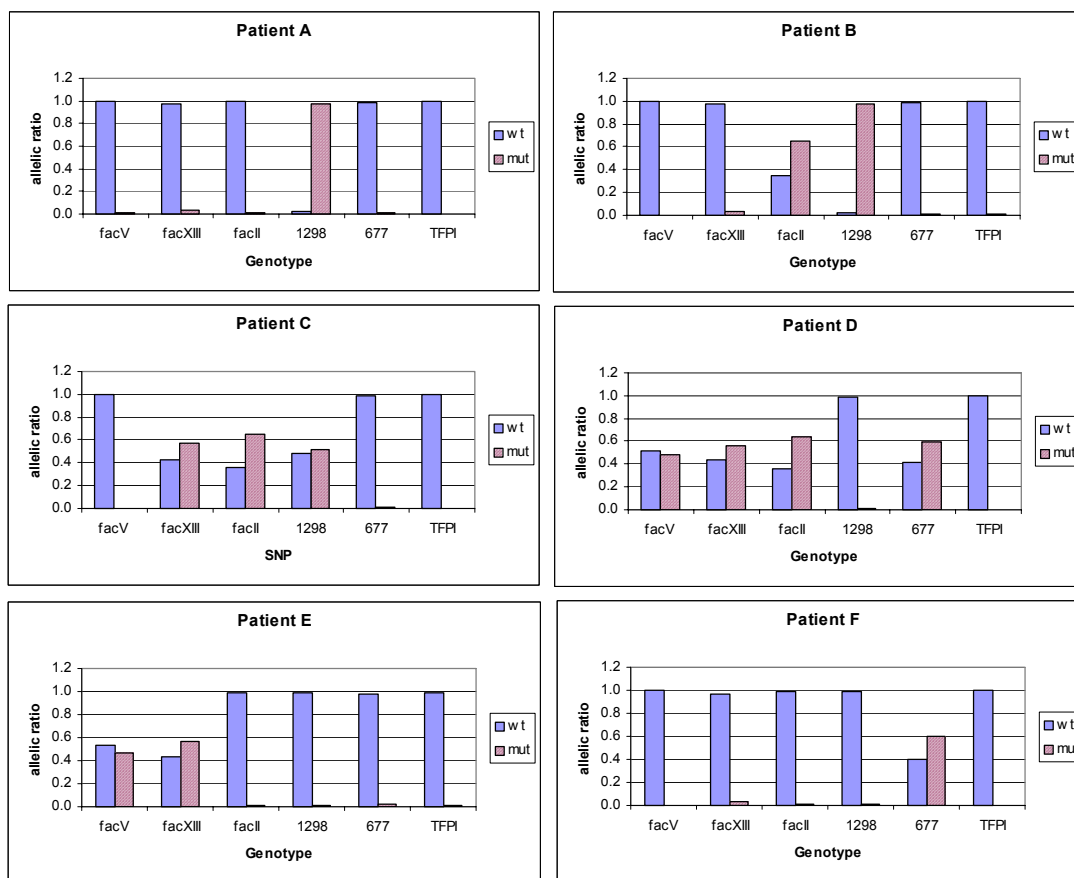
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### c) Hybridization on the FlexMAP Microspheres and Results

Immediately following ASPE, aliquots of the reaction were added directly to microwells or reaction tubes containing the appropriate sets of FlexMAP Microspheres. Tagged, extended products labeled with biotin during ASPE were captured by their tag complements (anti-tags) on the FlexMAP microspheres by hybridization (37°C). A fluorescent reporter molecule (streptavidin-phycoerythrin) was used to indirectly detect the ASPE-incorporated biotin. Samples were analyzed with the Luminex100 system. The signals generated for each bead population were used to assess the sample genotype. In these examples, the median fluorescence intensities (MFI) were used to calculate allelic ratios for each of the biallelic markers as follows:

$$\text{Allelic Ratio} = \frac{\text{MFI}_{a_1}}{(\text{MFI}_{a_1} + \text{MFI}_{a_2} + \dots + \text{MFI}_{a_n})}$$

Where  $\text{MFI}_{a_1}$  = the median fluorescence intensity of allele  $a_1$  and  $\text{MFI}_{a_2}$  = median fluorescence intensity of allele  $a_2$ . For these assays, arbitrary cut-offs were set. To be homozygous for a particular allele, the allelic ratio must be at least 0.75. To be heterozygous, each allele of the SNP must have a ratio of between 0.25 and 0.75. Consequently, an allele with a ratio of 0.25 or less is considered negative (i.e. not present). Sample results are shown below.



