

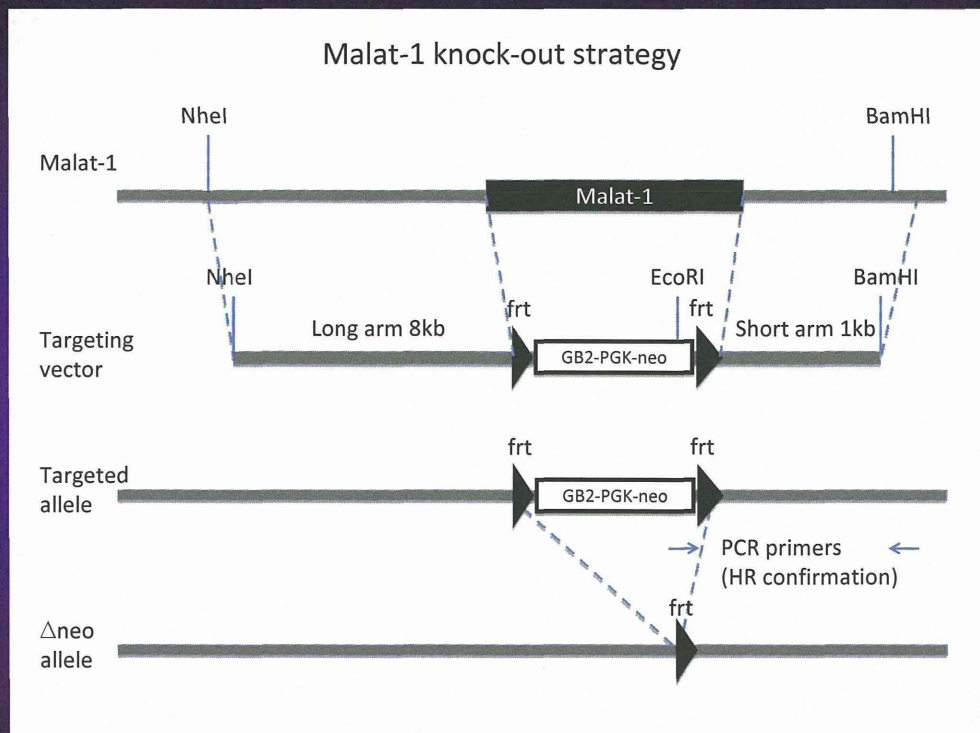
A long nuclear-retained non-coding RNA regulates synaptogenesis by modulating gene expression

Delphine Bernard^{1,5},
Kannanganattu V Prasanth^{2,3,5},
Vidisha Tripathi³, Sabrina Colasse¹,
Tetsuya Nakamura², Zhenyu Xuan²,
Michael Q Zhang², Frédéric Sedel^{1,6},
Laurent Jourden⁴, Fanny Couplier⁴,
Antoine Triller¹, David L Spector^{2,7,*}
and Alain Bessis^{1,7,*}

Introduction

A large portion of the eukaryotic genome is transcribed as non-coding RNAs (ncRNAs) of various sizes ranging from ~20 nucleotides to ~100 kb (reviewed in Mercer *et al*, 2009; Wilusz *et al*, 2009). Despite the increasing number of long ncRNAs (lncRNAs), very few have thus far been assigned a specific function (for review, see Mercer *et al*, 2009). Whether some of these ncRNAs represent transcriptional noise or are involved in important cellular functions remains a matter of

