

# Modification Form for Permit BIO-UWO-0178

**Permit Holder: Mark Daley**

**Approved Personnel**

**(Please stroke out any personnel to be removed)**

Beth Locke

**Additional Personnel**

**(Please list additional personnel here)**

	<b>Please stroke out any approved Biological Agent(s) to be removed</b>	<b>Write additional Biological Agent(s) for approval below. Give the full name</b>
<b>Approved Microorganisms</b>	Chilodonella uncinata, Metopus es, various protists	
<b>Approved Primary and Established Cells</b>	[primary]: Rodent Brain Bits LLC	
<b>Approved Use of Human Source Material</b>		Human saliva
<b>Approved Genetic Modifications (Plasmids/Vectors)</b>		
<b>Approved Use of Animals</b>		
<b>Approved Biological Toxin(s)</b>		
<b>Approved Gene Therapy</b>		
<b>Approved Plants and Insects</b>		

**\* PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOLOGICAL AGENTS.**  
**\*\* PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOLOGICAL AGENTS USED AND HOW THEY WILL BE STORED, USED AND DISPOSED OF..**

**As the Principal Investigator, I have ensured that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at <http://www.shs.uwo.ca/workplace/newposition.htm>**

Signature of Permit Holder:  \_\_\_\_\_

Current Classification: 1 Containment Level for Added Biohazards: 1

Date of Last Biohazardous Agents Registry Form: Jul 12, 2011

Date of Last Modification (if applicable): \_\_\_\_\_

BioSafety Officer(s): \_\_\_\_\_

Chair, Biohazards Subcommittee: \_\_\_\_\_ Date: \_\_\_\_\_

**Subject:** Re: Modification Form: Daley  
**From:** Mark Daley <daley@csd.uwo.ca>  
**Date:** Fri, 09 Dec 2011 17:43:01 -0500  
**To:** Jennifer Stanley <jstanle2@uwo.ca>

Hi Jennifer

HI Dr. Daley

Please call me Mark!

We happen to have a meeting on Friday. If by chance I had it by tomorrow morning at 9 am I could likely get it on the agenda for Friday. If not, there is a meeting mid-January.

I did my best, but the rest of my duties got in the way and I wanted to make sure that I had all the relevant information ready for the committee. Thanks so much for offering to rush it onto today's agenda nonetheless; mid-January will be no problem.

I've taken all the steps I could think of to ensure that the samples are devoid of infectious material and, honestly, they're certainly going to have fewer pathogens than your average restaurant table, but I also understand if the committee wishes to be conservative and treat all human fluids, regardless of treatment and origin, as Risk Group 2. In that case, I'll probably be asking for a meeting with you to determine what would be required to bring my lab up to Containment Level 2 compliance (there are so many items in the handbook that are "recommended" but not mandatory that I'd appreciate some guidance -- I want my lab to be as safe as possible, so I'm not keen to just ignore everything that isn't required).

Thanks for your time and happy holidays.

cheers  
Mark

<b>Daley_2011_full.pdf</b>	<b>Content-Type:</b> application/pdf <b>Content-Encoding:</b> base64
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# Project description: Developmental Imaging Genetics

Mark Daley

December 9, 2011

## 1 Brief Scientific Overview

In collaboration with the Cognitive Development and Neuroimaging Laboratory in the Department of Psychology we plan to undertake a study combining behavioural metrics, neuroimaging metrics and microarray-derived genotypes for a cohort of 8-year old children. The extraction of the collected subject DNA for genotyping is the portion of the project relevant to the current document.

## 2 Details of cells and tissue to be used

To facilitate microarray genotyping (SNP/CNV) we will collect a saliva sample from each child using the Oragene.DNA sample kit from DNA Genotek (<http://www.dnagenotek.com/>). Prior to saliva sample collection, children will be pre-screened for infectious disease by parental questionnaire (to exclude children with known, chronic, infectious disease) and physical examination by the investigator undertaking collection (to exclude children who are acutely ill with, e.g., cold or flu).

The saliva sampling kit is self-administered, ending with the subject closing a cap which punctures a membrane containing a fixative solution and inverting the tube to mix the fixative with the sample. The investigator collecting the samples will thus never contact subject saliva directly but will wear appropriate protective gear as an added precaution (latex and nitrile gloves and clothing which exposes no skin other than the head and neck).

The sealed saliva sample will be brought to the Daley Lab (WSC 124) and stored in a secure area. The nature of the fixative used in the Oragene.DNA kit is proprietary, but the relevant features are detailed in the

attached documents *Inactivation of viral and bacterial pathogens in OrageneDNA/saliva samples* and the fixative MSDS. To summarize: the fixative contains both ethyl alcohol and a very strong ionic detergent which is expected to effectively render most bacteria and enveloped viruses non-infectious.

As an additional precaution, all saliva samples will be “Super-Pasteurized” (as described in the attached document *Super-Pasteurization of OrageneDNA/Saliva samples*) at 72°C for 3 hours before being unsealed for DNA extraction.

At all times while handling samples in WSC 124, investigators will wear appropriate laboratory protective equipment including lab coat, safety glasses and latex or nitrile gloves. Excess sample will be stored at -20 in the biohazard-labelled freezer in WSC 124. Upon completion of the project, frozen samples will be destroyed by autoclaving and disposal as laboratory waste.

Given that the saliva samples will be:

- Taken from individuals pre-screened to be free of infectious disease
- Treated with a fixative including ethyl alcohol and a strong ionic detergent
- Pasteurized at 72°C for 3 hours

we request permission to undertake the DNA extraction step using Biosafety Level 1 protocols.

### **3 Summary of tissues to be used**

Pasteurized, fixed, human saliva    Human saliva    Risk Group 1

## Inactivation of viral and bacterial pathogens in Oragene•DNA/saliva samples

Oragene•DNA is a reliable and easy-to-use kit for the collection and stabilization of DNA in saliva. The popularity of Oragene•DNA for collecting DNA samples, even from remote parts of the world, has led some researchers to enquire about whether there is cause for concern about the possibility that viral and bacterial pathogens, if present in some Oragene•DNA/saliva samples, would remain viable.

Please note that DNA Genotek has not conducted any direct inactivation tests of viruses or bacteria in saliva/Oragene•DNA samples. The following discussion summarizes our current understanding of (i) shipping regulations that govern Oragene•DNA/saliva samples and (ii) the likelihood that microorganisms, even if present in the original saliva samples, would remain viable.

### **Shipping Oragene•DNA/saliva samples:**

Under IATA guidelines, Oragene•DNA/saliva samples typically fall under the 'Exempt Human Specimen' category. The IATA Dangerous Goods Regulations state, "In determining whether a patient specimen has a minimal likelihood that pathogens are present, an element of professional judgment is required to determine if a substance is exempt. That judgment should be based on the known medical history, symptoms and individual circumstances of the source, human or animal, and endemic local conditions".

In the event that it is determined that samples do not fall under the 'Exempt Human Specimen' category, special packaging and labeling procedures must be followed for Infectious Substances (e.g. leakproof packaging, rigid outer packaging, UN label). Additional information can be found directly through the current edition IATA Dangerous Goods Regulations (section 3.6.2.2), and on the DNA Genotek website at [http://www.dnagenotek.com/products\\_packagingoption.htm](http://www.dnagenotek.com/products_packagingoption.htm).

### **Likelihood that viruses or microorganisms would remain viable in Oragene•DNA**

1. Methods to inactivate enveloped viruses such as Influenza, HIV and Hepatitis C have been extensively studied and refined since the 1980's when they were discovered to be present in blood and blood products<sup>1,2</sup>. It has been proven that the lipid envelope (outer layer) of these viruses is very susceptible to treatment with mild non-ionic detergents and mild solvents. Oragene•DNA contains a very strong ionic detergent<sup>3</sup>, which is expected to effectively render enveloped viruses non-infectious.
2. Both enveloped viruses and bacteria are known to be inactivated or killed by heating.<sup>4-9</sup> If saliva samples are being collected from individuals suspected of being infected or are obtained from areas considered high risk and if the researcher deems that further precautions are warranted, the following procedure can be performed locally prior to shipment of the collected samples. The Oragene•DNA/saliva samples may be incubated in their original vials at elevated temperatures (72°C for up to 3 hours) without any detrimental effect on DNA quality<sup>10</sup>. It is the responsibility of researchers to determine whether this additional precaution is required, based on local circumstances.

