Product Code: 0407

OSTEO FG Box 1.0

Typing Kit

In vitro diagnostics disposal

Instructions Manual

geneBOX

DESENVOLVIMENTO E PRODUÇÃO
DE TESTES DE DIAGNÓSTICO

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DE TESTES DE DIAGNÓSTICO

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Presentation

This kit contains typing plates with dried primers mixes and PCR Master Mix for typing some polymorphisms of the following genes: COL1A1, CTR, ESR1 and VDRB.

Product Changes and Improvements

The OSTEO Box specificity and interpretation tables are constantly updated. This product can also be improved in order to increase the yield of the specific PCR product.

The primers exchanged, added or modified, compared to the previous lot, are detailed in the table below.

Tube	primers	Motivation
N/A		

Quality Control

The specificity of each primer solution of the kit has been tested using DNA samples from Genebox. Positive and negative results were obtained for each mutation. The OSTEO Box 1.0 Typing kit has the Genebox quality warranty.

OSTEO Box 1.0 Typing Kit Components

- **OSTEO typing plate**[+] (72 typings)

  12 plates (6 sample each) (Keep at -30º/-15ºC)

- **PCR Master Mix (With Taq DNA Polymerase)**

  12 X 310 µl (keep at -15 / -30ºC)

- **Plate sealers**

  1 PCR plate sealer

- **Instructions Manual**

  1 Instructions Manual

  [+] With dried specific primers pares.

PCR Master Mix Components

Nucleotides
Final concentration of each dNTP: 600 µM

PCR Buffer
Final concentration: 3,3x NH_4 Buffer; 2,0 mM $MgCl_2$ and 0,4 U/µl Taq DNA polymerase, pH 8.3.

Glycerol
Final concentration: 16,6%

Cresol Red
Final concentration: 300µg/ml

PCR amplification protocol

Reagents

- DNA Sample (100-200 ng/µl)
- PCR Master Mix
- $_{dd}H_2O$ (not supplied)

DNA Extraction

For SSP typing highly pure DNA is needed. We recommend isolation of DNA using any extraction kit with CE marking, which guarantees an OD ratio 260/280 higher than 1.6 and a 100ng – 200 ng/µl DNA concentration.
Alternatively, the DNA can be extracted using trimethyammoium-bromide salts (DTAB/CTAB) or by salting out, dissolving it in TE Buffer. The same OD and concentration values should be assured.
DO NOT USE HEPARINISED BLOOD WITH THIS METHOD

PCR Amplification

1. Spin briefly the DNA and Master Mix tubes.
2. Add:
   - **51 µl of PCR Master Mix,**
   - **106 µl of ddH_2O, and**

   to a 0,7 ml or 1,5 ml tube.
3. Vortex the tube vigorously for 15s.
4. Load **10 µl** of the mix into the negative well.
5. Add **14 µl of DNA sample (conc. 100-200 ng/µl)** into the PCR mix tube**.**
6. Vortex the tube vigorously for 15s.
7. Load **10 µl** of the PCR mix in each well of the plate (**14 primer pairs and a positive control**).
8. Repeat the previous steps to other DNA samples in order to complete the typing plate (6 samples each).

9. Close the typing plate with a self-adhesive lid and put it in a 96 well thermo cycler.

PCR Cycling Parameters

Step	Temperature	time	Cycle
Denaturation	96 ºC	1 min	1
Denaturation Annealing Extension	96 ºC 70 ºC 72 ºC	25 sec 45 sec 30 sec	5
Denaturation Annealing Extension	96 ºC 65 ºC 72 ºC	25 sec 45 sec 30 sec	21
Denaturation Annealing Extension	96 ºC 55 ºC 72 ºC	25 sec 1 min 2 min	4
Extension	72 ºC	10 min	1
Keep (optional)	4 ºC	Infinite	1

10. Keep the plate at 2-8 ºC after the PCR have finished.
11. Detect the PCR products with 2% agarose gel electrophoresis.