1. Malat-1ノックアウトマウス作製の戦略とターゲティングベクターの構造

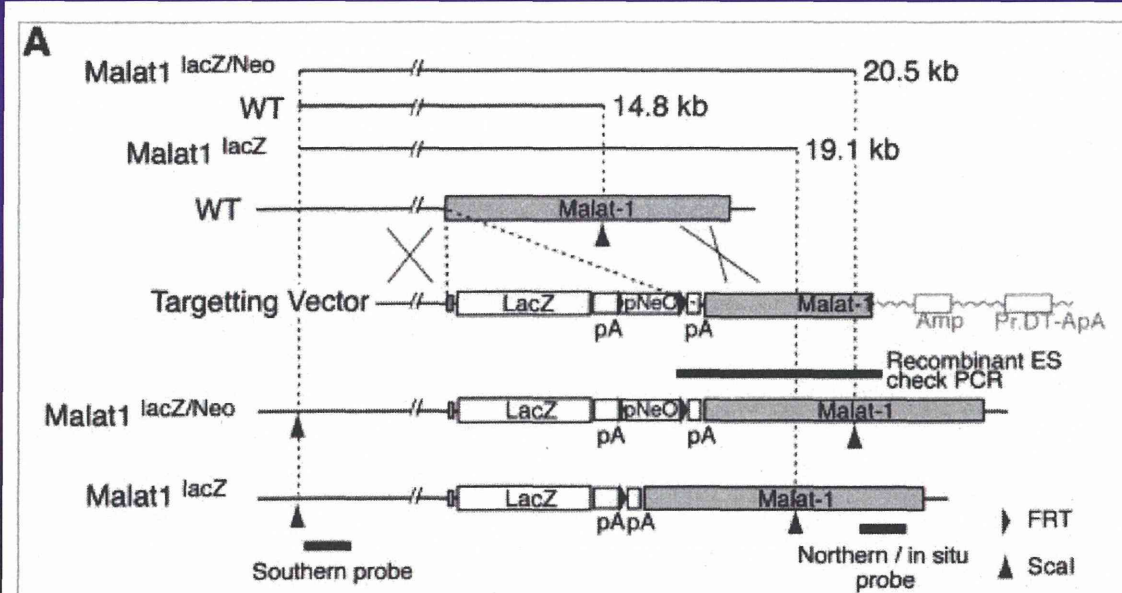


進捗: 132個のESコロニーをピックアップし、1個の組み換えクローンが得られた。

Malat1 is not an essential component of nuclear speckles in mice

SHINICHI NAKAGAWA,^{1,5} JOANNA Y. IP,¹ GO SHIOI,² VIDISHA TRIPATHI,³ XINYING ZONG,³ TETSURO HIROSE,⁴ and KANNANGANATTU V. PRASANTH³

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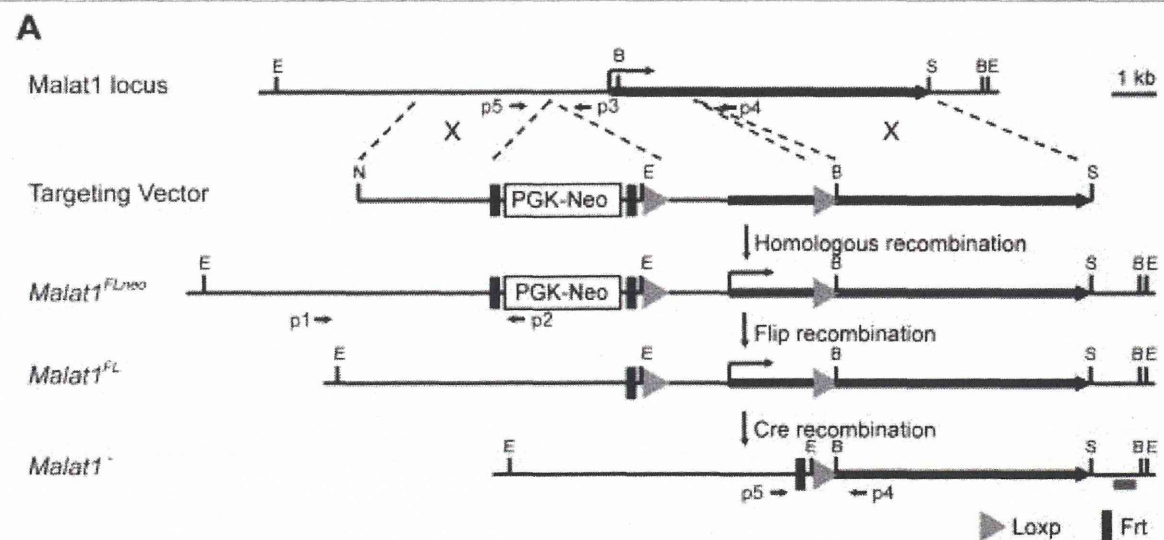
Cell Rep, 2, 111, 2012

