What are the sources of dsRNA?

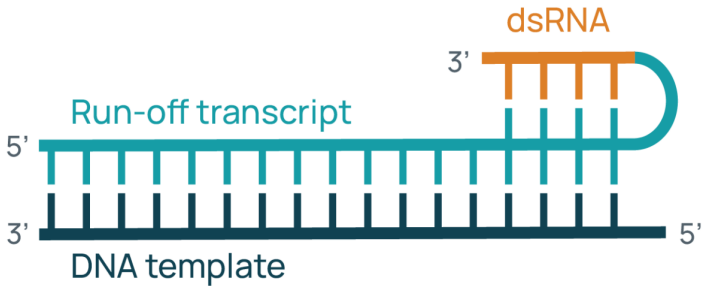
Double-stranded RNA (dsRNA) is a potential by-product of in vitro transcription (IVT). It is often associated with viruses, so when cells detect dsRNA, it triggers an immune response such as inflammation and targeted RNA degradation. When RNA-based therapeutics contain by-product dsRNA, cells may undergo harmful immune responses or even destroy the therapeutic before it can generate its intended effect.

Thus, understanding how dsRNA forms during IVT is critical for preventing dsRNA and improving RNA-based therapeutics' immunogenicity. In this FAQ, we will review three primary sources of dsRNA generation.

Intramolecular extension

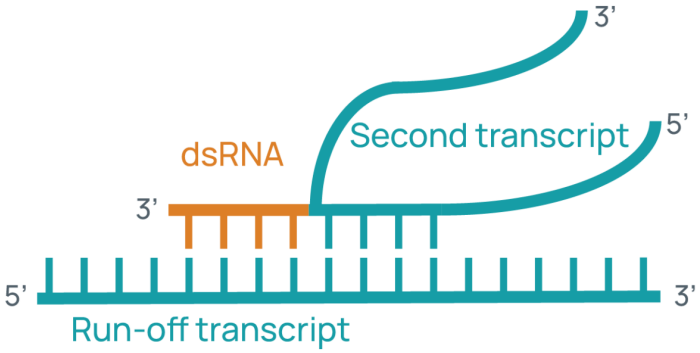
During a typical IVT reaction, RNA polymerase transcribes a strand of RNA with complementary bases to a DNA template, stopping when it reaches the 3' poly(A) tail. This single-stranded RNA is called the run-off transcript.

Sometimes, the run-off transcript folds over, looping back on top of itself into a hairpin structure that is connected through complementary base pairing. This loopback allows RNA polymerase to continue transcribing the run-off transcript beyond the poly(A) tail, forming dsRNA. Since this is the same RNA molecule interacting with itself, this reaction is considered to be an intramolecular source of dsRNA impurities that typically leads to higher molecular weight contaminants.



Intermolecular extension

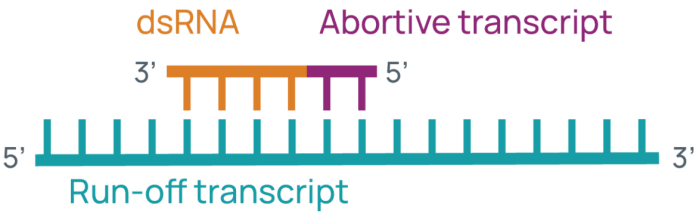
Instead of two regions interacting on the same molecule, complementary regions on two different RNA molecules can interact in solution enabling the polymerase to extend using one of the RNA molecules as a template. Similar to intramolecular extension, this leads to higher molecular weight dsRNA impurities.



Abortive transcription

When starting transcription, RNA polymerase can fall off shortly after transcription initiation, leading to the production of short RNA species called abortive transcripts.

Abortive transcripts can bind to the run-off transcript during an IVT reaction leading to dsRNA duplex formation and can trigger RNA polymerase re-engagement and dsRNA extension. This mechanism of dsRNA generation often leads to lower molecular weight impurities.



Reveal dsRNA with Eclipsebio

Fortunately, Eclipsebio's eSENSE dsRNA measures and locates dsRNA contamination. eSENSE dsRNA uses next-generation sequencing to determine the amount and location of dsRNA at single nucleotide resolution.

eSENSE dsRNA identifies exactly where RNA polymerase extension begins and ends, showing where dsRNA is present along the run-off transcript. The assay can also be combined with our eSHAPE structure profiling to offer insights into how RNA structure impacts dsRNA formation. These actionable insights offer suggestions on how to reduce dsRNA in RNA therapeutics, helping generate more effective RNA therapies.

Interested in measuring dsRNA contamination in your RNA therapeutic? Contact Eclipsebio to learn more.