

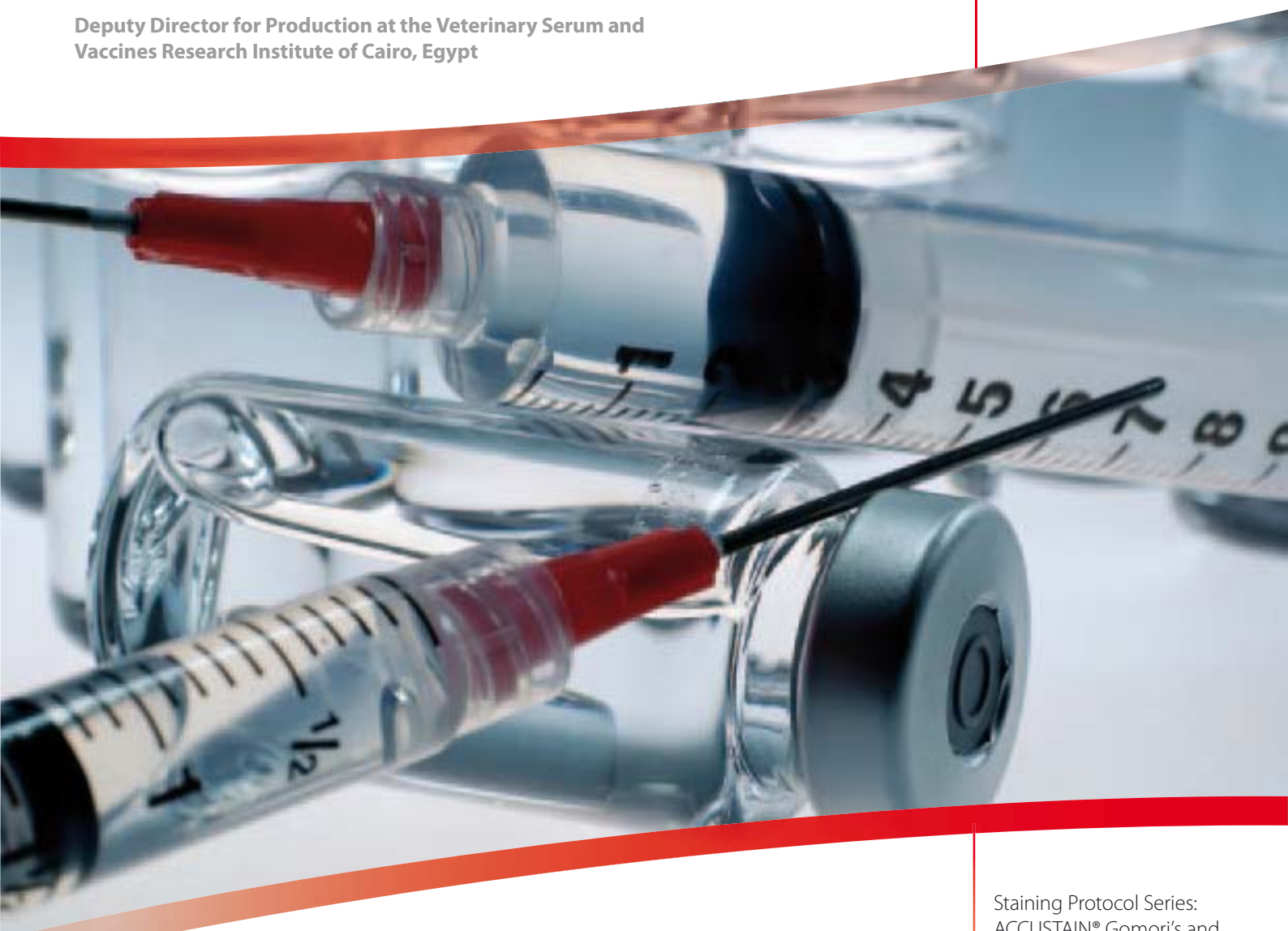
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# INVITRO

Issue 7 • 2010

Interview with Dr. Adel M. H. Azab

Deputy Director for Production at the Veterinary Serum and  
Vaccines Research Institute of Cairo, Egypt



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## Dear Researcher,



Sigma-Aldrich Chemie GmbH  
Walter Gmelin, PhD

It is our great pleasure to present an interview with Dr. Adel M. H. Azab, Deputy Director of The Veterinary Serum & Vaccine Research Institute (VSRI).

Dr. Adel Azab is an internationally renowned expert for veterinary viral vaccine research and production. The Egyptian scientist lives and works in Cairo, where he played a major role in developing The Veterinary Serum & Vaccine Research Institute to its high standards today. As a Deputy Director he helps the Institute to cover the vaccine production for his country, and to be prepared for future challenges arising from new epidemics in Africa.

Accurate and reliable detection in molecular biology and diagnostics is in the focus of this In Vitro issue. Constant improvement in chemistry has led to the development of Atto dyes with unprecedented brightness and photostability, which can be used in time saving in gel-staining protocols. One highlight of this issue is a scientific article describing how whole genome amplification was combined with comparative genome hybridisation to identify chromosomal abnormalities from limited amounts of DNA. Moreover, an update on Prestige Antibodies® and our regular BioFiles are included.

This BioFiles In Vitro series on classical histological staining protocols contains two protocols for Trichrome staining provided in accordance with the IVD product range, which can be collected in your lab work stain protocols.

Our Prestige Antibody® range, to which more than 2,500 antibodies are added per year, now covers more than 6,100 antibodies, with 5,200 human protein targets. The benefit of the validation of these antibodies

by the human protein atlas (HPA) programme is impressively demonstrated by detection of a highly specific tumour marker in a diagnostic application.

In this issue an article describes how the use of the GenomePlex® WGA kit for whole genome amplification (WGA) can be combined with comparative genome hybridisation to identify chromosomal abnormalities. The GenomePlex® WGA kit allows highly accurate conducting of the CGH analysis from a limited amount of DNA, providing a valuable tool for genetic research.

Wrapping up, In Vitro introduces a solution for selective detection of *Vibrio cholerae*, using the chromogenic medium HiCrome Vibrio Agar. The use of this medium allows the for differentiation of *Vibrio cholerae* in the presence of co-cultured contaminating bacteria.

We hope you enjoy our BioFiles In Vitro journal.

Kind regards,

Walter Gmelin, PhD  
European Marketing Manager Life Science

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# Interview with Dr. Adel M. H. Azab

Deputy Director for Production at the Veterinary Serum Vaccines Research Institute (VSVRI),  
Agriculture Research Center of Cairo, Egypt

## CURRICULUM VITAE

### Personal Information

Name: Dr. Adel M. H. Azab  
 Birth date: 10 December, 1949  
 Place of birth: Cairo, Egypt  
 Gender: Male  
 Social status: Married  
 Nationality: Egyptian  
 Work address: Vet-Ser. and Vacc. Res. Instit. (VSVRI)  
 P. O. Box No.: 131, Postal Code: 1 1381  
 Abbassia, Cairo, Egypt.



### Scientific Degrees

- 1982** Ph.D. in Microbiology, Faculty of Veterinary Medicine, Cairo University.
- 1979** Master of Veterinary Sciences, Microbiology, Faculty of Veterinary Medicine, Cairo University.
- 1973** Bachelor of Veterinary Sciences, Faculty of Veterinary Medicine, Cairo University.