Gel Electrophoresis protocol

PREPARING 2% AGAROSE GEL

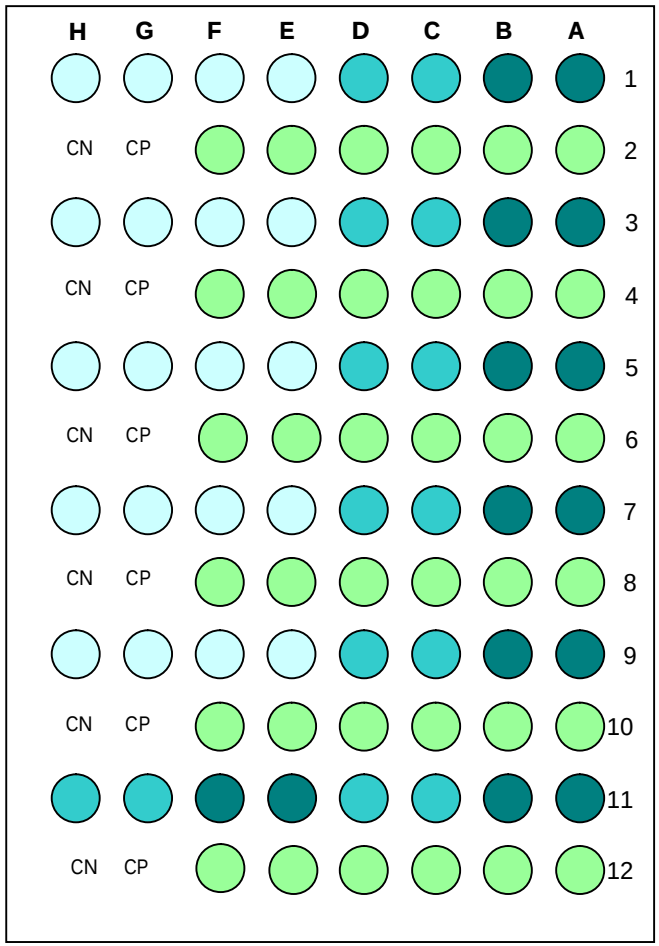
1. Dissolve **4 grams** of electrophoresis **grade agarose** powder in **200 ml** of **1X TAE buffer**.
2. Melt the agarose powder completely in a microwave oven.
3. Cool the heated agarose gel to **50°C.**
4. Add at least 10 µl of **ethidium bromide**[++] (10 mg/ml) or **Sybr Safe**[TM] (100000 x concentrate) to the heated agarose. Stir until it is thoroughly incorporated.
5. On a balanced surface, set up a gel plate with **96 wells**.
6. Cast a **5mm** thick gel on the plate.
7. Allow the gel to settle.

[++]**Caution**, this reagent is a strong mutagenic agent (read carefully its MSDS before using it).

GEL ELECTROPHORESIS

1. Submerge the gel in 1X TAE buffer in a gel box.
2. Gently remove the caps to avoid splashing of PCR products.
3. Load 10 µl into each well on the gel.
4. Connect the electric leads and turn on the power supply (115V). Electrophoresis for ~ 20 minutes, or until 2/3 of the lane.
5. Transfer the gel onto a UV transilluminator, document the result by photography.
6. Use the ***result interpretation sheet*** (1-4) to interpret results.

OSTEO Box 1.0 plate



OSTEO Box 1.0 plate identification

Position (sample 1)	Position (Sample 2)	Position (Sample 3)	Position (Sample 4)	Position (Sample 5)	Position (Sample 6)	Gene	Polymorphic site
1a	3a	5a	7a	9a	11a	COL1A1	G2046T
1b	3b	5b	7b	9b	11b	COL1A1	G2046T
1c	3c	5c	7c	9c	11c	CTR	Pro463Leu
1d	3d	5d	7d	9d	11d	CTR	Pro463Leu
1e	3e	5e	7e	9e	11e	ESR1	IVS-397 T>C (PvuII)
1f	3f	5f	7f	9f	11f	ESR1	IVS-397 T>C (PvuII)
1g	3g	5g	7g	9g	11g	ESR1	IVS-351A>G (XbaI)
1h	3h	5h	7h	9h	11h	ESR1	IVS-351A>G (XbaI)
2a	4a	6a	8a	10a	12a	VDR	I352I (TaqI)
2b	4b	6b	8b	10b	12b	VDR	I352I (TaqI)
2c	4c	6c	8c	10c	12c	VDR	M1T (FokI)
2d	4d	6d	8d	10d	12d	VDR	M1T (FokI)
2e	4e	6e	8e	10e	12e	VDR	IVS10 +354 G>A (BsmI)
2f	4f	6f	8f	10f	12f	VDR	IVS10 +354 G>A (BsmI)
2g	4g	6g	8g	10g	12g	CP	-----
2h	4h	6h	8h	10h	12h	CN	-----

Results Interpretation sheet (1/3)