Please contact your sales representative for more information, including about how DNA Genotek can provide further assistance in shipping your samples.

## References

1. Horowitz B, Prince AM, Hamman J, Watklevicz C. Viral safety of solvent/detergent-treated blood products. *Blood Coagul Fibrinolysis* 1994; 5 Suppl 3:S21-S28.
2. Korneyeva M, Hotta J, Lebing W, Rosenthal RS, Franks L, Petteway SR, Jr. Enveloped virus inactivation by caprylate: a robust alternative to solvent-detergent treatment in plasma derived intermediates. *Biologicals* 2002; 30(2):153-162 .
3. Material Safety Data Sheet, Oragene DNA (2009)
4. [http://www.vetmed.ucdavis.edu/vetext/INF-PO\\_Forum/howmuch.htm](http://www.vetmed.ucdavis.edu/vetext/INF-PO_Forum/howmuch.htm)
5. Swayne, D.E. and Beck J.R. (2004). Heat inactivation of avian influenza and Newcastle disease viruses in egg products. *Avian Pathol.* 33(5):512-8.
6. Swayne, D.E. (2006). Microassay for measuring thermal inactivation of H5N1 high pathogenicity avian influenza virus in naturally infected chicken meat. *Int J Food Microbiol.* 108(2):268-71
7. <http://www.foodsci.uoguelph.ca/dairyedu/pasteurization.html>
8. <http://www.cfis.agr.ca/english/regcode/hrt/juprodae.shtml>
9. Halvorson, D.A. (1986) . A Minnesota Cooperative Control Program. *Proceedings of the Second Annual International Symposium on Avian Influenza.* p. 327-336;
10. Iwasiew, R.M., Polan, M. and Birnboim, H.C. "Super-Pasteurization" of Oragene DNA/Saliva samples. (2006)



**OG-1xx, OG-2xx, OG-3xx, OG-500, OG-510, OG-575  
Small Volume Safety Data Sheet  
And  
OG-1xx, OG-2xx, OG-3xx, OG-500, OG-510, OG-575  
Material Safety Data Sheet**

**Guidance Statement**

The MSDS portion of this PDF has been prepared based on large volumes of chemistry. Adaptations have been made in the SVSDS portion to more accurately represent the small volume of chemistry in the kits.



# Small Volume Safety Data Sheet

OG-1xx, OG-2xx, OG-3xx, OG-500, OG-510, OG-575

## 1. Product and company identification

<b>Product name</b>	: OG-1xx, OG-2xx, OG-3xx, OG-500, OG-510, OG-575
<b>SVSDS #</b>	: PD-SVSDS-00004
<b>Material uses</b>	: Stabilization of DNA in collection kit.
<b>Supplier/Manufacturer</b>	: DNA Genotek Inc Ottawa, ON, Canada, K2K 1L1 Tel: +1-613-723-5757 Fax: +1-613-723-5057 Toll Free: +1-866-813-6354 (North America) Toll Free: +1-613-723-5757 (all other countries) Email: support@dnagenotek.com Web site: www.dnagenotek.com
<b>MSDS authored by</b>	: KMK Regulatory Services inc.
<b><u>In case of emergency</u></b>	: For Emergencies involving dangerous goods call Canutec's 24-hour number 613-996-6666
<b>Product type</b>	: Liquid.

This document provides safety information for the small volumes of stabilizing solution that are present in each DNA Genotek product, and should be used in conjunction with PD-MSDS-00004 when the substance is in bulk.

## 2. Hazards identification

<b>Physical state</b>	: Liquid.
<b>Signal word</b>	: WARNING!
<b>Hazard statements</b>	: FLAMMABLE LIQUID AND VAPOR. MAY CAUSE RESPIRATORY TRACT, EYE AND SKIN IRRITATION. CONTAINS MATERIAL THAT MAY CAUSE TARGET ORGAN DAMAGE, BASED ON ANIMAL DATA.
<b>Precautions</b>	: Follow good industrial hygiene practice. Wash thoroughly after handling.
<b>OSHA/HCS status</b>	: This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200).
<b><u>Potential acute health effects</u></b>	
<b>Inhalation</b>	: May cause respiratory tract irritation.
<b>Ingestion</b>	: No known significant effects or critical hazards.
<b>Skin</b>	: May cause skin irritation.
<b>Eyes</b>	: May cause eye irritation.
<b><u>Potential chronic health effects</u></b>	
<b>Chronic effects</b>	: No known significant effects or critical hazards.
<b>Carcinogenicity</b>	: No known significant effects or critical hazards.
<b>Mutagenicity</b>	: No known significant effects or critical hazards.
<b>Teratogenicity</b>	: No known significant effects or critical hazards.
<b>Developmental effects</b>	: No known significant effects or critical hazards.
<b>Fertility effects</b>	: No known significant effects or critical hazards.
<b><u>Target organs</u></b>	: No known significant effects or critical hazards.



## 2. Hazards identification

### Over-exposure signs/symptoms

- Inhalation** : Adverse symptoms may include the following:  
respiratory tract irritation
- Ingestion** : No specific data.
- Skin** : Adverse symptoms may include the following:  
irritation
- Eyes** : Adverse symptoms may include the following:  
irritation
- Medical conditions aggravated by over-exposure** : There is no known effect after over-exposure to this product.

See toxicological information (section 11)

## 3. Composition/information on ingredients

Refer to PD-MSDS-00004.

## 4. First aid measures

- Eye contact** : Immediately flush eyes with plenty of water for at least 20 minutes, occasionally lifting the upper and lower eyelids. Get medical attention if symptoms occur.
- Skin contact** : Wash with soap and water. Get medical attention if symptoms occur.
- Inhalation** : Supply fresh air. Get medical attention if symptoms occur.
- Ingestion** : Wash out mouth with water. Get medical attention if symptoms occur.
- Notes to physician** : No specific treatment.

## 5. Fire-fighting measures

**Flammability of the product** : Flammable liquid.

### Extinguishing media

- Suitable** : Use dry chemical, CO<sub>2</sub>, water spray (fog) or foam.
- Not suitable** : Do not use water jet.
- Special exposure hazards** : No specific hazard.
- Hazardous decomposition products** : Decomposition products may include the following materials:  
carbon dioxide  
carbon monoxide  
nitrogen oxides
- Special protective equipment for fire-fighters** : No special protection is required.

## 6. Accidental release measures

- Personal precautions** : No special precaution is required.
- Environmental precautions** : No special measures required.
- Methods for cleaning up**
- Small spill** : No special measures required.



## 7. Handling and storage

- Handling** : Put on appropriate personal protective equipment (see section 8). Use only with adequate ventilation. Keep away from heat, sparks and flame.
- Storage** : Store in accordance with local regulations. Store at room temperature. Store away from direct sunlight.

## 8. Exposure controls/personal protection

### United States

Ingredient	Exposure limits
Ethyl Alcohol	<b>ACGIH TLV (United States, 1/2009).</b> STEL: 1000 ppm 15 minute(s). <b>NIOSH REL (United States, 6/2009).</b> TWA: 1900 mg/m <sup>3</sup> 10 hour(s). TWA: 1000 ppm 10 hour(s). <b>OSHA PEL (United States, 11/2006).</b> TWA: 1900 mg/m <sup>3</sup> 8 hour(s). TWA: 1000 ppm 8 hour(s).