### Career History

- 2005 – today** Deputy Director for Production at the Veterinary Serum and Vaccine Research Institute (VSVRI), Agriculture Research Center.
- 1973 – 2003** Different positions at the VSVRI.
- 1991 – 2000** Expert in viral vaccine production at the Ministry of Agriculture and Water, Kingdom of Saudi Arabia. For production of: rinderpest, sheep pox, new castel rabies.
- 1983 – 1990** Associate Professor at the faculty of veterinary medicine, El-Mousel University, Iraq, teaching: virology, poultry viral disease.

### The Veterinary Serum and Vaccine Research Institute

The Veterinary Serum and Vaccine Research Institute (VSVRI) was established in 1903. It consists of 13 departments. Each one is responsible for the production of and research into specific vaccines. The departments of VSVRI include: FMD, Rift Valley fever, pet animals, new castel, sheep pox, rinderpest, African horse sickness, rinderpest like disease, aerobic, anaerobic, parasitic, antigens and sera, genetic engineering. The institute products satisfy Egyptian market needs in some vaccines, e.g. FMD, Rift Valley fever, sheep pox, some poultry vaccines, rabies etc.

The institute also includes a maintenance workshop, library, administration office, meeting hall and other supportive services. The institute employs about 1,200 people, 174 of them with M.Sc. and Ph.D.

I have a training course in Sandia National Laboratories Albuquerque NM-USA in:

- The transportation of infectious substances and diagnostic specimens. This training course meets or exceeds all IATA/ICAO requirements.
- Controlling laboratory: Biorisks, Biosafety, Biosecurity, Risk assessment.

### Projects (Recent)

- MP-12 RVF (F.E) contract No. 61751-05-M-0398 in cooperation with USA (NAMRU-3).
- TCPIEGY/3105 (E) Foot- and -Mouth FMD-like Disease Surveillance and vaccine evaluation in cooperation with FAO.

### Published Papers:

- Over 50 published papers and essays in the field of virology.



The Egyptian scientist Dr. Adel Azab is the Deputy Director of The Veterinary Serum & Vaccine Research Institute (VSVRI). It is one of the oldest institutes in the Middle East and the most prestigious in Africa. It started in 1903 with a laboratory that was established in Cairo. Since it was founded, the institute has seen a considerable laboratory expansion and new building construction in accordance with the demands of different services. Today, the institute is responsible for production and research on specific vaccines. The VSVRI has gained international recognition and has even exported its vaccines to Arab, African and Asian countries. The institute products satisfy Egyptian market needs in some vaccines, e.g. FMD, Rift Valley fever, sheep pox, some poultry vaccines and rabies. 1,200 people work for the institute, 174 of them have Master and Ph.D. degrees.

**IN VITRO:** You started in 1973 at the VSVRI. How did it develop and what are the challenges of your work?

**AA:** Well, during this period the institute developed in different ways: it increased the staff members qualified with Master and Ph.D. degrees. At the same time, the experience of the staff increased due to training courses in Egypt and abroad. We also increased the number of vaccines produced (new vaccines and number of doses) and improved the quality. Another development was the upgrading of facilities and of some laboratories. The main challenge of our institute is the changeable epidemiology of the country due the unique geographical position of Egypt.

**IN VITRO:** The VSVRI seems to be quite unique in respect of being a public institute and producer of biological goods and vaccines at the same time. Can other comparable institutes be found in Africa and is there any collaboration between you and other institutes?

**AA:** Yes, in Africa there are similarly institutes in South Africa, Kenya etc. And we already have cooperation through bilateral visits and conferences.

**IN VITRO:** Let's talk about research in Egypt. Is it predominantly financed by governmental money or private investments?

**AA:** We have both, the governmental is the biggest.

**IN VITRO:** From which source does the VSVRI obtain the pathogenic strains for vaccine production? Do you monitor antigenic drifts and collect (endemic) pathogen?

**AA:** We got the strains either from the local isolates after identification and characterisation in reference laboratories (e.g. FMDV) or directly from those labs (e.g. ND Lasota).

**IN VITRO:** FMD outbreaks are of major concern and have accounted for huge economic losses worldwide. What safety measures are in place at the VSVRI to prevent escape of highly contagious FMD pathogens which you propagate for vaccine production?

**AA:** We adopt biosafety and biosecurity measures, and VSVRI has started to build a new lab, which can be used for FMD vaccine production on BSL-3 bases.

**IN VITRO:** Why does your institute combine research and production?

**AA:** The research in VSVRI helps in vaccine production for Egypt and other countries in the Arab world and in Africa. At the same time, we have the possibility to improve quality.

**IN VITRO:** In the case of an outbreak, how fast can you deliver a sufficient amount of serum?

**AA:** The VSVRI makes an annual tender for serum, and the amount includes a strategic stock for emergencies.

**IN VITRO:** How has it come about that you also give training courses at the Sandia National Laboratories (S.N.L.) Albuquerque, USA?

**AA:** The staff of S.N.L. visits the VSVRI periodically and some of our staff visits the S.N.L. They have also given us advice about building the FMD BSL-3 Lab.

**IN VITRO:** There are three women with you on the board of directors. That is quite unique in the scientific world. How did it happen?

**AA:** The three scientists have very good experience in many fields. They've worked for a long time at the VSVRI and were appointed to the jobs by an order from the Minister of Agriculture.

**IN VITRO:** You're an internationally renowned expert for viral vaccine production ...

**AA:** Yes, I have a lot of experience. Many of my staff and I have worked a lot in the Middle East, in countries like Iraq and Saudi Arabia, and also in Africa, to help in viral vaccine production and viral diseases diagnosis.

**IN VITRO:** Are there any other scientists in your family?

**AA:** Yes, my wife is a scientist in the field of virology, specialised in FMD.

**IN VITRO:** What importance has Sigma-Aldrich for your research and production?

**AA:** Sigma-Aldrich supplies the VSVRI with a lot of items, e.g. media, trypsin, gel, saponin, etc. And it is our genetic and biological reagent supplier.



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41698	Atto 488-NHS ester	01464	Atto 633 NHS ester
00379	Atto 495-NHS ester	43429	Atto 635 NHS ester
77810	Atto 520 NHS ester	53394	Atto 637 NHS ester
88793	Atto 532 NHS ester	07376	Atto 647 NHS ester
61683	Atto 540 Q NHS ester	76508	Atto 647N NHS ester
92835	Atto 550 NHS ester	76245	Atto 655 NHS ester
92835	Atto 550 NHS ester	75999	Atto 680 NHS ester
72464	Atto 565 NHS ester	16986	Atto 700 NHS-ester
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79636	Atto 590 NHS ester	59808	Atto 740 NHS ester
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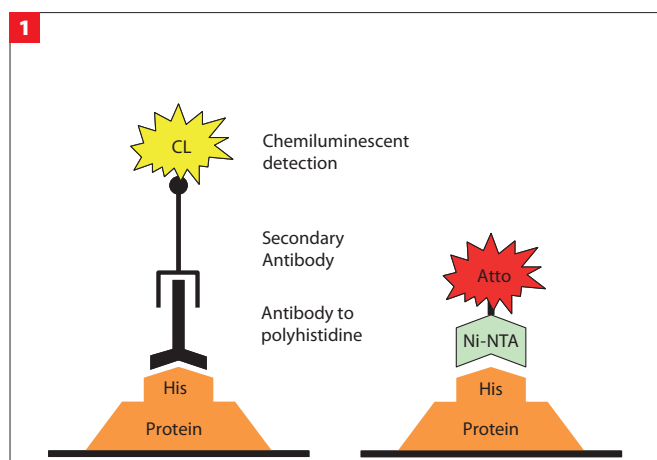