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The specific configurations and conditions for your assay applications can be established using this example as a general protocol. You may wish to refer to publications and other sources of information regarding multiplex PCR amplification, allele specific primer extension, and other laboratory methods. Additional product information is available on the Luminex Corporation website ([www.luminexcorp.com](http://www.luminexcorp.com)) and from Tm Bioscience ([www.universalarray.com](http://www.universalarray.com)).

### General References

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- Dunbar, S.A. and J.W. Jacobson. 2000. Application of the Luminex LabMAP in rapid screening for mutations in the cystic fibrosis transmembrane conductance regulator gene: A pilot study. *Clinical Chemistry* **46**: 1498-1500.
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- Ye, F., M.-S. Li, J.D. Taylor, Q. Nguyen, H.M. Colton, W.M. Casey, M. Wagner, M.P. Weiner, and J. Chen. 2001. Fluorescent microsphere-based readout technology for multiplexed human single nucleotide polymorphism analysis and bacterial identification. *Human Mutation* **17**: 305-316.



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## APPENDIX A

Bead	Anti-Tag (on the bead)	Tag (appended to primer)	Bead	Anti-Tag (on the bead)	Tag (appended to primer)
LUA-1	GATTGTATTGATTGAGATTAAG	CTTTAATCTCAATCAATACAAATC	LUA-51	ATTGTTGATGATTGATTGAAATGA	TCATTTCAATCAATCATCAACAAT
LUA-2	TGATTGTAGTATGTATTGATAAAG	CTTTATCAATACATACTACAATCA	LUA-52	ATTGTGAAGTATAAAGATGTTGA	TCAAATCATCTTTATACITTCACAAT
LUA-3	GATTGTAAGATTGATAAAGTGTA	TACACTTTATCAAATCTTACAATC	LUA-53	TGAGAAGATGAGATGATAATTA	TAATTATACATCTCATCTTCTACA
LUA-4	GATTTGAAGATTATTGGTAATGTA	TACATTAACCAATAATCTTCAAATC	LUA-54	ATGAATTGAAAAGTATTGAAAAG	CTTTTCAATCACTTTCAATTCAT
LUA-5	GATTGATTATTGTATTGAATTTG	CAATTCAAATCACAATAATCAATC	LUA-55	GTTAGTATTGAGAAGTGTATATA	TATATACACTTCTCAATAACTAAC
LUA-6	GATTGTATTGAAAAGATTGTTGA	TCAACAATCTTTACAATCAAAATC	LUA-56	AAAGTGATGTATATGAGTAAATTTG	CAATTTACTCATATACATCACTTT
LUA-7	ATTGGTAAATTTGGTAAATGAATTTG	CAATTCATTTACCAATTTACCAAT	LUA-57	GTAATGATAAAGATGATGATATTG	CAATATCATCATCTTTATCAATAC
LUA-8	GTAAGTAAATGAATGAAAAGGATT	AATCCTTTTACATTTACTTACTTAC	LUA-58	GTAGTAATGTTAATGAATTAGTAG	CTACTAATCTATTAACATTACTAC
LUA-9	GTAAGATGTTGATATAGAAGATTA	TAATCTTCTATATCAACATCTTAC	LUA-59	AAAGTAAAAAAGATTGATTGATGA	TCATCAATCAATCTTTTCACTTT