### Canada

Occupational exposure limits		TWA (8 hours)			STEL (15 mins)			Ceiling			
Ingredient	List name	ppm	mg/m <sup>3</sup>	Other	ppm	mg/m <sup>3</sup>	Other	ppm	mg/m <sup>3</sup>	Other	Notations
Ethyl Alcohol	US ACGIH 1/2009	-	-	-	1000	-	-	-	-	-	
	AB 4/2009	1000	1880	-	-	-	-	-	-	-	
	BC 9/2009	-	-	-	1000	-	-	-	-	-	
	ON 8/2008	1000	1900	-	-	-	-	-	-	-	
	QC 6/2008	1000	1880	-	-	-	-	-	-	-	

### Mexico

Ingredient	Exposure limits
Ethyl Alcohol	<b>NOM-010-STPS (Mexico, 9/2000).</b> LMPE-PPT: 1900 mg/m <sup>3</sup> 8 hour(s). LMPE-PPT: 1000 ppm 8 hour(s).

Consult local authorities for acceptable exposure limits.

- Recommended monitoring procedures** : Not required.
- Engineering measures** : No special measures required.
- Hygiene measures** : Follow good industrial hygiene practice.
- Personal protection**
- Respiratory** : Not required.
  - Hands** : Not required under normal conditions of use. Recommended: Disposable vinyl gloves.
  - Eyes** : Not required under normal conditions of use. Recommended: Safety glasses.
  - Skin** : Not required.
  - Environmental exposure controls** : None.



## 9 . Physical and chemical properties

Refer to PD-MSDS-00004.

## 10. Stability and reactivity

Refer to PD-MSDS-00004.

## 11. Toxicological information

Refer to PD-MSDS-00004.

## 12. Ecological information

Refer to PD-MSDS-00004.

## 13. Disposal considerations

**Waste disposal** : The generation of waste should be avoided or minimized wherever possible. This material and its container must be disposed of in a safe way. Dispose of surplus and non-recyclable products via a licensed waste disposal contractor.

Disposal should be in accordance with applicable regional, national and local laws and regulations.

Refer to Section 7: HANDLING AND STORAGE and Section 8: EXPOSURE CONTROLS/PERSONAL PROTECTION for additional handling information and protection of employees.

## 14 . Transport information

DOT/TDG/MXT/IMDG/IATA : Not regulated.  
Not restricted, Special Provision A58

## 15 . Regulatory information

Refer to PD-MSDS-00004.

## 16 . Other information

Refer to PD-MSDS-00004.



# Material Safety Data Sheet

OG-1xx, OG-2xx, OG-3xx, OG-500, OG-510, OG-575

## 1. Product and company identification

**Product name** : OG-1xx, OG-2xx, OG-3xx, OG-500, OG-510, OG-575  
**Material uses** : Stabilization of DNA in collection kit.  
**Supplier/Manufacturer** : DNA Genotek Inc  
 Ottawa, ON, Canada, K2K 1L1  
 Tel: +1-613-723-5757  
 Fax: +1-613-723-5057  
 Toll Free: +1-866-813-6354 (North America)  
 Toll Free: +1-613-723-5757 (all other countries)  
 Email: support@dnagenotek.com  
 Web site: www.dnagenotek.com

**MSDS authored by** : KMK Regulatory Services inc.  
**In case of emergency** : For Emergencies involving dangerous goods call  
 Canutec's 24-hour number 613-996-6666  
**Product type** : Liquid.

## 2. Hazards identification

**Physical state** : Liquid.  
**Signal word** : WARNING!  
**Hazard statements** : FLAMMABLE LIQUID AND VAPOR. CAUSES RESPIRATORY TRACT, EYE AND SKIN IRRITATION. CONTAINS MATERIAL THAT MAY CAUSE TARGET ORGAN DAMAGE, BASED ON ANIMAL DATA.

**Precautions** : Keep away from heat, sparks and flame. Do not breathe vapor or mist. Avoid contact with eyes, skin and clothing. Use only with adequate ventilation. Keep container tightly closed and sealed until ready for use. Wash thoroughly after handling.

**OSHA/HCS status** : This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200).

**Potential acute health effects**

**Inhalation** : Irritating to respiratory system. Exposure to decomposition products may cause a health hazard. Serious effects may be delayed following exposure.  
**Ingestion** : No known significant effects or critical hazards.  
**Skin** : May cause skin irritation.  
**Eyes** : May cause eye irritation.

**Potential chronic health effects**

**Chronic effects** : Contains material that may cause target organ damage, based on animal data.  
**Carcinogenicity** : No known significant effects or critical hazards.  
**Mutagenicity** : No known significant effects or critical hazards.  
**Teratogenicity** : No known significant effects or critical hazards.  
**Developmental effects** : No known significant effects or critical hazards.  
**Fertility effects** : No known significant effects or critical hazards.



## 2. Hazards identification

**Target organs** : Contains material which may cause damage to the following organs: blood, the reproductive system, liver, upper respiratory tract, skin, eyes, central nervous system (CNS).

### Over-exposure signs/symptoms

**Inhalation** : Adverse symptoms may include the following:  
respiratory tract irritation  
coughing

**Ingestion** : No specific data.

**Skin** : Adverse symptoms may include the following:  
irritation

**Eyes** : Adverse symptoms may include the following:  
irritation

**Medical conditions aggravated by over-exposure** : Pre-existing disorders involving any target organs mentioned in this MSDS as being at risk may be aggravated by over-exposure to this product.

See toxicological information (section 11)

## 3. Composition/information on ingredients

### United States

Name	CAS number	%
Ethyl Alcohol	64-17-5	10 - 30
1,3-Propanediol, 2-amino-2-(hydroxymethyl)-	77-86-1	1 - 5

### Canada

Name	CAS number	%
Ethyl Alcohol	64-17-5	10 - 30
1,3-Propanediol, 2-amino-2-(hydroxymethyl)-	77-86-1	1 - 5

### Mexico

Name	CAS number	UN number	%	IDLH	Classification			
					H	F	R	Special
Ethyl Alcohol	64-17-5	UN1170	10 - 30	3300 ppm	1	3	0	
1,3-Propanediol, 2-amino-2-(hydroxymethyl)-	77-86-1	Not regulated.	1 - 5	-	1	1	0	

There are no additional ingredients present which, within the current knowledge of the supplier and in the concentrations applicable, are classified as hazardous to health or the environment and hence require reporting in this section.

## 4. First aid measures

**Eye contact** : Immediately flush eyes with plenty of water for at least 20 minutes, occasionally lifting the upper and lower eyelids. Get medical attention if symptoms occur.

**Skin contact** : In case of contact, immediately flush skin with plenty of water for at least 20 minutes. Get medical attention if symptoms occur.

**Inhalation** : Move exposed person to fresh air. Get medical attention if symptoms occur.

**Ingestion** : Wash out mouth with water. Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if symptoms occur.



## 4. First aid measures

Notes to physician : No specific treatment.

## 5. Fire-fighting measures

Flammability of the product : Flammable liquid.

### Extinguishing media

Suitable : Use dry chemical, CO<sub>2</sub>, water spray (fog) or foam.

Not suitable : Do not use water jet.

Special exposure hazards : Move containers from fire area if this can be done without risk. Use water spray to keep fire-exposed containers cool.

Hazardous decomposition products : Decomposition products may include the following materials:  
carbon dioxide  
carbon monoxide  
nitrogen oxides

Special protective equipment for fire-fighters : Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode.

## 6. Accidental release measures

Personal precautions : Shut off all ignition sources. No flares, smoking or flames in hazard area. Provide adequate ventilation. Put on appropriate personal protective equipment (see section 8).

Environmental precautions : Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).

### Methods for cleaning up

Small spill : Dilute with water and mop up if water-soluble or absorb with an inert dry material and place in an appropriate waste disposal container. Use spark-proof tools and explosion-proof equipment. Dispose of via a licensed waste disposal contractor.