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# Ni-NTA-Atto conjugates for sensitive, specific detection of polyhistidine-tagged proteins

Authors: Monika Baeumle, Ph.D., Product Manager Biochemistry, monika.baeumle@sial.com  
Alex Rueck, Ph.D., Application Scientist R&D

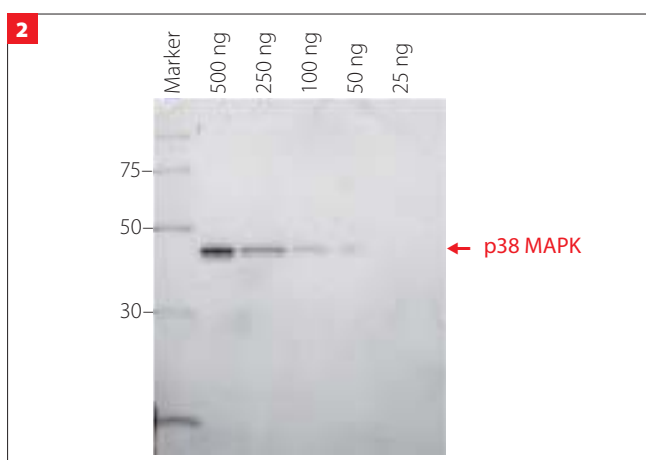
State-of-the-art recombinant protein applications require reliable methods of detection. Polyhistidine is one of the most popular affinity tags incorporated into recombinant proteins. It can be inserted either at the N- or C-terminus, and expressed in a variety of hosts. Due to its small size, the polyhistidine tag serves as an elegant tool for both protein purification and detection. Immunodetection of His-tagged fusion proteins on a Western blot typically uses an antibody to polyhistidine, followed by a secondary antibody conjugated to an enzyme such as horseradish peroxidase or alkaline phosphatase. The protein is then detected with an appropriate enzyme substrate. Ni-NTA-Atto conjugates provide specific and highly sensitive detection of His-tagged fusion proteins. The Ni-NTA-Atto complex (Ni,Na-bis(carboxymethyl)-L-lysine, Nickel(II) complex, conjugated to Atto dye) is specific for polyhistidine tags with minimal crossreactivity. Atto dyes deliver strong fluorescent signals at commonly available wavelengths and with little quenching. Ni-NTA-Atto conjugates can be directly applied either to an SDS-PAGE gel or Western blot membrane for fluorescence imaging, and have been successfully used in living cells.<sup>1,2</sup> Detection with Ni-NTA-Atto conjugates requires less incubation time than for protein-antibody binding. No secondary reaction is required, since the Ni-NTA complex is directly conjugated to the fluorophore. This results in a faster and more flexible procedure than the traditional immunodetection method (see **Figure 1**).



**Figure 1:** Comparison of protein detection by traditional antibody chemiluminescent immunodetection (left) to Ni-NTA-Atto conjugate detection (right). Immunodetection takes place on a transfer membrane after Western blotting, while the Ni-NTA-Atto conjugates may be used with either transfer membranes or directly on SDS-PAGE gels after fixing.

## Direct detection of histidine-tagged proteins on SDS-PAGE gels

Direct application of Ni-NTA-Atto conjugates to SDS-PAGE gels provides fast and easy detection for monitoring protein purification or protein expression at different points of time. A detection limit of 50 ng for His-tagged p38 MAPK was observed using fluorescence imaging (see **Figure 2**).

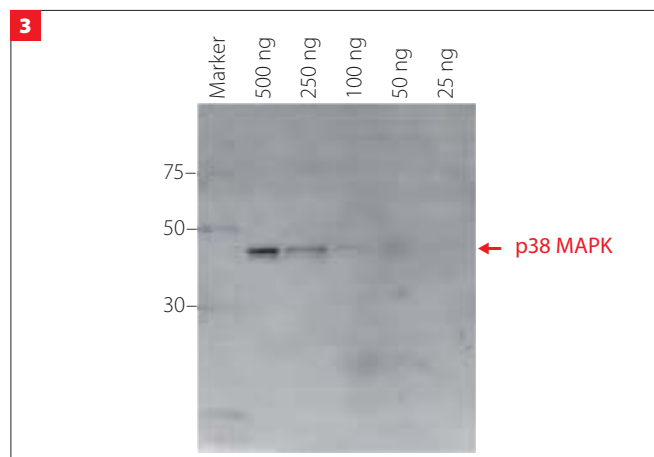


**Figure 2:** His-tagged p38 MAPK protein (500 ng–25 ng) was separated on a 4–20% Tris-Glycine SDS-PAGE gel. The gel was fixed overnight in 40% ethanol/10% acetic acid, washed in water and incubated with Ni-NTA-Atto 647N (1:1000) in the dark. The gel was washed and then imaged using a FLA-3000 Fuji® laser scanner with 633 nm excitation and a 675 nm emission filter. Ni-NTA-Atto 647N ( $\lambda_{ex}$  647 nm,  $\lambda_{em}$  669 nm) is excited in the red region of the spectrum.

### Detection of histidine-tagged proteins after Western blot transfer

After electrophoresis, proteins may be transferred from SDS-PAGE gels to low-fluorescence PVDF-membranes, which are more robust to handling. Traditional Western blots work well for polyhistidine tags, but are time consuming. Ni-NTA-Atto conjugates combine the advantages of highly specific detection with a more rapid procedure.

Application of Ni-NTA-Atto conjugates to the membrane yields sensitivity comparable to direct gel detection. Drying the membrane enhances the signal intensity (see **Figure 3**). In addition, the Ni-NTA-Atto conjugates may be stripped from the used membrane with a 60-90-minute incubation in 20 mM EDTA.



**Figure 3:** His-tagged p38 MAPK protein (500 ng–25 ng) was separated on a 4–20% SDS-PAGE gel. The protein was transferred to a low-fluorescence PVDF-membrane, blocked overnight with 5% BSA in PBS, rinsed with PBS-T, and incubated with Ni-NTA-Atto 647N (1:1000) in the dark. The membrane was washed and then imaged using a FLA-3000 Fuji laser scanner with 633 nm excitation and a 675 nm emission filter.

Comparison of procedures for the traditional Western immunoblot and Ni-NTA-Atto conjugates is shown in **Table 1**. Direct SDS-PAGE gel detection is the most convenient as it omits the transfer step. Similar detection limits with Ni-NTA-Atto conjugates are observed for Western blot membranes compared to SDS-PAGE gels, but without risk of gel damage. In either case, the hands-on time is less than for the traditional antibody technique.

Ni-NTA-Atto conjugates provide a valuable alternative to traditional antibody immunodetection for specific and sensitive detection of His-tagged fusion proteins. Use of Ni-NTA-Atto reduces experiment time and eliminates time-consuming antibody validation experiments, saving both time and expense.