Mix						Gene	Polymorphism	Allele	Specific Band	Control Band **
1a	3a	5a	7a	9a	11a	COL1A1	G2046T	G	80	790
1b	3b	5b	7b	9b	11b	COL1A1	G2046T	T	80	790
1c	3c	5c	7c	9c	11c	CTR	Pro463Leu	Pro (C)	142	790
1d	3d	5d	7d	9d	11d	CTR	Pro463Leu	Leu (T)	142	790
1e	3e	5e	7e	9e	11e	ESR1	IVS-397 (PvuII)	C	203	790
1f	3f	5f	7f	9f	11f	ESR1	IVS-397 (PvuII)	T	203	790
1g	3g	5g	7g	9g	11g	ESR1	IVS-351 (XbaI)	A	157	790
1h	3h	5h	7h	9h	11h	ESR1	IVS-351 (XbaI)	G	157	790
2a	4a	6a	8a	10a	12a	VDR	I352I (TaqI)	T	224	790
2b	4b	6b	8b	10b	12b	VDR	I352I (TaqI)	C	224	790
2c	4c	6c	8c	10c	12c	VDR	M1T (FokI)	C	163	790
2d	4d	6d	8d	10d	12d	VDR	M1T (FokI)	T	163	790
2e	4e	6e	8e	10e	12e	VDR	IVS10 +354 G>A (BsmI)	A	219	790
2f	4f	6f	8f	10f	12f	VDR	IVS10 +354 G>A (BsmI)	G	219	790
2g	4g	6g	8g	10g	12g	Controlo positivo				790
2h	4h	6h	8h	10h	12h	Controlo negativo				---
DNA 1	DNA 2	DNA 3	DNA 4	DNA 5	DNA 6					

****Control primer pairs match with non-allelic sequences. The internal positive control primer pairs amplify segments of the gene PIC1, giving rise to 790 base pair fragments.**

In the presence of the specific band amplification the control band intensity often decreases.

The PCR reaction is only valid in the presence of control band or, in some cases, in the presence of the specific band. In the absence of the control band, please repeat the typing.

If the PCR reaction results in fragments different from the specific or con troll band please do not consider, they are unspecific fragments.

Troubleshooting Guide

PROBLEMS	POSSIBLE CAUSES	SUGGESTIONS
The control and specific bands are weak.	Concentration of DNA sample is too low.	Check DNA quality and concentration
		Re-extract the DNA sample or try not add water into the PCR Mix
		Repeat typing with a good quality DNA sample
	DNA polymerase inhibitors in the DNA sample	Re-purify the sample DNA
		Repeat typing with a good quality DNA sample
Missing internal control bands in one or several lanes.	DNA polymerase inhibitors in the DNA sample.	Re-purify the sample DNA
		Repeat typing with a good quality DNA sample
	Dried PCR amplification products	Check the plate sealing
		Repeat the typing using a PCR MicroMat and/or overlay the PCR reaction mix with mineral oil
False negative of a specific band while the internal control appears normal	Degradation of DNA sample	Re-extract the DNA sample with fresh material
		Repeat typing with a good quality DNA sample
More than two specific alleles are detected/ Ambiguous results	Excess of template DNA	Check DNA quality and concentration
		Dissolve the DNA sample in $_{dd}$H2O in order to have the proper concentration
		Repeat typing with a good quality DNA sample
	Contamination with previously amplified PCR products or with other DNA samples during the DNA extraction or PCR preparation steps	Clean the working area
		Work in separated pre-PCR and post-PCR rooms
		Keep different lab coats in pre-PCR and post-PCR rooms
		Change protective gloves frequently
		Repeat typing with a good quality DNA sample
Blurred bands	Degradation of DNA sample	Re-extract the DNA sample with fresh material
		Repeat typing with a good quality DNA sample
	Excess of template DNA	Check DNA quality and concentration
		Dissolve the DNA sample in $_{dd}$H2O in order to have the proper concentration
		Repeat typing with a good quality DNA sample
	Electrophoresis Buffer Problems: wrong buffer or older buffer	Use a fresh recommended buffer

Precautions and Warnings

PCR amplification allows the amplification of small quantities of sample DNA in an exponential way. However, this is also true for foreign DNA, which can contaminate our PCR method. Consequently, special laboratory practices are necessary in order to avoid false positive amplifications. Bellow is listed Genebox recommendations to circumvent contaminations:

- Work in separated pre-PCR and post-PCR rooms.
- Laboratory workflow must be unidirectional, from pre-PCR to post-PCR area.
- Specific equipment for each working area must be used (sample preparation, amplification and preamplification).
- All equipment used in post-PCR should not leave this area.
- Use dedicated micropipettes, gloves and lab coats in each area.
- Use non talcum powder gloves (since talcum could inhibit the PCR reaction).
- Use filter tips in order to avoid cross contamination.
- Check regularly micropipettes, in order to ensure that they are accurate within 5 % of fixed volume.
- Use different micropipettes depending on the volume we wish to load.
- Check regularly thermocyclers, in order to ensure that they are accurate within 1% of fixed temperature.
- Open and close reagent vials carefully. After use, close vials and store at indicated temperatures.
- Do not use a kit after its expiration date.
- packaging material included within the kit is resistant to the indicated storage conditions. Storage at different conditions can cause breakage of the material, and possible contamination of the kit reagents.
- plastic material included within the kit is resistant under normal conditions of use. Use of plastic material in extreme conditions may cause its breakage, and therefore, the impossibility to use the kit.
- check suitability of DNA quantity and quality before use the kit.

General instructions for laboratory safety:

- do not eat, drink or smoke in laboratory work areas
- wear disposable gloves
- wear clean lab coats and eye protection
- wash hands thoroughly after handling specimens and test reagents
- clean the working area before and after kit handling
- Do not pipette with the mouth

Technical Guide

1. DNA Quality and Concentration

For optimal results with the OSTEO Box 1.0 Typing Kit™ the quality of DNA is critical. Good quality DNA means an OD ratio 260/280 higher than 1.6 and the major portion of DNA should run higher than 9.4 kb on an agarose gel. Different quality and concentration values require DNA re-extraction.
The quantity of DNA should be 100ng – 200 ng/µl. Excess of DNA can cause unspecific amplification.
We recommend any DNA extraction kit which has CE marking, in order to obtain this highly DNA purity

2. Taq Polymerase

OSTEO Box 1.0 Typing Kit™ kits have been intensively tested with the Taq DNA Reagente 5 (Reagente 5, Lisboa, Portugal).

3. PCR Master Mix

For optimal results with the OSTEO Box 1.0 Typing Kit™ the use of master mix supplied is obligatory.

4. Amplification Procedure

At the end of PCR, examine the degree of evaporation and condensation of PCR reaction mixture. If there is more than 20% volume loss do not validate the results. In order to prevent this you should overlay the PCR reaction mixture with mineral oil or use a MicroMat. It is also a good practice to maintain QC records on the heating lid.
If the temperature of the heating lid is not high enough, it will cause condensation problems on the lid.

5. Thermal Cycler

We recommend the use of any thermocycler with the following characteristics:
- heating rate up to 2.5ºC/sec; cooling rate up to 1.5ºC/sec; temperature range 4-100ºC; temperature uniformity ±0.5ºC; heated lid up to 100ºC.

6. Expiring Date

As specified in the package labels

If your problems persist, do not hesitate to contact our technical support

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Guarantee

geneBOX - R&D Diagnostic Tests guarantees that the primers in OSTEO Box typing kit have the specificities given in the Results Interpretations Sheet/Tables of the product insert.

1. Typing plate

When stored at -20ºC, the dried primers are stable for 12 to 19 months from the date of manufacture (see lot validity in the package).
When stored at 4ºC, the dried primers are stable for 12 months from the date of manufacture (see lot validity in the package).
At room temperature, the dried primers are stable for 3 to 4 weeks from the date of the reception.
When the sealer is removed the dried primers steal be stable for 2 days, maximum, in dried conditions.

2. PCR Master Mix

When stored at -20ºC, the PCR Master Mix is stable for 18 months from the date of manufacture (see lot validity in the package).
When stored at 4ºC, the Master mix is stable for 15 days from the date of the reception.
At room temperature, the master mix steal be stable for 3 days from the date of the reception.
The master mix should not be left or stored with the cap open.

3. DNA

Using extracted DNA from salting out or any kit procedure the samples should be stored at 4ºC or -20ºC. If you chose to freeze the samples you must avoid repeated cycles of heating/freezing, in order to preserve your sample stability.
The DNA samples stored in dH_2O are stable for at least 2 to 4 weeks (at 4ºC) or 24 months (at -20ºC).
The DNA samples stored in buffer are stable for at least 12 months (at 4ºC) or 5 years (at -20ºC).

Warranty

geneBOX - R&D Diagnostic Tests warrants its products to the client against defects in materials and contents under normal application. The company products under this warranty shall be replaced, at no charge, to the damaged client.

This warranty applies only to products that have been handled and stored in accordance with its recommendations/specifications.

The claims must be posted directly to geneBOX in writing and must be accompanied by a copy of the purchaser's invoice.

This product may not be reformulated, repacked or resold in any form without geneBOX - R&D Diagnostic Tests consent.