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Article



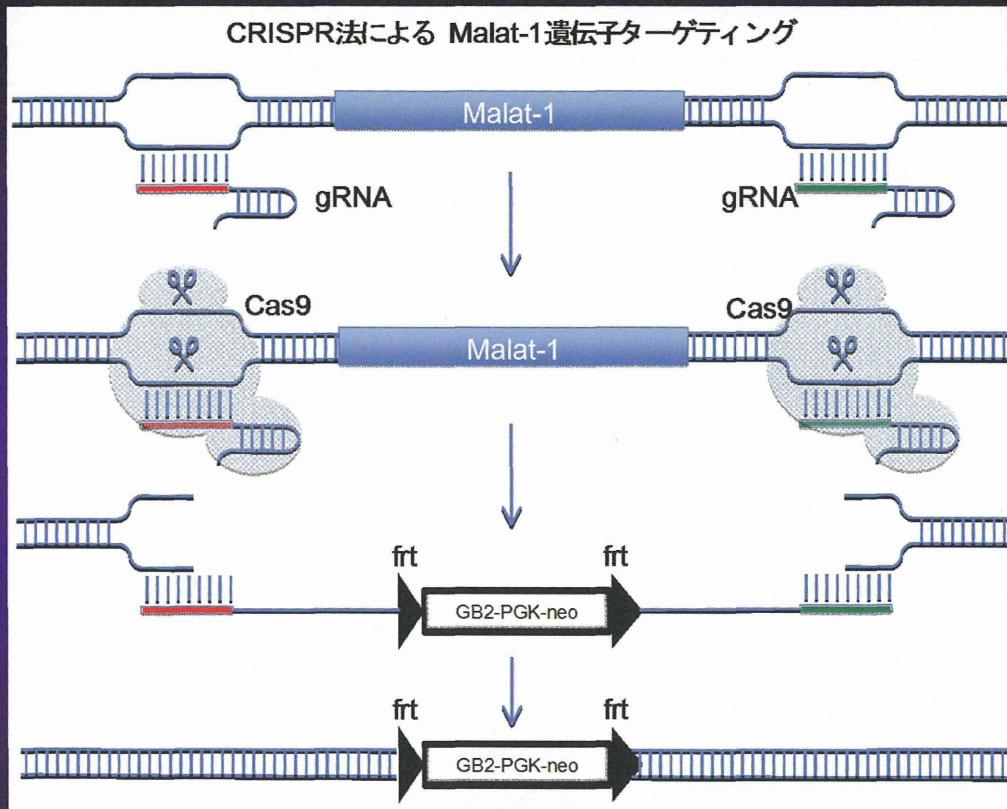
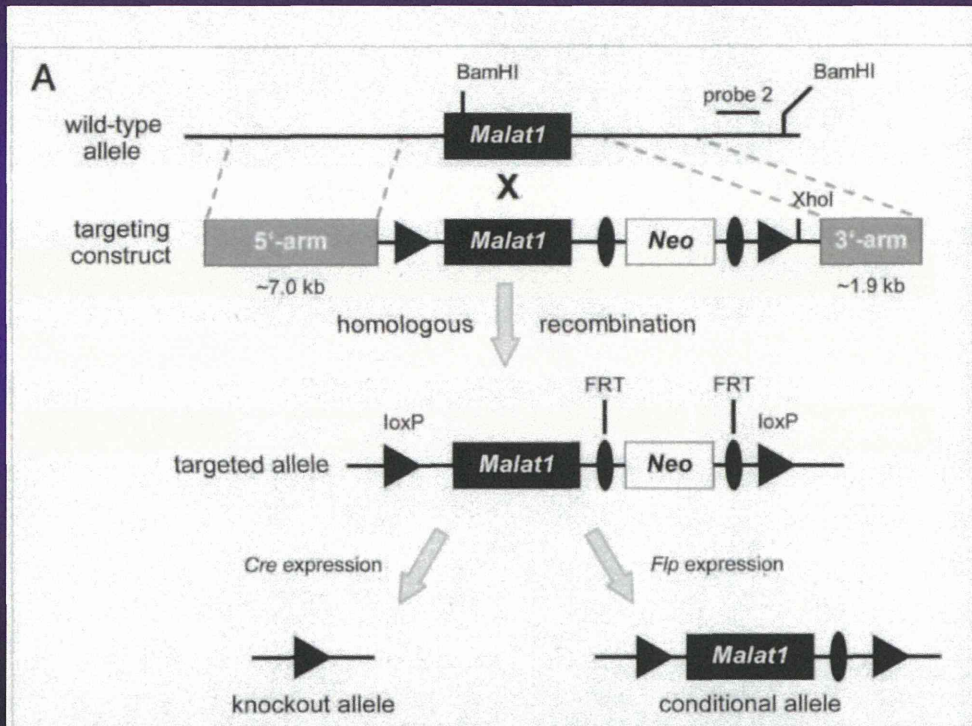
The lncRNA *Malat1* Is Dispensable for Mouse Development but Its Transcription Plays a *cis*-Regulatory Role in the Adult

Bin Zhang,¹ Gayatri Arun,¹ Yuntao S. Mao,^{1,6} Zsolt Lazar,¹ Gene Hung,² Gourab Bhattacharjee,² Xiaokun Xiao,² Carmen J. Booth,³ Jie Wu,^{1,4} Chaolin Zhang,⁵ and David L. Spector^{1,*}



Loss of the abundant nuclear non-coding RNA *MALAT1* is compatible with life and development

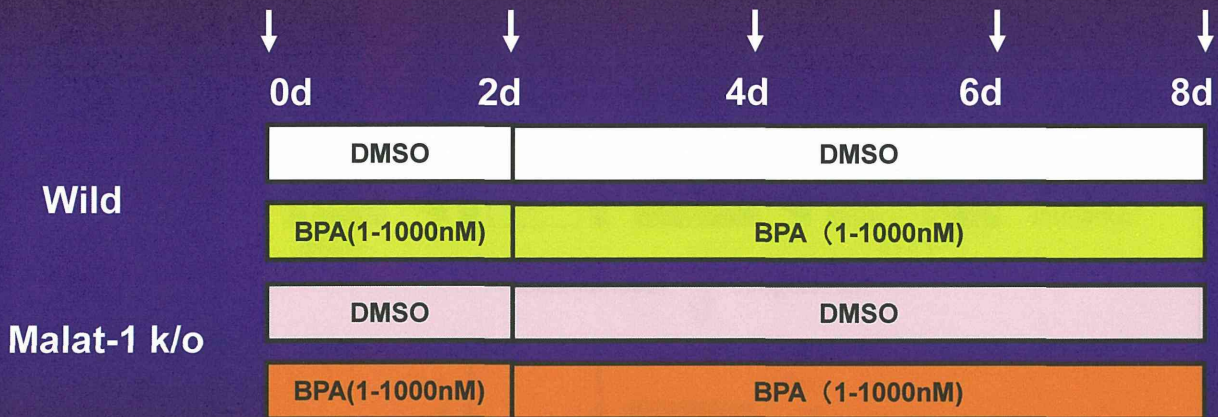
Moritz Eißmann,^{1,2} Tony Gutschner,^{2,1} Monika Hämmerle,^{2,3} Stefan Günther,⁴ Maiwen Caudron-Herger,⁵ Matthias Groß,² Peter Schirmacher,³ Karsten Rippe,⁵ Thomas Braun,⁴ Martin Zörnig^{1,*} and Sven Diederichs^{2,*}



標的DNA結合ガイドRNA (gRNA) とエンドヌクレアーゼ Cas9 により *Malat-1* 遺伝子部分をネオマイシン耐性遺伝子と置換することにより完全に欠損させる。さらに、導入されたネオマイシン耐性遺伝子も最終的に DNA 組み替え酵素にて除去される。