For additional information on related products for histidine-tagged proteins, visit our website at [sigma.com/hisselect](http://sigma.com/hisselect)

Traditional Immunoblotting procedure	Western Blot using Ni-NTA-Atto conjugate	Direct Detection using Ni-NTA-Atto conjugate
SDS-PAGE	SDS-PAGE	SDS-PAGE
↓	↓	↓
Membrane transfer (1–2 hr)	Membrane transfer (1–2 hr)	Gel fixation in 40% ethanol: 10% acetic acid (1–14 hr [overnight])
↓	↓	↓
Blocking with 5% BSA in PBS (1–14 hr [overnight])	Blocking with 5% BSA in PBS (1–14 hr [overnight])	Wash with water (2x30 min)
↓	↓	↓
Rinse with PBST	Rinse with PBST	Incubate with Ni-NTA-Atto 1:1000–1:2500 in PBST (1 hr, dark)
↓	↓	↓
Incubate with primary antibody (2–3 hr)	Incubate with Ni-NTA-Atto 1:1000–1:2500 in PBST (1 hr, dark)	Wash with water (1–2 hr, dark)
↓	↓	↓
Wash with PBST (3x5 min)	Wash with PBST (0–1 hr, dark)	Fluorescent imaging
↓	↓	
Incubate with secondary antibody (1 hr)	Fluorescent imaging	
↓		
Wash with PBST (3x5 min)		
↓		
Chemiluminescent detection		
<b>Total Time 5.5–20.5 hours</b>	<b>Total Time 3–18 hours</b>	<b>Total Time 4–18 hours</b>
<b>Number of Steps 8</b>	<b>Number of Steps 6</b>	<b>Number of Steps 5</b>

**Table 1:** Comparison of the protocols for traditional Western immunoblot, Western blot using Ni-NTA-Atto conjugate, and direct detection on an SDS-PAGE gel using Ni-NTA-Atto conjugate. Ni-NTA-Atto stock solution is prepared by dissolving 250 µg in 250 µL PBS.

### Ordering information

Cat. no.	Name	Pack size
02175	NTA-Atto 647N	250 µg
94159	NTA-Atto 550	250 µg
39625	NTA-Atto 488	250 µg
55183	NTA-Tracy 652	250 µg

### Related products

Cat. no.	Name	Pack size
05317	Immobilon-FL PVDF membrane	10 ea

### References

- 1) Tracy 645 and Tracy 652, BioFiles, Issue 4.1, pp. 6–8, Sigma-Aldrich (2009)
- 2) Ni-NTA-Atto Conjugates BioFiles, Issue 2.5, pp. 12–13, Sigma-Aldrich (2007)
- 3) Single Molecule Detection with Atto 647N NTA, BioFiles, Issue 3, pp. 8–9, Sigma-Aldrich (2006).
- 4) Guignet, E.G., et al., Reversible site-selective labelling of membrane proteins in live cells. *Nat. Biotechnol.*, 22, 440–4 (2004).

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# ACCUSTAIN® Gomori's Trichrome Stain

## Introduction

Trichrome stains are used primarily for distinguishing collagen from muscle tissue. In general, they consist of nuclear, collagenous and cytoplasmic dyes in mordants such as phosphotungstic or phosphomolybdic acid. Historically, the first trichrome system was attributed to Mallory. Further modifications were introduced by Masson, Foot and Gomori. The Sigma-Aldrich Gomori Trichrome Stain is intended for use in the study of connective tissue, muscle and collagen fibres. The procedure described here is based on the work of Gomori and is a one-step system combining the cytoplasmic and connective-fibre stain in a phosphotungstic acid/acetic acid solution. Tissue sections are treated with Bouin's solution to intensify the final coloration. Nuclei are then stained with Weigert's iron hematoxylin. The cytoplasm and muscle fibres are stained with chromotrope 2R; fast green FCF or aniline blue stains the collagen fibres. Rinsing in acetic acid after staining makes the shades of colour more delicate and transparent. Sigma-Aldrich also includes a microwave procedure in the package insert for rapid staining.

## Reagents

**Trichrome Stain LG Solution** Cat. no. HT10-3-16, chromotrope 2R, 0.6% (w/v), fast green FCF, (certified), 0.1% (w/v), phosphotungstic acid, 0.8% (w/v) and acetic acid, 1.0% (v/v).

## Trichrome Stain AB Solution

Cat. no. HT10-5-16, chromotrope 2R, 0.6% (w/v), aniline blue, (certified), 0.3% (w/v), phosphotungstic acid, 0.8% (w/v) and acetic acid, 1.0% (v/v).

## Weigert's Iron Hematoxylin Set

Cat. no. HT10-79 (Part A and Part B).

## Reagent preparation

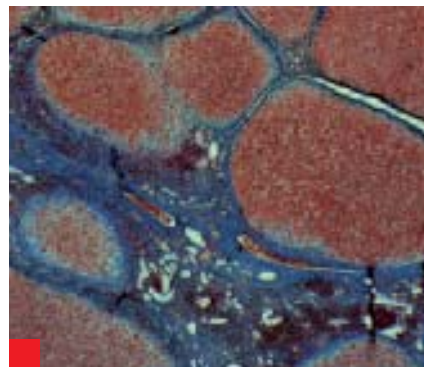
Prepare working Weigert's iron hematoxylin solution (Cat. no. HT10-79) by adding equal amounts of Part A and Part B.

## ACCUSTAIN® Gomori's Trichrome Stain

### Procedure

1. Deparaffinise tissue sections and hydrate to deionised water.
2. Mordant in preheated Bouin's solution (Cat. no. HT10-1) at 56 °C for 15 minutes.
3. Wash in running tap water until yellow colour is removed.
4. Stain in working Weigert's iron hematoxylin solution for 5 minutes.
5. Wash in running tap water for 5 minutes.
6. Stain in Trichrome Stain (LG or AB) solution for 5 minutes.
7. Place in 0.5% acetic acid for 1 minute.
8. Rinse slide in water and dehydrate in alcohol.
9. Clear in xylene and mount.

### Results



**Nuclei**  
**Muscle fibres**  
**Collagen**

**Black**  
**Red**  
**(AB) Blue**  
**(LG) Green**

**SIGMA**  
Life Science

# ACCUSTAIN® Masson's Trichrome Stain

## Introduction

The Sigma-Aldrich Masson's Trichrome Stain is intended for use in the study of connective tissue, muscle and collagen fibres. Trichrome stains are used primarily for distinguishing collagen from muscle tissue. In general, they consist of nuclear, collagenous and cytoplasmic dyes in mordants such as phosphotungstic or phosphomolybdic acid. The procedure described here is based on the work of Masson as modified by Lillie using aniline blue as a collagen stain instead of a green dye. Tissue sections are treated with Bouin's solution to intensify the final coloration. Nuclei are stained with Weigert's iron hematoxylin, and cytoplasm and muscle are then stained with Biebrich scarlet-acid fuchsin. After treatment with phosphotungstic and phosphomolybdic acid, collagen is demonstrated by staining with aniline blue. Rinsing in acetic acid after staining renders the shades of colour more delicate and transparent. Sigma-Aldrich also includes a microwave procedure in the package insert for rapid staining.

## Reagents

**Biebrich Scarlet Acid Fuchsin Solution** Cat. no. HT15-1, Biebrich scarlet, 0.9%, acid fuchsin 0.1%, in acetic acid, 1.0%.

### Phosphotungstic Acid Solution

Cat. no. HT15-2, phosphotungstic acid, 10%.

### Phosphomolybdic Acid Solution

Cat. no. HT15-3, phosphomolybdic acid, 10%.

### Aniline Blue Solution

Cat. no. HT15-4, aniline blue, 2.4% and acetic acid, 2%.