LUA-10	TGTAGATTGTATGTATGTATGAT	ATCATAACATACATAAATCTACA	LUA-60	ATGAGATTATTGGATTGTAGATT	AACTTACAAAATCAATAATCTCAT
LUA-11	GATTAAGATGATTGATGATTGTTGA	TACAATCATCAATCACTTTAATC	LUA-61	TGAAGATTATGAAATTTGGTAAAGATT	AACTTACCAATTCATAATCTTCA
LUA-12	AAAGAAAAGAAAAGAAAAGTGTA	TACACTTTCTTTCTTTCTTTCTTT	LUA-62	ATTGGATTATGAGATTATGATTGA	TCAAATCAATCTCATAATCCAAT
LUA-13	TTAGTGAAGAAAGTATAGTTTATTG	CAATAAACTATACCTTCTCACTAA	LUA-63	TGTAGTATAAAGTATATGAAGTAG	CTACTTCATATCTTTACTATACA
LUA-14	AAAGTATAGTAAGATGTATAGTAG	CTACTATACATCTTACTATACITTT	LUA-64	GTAAGTAGTAATTTGAATATGATG	CTACATATTCAAATTTACTACTTAC
LUA-15	TGAATTTGATGAATGAATGAAGTAT	ATACTTCATTCATTCATCAATTTCA	LUA-65	AAAGGTAAGATTATTGATGAAAAG	CTTTTCTCAATAATCTTACTCTTT
LUA-16	TGATGATTTGAAATGAAGATTGATT	AATCAATCTTCAATCAAAATCATCA	LUA-66	GTAGATAGTATAGTTGTAATGTTA	TAACATTAACAATTAACATCTAC
LUA-17	TGATAAAGTGATAAAGGTTAAAG	CTTTAATCCTTTTATCACTTTATCA	LUA-67	GATTTGTAATTTGTTGAGTAAATGA	CTACTTACTCAACAATTAACAATC
LUA-18	TGATTTGAGTATTGAGATTTTGA	TCAAAAATCTCAAATCTCAAATCA	LUA-68	AAAGAAAAGATTGTTGAGATTATGA	TCATAATCTCAACAATCTTTCTTT
LUA-19	GATTTTGAGTAAAGTAAATGATTTGA	TCAATCAATTTACTTACTCAAATAC	LUA-69	GATGTGAATGTAATATGTTTATAG	CTATAAACAATTAACATCTCACAIC
LUA-20	GATTGTATTGAAGTATTGAAAAG	CTTTTACAATCTTCAAATCAAAATC	LUA-70	TGATATGAATTTGGATTATTGGTAT	AATCCAATTAATCCAATTCATATCA
LUA-21	TGATTTGAGATTAAAGAAAAGGATT	AATCCTTTCTTTAATCTCAAATCA	LUA-71	ATGAATTTGATTGGATTGTAATGAT	ATCATTAACAATCCAATTAATTCAT
LUA-22	TGATTTGAATTTGAGTAAAAGGATT	AATCCTTTTACTCAATTTCAATCA	LUA-72	GATATTGGATTAAAGGTAATGA	TCATTTACTTTAATCCAATTAATC
LUA-23	AAAGTTGAGATTGAAATGATTGAA	TTCAATCATTCAAATCTCAACTTT	LUA-73	ATTGTTGAATTTGATGAGATTGAT	ATCAAAATCTCATCAATTCACAAT
LUA-24	GATTTGTATTGAAAAGGTAATTTGA	TCAATTTACTTTTCAATACAATAC	LUA-74	TGAAATTTAGTTTGAAGATGTTGA	TACACATCTTACAACAATTAATTTCA
LUA-25	TGAAGATTGGAAGTAAATGAAAAG	CTTTTCAAATCTTCAAATCTTCA	LUA-75	TGAAAAGATTGAAAAGTATGATT	AATCAACCTTTCAATCTTTTACA
LUA-26	TGAAAAGTTGATGATTTTGAAGTAA	TTACTCAAAAATCTACACTTTTCA	LUA-76	GTATTTAGATGAGTTTGTAGATT	AACTTAACAAAATCATCTAAATAC
LUA-27	AAAGTTGAGTATTGATTGAAAAG	CTTTTCAAATCAATACTCAACTTT	LUA-77	GTATGTATTGTATGATGTTAATTTG	CAATTAACATACATAAATACATAC
LUA-28	TTGATAATGTTTGTGTTTGTAG	CTACAACAACAACAATATATCAA	LUA-78	TGATATAGATAGTTAGATAGATAG	CTATCTATCAACTATCTATATCA
LUA-29	AAAGAAAAGGATTTGTAGTAAAGATT	AATCTTACTACAATCTTCTTTCTTT	LUA-79	ATGATGATGATTTGATGATGAA	TTCAATCAACAATCATCATCAT
LUA-30	TGAAAAGAAAAGGATATAAAGGTAA	TTACCTTTTATACCTTTCTTTTAC	LUA-80	GTTAGTTAGATTATTGTTAGTTAG	CTAACAATAAATCTAACAATAC