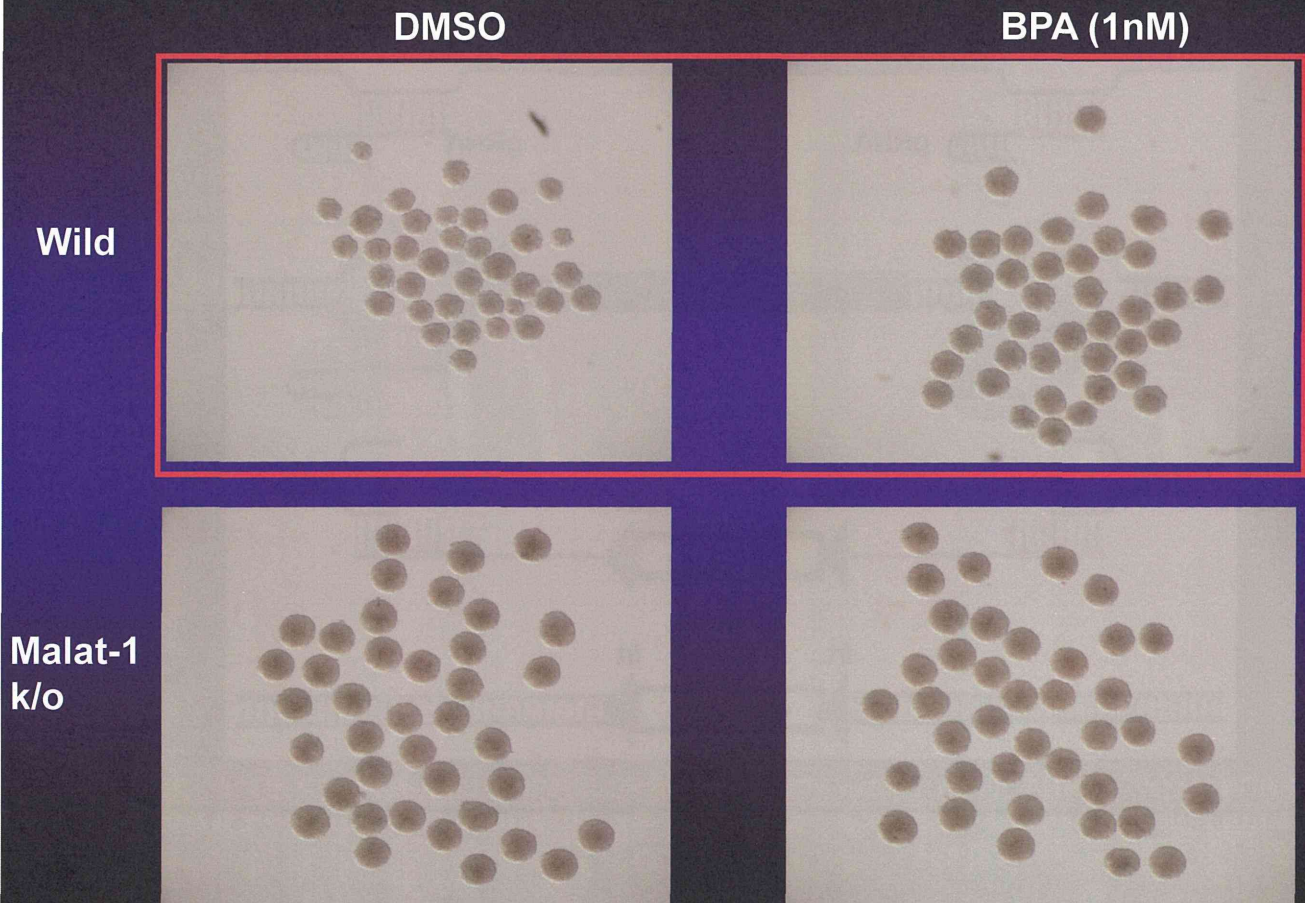
実験プロトコール3

- 細胞 : ES 細胞 (TT2)、Malat-1 k/o ES細胞
- 培養 : 8日間の浮遊培養 (天井培養2日間を含む)



- 培地 : LIF(-)のES細胞培養用培地
- 化合物 : Bisphenol-A (1、10、100、1000nM)
- 溶媒 : DMSO (final 0.1%)
- サンプリング : 2日ごとに8日目までのEmbryoid body (EB)を採取。

EBの実体顕微鏡写真(2d)

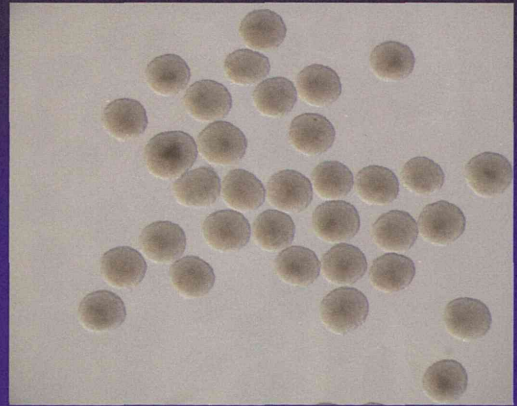
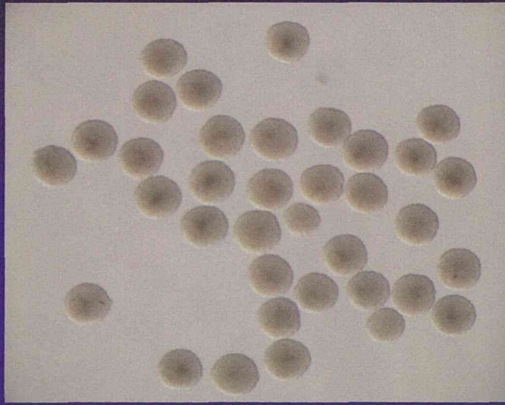