## Reagent preparation

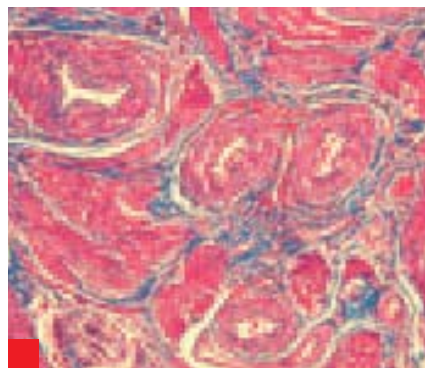
Prepare working Weigert's iron hematoxylin solution (Cat. no. HT10-79) by adding equal amounts of Part A and Part B. Prepare working phosphotungstic-phosphomolybdic acid solution by mixing 1 volume of phosphotungstic acid solution with 1 volume of phosphomolybdic acid solution with two volumes of deionised water. Use once and discard.

## ACCUSTAIN® Masson's Trichrome Stain

### Procedure

1. Deparaffinise tissue sections and hydrate to deionised water.
2. Mordant in Bouin's solution (Cat. no. HT10-1) at 56 °C for 15 minutes.
3. Wash in running tap water until yellow colour is removed.
4. Stain in working Weigert's iron hematoxylin solution for 5 minutes.
5. Wash in running tap water for 5 minutes.
6. Rinse in deionised water.
7. Stain in Biebrich scarlet acid fuchsin solution for 5 minutes.
8. Rinse in deionised water.
9. Place slides in working phosphotungstic-phosphomolybdic acid solution for 5 minutes.
10. Stain in aniline blue solution for 5 minutes.
11. Place slides in 1% acetic acid solution for 2 minutes.
12. Rinse slides, dehydrate through alcohol, clear in xylene and mount.

### Results



<b>Nuclei</b>	<b>Black</b>
<b>Muscle fibres</b>	<b>Red</b>
<b>Collagen</b>	<b>Blue</b>
<b>Cytoplasm</b>	<b>Red</b>



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With all characterisation data published on the Human Protein Atlas (HPA) portal ([www.proteinatlas.org](http://www.proteinatlas.org)), Prestige Antibodies® are the most highly characterised antibodies in the industry today. At the moment, there are over 6,100 Prestige Antibodies® available, covering 5,300 human protein targets. This corresponds to 25% of the human proteome. Each year, more than 2,500 new Prestige Antibodies® are made available to the scientific community. The antibodies are directed against both new and previously unknown proteins as well as established protein families.

Each Prestige Antibody has been analysed and annotated in a large variety of normal and diseased human tissues, and the expression profiles are conveniently searchable online. Immunohistochemical (IHC) staining has been performed in 48 normal human tissues, the 20 most common cancer types, 47 cell lines and 12 primary cell types. In addition, all antibodies have been tested for performance in Immunofluorescence (IF) and Western Blot (WB) applications. In total, more than 700 IHC, as well as IF and WB images per antibody, are shown online.

The Human Protein Atlas Program systematically generates antibodies against the human proteome,<sup>1,2</sup> and the resulting Human Protein Atlas provides a useful platform for biomarker discovery efforts.<sup>3</sup>

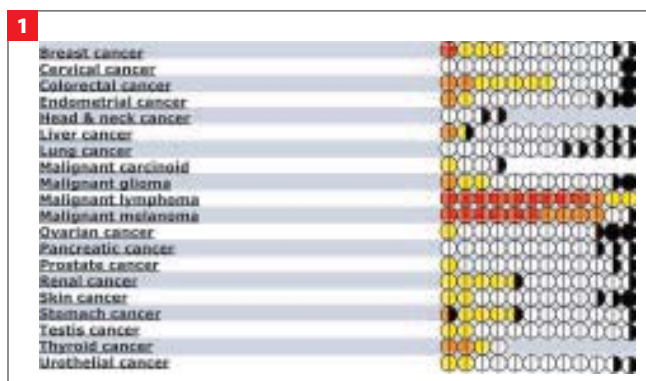
### ■ Malignant melanoma

Malignant melanoma is a common skin tumour with a rapidly increasing incidence rate. Survival rates are high, but melanomas tend to metastasise relatively early, and for patients with metastatic melanoma prognosis is poor, with a five-year survival rate of less than 10%. Today, there are no validated biomarkers for use in a clinical setting that are able to give prognostic information and estimate the risk for metastatic disease.

### ■ Syntaxin-7

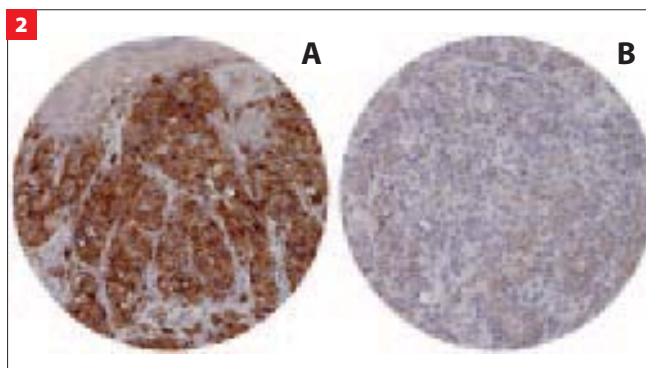
Syntaxin-7 (STX7) was identified on the Human Protein Atlas as a protein showing a selective expression profile in cancer tissue as shown in **Figure 1**.

Syntaxin-7 is a protein that belongs to the SNARE family, proposed to be mediators of all intracellular fusion events. Syntaxin-7 is believed to be a regulator of membrane vesicular trafficking between late endosomes and lysosomes.



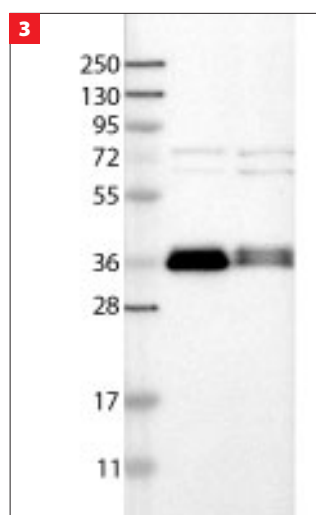
**Figure 1:** Detection of STX7 in HPA screening tissue micro arrays (TMAs). This schematic overview shows the staining results in cancer tissue with the colour codes representing different levels of immunoreactivity (red = strong staining, orange = moderate staining, yellow = weak staining, white = negative staining and black = missing or non-representative tissues). Duplicate samples are displayed from each individual.

The Prestige Antibody HPA001467, directed against Syntaxin-7 was used to evaluate the potential of Syntaxin-7 as a marker for malignant melanoma prognosis.<sup>4</sup> The antibody was successfully used in IHC, WB and IF applications. IHC staining was performed on tissue microarrays as well as on two extended melanoma cohorts. As can be seen in **Figure 2**, there was a clear differentiation in expression in different melanomas. This differential expression could be correlated to tumour aggressiveness. It was shown that high expression of STX7 indicates a good prognosis.



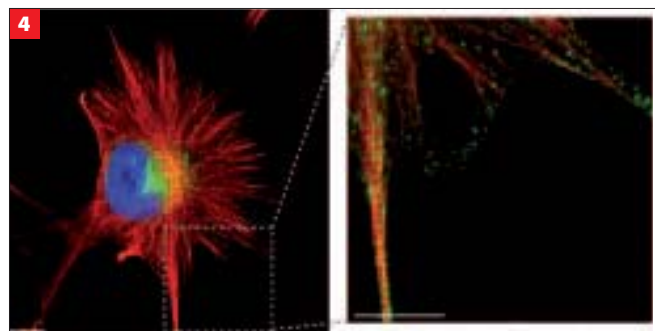
**Figure 2:** Immunohistochemical staining of malignant melanoma using the Anti-STX7 antibody. Strong staining is illustrated in A and negative/weak staining in B.