LUA-31	GATTTAAAGTTGATTGAAAAGTGA	TTCACTTTTCAATCAACTTTAATC	LUA-81	ATTGTTAGAAAAGTGTAGATTAAG	CITTAATCTACACTTTCTAACAAT
LUA-32	GTAGATAGTTTGAAGTGAATAAT	ATTATTCACCTTCAAATTAATCTAC	LUA-82	ATGAGTATGTTATAGTGTATGTA	TACATACACTAATAACAATCACTCAT
LUA-33	AAAGGATTAAGTGAAGTAAATTTGA	TCAATTTACTTCACTTTAATCCTTT	LUA-83	TGTAATAGTGAAGTTAGATTGTAT	ATACAATCAACTTCACTAATTACA
LUA-34	ATGAATTTGGTATGTATGAAATGA	TCATTTCAATACATACCAATTTCAAT	LUA-84	ATTGATAGATGATTAGTTAGTTGA	TCAACTAACAATCATCTATCAAT
LUA-35	TGAAATGAATGAATGAATGAAATTTG	CAATTTCAATCAATCTTCAATTTCA	LUA-85	TGATTTTGTATTGATGATGATAT	ATACTACATCAATCAACAATCA
LUA-36	ATTGATTGGAATGAATGAAATTTG	CAATTTCAATCTTCAACAATCAAT	LUA-86	ATTGTTAGTATGTTAGTAAATTTAG	CTAATTTACTAACAATCAACAAT
LUA-37	ATTGAAAAGTGAAGATGAAAAG	CTTTTCACTTTTCACTTTTCAAT	LUA-87	TGATGTAAGTATTGATGTTAGTTT	AAACTAACAATCAACTTACATCA
LUA-38	ATTGTTGAAAAGTGAATGATTGA	TCAATTCATACACTTTTCAACAAT	LUA-88	GATTTGAAAATGAAAAGTGAAGTAA	TTACTTCACTTTCTATTTACAATC
LUA-39	ATGATGTAATGAAAAGATTGTTGA	TACAACAATCTTTCAATCAATCAT	LUA-89	ATATGTTGTTGAGTTGATAGTATA	TATACTATCAACTCAACAACATAT
LUA-40	TAATGTTGTGAATAATGTAGAAAAG	CTTTTCACTAATTTCAACAACATTA	LUA-90	TTAGATGAATTTGGAAGTATTTAG	CTAAAATCTTCAACAATTCATCTAA
LUA-41	ATTGATGAGTATATTGTGATGTA	TTACTACACAATATACTCATCAAT	LUA-91	TGAAGTTATGATTGATGTTATGAA	TTCAATAACATCAATCATAACTTAC
LUA-42	GTTTATAGTGAATAATGAAAGTAAAG	CTATCTTCAATTTTCACTATAAATC	LUA-92	GTATTTGATGTTAAAAGTGAATAG	CTATTACACTTTAAACATCAATAC
LUA-43	TGTAATGAGTATTGTAATTTGAAAG	CTTTCAATTAACAATCTCAATACA	LUA-93	GTTTGTATTTAGATGAATGAAAAG	CITTTCTATCTCAATTAACAAC
LUA-44	GTATAAAGAAAAGATTGGTAAATGA	TCATTTACCAATCTTTCTTTATAC	LUA-94	ATTATTGAGTGAAGATGAAAAG	CITTTCTATCTTTACTCAATAAT
LUA-45	TTGAGTAAATTTGAATTTGAAAAGTGA	TCATTTCAACAATTTCAATTTACTCAA	LUA-95	TTAGTGTAGTAAAGTTAAAAGTGA	TACACTTTAAACTTACTACACTAA
LUA-46	TGATTTGAATGAATTTGATGTA	TACATCAACAATTTCAATCAATACA	LUA-96	GTTTAAAAGTTAGTTGAGTTAGTAT	ATACTAACTCAACTAATCTTAAAC
LUA-47	ATTATGAAGTAAAGTAAATGAGAAG	CTTCTCAATTAACCTTACTTCAATAAT	LUA-97	ATAGTGTATGATGATTTAGATGATT	AACTTCATAATCTACATCACTAT
LUA-48	ATTATTGAGATGGAAGTTGTTT	AAACAACCTTCAACTCTCAATAAT	LUA-98	TTGAATGATTAGTTGAGTATGATT	AATCACTCAACTAATCATCTCAA
LUA-49	GTAAGTAAATTTGAAAAGTATGATGA	TCATCAACTTTTCAATTTACTTAC	LUA-99	GTTATGAAATTTGTTAGTGTAGATT	AATCTACAAATCAACTTCAATAC
LUA-50	GTAATGATGATATTGGTATATTG	CAATATACCAATATCATCAATTTAC	LUA-100	GTTAGATTTGATGTTAAAAGTAG	CTATCTTTAAACTCAAAATCTAAC

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