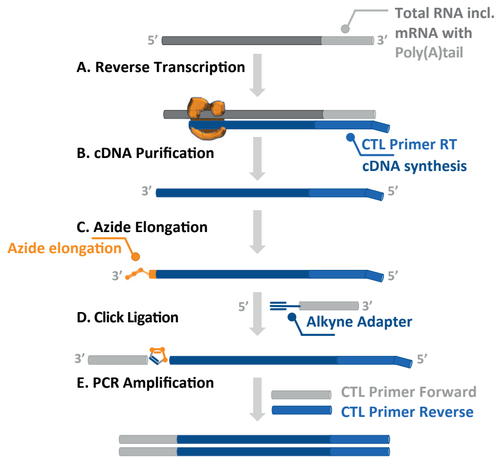
The Workflow: 5 Steps to success



baseclick's ClickTech Library Kit full-length mRNA_Seq has been developed as method for e.g. sequencing of total mRNA or the whole transcriptome of cells for genetic diagnosis. The kit provides reagents for cDNA synthesis of any RNA pool and introduces a single azido-nucleotide at the 3' END of cDNA.

This azido-modified cDNA can react with an alkyne-adapter sequence in a highly selective fashion under benign click reaction conditions. With the included special designed Primer, the adapter clicked cDNA can be used for PCR amplification of the cDNA pool as such or for targeted genes amplification. Sanger Sequencing of specific sequences achieved a high accuracy of 99.999% certainty.

The Proof: Why the ClickTech RNA Library Kit is superior to all other commercially available RNA Library kits!

- No inaccessible regions – full-length and complete transcriptome analysis
- Generated cDNA libraries can be amplified several times by PCR
- To determine alternative splicing and exon – intron organization of genes
- Quantification of splice variants and their true transcription start – and end sites
- mRNA-Seq is more efficient and inexpensive method for sequencing coding regions than total RNA sequencing

baseclick - Quantum leap solutions for New Generation Sequencing

Product Name	Product Number	Application	Price
ClickTech Library Kit full-length mRNA_Seq	BCK-CTL-FRS_10	Transcriptome Sequencing	720 €

Order at www.baseclick.eu

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Proprietary Technology | MADE IN GERMANY

ClickTech Library Kit full-length mRNA_Seq

The power of click technology opens new dimensions in Diagnostic, Research & Drug Development

Don't be satisfied with less!

baseclick DNA & RNA EXPERTS

Today: In RNA Library Kits enzymatic ligation is a limiting step, delivers only max. 40% efficiency and causes major problems

Mutations of exonic splicing enhancer motifs are significant contributors to genetic disorders.

Problem 1

Simple point mutations can inhibit affinity for splicing factors and result in alternative splicing, leading to altered mRNA sequences and protein translation. Today's conventional RNA library preparation kits are not able to detect this alternative splicing.

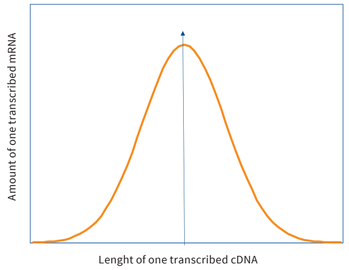
Problem 2

Randomized primers lead automatically to a statistical cDNA length distribution and to major downstream problems with analytical software. The rates of aberrant and pre- mRNA splicing cannot be evaluated at the nucleotide level to determine the quantity and identity of these events across splicing junctions. Today's conventional RNA Library kits use randomized primers and therefore are not able to identify each artifact and where the aligned reads differ from reference genomes.

Problem 3

In order to provide complete diagnostic information to the end user, full-length sequencing (from 5'End to 3'End) is absolutely essential e.g. for cancer tissue.

Today's conventional RNA library kits do not reverse transcribe the complete cDNA significantly from the 3'End to the 5'End especially the very important 5'End is inaccessible for these kits.