The expression of syntaxin-7 in two melanoma cell lines was confirmed by Western Blot analysis (**Figure 3**).



**Figure 3:** Western Blot analysis of STX7 in the melanoma cell lines SK-MEL-30 (middle lane) and WM-115 (right lane). The left lane shows the molecular weight markers. A strong band within the predicted size range (30 kDa) could be detected in the melanoma cell lines.

Immunofluorescence and confocal microscopy was used to determine the subcellular localisation of syntaxin-7 in SK-MEL-30 cells. As shown in **Figure 4**, vesicles along the plasma membrane with accumulation of vesicles in the juxtannuclear region are stained.



**Figure 4:** Confocal images of immunofluorescently stained SK-MEL-30 cells. STX7 antibody staining is shown in green, tubulin staining in red and nuclear staining in blue. The microscope settings have been adjusted to visualise the intense staining of vesicles in the outer region of the cytoplasm. Scale bar=10  $\mu$ m.

### Summary

- The Human Protein Atlas was used to identify STX7 as a potential prognostic marker for malignant melanoma.
- It was shown that reduced levels of STX7 are associated with more aggressive tumours.
- The Prestige Antibody HPA001467 has been validated as an excellent tool for biomarker detection.

### Use of Prestige Antibodies® in detection studies

Prestige Antibodies® are excellent tools for detection studies. These are highly characterised antibodies evaluated in several applications, such as IHC, IF and WB, and tested on a large variety of normal and diseased human tissues. Standardised universal protocols can be used and all characterisation data are conveniently searchable online ([www.proteinatlas.org](http://www.proteinatlas.org))

Each year the Human Protein Atlas portal is updated with characterisation data from 2,500 new human antibodies. During 2010, The Human Protein Atlas portal will contain expression profiles and localisation data for more than 50% of the human proteome.

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# Comparative genomic hybridisation of DNA amplified with GenomePlex® technology for detection of trisomy chromosomal abnormalities from limited patient source material

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## Introduction

Comparative Genomic Hybridisation (CGH) has been refined to find chromosomal abnormalities at progressively smaller resolution. Initial studies were limited to trisomies and some terminal deletions, but recent work has been successful in finding microdeletions or duplications, for instance, in the telomeric regions. This growing technique, however, is somewhat hampered by the large DNA input requirement: over 75,000 copies of a human genome are needed to perform one CGH assay. Whole Genome Amplification represents a means to decrease the amount of required DNA for CGH, which would open the technology for analysis of small tissue biopsies, or perhaps individual organisms with smaller genomes. In this study, GenomePlex® Whole Genome Amplification (WGA) technology was combined with CGH arrays to detect chromosomal abnormalities in patients with mental and developmental disabilities, such as Down's (Trisomy 21) and Edward's (Trisomy 18) syndromes. The use of GenomePlex® WGA Kit allowed for the identification of genome-wide copy number and chromosomal abnormalities without any bias detected in the array. The technique is simple to use and can be applied to academic and clinical research.

## Materials and methods

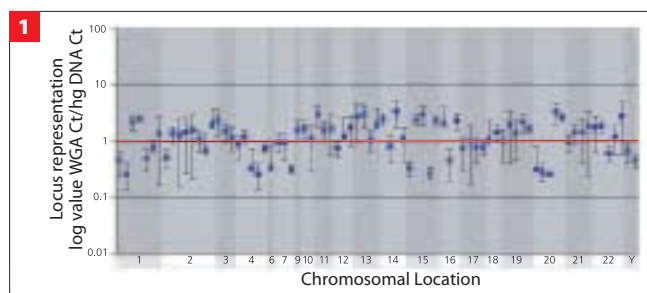
### Representation assay

Human genomic DNA was extracted using GenElute™ Mammalian Genomic DNA Purification Kit (Cat. No. **G1N**) and subjected to GenomePlex® amplification. The samples were then purified using GenElute™ PCR Cleanup Kit (Cat. no. **NA1020**). This DNA was aliquotted and subjected to 79 different SYBR® Green quantitative PCR analyses (Cat. no. **S4438**), using different UniSTS primers. These independently designed primers targeted the majority of human chromosomes. Reactions were run using three replicates and compared to a similar number of reactions on an MJ Opticon 2 instrument. Results were plotted as a ratio of amplified and unamplified hg DNA after qPCR analyses.

## Chromosomal microarray

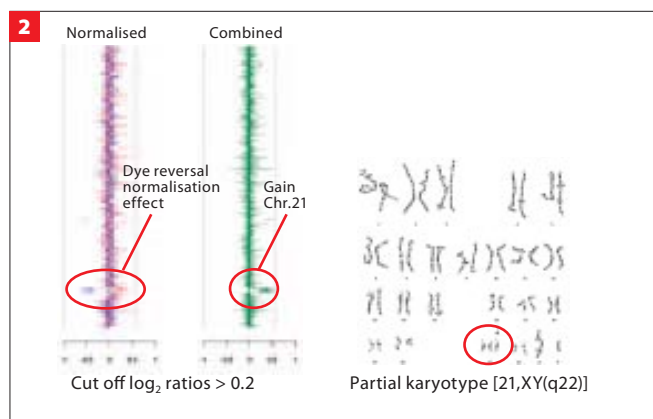
Analysis DNA was chemically modified and attached to an unmodified glass surface to produce arrays.<sup>1</sup> This method has been validated as a simultaneous screening method for DNA copy number changes in chromosomal regions associated with 41 well-established genomic disorders.<sup>4</sup> Genomic DNA from Down's and Edward's patients and healthy individuals were isolated from peripheral blood using PureGene® DNA-Purification Kit (Gentra Systems, Minneapolis, MN) and used in whole genome amplification with Sigma's WGA Kit. DNA from patient samples and controls were differentially labelled with cyanin-3 (Cy3) and Cy5 (PerkinElmer, Boston, MA) as described in the literature.<sup>1</sup> Labelled DNA was hybridised onto array at 37 °C for 24 hours. Fluorescent signals on the slides were scanned into image files using an Axon microarray scanner (Axon Instruments, Union City, CA).

## Results



**Figure 1: Highly representative whole genome amplification**

Real-time quantitative PCR was performed targeting 79 loci on human genomic DNA and whole genome amplified DNA. Thirty-six of the 79 loci are represented within two-fold when comparing genomic DNA to amplified DNA. The maximum bias for all samples was less than or equal to four-fold.