EBの実体顕微鏡写真(4d)

DMSO

BPA (1nM)

Wild



Malat-1
k/o

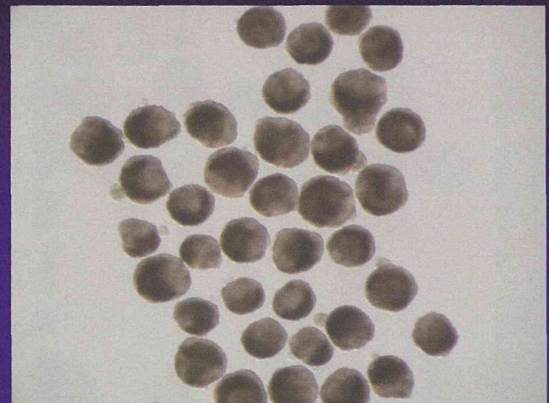
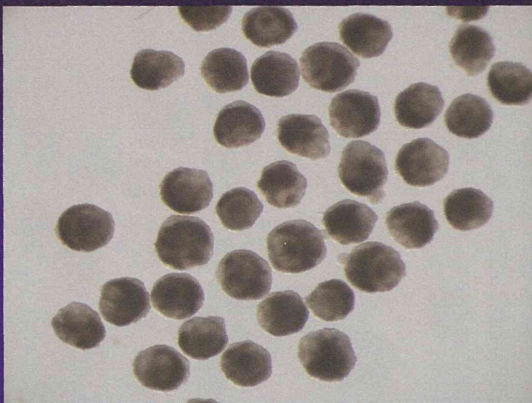


EBの実体顕微鏡写真(6d)

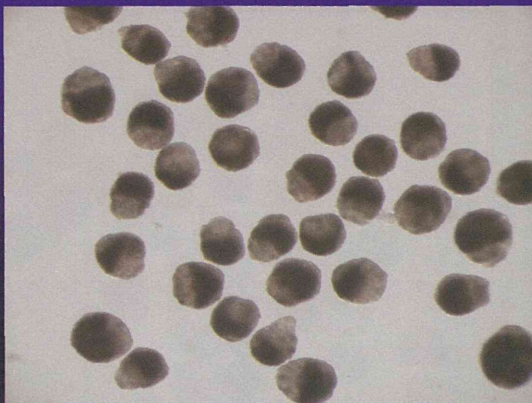
DMSO

BPA (1nM)

Wild



Malat-1
k/o

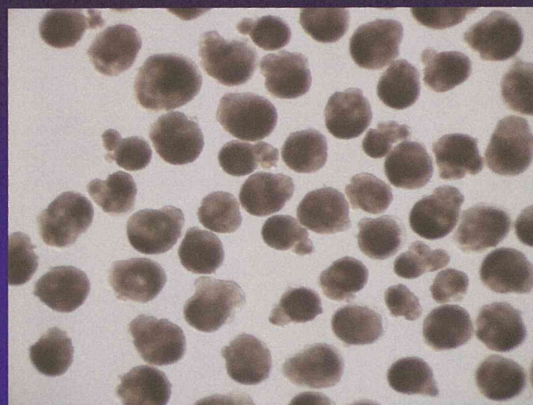
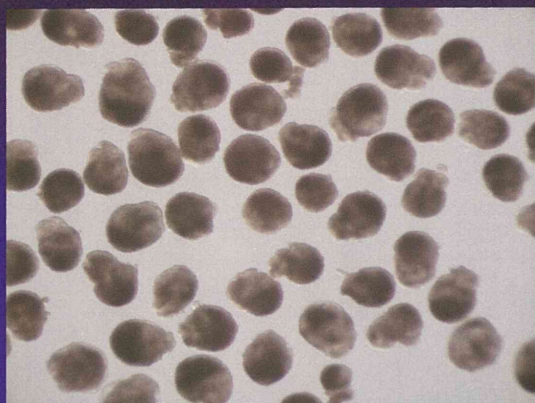


EBの実体顕微鏡写真(8d)

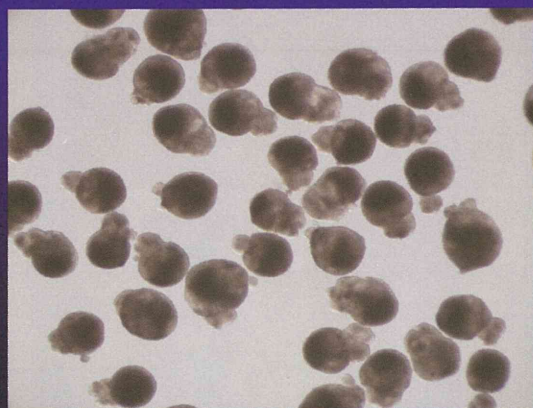
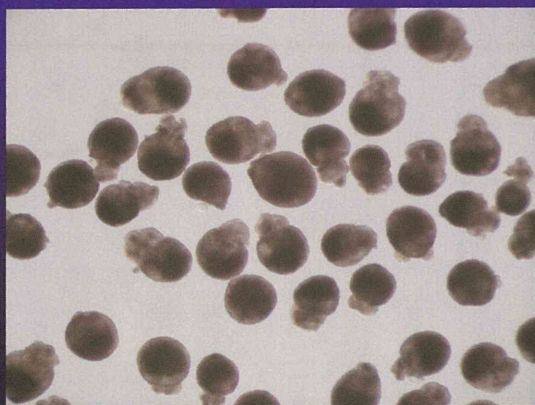
DMSO

