Yield Rescue Field Guide

A practical troubleshooting cheat-sheet for in-house oligo synthesis.

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Prepared for giveaway use (diagnostic credibility piece).

How to use this guide

This is a compact troubleshooting reference for diagnosing yield issues from common LC-MS / chromatogram patterns. Start by classifying your dominant impurity pattern (n-1 ladder, 5'-failure ladder, phosphate-attached failures, or specific mass-offset peaks), then apply the corrective actions listed below.

What to include in your LC-MS target list

All 5'-deletions (traditional "failure sequences" - truncated from the 5' end)

Example for 5'-CATGT-3': 5'-ATGT-3', 5'-TGT-3', 5'-GT-3' (typically exclude monomers).

All 3'-deletions (truncated from the 3' end)

Example for 5'-CATGT-3': 5'-CATG-3', 5'-CAT-3', 5'-CA-3'.

All 5'-failures with phosphate attached

Example for 5'-CATGT-3': 5'-P-ATGT-3', 5'-P-TGT-3', 5'-P-GT-3'.

All 3'-failures with phosphate attached

Example for 5'-CATGT-3': 5'-P-CATG-3', 5'-P-CAT-3', 5'-P-CA-3'.

Quick pattern index

- High n-1 ladder -> detritylation / coupling / capping / oxidation / wet reagents.
- High 5'-failure ladder -> coupling (most common) or oxidation (rare).
- Phosphate-attached failures + phosphate-attached 3'-deletions -> depurination signal.
- n+1 -> activator too acidic. n+x (x>=2) after FLP -> amidite decomposition / branching.
- m+275/m+261 (universal linker) or m+302 (DMT) -> cleavage/deprotection too mild.
- m+366/m+286 -> incomplete oxidation.
- m+41 -> capping behavior. m+53 just after FLP -> incomplete DEA reaction.

Diagnostic playbook

Match your pattern, then run the fixes in order (fast -> slow).

High n-1 ladder (truncations dominate)

Bad detritylation yields

• Double deblock times, adjust pulse times, and/or increase the number of reps to increase the time deblock is in contact with (or flowing through) the resin.

Bad coupling and bad capping

• Double coupling times or number of reps to greatly increase the time phosphoramidite/activator and capping reagents are in contact with (or flowing through) the resin.

Bad oxidation yields

• Increase oxidation contact time (time oxidizer is in contact with/flowing through the resin).

• Try reversing the Ox/Cap steps, or use Cap/Ox/Cap or Cap/Ox/Ox/Cap.

Old or wet reagents (especially phosphoramidites + activator)

• Replace phosphoramidites with freshly prepared reagents made with very dry ACN.

• Add drying traps (e.g., ChemGenes, BioSearch, AIC, etc.) to: all phosphoramidites, the ACN bottle, and the activator bottle.

• Note: drying traps take ~24 hours to remove ~80% of H2O-plan ahead.

First cycle kinetics are slower

• Double detritylation time and coupling time for the first cycle only.

High 5'-failure sequences (n-1, n-2, n-3…)

Poor coupling

• Double coupling times or number of reps to greatly increase the time phosphoramidite/activator is in contact with (or flowing through) the resin.

Poor oxidation (rare)

• If oxidation is the driver, the 5'-failure ladder is often accompanied by m+366 and/or m+286 peaks (eluting very late).

• Increase oxidation time.

• Increase I2 concentration above 20 mM (if not using dmf-dG phosphoramidite).

High 5'-failures with phosphate attached + significant 3'-deletions with phosphate (or phosphate +98)

Excessive depurination

• Reduce deblock exposure time (limit to 60 s, especially if using TCA).

• Test different deblocks (concentration and/or acid identity) and tune exposure to reduce depurination while maintaining detritylation yield.

High n-1s with very little 5'-failure ladder

Bad capping yields

• Replace capping solutions.

• Increase capping times.

• Test different capping formulations.

n+1 impurity

Activator is too acidic (DMT removed → dimer → adds to 5'-OH during coupling)

• Switch to a less acidic activator (e.g., DCI or tetrazole).

n+x (x = 2, 3, 4…) impurities after the FLP peak

Protecting groups coming off phosphoramidites in the bottle (pre-synthesis) → branching off exocyclic amines

• Replace phosphoramidites.

m+275 and/or m+261 (universal linker) and/or m+302 (DMT) - peaks after FLP

Cleavage/deprotection too mild

• Longer or otherwise harsher cleavage/deprotection conditions are required.

Note

• Molecular weights can vary depending on Me vs Ph on the universal linker, and how far succinimide ring degradation has progressed.

m+366 and/or m+286 - elute long after FLP

Trityl adds to remaining P(III) if oligo was not fully oxidized to P(V)

• Increase oxidation time.

• Increase I2 concentration above 20 mM (if not using dmf-dG phosphoramidite).

Large m+41 peak

Bad capping yields

• Tune capping times to minimize m+41 without increasing n-1 impurities (from incomplete capping).

• Consider alternative capping solutions.

Excessive m+53 peak (just after FLP)

Incomplete DEA reaction

• Use longer reaction times with DEA (post-synthesis, pre-cleavage).

• For the first few DEA additions (or other hindered amine), push solution through columns quickly (<10 s) to rinse acrylonitrile away from the oligo.

Mass-difference cheat sheet

Common annotations observed in crude LC-MS. Use as a quick reference when assigning peaks.

Common impurity / annotation	Mass Δ (Da)	Formula (as noted)
Cyanoethyl	53.0265	C3H3N
CE2	106.0530	C6H6N2
m+linker-1	275.0195	C9H10N1O7P1
m+linker-2	261.0039	C8H8N1O7P1
10mer-P	79.9663	PO3H
DMT	302.1307	C21H18O2
Transamination	14.0156	CH2
DMF	55.0422	C3H5N
BZ	104.0262	C7H4O
Ac	42.0106	C2H2O
m+FAM	537.1190	-
n+T	304.0460	-
n+G	329.0525	-
n+A	313.0576	-
n+C	289.0464	-
-A+OH	-115.0284	C5H3N5, +O
-G+OH	-131.0233	C5H3N5
-C+OH	-91.0171	C4H3N3
-T+OH	-106.0168	C5H4N2O
-T (in source)	-125.0352	C5H5N2O2
-G (in source)	-150.0417	C5H4N5O1
-A (in source)	-134.0468	C5H4N5
-C (in source)	-110.0355	C4H4N3O

Notes

• Keep naming conventions consistent inside your team; the exact naming scheme is less important than consistency.
• FLP = full-length product.
• Want a second set of eyes on a chromatogram? Reply with an exported trace + crude LC-MS and we'll point you to the most likely constraint.