The use of randomized primers automatically leads to a statistical distribution of different length cDNAs. The complete cDNA from the 5'End to the 3'End is scarcely reverse transcribed.

baseclick's ClickTech mRNA Library Kit solves this problem.

NEW: ClickTech Ligation makes the difference in full-length RNA Library kits, has no limits and delivers up to 95% efficiency

baseclick's ClickTech Library Kit full - length mRNA Seq has been developed for sequencing of total mRNA or the whole transcriptome of cells for genetic diagnosis. The kit provides reagents for cDNA synthesis of any RNA pool and introduces a single 3'-azido nucleotide at the 3'End of cDNA.

This azido-modified cDNA can react with an alkyne-adapter sequence in a highly selective fashion under benign click reaction conditions. With the included specially designed primer, the adapter-clicked cDNA can be used for PCR amplification of the cDNA pool as such or for targeted genes amplification.

Sanger Sequencing of specific sequences achieved a high accuracy of 99.999% certainty.

ClickTech Ligation
- More Specificity
- More Sensitivity

More and precise diagnostic information

Prof. Dr. Afshin Samali, from the School of Natural Sciences, NUI Galway, Ireland states:"The new ClickTech Library Kit full-length mRNA_Seq developed by baseclick is the first true mRNA preparation kit covering the whole sequence of transcripts. Even for long transcripts, it results in full sequence coverage from 5'-UTR to 3'-poly A tail."

More therapeutic options for precision medicine

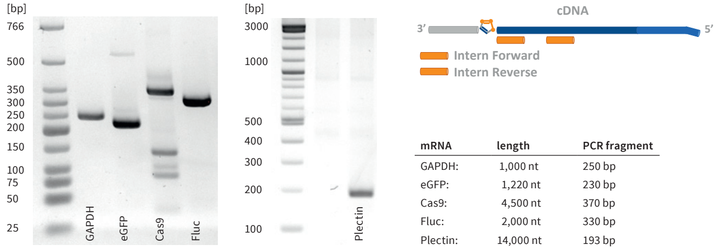
Prof. Dr. Afshin Samali: "This is particularly valuable for stratification of cancer patients, identifying those with high IRE1 activity and XBP1 splicing events, who would best respond to an IRE1 intervention therapy. It can also serve as a companion diagnostic to evaluate efficacy of such treatments in a wide range of malignancies."

Better clinical scientific success

ClickTech mRNA Library Kits full-length: Don`t be satisfied with less!

- ClickTech mRNA Library Kits are ideal for determining the location of both simple point mutations and alternative splicing and shows no limitation by exon – intron organization of genes
- No recurrent functional misinterpretations of RNA -seq data caused by sample specific gene length bias
- ClickTech mRNA Library Kits enable full-length and complete transcriptome analysis of cancer and other samples
- No inaccessible regions, nothing left out, from 5'End to 3'End complete diagnostic information
- ClickTech mRNA Library Kits enable the detection and correct quantification of splice variants and their true transcription start – and end sites, in both short and long mRNA molecules
- ClickTech mRNA library kits do not use randomized primers, nor problematic template switching oligonucleotides.
- No artefacts and clear information where the aligned reads differ from reference genomes

You can easily check if one or even more mRNAs were in your pool



mRNA	length	PCR fragment
GAPDH:	1,000 nt	250 bp
eGFP:	1,220 nt	230 bp
Cas9:	4,500 nt	370 bp
Fluc:	2,000 nt	330 bp
Plectin:	14,000 nt	193 bp

Figure shows PCR fragments that were amplified from a click ligated cDNA pool generated by the kit. The Intern Forward Primer (not provided within the kit) cover the very 5'End of the transcript and the second primer (Intern Reverse) binds to a gene specific reverse complement. Next to house-keeping genes like plectin, GAPDH, mRNAs for eGFP, Cas9 and Fluc were spiked into a Jurkat cell total mRNA pool for internal control. All PCR products have the desired fragment size.