BPA (1nM)

Wild



Malat-1
k/o



EBの実体顕微鏡写真(8d)

BPA (10nM)

BPA (100nM)

Wild



Malat-1
k/o

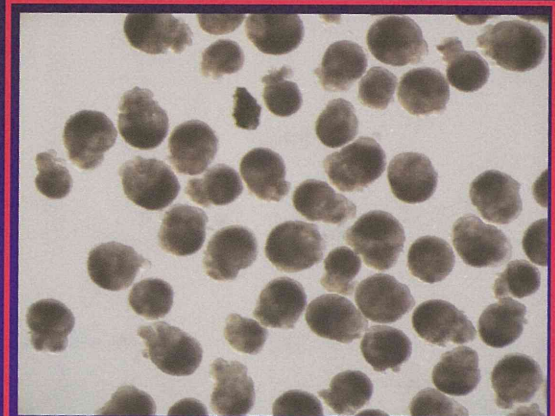
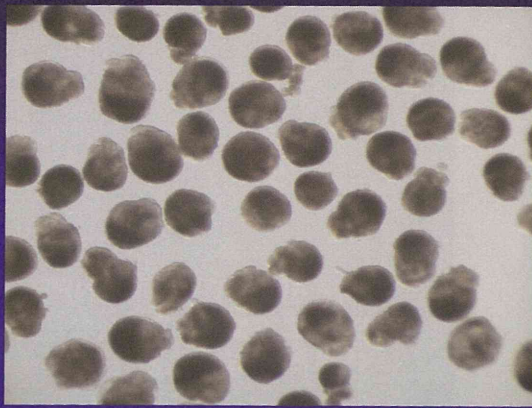


EBの実体顕微鏡写真(8d)

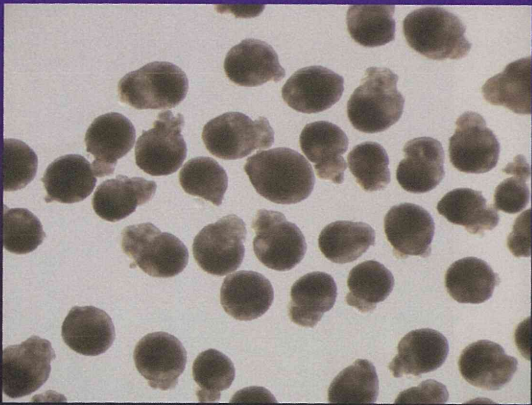
DMSO

BPA (1000nM)

Wild



Malat-1
k/o

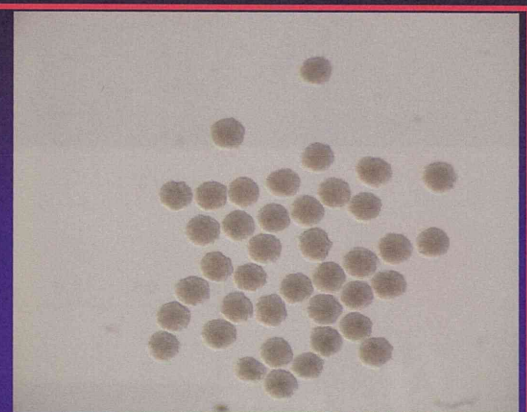
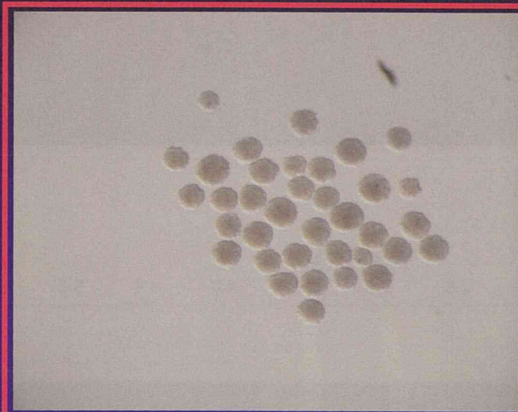


EBの実体顕微鏡写真(2d)