Large spill : Prevent entry into sewers, water courses, basements or confined areas. Wash spillages into an effluent treatment plant or proceed as follows. Contain and collect spillage with non-combustible, absorbent material e.g. sand, earth, vermiculite or diatomaceous earth and place in container for disposal according to local regulations (see section 13). Use spark-proof tools and explosion-proof equipment. Dispose of via a licensed waste disposal contractor. Contaminated absorbent material may pose the same hazard as the spilled product. Note: see section 1 for emergency contact information and section 13 for waste disposal.

## 7. Handling and storage

Handling : Put on appropriate personal protective equipment (see section 8). Eating, drinking and smoking should be prohibited in areas where this material is handled, stored and processed. Workers should wash hands and face before eating, drinking and smoking. Avoid contact with eyes, skin and clothing. Use only with adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Do not enter storage areas and confined spaces unless adequately ventilated. Use explosion-proof electrical (ventilating, lighting and material handling) equipment. Use non-sparking tools. Take precautionary measures against electrostatic discharges. To avoid fire or explosion, dissipate static electricity during transfer by grounding and bonding containers and equipment before transferring material. Empty containers retain product residue and can be hazardous. Keep away from heat, sparks and flame.

Storage : Store in accordance with local regulations. Store at room temperature. Store away from direct sunlight.



## 8. Exposure controls/personal protection

### United States

Ingredient	Exposure limits
Ethyl Alcohol	<p><b>ACGIH TLV (United States, 1/2009).</b>                      STEL: 1000 ppm 15 minute(s).</p> <p><b>NIOSH REL (United States, 6/2009).</b>                      TWA: 1900 mg/m<sup>3</sup> 10 hour(s).                      TWA: 1000 ppm 10 hour(s).</p> <p><b>OSHA PEL (United States, 11/2006).</b>                      TWA: 1900 mg/m<sup>3</sup> 8 hour(s).                      TWA: 1000 ppm 8 hour(s).</p>

### Canada

Occupational exposure limits		TWA (8 hours)			STEL (15 mins)			Ceiling			Notations
Ingredient	List name	ppm	mg/m <sup>3</sup>	Other	ppm	mg/m <sup>3</sup>	Other	ppm	mg/m <sup>3</sup>	Other	
Ethyl Alcohol	US ACGIH 1/2009	-	-	-	1000	-	-	-	-	-	
	AB 4/2009	1000	1880	-	-	-	-	-	-	-	
	BC 9/2009	-	-	-	1000	-	-	-	-	-	
	ON 8/2008	1000	1900	-	-	-	-	-	-	-	
	QC 6/2008	1000	1880	-	-	-	-	-	-	-	

### Mexico

Ingredient	Exposure limits
Ethyl Alcohol	<p><b>NOM-010-STPS (Mexico, 9/2000).</b>                      LMPE-PPT: 1900 mg/m<sup>3</sup> 8 hour(s).                      LMPE-PPT: 1000 ppm 8 hour(s).</p>

Consult local authorities for acceptable exposure limits.

**Recommended monitoring procedures** : Personal, workplace atmosphere or biological monitoring may be required to determine the effectiveness of the ventilation or other control measures and/or the necessity to use respiratory protective equipment.

**Engineering measures** : Use only with adequate ventilation. Use process enclosures, local exhaust ventilation or other engineering controls to keep worker exposure to airborne contaminants below any recommended or statutory limits. Use explosion-proof ventilation equipment.

**Hygiene measures** : Ensure that eyewash stations and safety showers are close to the workstation location. Wash hands, forearms and face thoroughly after handling chemical products, before eating, smoking and using the lavatory and at the end of the working period.

### Personal protection

**Respiratory** : Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirator. Not required under normal conditions of use. Recommended: Wear an appropriate NIOSH approved respirator if concentration levels exceed the safe exposure limits.

**Hands** : Use gloves appropriate for work or task being performed. Not required under normal conditions of use. Recommended: Natural rubber (latex).

**Eyes** : Safety eyewear should be used when there is a likelihood of exposure. Not required under normal conditions of use. Recommended: Safety glasses with side shields.

**Skin** : Personal protective equipment for the body should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product. No special protective clothing is required. Recommended: Lab coat.

**Environmental exposure controls** : None.



## 9. Physical and chemical properties

Physical state	: Liquid.
Flash point	: Closed cup: 29°C (84.2°F) [Pensky-Martens.]
pH	: 9.5
VOC	: 29.7 % (w/w)

## 10. Stability and reactivity

Chemical stability	: The product is stable.
Conditions to avoid	: Avoid all possible sources of ignition (spark or flame).
Materials to avoid	: Reactive or incompatible with the following materials: oxidizing materials
Hazardous decomposition products	: Decomposition products may include the following materials: carbon dioxide carbon monoxide nitrogen oxides
Possibility of hazardous reactions	: Under normal conditions of storage and use, hazardous reactions will not occur.
Hazardous polymerization	: Under normal conditions of storage and use, hazardous polymerization will not occur.

## 11. Toxicological information

### Acute toxicity

Product/ingredient name	Result	Species	Dose	Exposure
Ethyl Alcohol	LC50 Inhalation Vapor	Rat	124700 mg/m <sup>3</sup>	4 hours
	LD50 Oral	Rat	7 g/kg	-
1,3-Propanediol, 2-amino-2-(hydroxymethyl)-	LD50 Oral	Rat	>3000 mg/kg	-

### Chronic toxicity

#### Classification

Product/ingredient name	ACGIH	IARC	EPA	NIOSH	NTP	OSHA
Ethyl Alcohol	A4	-	-	-	-	-

## 12. Ecological information

Environmental effects : Not established

### Aquatic ecotoxicity

Product/ingredient name	Result	Species	Exposure
Ethyl Alcohol	Acute EC50 2000 ug/L Fresh water	Daphnia - Daphnia magna	48 hours
	Acute LC50 25500 ug/L Marine water	Crustaceans - Artemia franchiscana - LARVAE	48 hours
	Acute LC50 42000 ug/L Fresh water	Fish - Oncorhynchus mykiss	4 days
	Chronic NOEC <6.3 g/L Fresh water	Daphnia - Daphnia magna	48 hours



### 13. Disposal considerations

**Waste disposal** : The generation of waste should be avoided or minimized wherever possible. This material and its container must be disposed of in a safe way. Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Empty containers or liners may retain some product residues. Dispose of surplus and non-recyclable products via a licensed waste disposal contractor.

Disposal should be in accordance with applicable regional, national and local laws and regulations.

Refer to Section 7: HANDLING AND STORAGE and Section 8: EXPOSURE CONTROLS/PERSONAL PROTECTION for additional handling information and protection of employees.

### 14. Transport information

Regulatory information	UN number	Proper shipping name	Classes	PG*	Label	Additional information
<b>DOT Classification</b>	Not regulated.	FLAMMABLE LIQUIDS, N.O.S. (Ethyl Alcohol)	3	II		-
<b>TDG Classification</b>	UN1993	FLAMMABLE LIQUIDS, N.O.S. (Ethyl Alcohol)	3	II		-
<b>Mexico Classification</b>	UN1993	FLAMMABLE LIQUIDS, N.O.S. (Ethyl Alcohol)	3	II		-
<b>IMDG Class</b>	UN1993	FLAMMABLE LIQUIDS, N.O.S. (Ethyl Alcohol)	3	II		-
<b>IATA-DGR Class</b>	UN1993	FLAMMABLE LIQUIDS, N.O.S. (Ethyl Alcohol)	3	II		-

PG\* : Packing group

Exemption to the above classification may apply.

**AERG** : 128

### 15. Regulatory information

**United States**

**HCS Classification** : Flammable liquid  
Irritating material  
Target organ effects

**U.S. Federal regulations** : **United States inventory (TSCA 8b)**: All components are listed or exempted.  
**SARA 302/304/311/312 extremely hazardous substances**: No products were found.  
**SARA 302/304 emergency planning and notification**: No products were found.  
**SARA 302/304/311/312 hazardous chemicals**: Ethyl Alcohol; Acetic acid, sodium salt, trihydrate  
**SARA 311/312 MSDS distribution - chemical inventory - hazard identification**: Ethyl Alcohol: Fire hazard, Immediate (acute) health hazard, Delayed (chronic) health hazard; Acetic acid, sodium salt, trihydrate: Delayed (chronic) health hazard  
**Clean Water Act (CWA) 307**: No products were found.  
**Clean Water Act (CWA) 311**: No products were found.  
**Clean Air Act (CAA) 112 accidental release prevention**: No products were found.



