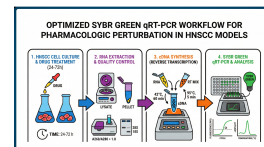


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Optimized SYBR Green qRT-PCR Workflow for Pharmacologic Perturbation in Head and Neck Squamous Cell Carcinoma (HNSCC) Models (TR146 and SCC58)



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We use this protocol and it's working

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Abstract

This protocol details a standardized SYBR Green-based qRT-PCR workflow for assessing transcriptional responses to pharmacologic perturbations (e.g., 4NQO, BaP) in head and neck squamous cell carcinoma (HNSCC) lines (TR146 and SCC58). Unlike general qPCR methods, this workflow is optimized for comparative toxicology, emphasizing strict matched-vehicle controls, light-sensitive handling for polycyclic aromatic hydrocarbons (PAHs), and plate-level normalization. It utilizes the $\Delta\Delta C_t$ method with GAPDH normalization to quantify relative gene expression changes following DNA damage or AHR pathway activation.

Image Attribution

Created by the author (Boluwatife OLU Afolabi) using SciDraw

Guidelines

Suitable for:

- Dose-response and time-course analysis of environmental toxins.
- Determining transcriptional signatures.
- Evaluating AHR antagonist efficacy (e.g., CH223191).

Not suitable for:

- Absolute quantification (copy number).
- Clinical diagnostic tissue samples without modification.



Materials

Cell Models

- **TR146 Cells:** Oral squamous carcinoma.
- **SCC58 Cells:** Oral squamous carcinoma.
- **Culture Media:** (e.g., DMEM/F12 or user-specific formulation) supplemented with FBS; PBS

Chemical Treatments

- **4-nitroquinoline 1-oxide (4NQO):** (Stock dissolved in DMSO; Store at -20°C).
- **Benzo[a]pyrene (BaP):** (Stock dissolved in DMSO; **Light Sensitive**; Store at -20°C).
- **CH223191 (AHR Antagonist):** (Stock dissolved in DMSO; **Light Sensitive**; Store at -20°C).
- **Vehicle Control:** Dimethyl sulfoxide (DMSO), PCR grade.

Molecular Biology Reagents

- **RNA Extraction:** Zymo Quick-RNA Kit (or equivalent).
- **cDNA Synthesis:** Bio-Rad cDNA Synthesis Kit.
- **qPCR Master Mix:** SYBR Green qPCR Master Mix (e.g., Bio-Rad SsoAdvanced).
- **Primers:** Gene-specific forward/reverse primers (validated for ~60°C T_m).
- **Plates:** 96-well 0.2 mL PCR plates (e.g., Bio-Rad Hard-Shell) and optical seals.

Safety warnings

- ❗ 1. **Carcinogen Hazard:** 4NQO and BaP are potent mutagens and carcinogens. Handle only in a certified biosafety cabinet while wearing appropriate PPE (double gloves, safety glasses).
- 2. **Waste Disposal:** All tips, tubes, and media contacting these chemicals must be disposed of as hazardous chemical waste, not biohazard trash.
- 3. **Light Sensitivity:** BaP and CH223191 are photo-reactive or prone to degradation. Perform treatments in low light and wrap tubes/plates in foil.

Step 1: Cell Seeding and Treatment

- 1 **Seeding:** Seed TR146 or SCC58 cells into 6-well plates (approx. $2-5 \times 10^5$ cells/well) to reach ~70-80% confluency at the time of treatment. 
- 2 **Stock Preparation:** Prepare 1000x stock solutions of 4NQO, BaP, or CH223191 in sterile DMSO. 
- 3 **Treatment Media:** Dilute stocks 1:1000 into fresh, pre-warmed media.
 - *Critical:* Ensure the final DMSO concentration is $\leq 0.1\%$ in all wells, including the Vehicle Control.
- 4 **Exposure:** Aspirate old media, wash with PBS and apply treatment media. 
 - **For BaP/CH223191:** Immediately wrap plates in aluminum foil to protect from light.
- 5 **Incubation:** Incubate at 37°C / $5\% \text{CO}_2$ for the designated time course (e.g., 6h, 12h, 24h).

Step 2: RNA Extraction & cDNA Synthesis

- 6 **Harvest:** Aspirate media and wash cells once with PBS.
- 7 **Lysis:** Add lysis buffer directly to the well (per Zymo kit instructions) and scrape cells.
 - *QC Check:* Assess cell morphology prior to lysis. If significant cell death is visible ($>20\%$), data may be confounded by apoptosis rather than specific signaling.
- 8 **Extraction:** Proceed with Zymo Quick-RNA protocol including the in-column DNase I treatment step to remove genomic DNA.
- 9 **Quantification:** Measure RNA concentration (Nanodrop).
A260/280 ratio should be > 2.0 .
- 10 **cDNA Synthesis:** Normalize all samples to **500 ng** total RNA input. Synthesize cDNA using the Bio-Rad iScript/cDNA kit (20 μL reaction volume). 
 - Store cDNA at -20°C .



Step 3: qRT-PCR Plate Setup

- 11 **Primer Mix:** Prepare a working stock of Forward/Reverse primers (final reaction concentration usually 300–500 nM).
- 12 **Reaction Mix (per well):**
 - **SYBR Green Master Mix:** 5.0 µL
 - **Forward Primer:** 1.0 µL
 - **Reverse Primer:** 1.0 µL
 - **Nuclease-Free Water:** 1.0 µL
 - **cDNA Template:** 2.0 µL (diluted 1:5 to 1:10 if necessary)
 - **Total Volume:** 10.0 µL
- 13 **Plating:** Pipette into 96-well plate. Run technical duplicates or triplicates. 
- 14 **Sealing:** Apply optical seal and briefly centrifuge (1 min at 1000 x g) to remove bubbles.

Step 4: Thermal Cycling Conditions

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	Step	Temperature	Time	Note
	Activation	95.0°C	3:00	Polymerase activation
	Denature	95.0°C	0:15	
	Anneal/Ext end	55.0°C	0:30	
	Repeat	GOTO Step 2	39x	Total 40 cycles
	Melt Curve	65.0°C – 95.0°C	0.5°C inc	0:05 per step

Optimization Note: 55°C is used here for specific primer compatibility. Standard Bio-Rad cycling often uses 60°C for Step 3. Ensure your primers are validated for 55°C.

Step 5: Data Analysis (Microsoft Excel / GraphPad Prism)



- 16 **Export:** Export quantization data (C_t values) to Excel.
- 17 **QC Filter:** Flag any replicates where C_t variance > 0.5.
- 18 **Normalize (ΔC_t):** Calculate $\Delta C_t = C_t(\text{Target}) - C_t(\text{GAPDH})$ for each sample.
- 19 **Normalize to Control ($\Delta\Delta C_t$):** Calculate $\Delta\Delta C_t = \Delta C_t(\text{Treated}) - \Delta C_t(\text{Vehicle Control})$
- 20 **Fold Change:** Calculate relative expression using the formula:
$$\text{Fold Change} = 2^{-\Delta\Delta C_t}$$
- 21 **Statistics:** Perform one-way ANOVA (for dose-response) or t-tests (paired by plate) in GraphPad Prism.

Protocol references

The Methodological Standard (The "Pfaffl" or Livak Method)

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4NQO as an Oral Cancer Model

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