

**Biomakespace COSHH Assessment – CRISPR intermediary work COSHH**

Important: Please complete this after reading the [BMS15 Chemical Safety Policy](https://docs.google.com/document/d/1jqt7U67YcpqbM-yku7-O4V3EzZd7p3cp1H4lFuTk4Nk/edit#heading=h.pdre7kyhkn2a) and any other [relevant safety policies](https://drive.google.com/drive/folders/0B9V3tIqIu0UZdFRIdzBOWDE5OFk).

This COSHH covers preparation of yeast media, preparation of chemicals for transformation, E coli culturing and minipreps to prepare plasmid DNA, DNA quantification, yeast culturing and testing of transformation protocol, assessment of DNA digest success using agarose gels, and use of an NEB Monarch DNA extraction kit to prepare DNA for sequencing.

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| **Chemical**  *Or biological agent* | **Chemical risk and hazard categories (R-phrases) Hazard and Work Place Exposure Limits (WEL)** |
| YPD media (broth)   YPD plates (agar)   Kanamycin Solution (in broth or plates)   Minimal Yeast Media, supplemented with sugars and to exclude 1 nutrient (e.g. Ura-) (agar)   50 % PEG-4000   50% PEG-3350   1 M / 0.1 M LiOAc   Salmon Sperm DNA (10 mg/mL)    Ampicillin Solution (in plate)    Kanamycin Solution (in plate)   LB broth   LB Agar plates    Restriction enzymes   BioBasic Miniprep Kit    Monarch DNA extraction kit   R-Zymolyase  Ethidium bromide  SYBR-based DNA dyes inc SYBR Safe    Agarose dry  Agarose molten | None  None   H360 – may damage to fertility or unborn child  R61 – may cause harm to unborn child  None    None   None   None   None    H317 – may cause allergic skin reactions H334 – may cause breathing difficulties, allergy or asthma symptoms if inhaled  H360 – may damage to fertility or unborn child  R61 – may cause harm to unborn child  None   None   None   None   None   None   H341 - Suspected of causing genetic defects  H331 Toxic if inhaled  H302 - Harmful if swallowed --> Absorption  R36/37/38: Irritating to eyes, respiratory system, and skin --> Inhalation  Causes burns --> Skin |
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| **Control Measures** [*Fume Cupboard, glove box, safety cabinet, local exhaust ventilation*]  No requirement for specific control measures for most listed, use in well-ventilated space. Use of lab coat and gloves at all times when handling ethidium bromide.  Good ventilation should be ensured when pouring gels.  Gels should be poured in the gel area to limit potential contamination of other areas of the lab. | |
| **Flammables and explosives**  *Is there a substance used or formed that might give rise to a fire or explosion?* **No** | |
| **Personal Protective Equipment** [*Lab coat/overalls, gloves, eye/hearing/respiratory protection*]  Use of lab coat and gloves while handling chemicals and biological material. Eye protection should be worn while handling hot liquids.  Heat resistant gloves should used while handling molten agarose in bottles. | |
| **Monitoring** [*Chemical, gas, oxygen depletion etc.*]  None | |
| **Health surveillance required** [*E.g. Carcinogen, mutagen, toxic to reproduction, sensitizer*]  EtBr is suspected of causing genetic defects so work should be carried out with extra care to avoid any skin contact or inhalation but no health surveillance is required.  As per Biomakespace Chemical Safety Policy BMS15, anyone who is pregnant is encouraged to report to the Biomakespace Safety Officer so a personal risk assessment can be performed. | |
| **Storage**  Kits are stored in a dry, cool and well-ventilated area  LB agar plates should be kept at 4c  Glycerol stocks should be kept at -80C  Biological components/buffers should be kept at -20C  Small aliquots of working solution should be stored to avoid frequent handling of concentrated stock solution.  SYBR-based dyes should be kept in the freezer if advised by manufacturer. | |
| **Waste disposal** [*Contractor, chlorinated, non-chlorinated, non-hazardous aqueous, general waste*]  Used/unused LB agar plates should be autoclaved and disposed of as standard waste  Kits should be disposed of as hazardous waste  Used growth cultures should have liquid decanted into chemgene, with tubes autoclaved and disposed of as standard waste Gels must be wrapped in a double plastic bag and can be disposed in the normal general waste.  Running buffer containing traces of EtBr must be decontaminated using activated charcoal as per waste policy and can then be disposed of down the sink.  SYBR-containing running buffer will be disposed off as chemical waste. | |
| **Emergency Procedure**  Follow emergency procedures as per BMS08 - Accident at Work Emergency Plan.  In the event of a fire, supply emergency services with chemicals register as necessary.  For small spillages, clean up with tissues and general surface cleaner, dispose of the tissues as contaminated waste. For larger spillages use spill kit and dispose of pads as contaminated waste | |
| **First Aid**  Inhalation: remove to fresh air. Seek medical advice. If not breathing, give CPR. Do not use mouth-to-mouth method if victim ingested or inhaled the substance  Skin: wash thoroughly. If irritation occurs seek medical advice  Eyes: flush with plenty of soap and water, and seek medical advice.  Ingestion: Rinse mouth with water and seek medical attention immediately | |
| **Out of hours/lone working (any specific risks)**  No significant risks – CRISPR Intermediary work is allowable out of hours and when lone working for individuals approved by the Safety Officer. | |

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| **Assessment Summary** Ethidium bromide and SYBR-based dyes require handling with extra care and gels should be disposed of properly in a double bag.  Molten agarose gel should be handled with care and using heat resistant gloves and eye protection to avoid burns risks. |