## 15 . Regulatory information

**Clean Air Act (CAA) 112 regulated flammable substances:** No products were found.

**Clean Air Act (CAA) 112 regulated toxic substances:** No products were found.

**Clean Air Act Section 112(b) Hazardous Air Pollutants (HAPs)** : Not listed

**Clean Air Act Section 602 Class I Substances** : Not listed

**Clean Air Act Section 602 Class II Substances** : Not listed

**DEA List I Chemicals (Precursor Chemicals)** : Not listed

**DEA List II Chemicals (Essential Chemicals)** : Not listed

**State regulations** :

- Connecticut Carcinogen Reporting:** None of the components are listed.
- Connecticut Hazardous Material Survey:** None of the components are listed.
- Florida substances:** None of the components are listed.
- Illinois Chemical Safety Act:** None of the components are listed.
- Illinois Toxic Substances Disclosure to Employee Act:** None of the components are listed.
- Louisiana Reporting:** None of the components are listed.
- Louisiana Spill:** None of the components are listed.
- Massachusetts Spill:** None of the components are listed.
- Massachusetts Substances:** The following components are listed: Ethyl Alcohol
- Michigan Critical Material:** None of the components are listed.
- Minnesota Hazardous Substances:** None of the components are listed.
- New Jersey Hazardous Substances:** The following components are listed: Ethyl Alcohol
- New Jersey Spill:** None of the components are listed.
- New Jersey Toxic Catastrophe Prevention Act:** None of the components are listed.
- New York Acutely Hazardous Substances:** None of the components are listed.
- New York Toxic Chemical Release Reporting:** None of the components are listed.
- Pennsylvania RTK Hazardous Substances:** The following components are listed: Ethyl Alcohol
- Rhode Island Hazardous Substances:** None of the components are listed.

### Canada

**WHMIS (Canada)** : Class B-2: Flammable liquid  
Class D-2B: Material causing other toxic effects (Toxic).

**Canadian lists** :

- CEPA Toxic substances:** None of the components are listed.
- Canadian ARET:** None of the components are listed.
- Canadian NPRI:** The following components are listed: Ethyl Alcohol
- Alberta Designated Substances:** None of the components are listed.
- Ontario Designated Substances:** None of the components are listed.
- Quebec Designated Substances:** None of the components are listed.

**Canada inventory** : All components are listed or exempted.

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all the information required by the Controlled Products Regulations.

### Mexico



## 15 . Regulatory information

Classification :



### International regulations

**International lists** :

- Australia inventory (AICS):** Not determined.
- China inventory (IECSC):** All components are listed or exempted.
- Japan inventory:** Not determined.
- Korea inventory:** Not determined.
- New Zealand Inventory of Chemicals (NZIoC):** All components are listed or exempted.
- Philippines inventory (PICCS):** Not determined.

## 16 . Other information

### United States

**Hazardous Material Information System (U.S.A.)** : Health : 1 \* Flammability : 2 Physical hazards : 0

Caution: HMIS® ratings are based on a 0-4 rating scale, with 0 representing minimal hazards or risks, and 4 representing significant hazards or risks. Although HMIS® ratings are not required on MSDSs under 29 CFR 1910.1200, the preparer may choose to provide them. HMIS® ratings are to be used with a fully implemented HMIS® program. HMIS® is a registered mark of the National Paint & Coatings Association (NPCA). HMIS® materials may be purchased exclusively from J. J. Keller (800) 327-6868.

The customer is responsible for determining the PPE code for this material.

**National Fire Protection Association (U.S.A.)** : Health : 1 Flammability : 2 Instability : 0

### Canada

**WHMIS (Canada)** :



**References** :

- ANSI Z400.1, MSDS Standard, 2004. - Manufacturer's Material Safety Data Sheet. - 29CFR Part1910.1200 OSHA MSDS Requirements. - 49CFR Table List of Hazardous Materials, UN#, Proper Shipping Names, PG. - Canada Gazette Part II, Vol. 122, No. 2. Registration SOR/88-64, 31 December 1987. Hazardous Products Act "Ingredient Disclosure List" - Canadian Transport of Dangerous Goods, Regulations and Schedules, Clear Language version 2005. - Official Mexican Standards NOM-018-STPS-2000 and NOM-004-SCT2-1994.

**Date of issue** : 06/30/2010

**Version** : 1

### Notice to reader

To the best of our knowledge, the information contained herein is accurate. However, neither the above-named supplier, nor any of its subsidiaries, assumes any liability whatsoever for the accuracy or completeness of the information contained herein.

Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist.

## “Super-Pasteurization” of Oragene®•DNA/Saliva samples

R.M. Iwaszow, M. Polan, and H.C. Birnboim  
DNA Genotek, Ottawa, Ontario, Canada

Some Oragene•DNA users have inquired about the possible presence of infectious agents in saliva samples shipped under routine conditions. To address this question and to further prove the robustness and reliability of Oragene•DNA/saliva samples, experiments were conducted to demonstrate that Oragene•DNA/saliva can be “super-pasteurized” (treated for up to 3 hours at 72°C) with no effect on the quality and quantity of DNA recovered.

### Introduction

The Oragene•DNA Self-Collection Kit is a non-invasive method for collecting large amounts of DNA. The ability for Oragene•DNA to release and stabilize DNA from saliva for long periods of time at room temperature makes it an ideal collection method. (The relative ease and success with which Oragene•DNA is increasingly being used to collect DNA samples around the world has led to questions regarding potential pathogens in oral samples.) Pasteurization, an industrial process typically associated with the dairy industry, has been documented to efficiently kill pathogens (ref. 1, 2 and 3). Pathogens can be inactivated by heat, given sufficient temperature and time of exposure (ref. 4).

Oragene•DNA contains agents that are bactericidal for many microorganisms even at room temperature; this bactericidal effect will be even more effective at elevated temperatures. In this report, we describe a series of experiments conducted to further demonstrate the safety and integrity of transport and handling of saliva samples collected with Oragene•DNA. In these experiments, Oragene•DNA/saliva samples were subjected to “super-pasteurization” conditions that are likely to be strongly bactericidal.

### Materials and Methods

#### Saliva Collection

Two milliliters of saliva was collected from two donors using the Oragene•DNA Self-Collection Kit. The Oragene•DNA vial was capped and the entire sample was mixed by inversion, allowing the DNA to be released and stabilized.

#### Sample Treatment

As described in the standard Oragene•DNA protocol, samples were incubated in a water bath for 1 hour at 50°C. A 250 µL aliquot was immediately removed from each donor's sample. The remainder was placed in an air incubator and incubated at 72°C. From each donor's sample at 72°C, a 250 µL aliquot was taken at 30 minutes, 1 hour, 2 hours, and 3 hours.

#### Monitoring of Sample Temperature

A control Oragene•DNA/saliva sample was used to monitor the temperature within the collection vial. To monitor temperature, a remote temperature sensor was inserted through a modified cap.

#### DNA Purification

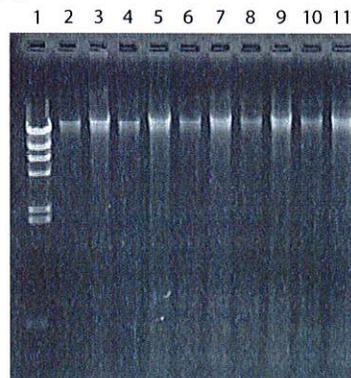
The standard Oragene•DNA purification protocol was followed. In brief, 10 µL (1/25th volume) of purifier was added to each 250 µL sample. Each tube was mixed well and incubated on ice for 10 minutes. The tubes were centrifuged at 15,000×g for 5 minutes. The supernatant was removed and transferred to a fresh tube; an equal volume of 95% EtOH at room temperature was added. The sample was mixed by inversion and allowed to stand 10 minutes at room temperature. The sample was centrifuged at 15,000×g for 2 minutes and the supernatant was discarded. The DNA pellet was dissolved in 50 µL of TE buffer.

