Infographic

Common approaches to bioanalysis of oligonucleotides

Oligonucleotides (OGNTs) are short, single- or double-stranded DNA or RNA molecules consisting of strands with 10–15 nucleotides. By manipulating gene expression, OGNTs provide the opportunity to target diseases at their molecular level.

There are five main approaches to bioanalysis of OGNTs, each of which has its advantages and disadvantages. To help you determine which approach is right for you, check out the pros and cons listed below.

APPROACH TYPE	PROS	CONS
LC/UV	• Simple and straightforward since no extraction is needed • Inexpensive instruments • No IS • Method development is fairly straightforward as long as you have the reference standards available • Able to detect multiple analytes (antisense, sense, and metabolites in a single assay) if separation can be achieved and reference standards are available	• None selective, all components are identified based on reference material • Need reference material for all peaks to be cross-identified to the *in vivo* sample peaks • Not very sensitive • Sample analysis can be convoluted if there are multiple peaks in the sample that cannot be identified with known reference standards • Longer run times
LC/MS/MS	• Very selective • Much lower LLOQs than UV detection • Able to detect multiple analytes (antisense, sense, and metabolites in a single assay) • Sample is extracted so it can be concentrated to increase sensitivity if necessary • Faster run times than LC/FD or LC/UV assays	• Much more expensive instruments • Need analogue internal standard • Longer OGNTs have lower sensitivity • ESI (–) mode is not as sensitive as ESI (+) • Need to maintain LC and MS systems
HYBRIDIZATION LF/FD	• Very sensitive with a small aliquot • Specificity increases with the size of the OGNT • Rugged with modified OGNTs since you typically melt away the conjugated strand • If reference standards are available, you may be able to establish conditions to separate n, n-1, n-2.... metabolites	• Specificity is not as good as LC/MS • Long run times (~20 mins) • Longer MD times due to the longer run times • If metabolites are not fully separated the *in vivo* samples may have interfering peaks • Since LC is SAX based, it does not work with positive backbone OGNTs • If extractions are necessary, there is no internal standard to track well to well variances
LC/HRMS	• Very selective • Selectivity can be based on accurate mass of intact OGNT or on accurate mass of a fragment • Much lower LLOQs than UV detection • Able to detect multiple analytes (antisense, sense, and metabolites in a single assay) • The sample is extracted so it can be concentrated to increase sensitivity if necessary • Faster run times than LC/FD or LC/UV assays • With full scan analysis you can mine the data post-analysis	• Much, much more expensive instruments • Slower cycle time than triple quadropole mass spectrometers • Need analogue internal standard • Longer OGNTs have lower sensitivity • ESI (–) mode is not as sensitive as ESI (+) • Need to maintain LC and MS systems
HYBRIDIZATION ELISA	• Fast sample prep • Very, very fast run analysis • Works with modified oligonucleotides • Sensitivity is not impacted by the length of the oligonucleotide • The necessary instruments are not as expensive as mass spectrometers • Very sensitive	• Not as selective as mass spectrometry • Can only detect single strands (i.e., cannot detect sense and antisense strands in a single run) • Troubleshooting can be difficult • Critical reagents may be necessary • Lower linear range

Learn how our technical experts can help you find an approach that most closely aligns with your goals and get you the data you need fast.

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