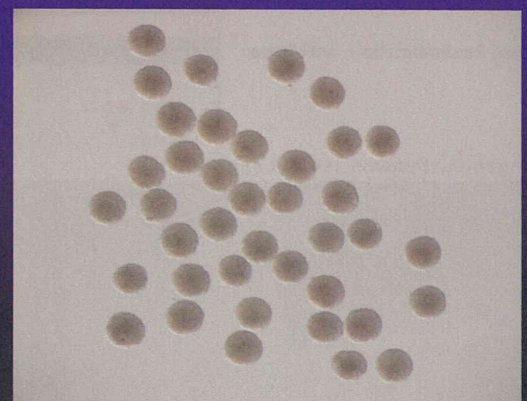
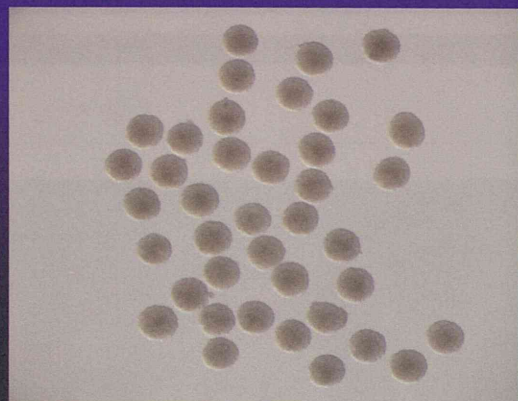
DMSO

BPA (1nM)

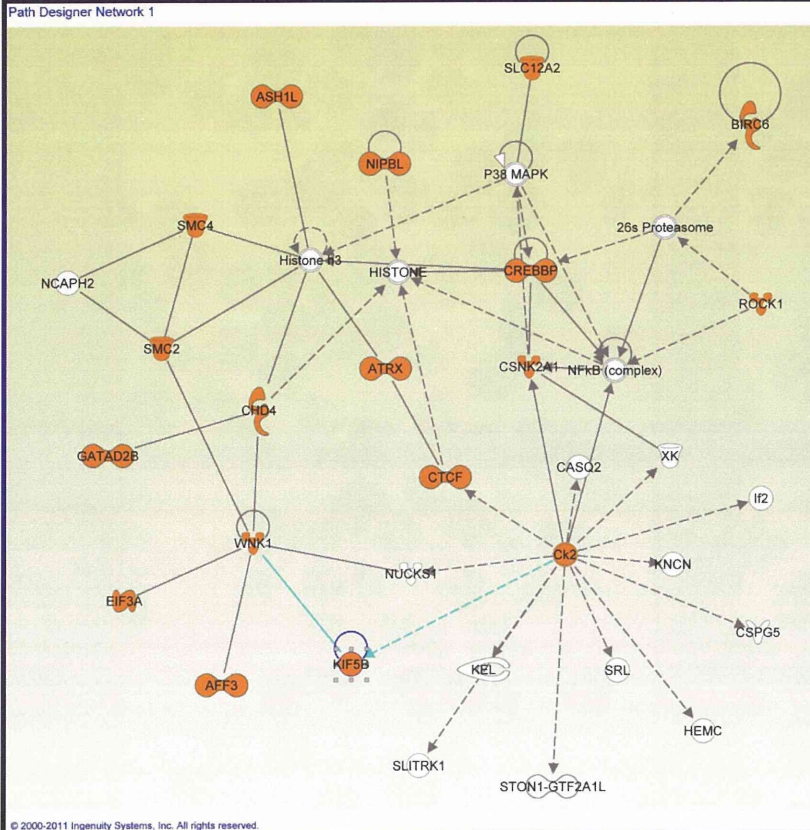
Wild



Malat-1
k/o

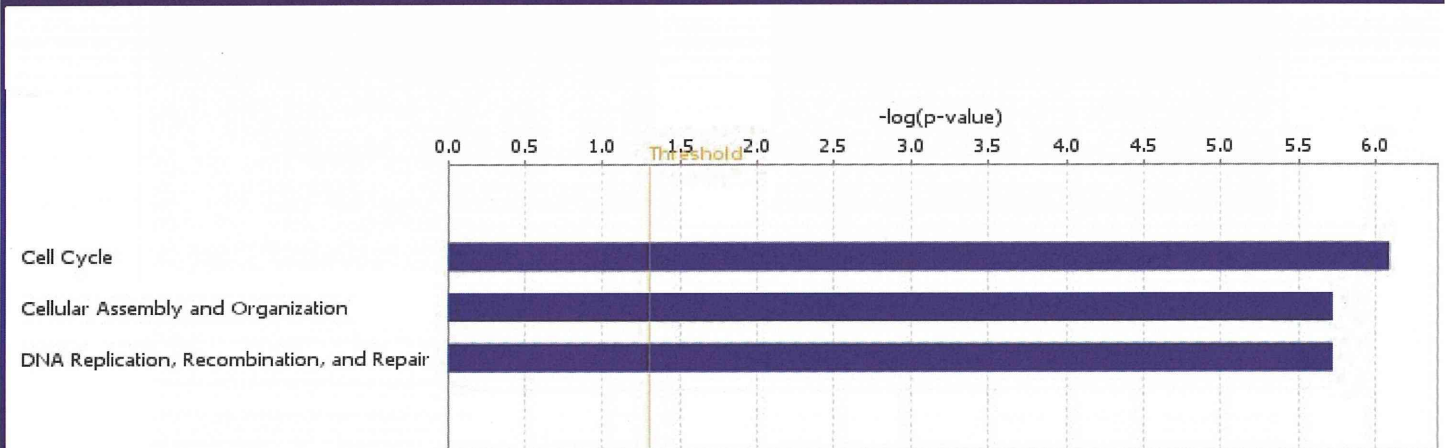


選択した遺伝子のIPAによるパスウェイ解析結果



→ AKAP9, ATRX, CENPE, ESCO1, SMC2, SMC4, WNKなど染色体分離に関与する遺伝子が多い

選択した遺伝子のIPAによるパスウェイ解析結果



ヒト-マウス間で
保存性の高い上流配列
約10kb
(制御配列がある可能性高い)