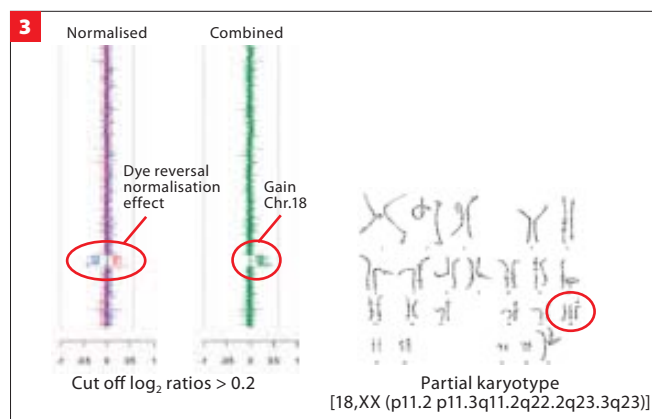
**Figure 2: CGH analysis and karyotype comparison in Trisomy 21 male (Down's syndrome)**

The karyotype shown indicates Trisomy 21. Microarray hybridisation was performed simultaneously using patient DNA and a DNA sample from a healthy individual as reference DNA. The effect of normalisation is shown by comparing data marked "normalised" with the "raw" data (not shown). The normalised data shows a number of clones from chromosome 21 that were displaced to the left in blue and to the right in the dye reversal, both indicating a gain of chromosomal material in the patient versus the female reference DNA. In the "combined" column, the sign of one of the two reversed hybridisations is changed and the data are averaged with gains to the right and losses to the left. For the combined data of WGA DNA, there is a strong indication of gain of 14 clones corresponding to the 21q22 region specified in **Table 1**. The circled regions show the increase in the total genomic data and indicate the presence of an extra chromosome 21.

## Discussion

To prove unbiased amplification using GenomePlex® Whole Genome Amplification Kit, 79 primers were selected across a spectrum of loci in the human chromosome. Quantitative PCR was performed using these primers to amplify human genomic DNA before and after GenomePlex® amplification. The cycle number from the qPCR amplification with template prior to WGA amplification was normalised to one, and the bias for the cycle numbers from the DNA template after WGA treatment was calculated for three replicates. The data demonstrates that all 79 primers were successfully amplified after WGA without any drop-outs, indicating that GenomePlex™ amplifies the whole genome without any bias.

To perform the CGH assay, probe samples were prepared using 250 ng of control DNA and the same amount of patient DNA combined together. Two microarray slides were run for each trisomy case, one with unamplified genomic DNA and another with whole genome amplified DNA. Only 10 ng of starting material, or 4% of the normal amount, was used for whole genome amplification.<sup>2</sup> Each pair of patient and control DNA was labelled twice with the dyes reversed, hybridised to the array, and analysed as previously described.<sup>2</sup> The threshold for the designation of over- or under-representation in these experiments was determined to be 0.2 and -0.2 respectively.<sup>4</sup>



**Figure 3: CGH analysis and karyotype comparison in Trisomy 18 female (Edward's syndrome)**

The karyotype shown indicates Trisomy 18 in an Edward's syndrome female DNA sample. The hybridisations and normalisation of data were performed in the same manner as indicated in **Figure 2**. Unamplified genomic DNA showed a gain in 30 clone locations of 33 possible test clones, while whole genome amplified DNA showed a gain in 27 clone locations of 33 possible clones. The combined data represents a strong indication of gain in the 18 p11.2p11.3q11.2q22.2q23.3q23 regions as indicated in **Table 2**. The circled regions show the increase in the total genomic data and indicate the presence of an extra chromosome 18.

The data (**Figure 2**) shows that CGH microarray analysis of the Down's syndrome patient is consistent with a genomic gain as detected by 15 target clones in the array for unamplified gDNA and the whole genome amplified DNA, reflecting the presence of a third chromosome corresponding to region 21q22. Furthermore, the array for Edward's syndrome (**Figure 3**) showed a genomic gain in 30 of 33 possible test clones for the unamplified genomic DNA sample and a gain of 27 target clones from 33 in the whole genome amplified DNA sample in 18p11.2 p11.3q11.2q22.2q23.3q23 regions. This represents strong indication of trisomy 18. The CGH array analysis was accurate in detecting clones displaying a gain for each patient tested and confirms that the GenomePlex® Whole Genome amplification method provides a sufficient amount of DNA without any bias.

## Tables of results listing signal quantities for loci showing major differences

Signal values for critical diagnostic clones are listed in the tables below. The threshold for designation of over-representation in these experiments was 0.2.

Clone	Location	Genomic combined	WGA combined
RP11-625C23	21:q11.2	0.251	0.278
RP11-840D8	21:q21.1	0.26	0.367
RP11-143A3	21:q21	0.276	0.258
RP11-108H5	21:q21	0.384	0.312
RP11-17O20	21:q22.1	0.403	0.393
RP11-166F15	21:q22.1	0.36	0.352
RP11-401I23	21:q22.1	0.497	0.356
RP11-35C4	21:q22.3	0.364	0.287
RP11-92D3	21:q22.3	0.259	0.29
RP11-88N2	21:q22.3	0.352	0.34
RP11-190A24	21:q22.3	0.353	0.36
RP11-40L10	21:q22.3	0.31	0.189
RP11-16B19	21:q22.3	0.369	0.276
RP11-640F21	21:q22.3	0.358	0.273
GS-63H24	21:qter	0.31	0.229

**Table 1:** Shows quantified data for trisomy 21, where a copy number gain was detected in all possible clones for the unamplified genomic DNA and 14 of 15 clones were detected for the WGA product. All clones were detected in the expected 21q22 region, ranging from 0.251 to 0.497.

Clone	Location	Genomic combined	WGA combined
RP11-70501	18:p11.3	0.191	0.197
RP11-14P20	18:p11.3	0.224	0.243
RP11-607C2	18:p11.3	0.29	0.213
RP11-78H1	18:p11.3	0.294	0.243
RP11-55N14	18:p11.3	0.193	0.174
RP11-193E15	18:p11.3	0.239	0.217
RP11-838N2	18:p11.3	0.208	0.188
RP11-874J12	18:p11.3	0.271	0.15
RP11-183C12	18:p11.3	0.33	0.153
RP11-105C15	18:p11.3	0.339	0.356
RP11-781P6	18:p11.3	0.308	0.257
RP11-931H21	18:p11.2	0.244	0.231
RP11-772F18	18:p11.2	0.213	0.21
RP11-752I5	18:p11.2	0.38	0.272
RP11-807E13	18:p11.2	0.305	0.235
RP11-411B10	18:p11.2	0.203	0.201
RP11-380C8	18:q11.1	0.168	0.169
RP11-459H24	18:p11.2	0.219	0.163
RP11-758N17	18:p11.2	0.336	0.245
RP11-540M4	18:p11.2	0.326	0.256
RP11-90G7	18:p11.2	0.33	0.221
RP11-704G7	18:q22.2	0.246	0.191
RP11-47G4	18:q22.3	0.306	0.204
RP11-669I1	18:q22.3	0.299	0.214
RP11-27C7	18:q22.3	0.373	0.26
RP11-357H3	18:q23	0.402	0.345
RP11-162A12	18:q23	0.2	0.199
RP11-90L3	18:q23	0.311	0.31
RP11-451L19	18:q23	0.354	0.342
RP11-91C19	18:q23	0.322	0.219
RP11-154H12	18:q23	0.325	0.427
GS-964M9	18:qter	0.23	0.241
RP11-89N1	18:q23	0.298	0.34

**Table 2:** Shows quantified data for trisomy 18, where a copy number gain was detected in 30 of 33 possible clones for the unamplified genomic DNA, while whole genome amplified DNA showed a gain in 27 of 36 possible clones. Clones were detected in expected regions, ranging from 0.2 to 0.427 compared to the selected gain threshold of 0.2.

## Conclusions

The data presented proves that whole genome amplification using the GenomePlex® Whole Genome Amplification Kit in conjunction with Comparative Genomic Hybridisation array allows for the identification of genome-wide copy number and chromosomal abnormalities amplifying limited DNA without bias.

