Molecular decontamination of micropipettes and work surfaces

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PCR and RT-PCR are sensitive methods for detecting the presence of either DNA or RNA for research and diagnostic applications. The most significant threat to reliable results is carry-over contamination by products of previous PCR and cloning steps. UV-irradiation at of 254 nm damages double-stranded DNA by forming pyrimidine-pyrimidine photoadducts, cyclobutyl pyrimidine dimers, by oxidizing bases and by introducing single and double-strand breaks (SSB & DSB), all of which stall the Taq DNA polymerase and prevent PCR and RT-PCR reactions to take place. UV irradiation has been shown to decrease carry-over contamination by 1,000 to 100,000-fold, especially on DNA fragments > 150 bp. However, the extent of UV decontamination decreases with the square of the distance between the light source and the irradiated material (rendering roof mounted or handheld irradiators virtually useless for surface decontamination).

Wiping surfaces with detergent has the same effect as using a commercial DNAse solution lie DNA away®, eliminating nearly 30% of all DNA present. Although wiping surfaces down with 0.1% NaOCl has been found to be sufficient for DNA decontamination (assessed through qPCR), exposure to 254 nm UV light for up to an hour (at less than 10 cm distance and with clean lamp tubes) yields even better decontamination of materials.

This protocol focuses on the molecular decontamination procedure as well as surface decontamination of workareas. As NaOCl is also used, biological decontamination is also ensured.

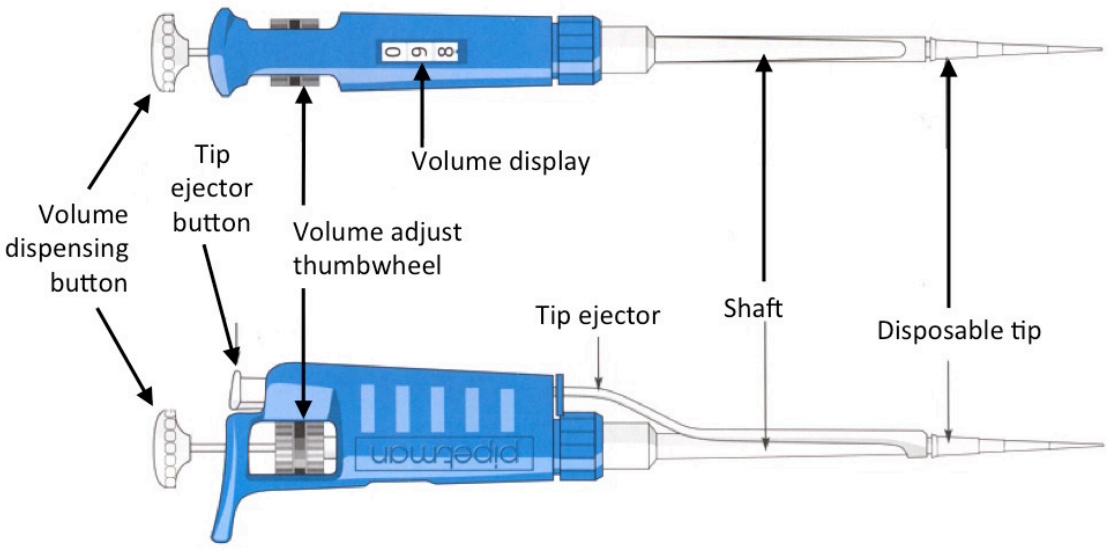
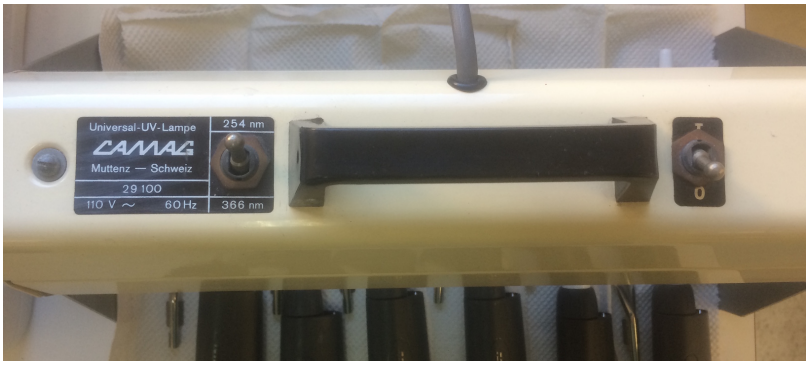


Diagram showing micropipette parts and their names

Micropipette decontamination

1. Disassemble micropipettes to remove tip ejector, if possible.
2. Wipe down tip ejector and pipette shaft with soap and water.
3. Wipe down tip ejector and pipette shaft with 0.1% NaOCl.
4. Wipe down tip ejector and pipette shaft with 70% ethanol.
5. Wipe down entire pipette body and buttons with 70% ethanol.
6. Clean the 254 nm UV lamp tube (the transparent one not the black–light one) with 70% ethanol.



Camag universal UV light source set for 254 nm in the off (0) position.

7. Place absorbent paper towels under light source and arrange micropipette shafts and tip-ejectors left-side up, irradiate for 20 to 40 minutes.


Micropippetes and ejector-tip shafts under UV light source.

8. Turn micropipette shafts and tip-ejectors right-side up and irradiate for 20 to 40 minutes.

9. Place UV warning sign in front of light source as well as in the RT-PCR room's door and the RT-PCR workstations inside the RT-PCR room where the irradiation is taking place.

"UV light in operation" warning signs in front of light source and room entrance.

"UV light in operation" warning signs placed in workstations next to light source.

10. After UV irradiation has been accomplished, re-assemble micropipettes and remove all warning signs.

11. Document micropipette decontamination in the corresponding laboratory log.

Bitácora general
Laboratorio de Genómica Viral y Humana
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Bitacora General
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Bitácora de descontaminación de micropipetas

Fecha	Ubicación	Descontaminación	Responsable	Notas

Laboratorio de Genómica Viral y Humana - Facultad de Medicina - Universidad Autónoma de San Luis Potosí

Micropipette decontamination log example (in Spanish)

Laboratory surface molecular decontamination

1. Wash all surfaces with soap and water, preferably using diluted extran.

2. Wipe down work surface with fresh 0.1% NaOCl solution.

3. Wipe down work surface with 70% Ethanol to prevent NaOCl residue accumulation and oxidation of metal parts.

Notes

1. Use of the DNA Away trade name is for identification only and does not imply endorsement nor preference by our lab.

2. Avoid attempts at UV-irradiating solutions contained in plastic recipients as many plastics used for molecular biology absorb UV rays.

3. Do not submerge micropipettes or thumbwheel mechanism in any liquid unless otherwise stated by the manufacturer.

4. Sterilization in autoclaves provides no additional benefit to the molecular decontamination of micropipettes, but cell biology technique might require this.

References

1. An efficient multistrategy DNA decontamination procedure of PCR reagents for hypersensitive PCR applications. Champlot S, Berthelot C, Pruvost M, Bennett EA, Grange T, Geigl EM. PLoS One. 2010 Sep 28;5(9). PMID: 20927390.

2. Photosensitized reactions of nucleic acids. Cadet J, Berger M, Decarroz C, Wagner JR, van Lier JE, Ginot YM, Vigny P. Biochimie. 1986 Jun;68(6):813-34. Review. PMID: 3092878.

Revision history

1.0 Original document.