#### DNA Analysis

DNA from the purified samples was quantified using a fluorescent dye, SYBR Green I™ dye (Molecular Probes, Inc.). The  $A_{260}/A_{280}$  ratio was corrected by subtracting the  $A_{320}$  value. The molecular weight of the DNA was determined by electrophoresis on a 0.8% agarose gel.

### Results and Discussion

The saliva from 2 donors was used for these experiments. The average amount of DNA present in 2 mL of saliva from donor A was 40.8 µg and from donor B was 116.0 µg. The initial and subsequent  $A_{260}/A_{280}$  ratios were between 1.8 and 1.9 for all samples. Representative results of agarose gel electrophoresis analysis are shown in Figure 1.



**Figure 1.** Samples were incubated at 72°C for 0 hr (lanes 2,3); 0.5 hr (lanes 4,5); 1 hr (lanes 6,7); 2 hr (lanes 8,9); 3 hr (lanes 10,11). Donor A (lanes 2,4,6,8,10); donor B (lanes 3,5,7,9,11). Lane 1 – Lambda-Hind III marker.

Pasteurization is a process for removing pathogens by the heating of a solution to a specific temperature for a defined time without allowing recontamination during the process. The accepted minimum conditions required for standard pasteurization are heating at 72°C for 16 seconds. These conditions are accepted by the dairy industry as being sufficient to destroy most pathogens in milk (ref. 2 and 3). Essentially all known pathogens are inactivated by heat, given sufficient temperature and exposure (ref. 4). Because of recent concerns about avian influenza virus, several reports have studied thermal inactivation of the virus. The authors report that the virus is heat-sensitive and can be destroyed by standard pasteurization conditions (ref. 5 and 6). It is important to emphasize that the “super-pasteurization” conditions used in our experiments involved heating for up to 3 hours, that is, about 600 times longer than standard pasteurization and was carried out in the presence of strong detergents and alcohol.

### Conclusions

Heating Oragene•DNA/saliva samples at 72°C for as long as 3 hours (“super-pasteurization”) does not degrade DNA. Indicating that, where warranted, heat treatment could be used to further reduce risk from pathogens potentially present in an Oragene•DNA/saliva sample.

### References

1. <http://www.foodsci.uoguelph.ca/dairyedu/pasteurization.html>
2. <http://www.milk.org/faqs/pasteurization.html>
3. <http://www.cfsis.agr.ca/english/regcode/hrt/juprodae.shtml>
4. Halvorson, D.A. (1986) . A Minnesota Cooperative Control Program. Proceedings of the Second Annual International Symposium on Avian Influenza. p. 327-336;  
[http://www.vetmed.ucdavis.edu/vetext/INF-PO\\_Forum/howmuch.htm](http://www.vetmed.ucdavis.edu/vetext/INF-PO_Forum/howmuch.htm)
5. Swayne, D.E. and Beck J.R. (2004). Heat inactivation of avian influenza and Newcastle disease viruses in egg products. *Avian Pathol.* 33(5):512-8.
6. Swayne, D.E. (2006). Microassay for measuring thermal inactivation of H5N1 high pathogenicity avian influenza virus in naturally infected chicken meat. *Int J Food Microbiol.* 108(2):268-71

**THE UNIVERSITY OF WESTERN ONTARIO  
BIOLOGICAL AGENTS REGISTRY FORM  
Approved Biohazards Subcommittee: October 14, 2010  
Biosafety Website: [www.uwo.ca/humanresources/biosafety/](http://www.uwo.ca/humanresources/biosafety/)**

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biological agents being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1<sup>st</sup> edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or [biosafety@uwo.ca](mailto:biosafety@uwo.ca). If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: [www.uwo.ca/humanresources/biosafety/](http://www.uwo.ca/humanresources/biosafety/)

PRINCIPAL INVESTIGATOR Mark Daley  
 DEPARTMENT Computer Science & Biology  
 ADDRESS Middlesex College  
 PHONE NUMBER +87897  
 EMERGENCY PHONE NUMBER(S) 519 672 0712  
 EMAIL daley@csd.uwo.ca

Location of experimental work to be carried out: Building(s) WSC Room(s) 124

\*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).

FUNDING AGENCY/AGENCIES: NSERC  
 GRANT TITLE(S): Competition in biological processes

List all personnel working under Principal Investigators supervision in this location:

Name	UWO E-mail Address	Date of Biosafety Training
<u>Beth Locke</u>	<u>mlocke2@uwo.ca</u>	<u>9/2006</u>

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Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.

See attached.

Please include a one page research summary or teaching protocol.

See attached.

## 1.0 Microorganisms

1.1 Does your work involve the use of biological agents?  YES  NO  
 (non-pathogenic and pathogenic biological agents including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA?  YES  NO

If YES, please give the name of the species. \_\_\_\_\_

What is the origin of the microorganism(s)? \_\_\_\_\_

Please describe the risk (if any) of escape and how this will be mitigated:

\_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

\_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

1.2 Please complete the table below:

Name of Biological Agent(s)* (Be specific)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/Supplier	PHAC or CFIA Containment Level
Chilodonella uncinata Metopus es				.25	Zuh4 lab	<input checked="" type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
				.25	ATCC	<input checked="" type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
<i>Polyspora</i> <i>Neurospora</i>	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Various Protists	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	.25	Freshwater Ponds	<input checked="" type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3

\*Please attach a Material Safety Data Sheet or equivalent from the supplier.

## 2.0 Cell Culture

2.1 Does your work involve the use of cell cultures?  YES  NO  
 If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	<input type="radio"/> Yes <input checked="" type="radio"/> No		Not applicable
Rodent	<input checked="" type="radio"/> Yes <input type="radio"/> No	Brain Bits LLC	N/A
Non-human primate	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input checked="" type="radio"/> No		

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	<input type="radio"/> Yes <input checked="" type="radio"/> No			
Rodent	<input checked="" type="radio"/> Yes <input type="radio"/> No			
Non-human primate	<input type="radio"/> Yes <input checked="" type="radio"/> No			
Other (specify)	<input type="radio"/> Yes <input checked="" type="radio"/> No			

\*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required  1  2  2+  3

### 3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials?  YES  NO

If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	Source/Supplier /Company Name	Is Human Source Material Infected With An Infectious Agent? YES/UNKNOWN	Name of Infectious Agent (If applicable)	PHAC or CFIA Containment Level (Select one)
Human Blood (whole) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Blood (fraction) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Organs or Tissues (unpreserved)		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

### 4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0?  YES  NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done?  YES, complete table below  NO

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection

\* Please attach a Material Data Sheet or equivalent if available.

\*\* Please attach a plasmid map.

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?

YES, complete table below  NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction

\* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- ◆ HIV  YES, please specify \_\_\_\_\_  NO
- ◆ HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens  YES, specify \_\_\_\_\_  NO
- ◆ SV 40 Large T antigen  YES  NO
- ◆ E1A oncogene  YES  NO
- ◆ Known oncogenes  YES, please specify \_\_\_\_\_  NO
- ◆ Other human or animal pathogen and or their toxins  YES, please specify \_\_\_\_\_  NO

4.5 Will virus be replication defective?  YES  NO

4.6 Will virus be infectious to humans or animals?  YES  NO

4.7 Will this be expected to increase the containment level required?  YES  NO

### 5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent?  YES  NO  
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)  
If no, please proceed to Section 6.0

5.2 If YES, please specify which biological agent will be used: \_\_\_\_\_  
Please attach a full description of the biological agent.