マウス19番染色体

Malat-1

Malat-1-ルシフェラーゼレポーター
(培養細胞へ導入)

Luciferase

BPA添加によりルシフェラーゼ発現が
上昇することを確認

BPA

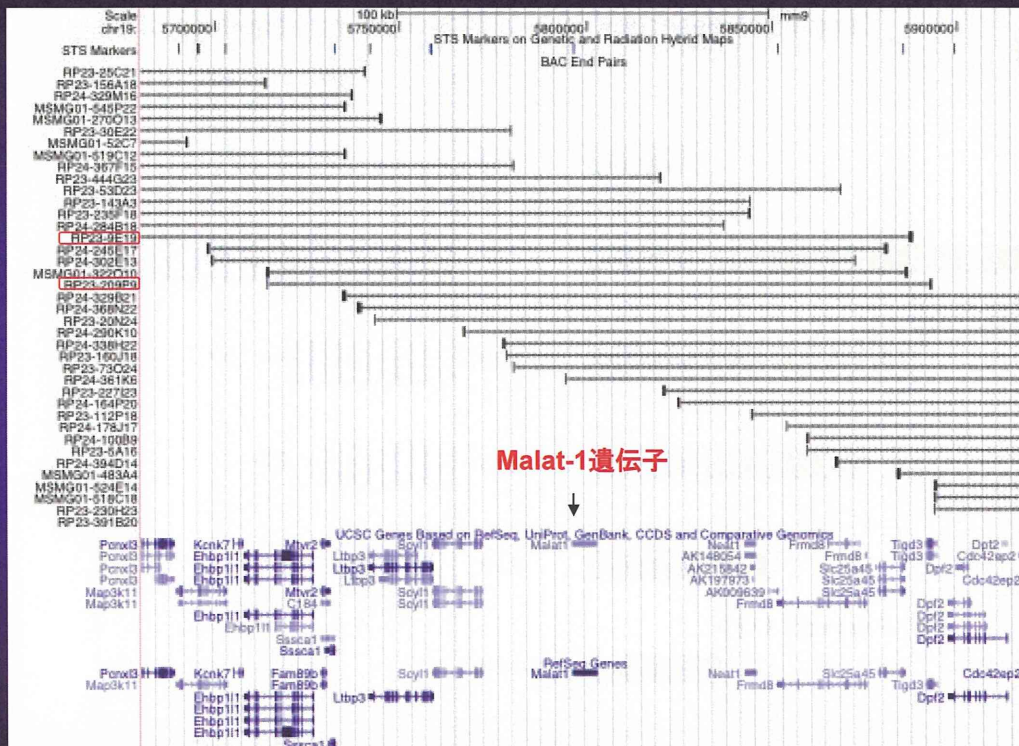
Luciferase

欠失, 変異導入により
BPA応答配列を同定

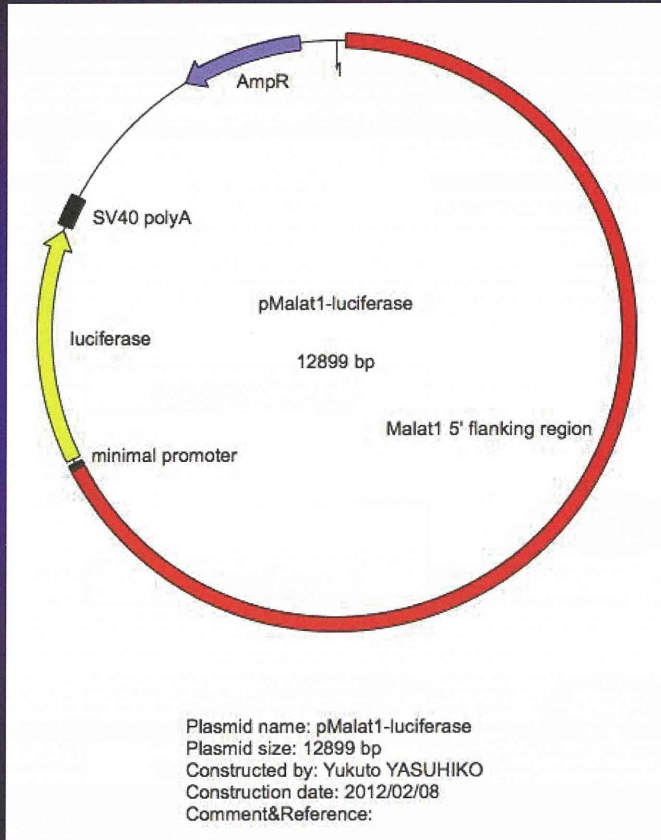
BPA

Luciferase

UCSC Genome Browser からBAC cloneの選択



完成したconstruct



C57BL/6マウスのMalat-1遺伝子およびその上流・下流領域を含むBAC(細菌人工染色体)クローン(RP23-9E19)をIn vitrogen社から入手し、Malat-1上流9kbを制限酵素EcoRIおよびNheIで切り出してレポーターベクターpGL4-23(プロメガ社)に挿入し、ベクターを構築した。

Cos-7細胞を用いたルシフェラーゼアッセイの結果

Sequential dissection of 5'- and 3'- flanking sequence of Malat-1