Declaration of conformity

Product Name: OSTEO Box

Product Number: GB.0407

Intended use: Genotyping of: COL1A1, CTR, ESR1, and VDR genes.

Manufacturer:

geneBOX - R&D Diagnostic Tests,
Biocant – centro de inovação em biotecnologia
núcleo 4, lote 3
3060-197 Cantanhede,
Portugal

We, geneBOX - R&D Diagnostic Tests, hereby declare that this product, to which this declaration of conformity relates, is in conformity with the following standards and other normative documents ISO 9001:2008 and ISO 13485:2003, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices as transposed into the national laws of the Member States of the European Union.

The technical file of the product is maintained at geneBOX - R&D Diagnostic Tests, Biocant Park, Parque tecnológico de Cantanhede, 3060-197 Cantanhede, Portugal.

Sandra Balseiro
Technical Director

Material Safety Data Sheet (MSDS) (1/3)

geneBOX - R&D Diagnostic Tests™ PCR-SSP Kits

geneBOX ™ PCR-SSP typing products

This Material Safety Data Sheet (MSDS) applies to all geneBOX - R&D Diagnostic Tests SSP™ typing kits

1. Chemical products and company identification

Date of Issue:	May 2010
Product group:	geneBOX™ PCR-SSP Typing Products
Manufacturer:	geneBOX - R&D Diagnostic Tests, biocant – centro de inovação em biotecnologia núcleo 4, lote 3 3060-197 cantanhede, portugal
tel/fax:	+351 231 410 946/ +351 231 410 947
e-mail:	info@genebox.com

2. Composition and reagents information

Component	Chemical	Common Name
Plate	Deoxyribonucleic acid	Oligonucleotide
	Cresol Red	
PCR Master Mix	Deoxyribonucleotides	Nucleotides
	NH_4 Buffer	
	Magnesium chloride	MgCl2
	Cresol Red	
	Glycerol	Glycerine

3. Physic-chemical properties:

Components	Appearance	Colour	Odour
Plate	dried, in plate wells	Red	none
Master Mix	liquid	Pink/red	none

4. Toxicological information

Chemical	Toxicities
Glycerol	LD50= oral 4090 mg/kg (mouse) LD50= oral 12600 mg/kg (rat) LD50= oral 1480 mg/kg (human)

5. Stability and reactivity

Conditions to avoid: Heat and moisture.
Incompatibilities: Strong oxidizing agents, strong bases.

6. Personal protection.

Hand protection: Wear appropriate chemically resistant gloves.
Eye protection: Chemical safety goggles are recommended.
Skin protection: Wear laboratory coat.

7. Handling and storage

Handling: Avoid substance contact.
Storage: Protect from light. Store at temperature indicated on package.
Package Damage: reject damaged components.

Material Safety Data Sheet (MSDS) (2/3)

8. Hazards

Master Mix Components: may be harmful by **inhalation, ingestion or skin absorption**. May cause eye and skin irritation. Material is irritating to mucous membranes and upper respiratory tract. **Ingestion** of large amounts can cause stomach pains, vomiting or diarrhoea.

9. First aid measures

In case of eye contact: Immediately flush eyes with large amounts of water for at least 15 minutes. Call a physician.
In case of skin contact: Immediately wash skin with soap and large amounts of water. Wash contaminated clothing before re-use.
In case of ingestion: Wash out mouth with water provided person is conscious. Call a physician if needed.
In case of inhalation: remove to fresh air, if not breathing give artificial respiration. If breathing difficult, give oxygen. Call a physician.

10. Fire fighting measures

Extinguishing media: Water, carbon dioxide, dry chemical powder or appropriate foam.
Extinguishing media NOT to use: None are known.
Special exposure hazards: May emit toxic fumes of carbon dioxide, carbon monoxide, nitrogen, phosphorus, hydrogen chloride, and hydrogen gas under fire conditions.
Special fire-fighting equipment: When large amounts of substances are released work only with self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

11. Accidental release measures

Personal Precautions: Avoid substance contact. No further requirements.
Cleaning Method: Clean up affected area. No further requirements.

12. Ecological information

No data available.

13. Waste disposal information

Waste disposes in accordance with all applicable regulations (the disposals should be incinerated).

14. Transport information

During transportation the temperature could not exceed 25ºC.
Transportation should not exceed 3 days.

15. Other information

The above information is based on our current level of knowledge, but does not purport to be all-inclusive and shall be used only as a guide. *geneBOX - R&D Diagnostic Tests* shall not be held liable for any damage resulting from handling or from contact with the above products.

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