5.2 Will the biological agent be able to replicate in the host?  YES  NO

5.3 How will the biological agent be administered? \_\_\_\_\_

5.4 Please give the Health Care Facility where the clinical trial will be conducted: \_\_\_\_\_

5.5 Has human ethics approval been obtained?  YES, number: \_\_\_\_\_  NO  PENDING

### 6.0 Animal Experiments

6.1 Will live animals be used?  YES  NO If no, please proceed to section 7.0

6.2 Name of animal species to be used \_\_\_\_\_

6.3 AUS protocol # \_\_\_\_\_

6.4 Will any of the agents listed in section 4.0 be used in live animals  YES, specify: \_\_\_\_\_  NO

6.5 Will the agent(s) be shed by the animal:  YES  NO, please justify:  
\_\_\_\_\_  
\_\_\_\_\_

## 7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)?  YES  No If no, please proceed to section 8.0

7.2 Will live animals be used?  YES  No

7.3 If yes, please specify the animal(s) used:

- ◆ Pound source dogs  YES  NO
- ◆ Pound source cats  YES  NO
- ◆ Cattle, sheep or goats  YES, please specify species \_\_\_\_\_  NO
- ◆ Non-human primates  YES, please specify species \_\_\_\_\_  NO
- ◆ Wild caught animals  YES, please specify species & colony # \_\_\_\_\_  NO
- ◆ Birds  YES, please specify species \_\_\_\_\_  NO
- ◆ Others (wild or domestic)  YES, please specify \_\_\_\_\_  NO

7.4 If no live animals are used, please specify the source of the specimens:  
\_\_\_\_\_

## 8.0 Biological Toxins

8.1 Will toxins of biological origin be used?  YES  NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s) \_\_\_\_\_  
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD<sub>50</sub> (specify species) of the toxin \_\_\_\_\_

8.4 How much of the toxin is handled at one time\*? \_\_\_\_\_

8.5 How much of the toxin is stored\*? \_\_\_\_\_

8.6 Will any biological toxins be used in live animals?  YES, Please provide details: \_\_\_\_\_  NO

\*For information on biosecurity requirements, please see:

[http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity\\_Requirements.pdf](http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf)

## 9.0 Insects

9.1 Do you use insects?  YES  NO If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. \_\_\_\_\_

9.3 What is the origin of the insect? \_\_\_\_\_

9.4 What is the life stage of the insect? \_\_\_\_\_

9.5 What is your intention?  Initiate and maintain colony, give location: \_\_\_\_\_  
 "One-time" use, give location: \_\_\_\_\_

9.6 Please describe the risk (if any) of escape and how this will be mitigated:  
\_\_\_\_\_  
\_\_\_\_\_

9.7 Do you use insects that require a permit from the CFIA permit?  YES  NO

If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

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### 10.0 Plants

10.1 Do you use plants?  YES  NO If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. \_\_\_\_\_

10.3 What is the origin of the plant? \_\_\_\_\_

10.4 What is the form of the plant (seed, seedling, plant, tree...)? \_\_\_\_\_

10.5 What is your intention?  Grow and maintain a crop  "One-time" use

10.6 Do you do any modifications to the plant?  YES  NO

If yes, please describe: \_\_\_\_\_  
\_\_\_\_\_

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:

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10.8 Is the CFIA permit attached?  YES  NO

If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

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### 11.0 Import Requirements

11.1 Will any of the above agents be imported?  YES, please give country of origin \_\_\_\_\_  NO  
If no, please proceed to Section 12.0

11.2 Has an Import Permit been obtained from HC for human pathogens?  YES  NO

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens?  YES  NO

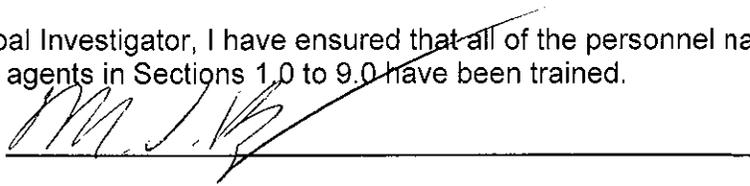
11.4 Has the import permit been sent to OHS?  YES, please provide permit # \_\_\_\_\_  NO

### 12.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- ◆ Biosafety
- ◆ Laboratory and Environmental/Waste Management Safety
- ◆ WHMIS (Western or equivalent)
- ◆ Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biological agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE  \_\_\_\_\_

**13.0 Containment Levels**

13.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required.  1  2  2+  3

13.2 Has the facility been certified by OHS for this level of containment?  
 YES, date of most recent biosafety inspection: July 11/11 WSC 124 will be inspected per AMM (lab safety) JS  
 NO, please certify  
 NOT REQUIRED for Level 1 containment

13.3 Please indicate permit number (not applicable for first time applicants): BIO-UWO-D178

**14.0 Procedures to be Followed**

14.1 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.  
None.

14.2 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury or an accidental splash:  
Needling not used. Splashes dealt with by washing affected areas. (JS) disinfectant soap recommended.

14.3 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at <http://www.wph.uwo.ca/>

SIGNATURE [Signature] Date: 1 March 2011

**15.0 Approvals**

1) UWO Biohazards Subcommittee: SIGNATURE: [Signature]  
Date: July 12 2011

2) Safety Officer for the University of Western Ontario  
SIGNATURE: [Signature]  
Date: July 11, 2011

3) Safety Officer for Institution where experiments will take place (if not UWO):  
SIGNATURE: \_\_\_\_\_  
Date: \_\_\_\_\_

Approval Number: \_\_\_\_\_ Expiry Date (3 years from Approval): \_\_\_\_\_

Special Conditions of Approval:

## Protozoa and Algae

ATCC® Number: **50194™** [Order this Item](#) Price: **\$155.00**

Organism: *Chilodonella uncinata* Ehrenberg  
 Designations: ATCC :0189:1  
 Isolation: contaminant of Euplotes gracilis culture, ATCC [50191](#), 1988  
 Depositors: TA Nerad  
 Biosafety Level: 1  
 Shipped: frozen  
 Growth Conditions: [ATCC medium 802](#): Sonneborn's Paramecium medium  
**Temperature:** 25.0°C  
 Duration: grown with Enterobacter aerogenes ATCC [13048](#) and mixed bacteria  
 In addition to the [MTA](#) mentioned above, other [ATCC and/or regulatory permits](#) may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please [click here](#) for information regarding the specific requirements for shipment to your location.  
 Permits/Forms:  
 Classification: KINGDOM: Protozoa

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# Project descriptions

Mark Daley

## 1 Brief Scientific Overview

### 1.1 Project 1

We propose to observe the structural evolution of information processing in networks of rat (and mouse) neurons. Recent work by Bassett et. al. [1] suggests that the underlying connection topology of neural networks is self-similar and scale invariant. While connectivity data can provide loose bounds for information-processing capabilities, it does not address this question directly.

In our initial pilot study we wish to begin to address the question of information-processing capabilities directly through the growth of rat and mouse neurons on Microelectrode Arrays (MEAs). The MEAs will be used to both stimulate, and record, the activity of neurons as they form associations in vitro (for a description of these techniques, see, e.g., [3]). Using stimulation, and recording, sequences suggested by theoretical analysis based on computability theory we intend to characterize the information processing capabilities of the forming neural network over time. It is our hypothesis that the information processing capabilities will increase with time and, moreover, that the resultant functional units will show processing behaviour that reflects the self-similar and scale invariant morphological organization described in [1].

### 1.2 Project 2

We propose to observe the morphology of macronuclear chromosomes in two species of ciliated protozoan using transmission electron microscopy. We will follow a procedure similar to that used in the characterization of the macronuclear chromosome structure of the spirotrichs *Stylonichia pustulata* and *Euplotes aediculatus* [2].

In one of these organisms, *Chilodonella uncinata*, we also wish to carry out DNA and RNA sequencing (with a particular focus on small RNA).