The results verify that the GenomePlex® WGA Kit is able to amplify DNA across >800 loci without detectable bias. When using DNA amplified with the GenomePlex® technology, four to five CGH arrays were run using a total of 10 ng genomic DNA isolated from the patients. Typical CGH arrays require 500 ng of genomic DNA for a single array. Using the GenomePlex® WGA Kit eliminates the need for obtaining a significant quantity of patient samples for CGH and other assay methodologies.

## Acknowledgements

The authors would like to thank Dr. Sau W. Cheung for providing Trisomy 21 and Trisomy 18 DNA samples along with normal control DNA for whole genome amplification; Dr. Xinyan Lu for performing chromosomal microarray analysis; and Steve D. Bland for his illustrations. We also would like to thank Dr. Arthur Beaudet for facilitating communication with the microarray facilities in the Department of Molecular and Human Genetics at Baylor College of Medicine, Houston, TX.

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# Vibrio detection

By Jvo Siegrist, Product Manager Microbiology, ivo.siegrist@sial.com

▣ ***Vibrio cholerae* causes cholera in humans, *Vibrio parahaemolyticus* and *Vibrio vulnificus* are the leading cause of seafood-associated gastroenteritis.**

Vibrios are motile, curved or comma-shaped bacilli and have a single polar flagella with sheet proteins. They are often found in open water, freshwater and saltwater. Vibrios are facultative aerobe and Gram-negative bacterium and do not form spores. The metabolism can be oxidative and fermentative. Most species are oxidase-positive, except *V. metschnikovii*. Some vibrios such as *Vibrio fischeri* exhibit bioluminescence (Quorum sensing) under certain conditions. In most ways, vibrios are close to Enterobacteriaceae, but also share some properties with pseudomonads. They can be differentiated from enteric bacteria by oxidase-positive reaction and motility. Differentiation from *Pseudomonas* can be made based on the ability of vibrios to undergo oxidative and fermentative metabolism.

Most vibrios are not fastidious and a simple C-source like glucose serves as an energy source. As it is a typically marine organism, most species require 2–4% NaCl or other salts and trace elements present in sea water for optimal growth. Some species are like *Pseudomonas* and can use diverse energy sources, and show great versatility in their metabolism.

The widely used media for *Vibrio* isolation are TCBS Agar and Alkaline Peptone Water. However, accompanying sucrose-fermenting bacteria may pose a problem in the identification of *Vibrio* species on TCBS Agar.<sup>3</sup> The TCBS Agar contains a mixed indicator of bromothymol blue and thymol. This system reacts upon acid production from sucrose fermentation. On a chromogenic medium like HiCrome™ *Vibrio* Agar (see **Table 2**), the colour development by *Vibrio* species is not affected by the presence of colonies of other bacteria. This is because the amount of colour developed depends on the reaction of the bacterial  $\beta$ -galactosidase with the substrate contained in the media. The TCBS Agar also contains a sodium thiosulphate and ferric citrate indicator system which detects the production of hydrogen sulphide.

Pepton from animal origin provides carbonaceous, nitrogenous and essential nutrients to the *Vibrio* species to promote growth. High concentrations of sodium chloride in the medium are used to get an inhibitory effect on the accompanying microflora. Sodium thiosulphate, sodium citrate and sodium cholate are used as well to inhibit the growth of gram-positive and some gram-negative bacteria, but not members of Enterobacteriaceae. The strongly alkaline pH of the medium is also an important tool to get selectivity for *Vibrio* species.



Kingdom:	Bacteria	Order:	Vibrionales
Phylum:	Proteobacteria	Family:	Vibrionaceae
Class:	Gamma Proteobacteria	Genus:	<b><i>Vibrio</i></b>

**Figure 1:** *Vibrio vulnificus* is the cause of seafood-related mortality. Scientists from Northwest Fisheries Science Center have isolated and characterised a key surface protein involved in the ability of *Vibrio vulnificus* to attach to shellfish, such as oysters (microscopic image from Northwest Fisheries Science Center, Seattle, USA).

*V. cholerae* is a non-invasive bacteria, affecting the small intestine by producing the cholera enterotoxins. The result is a life-threatening watery diarrhea because of activation of the adenylate cyclase in the intestinal cells. This reaction causes water and electrolytes from blood and tissues to be pumped into the intestinal tract. The rapid loss of fluids leads to dehydration, anuria, acidosis and shock. An additional loss of potassium ions may result in cardiac complications and circulatory failure. The mortality rate is very high (50–60%) if the disease is not treated. Infection source is the water or food contaminated with human faeces.

*V. parahaemolyticus* causes gastroenteritis. It is an invasive organism affecting primarily the colon tissue, and excretes a presently unidentified toxin. The origin of an infection leads in most cases back to contaminated raw and improper refrigerated seafood or a faecal contamination of water and food.

*V. vulnificus* lives in warm seawater and is halophile, meaning it requires salt for growth. Contaminated seafood which is eaten raw or is undercooked is in most cases the source of infections and causes gastroenteritis, or a syndrome known as “primary septicemia”. Also, open wounds that are exposed to seawater can lead to a wound infection.



**Figure 2:** HiCrome™ Vibrio Agar, a selective Agar with chromogenic system as differential system

	<i>V. cholerae</i>	<i>V. parahaemolyticus</i>	<i>V. vulnificus</i>
Growth in nutrient broth without NaCl	+	-	-
Growth in nutrient broth with 1% NaCl	+	+	+
Oxidase	+	+	+
Nitrate reduction	+	+	+
myo-Inositol fermentation	-	-	-
Arginine dihydrolase	-	-	-
Lysine decarboxylase	+	+	+
Ornithine decarboxylase	+	+	V
ONPG	+	+	+

**Table 1:** Typical biochemical reactions

	Brand	Cat. no.	Name	ISO
Nonselective media	Fluka	43856	Alkaline Peptone Water (ISO)	8914
	Fluka	77185	Peptone Water	
	Fluka	T2117	Thiol broth	
Nonselective media w/differential system	Fluka	62895	Lysine Decarboxylase Salt Broth	8914
	Fluka	75315	OF Test Nutrient Agar	
	Fluka	22091	Tryptic Soy Agar with supplement: TTC solution	
Selective media	Fluka	49281	Glucose Salt Teepol Broth	
Selective media w/differential system	Fluka	17134	CPC-Agar (Base)	8914
	Fluka	70135	DCLS Agar	
	Fluka	90035	DCLS Agar No. 2	
	Fluka	92323	HiCrome™ Vibrio Agar	
	Fluka	86348	TCBS Agar	

**Table 2:** Media for enrichment, detection and differentiation of *Vibrio* species

Brand	Cat. no.	Name
Fluka	P9602	Polymyxin B Selective Supplement
Fluka	17779	TTC Solution

**Table 3:** Supplements for *Vibrio* media

Brand	Cat. no.	Name
Fluka	1850	Oxidase Reagent acc. Gordon-McLeod
Fluka	40560	Oxidase Strips
Fluka	70439	Oxidase Test
Fluka	07345	Oxidase Reagent acc. Gaby-Hadley A
Fluka	07817	Oxidase Reagent acc. Gaby-Hadley B
Fluka	49940	ONPG Disks
Fluka	51138	Nitrate Reagent Disks Kit
Fluka	38497	Nitrate Reagent A
Fluka	39441	Nitrate Reagent B
Fluka	77730	Gram Staining Kit

**Table 4:** Biochemical products and kits for *Vibrio* identification and differentiation

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