Finally, we wish to undertake a collaborative effort combining computer vision and ecology: creating a system for the automated identification of protists, via light microscopy, in fresh water samples. The diversity of species present in a water sample is an important metric of ecosystem health and an automated screening and identification system would enable high-throughput testing.

## 2 Details of cells and tissue to be used

### 2.1 Project 1

Neurons will be obtained from E18 Sprague-Dawley rat primary neuronal tissue and E18 C57 mouse primary neuronal tissue supplied by BrainBits LLC, Springfield IL.

Culturing and experimental stimulation/observation will be carried out in the Daley lab (WSC 124) according to the Primary Neuron Cell Culture protocol provided by BrainBits LLC[4] and MEA protocols described in [3].

These non-mammalian, disease-free, animal tissues are classified as Risk Group 1 according to UWO's Biosafety standards.

### 2.2 Project 2

All work will be carried out in the Daley lab (WSC 124).

We will culture the species *Chilodonella uncinata* and *Metopus es* as representatives of the classes Phyllopharyngea and Armophorea, respectively. Both of these organisms are listed as Risk Group 1 by international regulatory authorities (CDC, NIH and others) and represent an extremely low risk to both laboratory workers and the community. All of these species are "native", and ubiquitous, in the sense that they are present, in great numbers, in every freshwater lake and pond (and probably most large puddles) in southwestern Ontario.

*Chilodonella* will be cultured in a plain, pH-adjusted, Cereal grass medium (Scholar Chemistry #9448604) and requires no organisms to be added as food.

*Metopus* will be cultured in cell culture flasks under anaerobic conditions. Previous experience has demonstrated that cultures can survive for up to 6 months without feeding and, as we need only a very modest number of organisms, this will be entirely sufficient for the purposes of this project; no other organisms will be required as food.

July 11 / 11  
- location confirmed per  
(JS)  
voicemail

Finally, for the computer vision based identification of protists, we require samples of a large variety of organisms. Water samples will be taken from sites around Southwestern Ontario and screened using light microscopy. Some organisms may be retained, and grown briefly in culture, to use as training data for computer vision algorithms. Any retained species will first be identified via morphological features or rDNA sequencing and only species belonging to Risk Group 1 shall be retained.

After experimental completion, all organisms will be destroyed with bleach and all equipment autoclaved.

### 3 Summary of tissues to be used

E18 Sprague-Dawley rat primary neuronal tissue	Animal tissue	Risk Group 1
E18 C57 mouse primary neuronal tissue	Animal tissue	Risk Group 1
<i>Metopus es</i>	Ciliated protozoan	Risk Group 1
<i>Chilonella uncinata</i>	Ciliated protozoan	Risk Group 1
Various	Protozoa	Risk Group 1

### References

- [1] Danielle S. Bassett, Daniel L. Greenfield, Andreas Meyer-Lindenberg, Daniel R. Weinberger, Simon W. Moore, Edward T. Bullmore (2010). Efficient Physical Embedding of Topologically Complex Information Processing Networks in Brains and Computer Circuits. *PLoS Computational Biology* 6 (4) p. e1000748.
- [2] Murti KG, Prescott DM (2002) Topological organization of DNA molecules in the macronucleus of hypotrichous ciliated protozoa. *Chromosome Res* 10: 165-173.
- [3] Daniel A Wagenaar, Steve M Potter (2004). A versatile all-channel stimulator for electrode arrays, with real-time control. *Journal of neural engineering* 1 (1) p. 39-45.
- [4] Protocol available at <http://www.brainbitsllc.com/neuronprotocol.aspx>; accessed 28 February 2011.

## Neurons

### Primary Rat Brain Neuron Cell Culture from Hibernate Tissue

Please find enclosed one 2 ml tube containing embryonic day 18 Sprague/Dawley or Fischer 344 rat hippocampus or cortex in 2 ml B27/Hibernate. Tissue can be stored at 4-8°C for one week. Also find 12 ml Neurobasal/B27/0.5 mM Glutamax culture medium (25 uM glutamate for hippocampus.). For cortex and other tissues, the glutamate is omitted. Prepare substrate by coating with poly-D-lysine (0.15 ml/cm<sup>2</sup>, 50 ug/ml water, 135 kD, 1-20 hr. e.g. Sigma P6407) and rinse one time with sterile 18 Mohm deionized water and let dry.

To prepare isolated neurons:

1. From the tube with the brain tissue, remove 1 ml medium and save for step 3, being careful not to remove the tissue.
2. With 1 ml pipettor with sterile blue plastic tip, or silanized 9 inch pasteur pipet with tip barely fire polished (preferable), suck the tissue with medium into the pipet and immediately dispense the contents back into the same container. Take care not to create bubbles. Repeat this trituration step about 10 times or until most all pieces of tissue are dispersed. Higher survival can be obtained by incubating the tissue for 30 min. at 30°C in 2 mg/ml papain (Worthington) in Hibernate E-Calcium without B27, followed by trituration in Hibernate/B27.
3. Add back 1 ml medium that you removed in step 1 and mix.
4. Let undispersed pieces settle by gravity for 1 min.
5. Transfer supernatant to a new sterile 15 ml tube.
6. Spin 1100rpm (200 x G), 1 min. Discard supernatant.
7. Flick the tube to disperse the pellet of cells. Resuspend pellet in 1 ml B27/Neurobasal+0.5 mM Glutamax +25 uM glutamate, for hippocampal neurons. For cortical neurons, glutamate is omitted. (12 mL provided; more available from [www.invitrogen.com](http://www.invitrogen.com), 1-800-955-6288)
8. Aliquot 20 ul and mix with 20 ul 0.4% trypan blue.
9. Count in hemacytometer: calculate cells/ml.
10. Dilute cells with B27/Neurobasal to plate  $32 \times 10^3$  cells/2 cm<sup>2</sup> of substrate in 0.4ml/2 cm<sup>2</sup> substrate (or whatever density you desire).
11. Incubate 37°C, 5% CO<sub>2</sub>, 9% oxygen (20% oxygen is O.K.)
12. After 4 days or longer, neurons are well differentiated. If further culture is desired, change one half of the medium with fresh, warm B27/Neurobasal + 0.5 mM Glutamax without glutamate. Change one half every 3 or 4 days.

Viability assay:  $\text{viability} = \frac{\text{green cells per unit area}}{\text{total cells plated per unit area}}$   
or survival ( $\frac{\text{green cells}}{\text{green} + \text{red cells}}$ ):

1. Rinse 2 times with PBS or HBSS/1 g/l glucose/1 mM pyruvate/1 mM  $\text{NaHCO}_3$ /10 mM Hepes, 0.4 ml/2  $\text{cm}^2$  of substrate
2. From an acetone stock of 15 mg/ml fluorescein diacetate (Sigma), add 15  $\mu\text{L}$  into 1.5 ml HBSS (1:100 dilution of the stock). From an aqueous stock of 4.6 mg/ml propidium iodide, add 15  $\mu\text{L}$  of the stock into the same 1.5 ml HBSS (1:100 dilution). Add 44  $\mu\text{L}$  of that dilution to each well with 0.4 ml HBSS (further 1:10 dilution).
3. After about 1 min., count using Nikon B1A filter or other blue excitation appropriate for fluorescein fluorescence. Green cells are live, small red nuclear stain are dead. Count dead cells using Nikon GIB or other green excitation to visualize small red nuclei.

If desired, fix and stain with 0.25% Coomassie blue R in ethanol/Acetic acid/water (45/10/45), 1 min., rinse with 10% Acetic acid, aspirate and dry.

Please report any questions or problems us:

voice: 217-789-9313  
fax: 217-789-9314  
[brainbits@brainbits.biz](mailto:brainbits@brainbits.biz)

Methods based on Brewer et al. (1993) J. Neurosci. Res. 35:567-576 and Brewer & Price (1996) Neuroreport 7:1509-1512

\*Hibernate, Neurobasal and B27 is for research purposes only and is manufactured by Invitrogen Corporation. Hibernate, Neurobasal, and B27 is a trademark of Invitrogen Corporation.